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# Maternal obesity: Associations between obesity, pregnancy complications, and maternal and newborn metabolome

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ACADEMIC DISSERTATION

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To all women

# Abstract

The prevalence of obesity is increasing and in this century has reached pandemic levels. Maternal obesity increases accordingly. In pregnancy, obesity is associated with a higher risk for numerous adverse outcomes, including gestational diabetes (GDM), hypertensive disorders, excessive fetal growth, and instrumental delivery. Both the mother and the offspring are also at risk for many long-term adverse outcomes, including type 2 diabetes and cardiovascular diseases. This thesis explores three areas: the association between maternal obesity and pregnancy disorders, the maternal metabolomic profile associated with obesity and pregnancy disorders, and the cord blood metabolomic profile associated with maternal obesity.

Study I assessed the association between maternal prepregnancy body mass index (pBMI) and GDM or hypertensive disorders in women's second pregnancy and evaluated how the complications of the first pregnancy modify this association. Nationwide data on all women with their first and second pregnancies in 2006–2013 (n=50 219) was extracted from the Medical Birth Register (MBR) and linked with the data of the Finnish Hospital Discharge Register. The risk of pregnancy complications increased with adiposity, even for those slightly exceeding normal weight. The magnitude of the risk for GDM or gestational hypertension (GH) was far higher, however, if the first pregnancy had been complicated. If pregnancy is the window to future health, the first pregnancy is a window to health in the second pregnancy.

Studies II–IV examined the maternal metabolomic profile of pregnancies complicated by obesity, GDM or hypertensive disorders, and the newborn cord blood metabolomic profile of maternal obesity. These studies are secondary analyses utilising data from three large Finnish studies, i.e. the Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO), the Finnish Gestational Diabetes Prevention (Raskaudiabetes ja elämäntavat, RADIEL), and the Intrauterine Sampling in Early Pregnancy (ITU) study. Study II included 741 women from PREDO and RADIEL, Study III included 755 women from PREDO and RADIEL and 490 from ITU, and Study IV included 1702 mother-child dyads from PREDO, RADIEL, and ITU. Maternal blood samples were available from three time points across pregnancy for PREDO and RADIEL, and for ITU, once during the pregnancy. Cord blood samples were collected at delivery. A nuclear magnetic resonance metabolomics platform analysed 225 metabolic measures, of which 68 (Study II), 66 (Study III), or 95 (Study IV) served as primary outcomes.

Study II assessed the longitudinal (i.e. the mean across three time points) metabolomic profile of maternal obesity, GDM, and hypertensive disorders. Compared to normal weight, maternal obesity was associated with broad perturbations. These perturbations were atherogenic, i.e. higher levels of many very-low-density lipoprotein (VLDL) -related measures, triglycerides, and apolipoprotein B (ApoB), smaller high-density lipoprotein (HDL) particles, and a lower degree of fatty acid (FA) unsaturation. The perturbations reflected insulin resistance, i.e. higher levels of branched-chain amino acids (BCAA) and aromatic amino acids (AAA), and indicated inflammation, i.e. a higher level of glycoprotein acetyls (GlycA). Compared to normal weight, the change across pregnancy in many metabolic measures with maternal obesity was smaller. The metabolomic profiles of both GDM and preeclampsia resembled the profile of obesity in terms of VLDL-related measures, triglycerides, and BCAAs. GDM and obesity were also associated with a lower degree of FA unsaturation and a higher level of GlycA. The similarities in the metabolomic profiles of maternal obesity and pregnancy disorders reflect the shared pathophysiologic mechanisms behind all of these conditions.

Study III compared the metabolomic profiles of three study groups to the profile of the control group. The control group were women with no obesity or GDM. The three study groups were women with 1) nonobesity and GDM (NOGDM), 2) obesity and GDM (OGDM) and 3) obesity alone. We were able to classify the metabolomic perturbations in three clusters: those similar in all three study groups, those shared between the groups with obesity (women with OGDM or obesity alone), and those distinguishing the two GDM subtypes (NOGDM and OGDM), but not explained by their difference in obesity. The last cluster included measures that likely reflect the differences in the pathophysiology of GDM in these women: insulin deficiency in women with no obesity and insulin resistance in women with obesity. The metabolomic perturbations were already present in early pregnancy, before the clinical diagnosis of GDM, offering the potential for detection of high-risk women for targeted interventions.

Study IV examined the associations between maternal pBMI and cord blood metabolome at delivery and between the maternal and the cord blood metabolome; it explored whether the association between maternal and respective newborn cord blood metabolic measures was modified by maternal pBMI. Higher maternal pBMI was associated in the cord blood with smaller HDL particles, a lower degree of FA unsaturation, and higher levels of BCAAs and one of the AAAs, in other words, metabolic perturbations associated with maternal obesity in Study II. Associations between 61 of 95 maternal and cord blood measures were significant. These measures spanned all different classes of metabolites, independent of transportation of the metabolite across the placenta. Maternal BMI modified the association between maternal and cord blood levels of many lipid measures. The association was stronger in maternal obesity. This may be of importance because lipids are strong determinants of fetal growth.

This study emphasises the significance of maternal obesity in the development of metabolic derangements and in determining the metabolic milieu of the fetus. Maternal metabolomic derangements associated with obesity are in part reflected in the cord blood metabolome. Some of these derangements may offer potential for interventions during pregnancy. However, the presence of the derangements already in early pregnancy highlights the importance of weight management before pregnancy.

# Tiivistelmä

Ylipaino ja lihavuus lisääntyvät jatkuvasti; pandemian mittasuhteet on saavutettu 2000-luvulla. Raskaudenaikainen lihavuus lisääntyy vastaavasti. Lihavuus lisää useiden raskaushäiriöiden, kuten raskausdiabeteksen, kohonneen verenpaineen, sikiön liiallisen kasvun ja toimenpidesynnytysten riskiä. Äidin ja lapsen pitkän aikavälin komplikaatioiden, kuten tyypin 2 diabeteksen ja sydän- ja verisuonisairauksien riski lisääntyy. Tämä väitöskirjatutkimus tarkastelee äidin lihavuuden yhteyttä raskaushäiriöihin, äidin metabolista profiilia lihavuudessa ja raskaushäiriöissä, sekä lapsen napaveren metabolista profiilia äidin lihavuuden yhteydessä.

I osatyössä tutkittiin äidin toisessa raskaudessa yhteyttä raskautta edeltävän painoindeksin ja raskausdiabeteksen sekä verenpainesairauksien välillä ja arvioitiin, kuinka edellisen raskauden komplikaatiot vaikuttavat tähän yhteyteen. Tutkimukseen otettiin mukaan kaikki Syntymärekisteriin kirjatut, vuosina 2006–2013 Suomessa sekä ensimmäisen että toisen lapsensa synnyttäneet naiset (n=50 219) ja tiedot linkitettiin sairaaloiden hoitoilmoitusrekisteriin (Hilmo). Raskaushäiriöiden riski kasvoi painoindeksin noustessa kaikilla naisilla, mutta raskausdiabeteksen ja raskaudenaikaisen verenpainetaudin riski oli huomattavasti korkeampi, jos näitä häiriöitä oli esiintynyt myös ensimmäisessä raskaudessa. Jos raskaus on ikkuna naiseen terveyteen, ensimmäinen raskaus on ikkuna terveyteen toisessa raskaudessa.

Osatöissä II-IV tarkasteltiin äidin metabolista profiilia lihavuuden, raskausdiabeteksen ja verenpainesairauksien komplisoimissa raskauksissa sekä napaveren metabolista profiilia äidin lihavuuden yhteydessä. Nämä tutkimukset ovat sekundäärianalyysejä kolmesta laajasta suomalaisesta tutkimuksesta, PREDO (Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction), Raskaudiabetes ja elämäntavat (RADIEL), ja ITU (Intrauterine Sampling in Early Pregnancy). Osatyössä II oli 741 naista PREDO- ja RADIEL-tutkimuksista, osatyössä III 755 naista PREDO- ja RADIEL-tutkimuksista ja 490 ITU-tutkimuksesta sekä osatyössä IV 1702 äiti-lapsi-paria PREDO-, RADIEL- ja ITU-tutkimuksista. Äideiltä kerättiin verinäytteet kolmesti (PREDO ja RADIEL) tai kerran (ITU) raskauden aikana. Napaverinäytteet kerättiin lapsen syntymän yhteydessä. Kaikki näytteet analysoitiin 225 metabolista muuttujaa tunnistavalla NMR-menetelmällä (nuclear magnetic resonance, ydinmagneettinen resonanssi). Näistä 225 muuttujasta 68 (osatyö II), 66 (osatyö III) ja 95 (osatyö IV) valittiin ensisijaisesti tarkasteltaviksi muuttujiksi.

II osatyössä tutkittiin äidin pitkittäistä (kolmen ajankohdan keskiarvo) metabolista profiilia lihavuuden, raskausdiabeteksen ja verenpainesairauksien komplisoimissa raskauksissa. Verrattuna äiteihin, jotka olivat normaalipainoisia, lihavuus oli yhteydessä laajoihin poikkeavuuksiin metabolisessa profiilissa. Näitä olivat aterogeeniset poikkeavuudet eli korkeammat VLDL (very-low-density lipoprotein) -partikkelien, triglyseridien ja apolipoproteiini B:n tasot, pienempi HDL (high-density lipoprotein) -partikkelien koko sekä tyydyttymättömien rasvahappojen matalampi taso. Poikkeavuudet kertoivat myös insuliiniresistenssistä, joka näkyi korkeampina haaraketjuisten ja aromaattisten aminohappojen tasoina, sekä matala-asteisesta tulehduksesta, jota kuvasti korkeampi GlycA:n (glycoprotein acetyls) taso. Äideillä, joilla oli lihavuutta, metabolinen profiili muuttui vähemmän raskauden aikana kuin äideillä, joiden paino oli normaali. Raskausdiabeteksessä ja pre-eklampsiassa nähtiin samanlaisia VLDL:n, triglyseridien ja haaraketjuisten aminohappojen tasoja kuin lihavuudessa. Raskausdiabeteksessä myös tyydyttymättömien rasvahappojen taso oli matalampi ja GlycA:n taso korkeampi samaan tapaan kuin lihavuudessa. Samankaltaisuudet lihavuuden ja raskaushäiriöiden metabolisessa profiilissa kuvastavat samoja patofysiologisia mekanismeja häiriöiden takana.

III osatyössä verrattiin kolmen tutkimusryhmän metabolista profiilia kontrolliryhmän profiilin. Kontrolliryhmän naisilla ei ollut lihavuutta eikä raskausdiabetesta. Tutkimusryhmät olivat naiset, joilla oli 1) raskausdiabetes mutta ei lihavuutta (NOGDM), 2) sekä raskausdiabetes että lihavuutta (OGDM) ja 3) vain lihavuutta, mutta ei raskausdiabetesta. Löydetyt metaboliset häiriöt oli mahdollista luokitella kolmeen ryhmään: häiriöt, jotka olivat yhteisiä kaikille kolmelle tutkimusryhmälle, häiriöt, joita ilmeni vain niissä kahdessa ryhmässä, joissa naisilla oli lihavuutta, ja häiriöt, jotka olivat erilaisia kahden raskausdiabetesta sairastavan ryhmän (NOGDM ja OGDM) välillä, mutta joita ei selittänyt ryhmien välinen ero lihavuudessa. Viimeksi mainittu ryhmä metabolisia muuttujia olivat niitä, jotka kuvastavat eroja raskausdiabeteksen patofysiologiassa: Naisilla, joilla ei ole lihavuutta, tautia aiheuttaa useammin riittämätön insuliinin eritys, kun taas naisilla, joilla on lihavuutta, tautia selittää insuliiniresistenssi. Metaboliset häiriöt ilmenivät jo alkuraskaudessa ennen raskausdiabeteksen diagnosointia. Tämä voi tarjota mahdollisuuksia varhaiselle diagnostiikalle ja interventioille.

IV osatyössä tutkittiin äidin raskautta edeltävän painoindeksin ja raskaudenaikaisen metabolomin yhteyttä lapsen napaveren metabolomiin. Lisäksi arvioitiin, muokkaako äidin raskautta edeltävä painoindeksi äidin metabolisten muuttujien yhteyttä lapsen vastaaviin. Korkeampi äidin painoindeksi oli yhteydessä lapsen pienempiin HDL-partikkeleihin, matalampaan tyydyttymättömien rasvahappojen tasoon sekä korkeampaan haaraketjuisten ja aromaattisten aminohappojen tasoon. Nämä häiriöt ovat samoja, joita todettiin äidillä lihavuuden metabolisessa profiilissa osatyössä II. Tutkituista 95 muuttujasta 61 muuttujaa oli samansuuntaisia äidin raskaudenaikaisessa ja lapsen napaveren metabolomissa. Samansuuntaisuutta todettiin kaikissa eri muuttujien alaluokissa, riippumatta siitä läpäisevätkö kyseiset metaboliitit istukan. Äidin painoindeksi muokkasi yhteyttä äidin ja lapsen rasva-aineisiin liittyvien muuttujien tasoissa. Tällä voi olla merkitystä, sillä rasva-aineet vaikuttavat voimakkaasti sikiön kasvuun.

Tutkimus korostaa äidin lihavuuden merkitystä metabolisten häiriöiden synnyssä ja sikiöaikaisen ympäristön muokkaamisessa. Äidin metaboliset häiriöt heijastuvat lapsen napaveren metabolomiin. Osa häiriöistä voi tarjota mahdollisuuksia interventioille. Metabolisten häiriöiden esiintyminen jo alkuraskaudessa korostaa kuitenkin painonhallinnan tärkeyttä ennen raskautta.

# List of original publications

This thesis is based on the following publications, which in the text are referred to by their Roman numerals:

I Sormunen-Harju H, Koivusalo S, Gissler M, Metsälä J. The risk of complications in second pregnancy by maternal BMI: The role of first-pregnancy complications, pregestational diabetes and chronic hypertension. Acta Obstet Gynecol Scand 2020;00:1-8

II Kivelä J, Sormunen-Harju H, Girchenko PV, Huvinen E, Stach-Lempinen B, Kajantie E, Villa PM, Reynolds RM, Hämäläinen EK, Lahti-Pulkkinen M, Murtoniemi KK, Laivuori H, Eriksson JG, Räikkönen K, Koivusalo SB. Longitudinal metabolic profiling of maternal obesity, gestational diabetes and hypertensive pregnancy disorders. J Clin Endocrinol Metab 2021;106(11):e4372-e4388

III Sormunen-Harju H, Huvinen E, Girchenko PV, Kajantie E, Villa PM, Hämäläinen EK, Lahti-Pulkkinen M, Laivuori H, Räikkönen K, Koivusalo SB. Metabolomic Profiles of Nonobese and Obese Women With Gestational Diabetes. J Clin Endocrinol Metab. 2023 Oct 18;108(11):2862-2870.

IV Sormunen-Harju H, Girchenko PV, Kajantie E, Villa PM, Hämäläinen EK, Huvinen E, Lahti-Pulkkinen M, Laivuori H, Räikkönen K, Koivusalo SB. Associations Between Maternal Prepregnancy Body Mass Index and Maternal and Cord Blood Metabolome. Submitted 2024.

# Abbreviations

| | |
|---|---|
| AA | Amino acid |
| AAA | Aromatic amino acid |
| ASA | Acetylsalisylic acid |
| ApoA-1 | Apolipoprotein A-1 |
| ApoB | Apolipoprotein B |
| BCAA | Branched-chain amino acid |
| BMI | Body mass index |
| CI | Confidence interval |
| DHA | Docosahexaenoic acid |
| FA | Fatty acid |
| FFA | Free fatty acid |
| GDM | Gestational diabetes (mellitus) |
| GH | Gestational hypertension |
| GlycA | Glycoprotein acetyls |
| GW | Gestational week |
| HAPO | Hyperglycemia and Adverse Pregnancy Outcome Study |
| HDL | High-density lipoprotein |
| HT | Chronic hypertension |
| IQR | Interquartile range |
| ITU | InTraUterine Sampling in Early Pregnancy Study |
| IUGR | Intrauterine growth restriction |
| LA | Linoleic acid |
| LDL | Low-density lipoprotein |
| MBR | Medical Birth Register |
| MUFA | Monounsaturated fatty acid |
| NMR | Nuclear magnetic resonance |
| NOGDM | Women with no obesity, but GDM (in Study III) |
| OGDM | Women with obesity and GDM (in Study III) |
| OGTT | Oral glucose tolerance test |
| OR | Odds ratio |
| pBMI | Prepregnancy body mass index |
| PE | Preeclampsia |
| PREDO | Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction Study |
| PUFA | Polyunsaturated fatty acids |
| RADIEL | Finnish Gestational Diabetes Prevention Study (Raskausdiabeteksen ennaltaehkäisy elintavoin) |
| SD | Stardard deviation |
| SFA | Saturated fatty acid |
| TNF-α | Tumour Necrosis Factor Alpha |
| VDAART | Vitamin D Antenatal Asthma Reduction Trial |
| VLDL | Very-low-density lipoprotein |

# 1 Introduction

Obesity is increasing at an alarming rate both globally [1] and in Finland. Lifestyle changes, availability of calorie-rich nutrition, diminished physical activity, genetic predisposition, and other factors, including stress and hormones, have resulted in nearly a third of Finnish adults living with obesity [2]. Obesity is not evenly distributed in society but is more common alongside lower socioeconomic status and among ethnic minorities [3]. Numerous factors contribute to the difference in prevalence between the socioeconomic groups; among them may be unequal opportunities to purchase healthy food, to exercise, or to receive treatment for obesity. The price gap between healthy and unhealthy food may be higher in resource-poor countries, resulting in their increasing rate of obesity [4]. Solutions to the growing epidemic of obesity must be sought at all levels, from individual to governmental. National policies involving advertising restrictions and implementation of carefully designed taxes to reduce the consumption of unhealthy food and drinks could potentially contribute to obesity prevention [5].

Included in the increase in overall obesity is maternal obesity. It is associated with a variety of pregnancy disorders [6], with lower health-related quality of life [7], and with long-term complications for both the mother and the developing child [8]. Obesity creates a vicious cycle: an increased risk of the offspring's already developing obesity in childhood, and girls' childhood obesity becoming first adolescent and then maternal obesity. Paternal obesity can also affect fetal growth and have an adverse impact on pregnancy outcomes [9]. The approach to preventing childhood obesity should not be limited by gender.

Exploring the association between obesity in pregnancy and the complications that it drives must start with viewing the enormous metabolic changes occurring in pregnancy. These changes arise from the needs of the growing, developing fetus; the mother must supply all the necessary nutrients across the placenta, a unique organ that both connects and separates these two living organisms. Both the placenta and the fetus rely on a continuous flux of nutrients from the mother. This flux of nutrients may, however, exceed the needs and become harmful to the fetus.

Metabolomics is a relatively new technological tool used to detect profiles and measure levels of low molecular weight metabolites in any tissue in response to genetic variation, to physiological condition, or to a disease process [10]. Various health outcomes and diseases in both non-pregnant and pregnant populations have been associated with changes in metabolomic profiles. Metabolomics may aid in finding predictive biomarkers or in recognising the mechanisms behind pathophysiology.

This thesis begins by exploring the association between maternal pBMI and pregnancy disorders in the second pregnancy (Study I). The association between maternal obesity and pregnancy disorders has been extensively studied, but data has been lacking as to how the presence of these disorders in the first pregnancy or pre-existing conditions like maternal type I diabetes modifies this association in the second pregnancy.

Studies II and III explore longitudinal metabolomic profiles of obesity and obesity-associated pregnancy complications, including GDM and hypertensive disorders. Many studies have been cross-sectional, but in Studies II and III, three maternal blood samples during pregnancy were available, and the change in the profiles relative to normal pregnancy is assessed in Study II. Study III introduces a novel setting in exploring metabolomic profiles, because it stratifies women with GDM in those with or without obesity.

In the last Study, this thesis explores how maternal obesity is reflected in the cord blood metabolomic profile. This involves a meta-analysis of three large Finnish cohorts, creating the largest study to explore the association between maternal adiposity and cord blood measures. The novelty of Study IV also arises from its aim to learn whether the association between maternal and cord blood measures varies according to maternal obesity.

# 2 Review of the literature

## 2.1 Obesity and pregnancy complications

### 2.1.1 Definition and epidemiology of obesity

Body mass index (BMI) is calculated as mass in kilograms divided by height in square meters. BMI correlates moderately with direct measures of body fat and strongly with metabolic risk factors and disease outcomes [11]. In adults, a BMI of 30 kg/m$^2$ is considered the threshold of obesity, and a BMI of 25 to 29.9 kg/m$^2$ is classified as overweight (**Table 1** [12]).

**Table 1.** *WHO body mass index (BMI) classification.*

| BMI (kg/m$^2$) | Classification |
|---|---|
| <18.5 | Underweight |
| 18.5–24.9 | Normal weight |
| 25–29.9 | Overweight |
| 30–34.9 | Class I obesity |
| 35–39.9 | Class II obesity |
| ≥40 | Class III obesity |

The prevalence of obesity is increasing, and it is predicted that by 2030, globally, 20% of women and 15% of men will be living with obesity, while in 2010, the figures were 14% and 9%. The increase in obesity is not limited to adults, but among children aged 5–9 years, globally, 11% is predicted to be living with obesity in 2030 [1]. Childhood obesity persists; 55% of children with obesity go on to live with obesity in adolescence, and 80% of adolescents will still be living with obesity in adulthood [13].

In many countries, population-wide statistics of maternal obesity are not available, and reported figures are estimates based on surveillance data of women of reproductive age or on cohort studies. A steady increase in the prevalence has been reported in Europe (**Figure 1**). In the United States, the Centers for Disease Control and Prevention reported in 2019 a prepregnancy obesity (BMI≥30 kg/m$^2$) rate of 29 % [14]. A National Maternity and Perinatal Audit in the UK in 2015–2017 presented a rate of 22% [15]. Maternal obesity is becoming a public health challenge also in low-resource countries; in India, a prevalence of 12% was estimated in 2015–2016 [16], and in Africa, up to 2014, prevalences were reported ranging from 6.5% to 50.7% [17].

The Medical Birth Register (MBR) collects information in Finland on all deliveries and on maternal BMI. The prevalence of prepregnancy obesity has increased between 2006 and 2022 from 11% to 19.5% (**Figure 2**). The increase has been faster in Finland than in Sweden, as the prevalence has increased from 2010 to 2022 from 13.2% to 19.5% in Finland and 13.7% to 18% in Sweden [18]. Obesity is not evenly distributed in the populations, but disparities in obesity rates exist based on ethnicity and socioeconomic status [3].

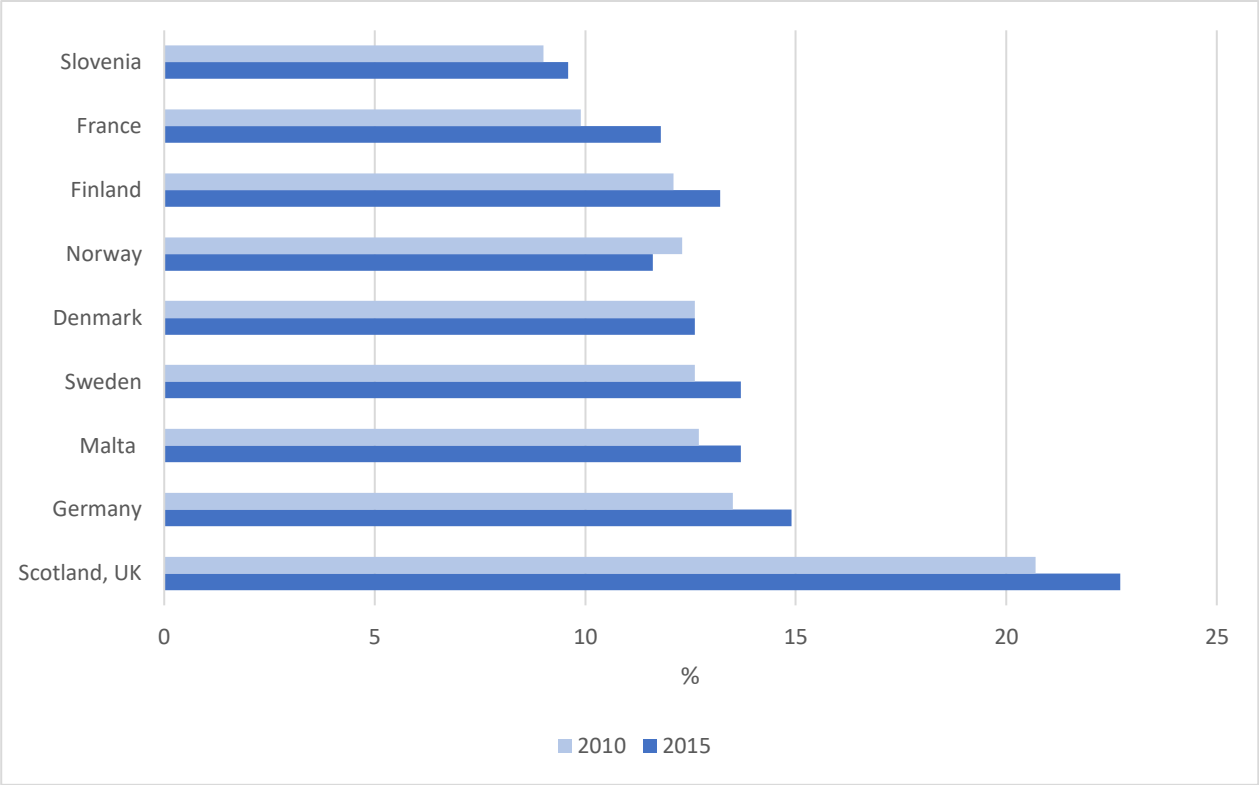



**Figure 1.** *Prevalence of prepregnancy obesity in selected European countries in 2010 and 2015 from Euro-Peristat database* [19].

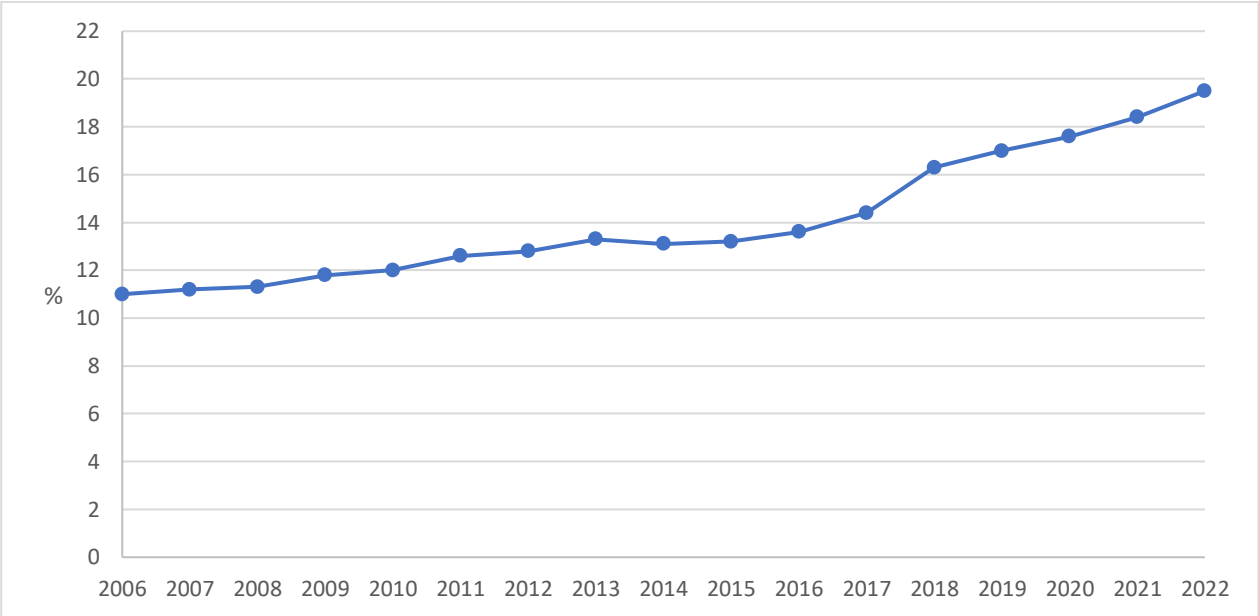



**Figure 2.** *Prevalence of prepregnancy obesity in Finland in 2006-2022 (Medical Birth Register, National Institute of Health and Welfare).*

### 2.1.2 Obesity in pregnancy

In pregnancy, BMI is calculated based on prepregnancy weight or weight measured at the first antenatal visit, and gestational weight gain is reported separately. Entering pregnancy with pre-existing obesity is associated with various complications, and if the weight gain in pregnancy is nonoptimal, the risk of pregnancy complications is further increased [20]. With normal weight, the recommended gestational weight gain is 11.5–16 kg; with overweight, 7–11.5 kg; and with any class obesity, 5–9 kg [21]. In addition to the growing fetus and the placenta, gestational weight gain consists of amniotic fluid, increased blood and fluid volume, and an increase in maternal fat mass. Weight gain above the guidelines increases fat mass [22]. Likelihood of postpartum weight retention increases with higher prepregnancy BMI (pBMI), [23, 24], and of women with obesity in pregnancy, only about 11% return to their prepregnancy weight within 5 years postpartum [25]. As postpartum BMI becomes the pBMI of subsequent pregnancy, a vicious cycle is created.

Preconception weight management improves metabolic health and fertility [26], but the benefit may not extend over the entire course of pregnancy as rebound weight gain often follows weight loss [27]. Clinical practice guidelines for the management of women with prepregnancy obesity recommend counselling on healthy diet and exercise in early pregnancy. Still, diet and lifestyle interventions aiming to control weight gain during pregnancy in women with overweight or obesity have often not been successful in improving pregnancy and birth outcomes [28].

## 2.2 Short-term pregnancy complications with obesity in pregnancy

The various short-term complications associated with maternal obesity during pregnancy, delivery, and neonatal period are listed in **Table 2**.

**Table 2.** *Pregnancy, intrapartum, postpartum, and neonatal complications associated with maternal obesity.*

| Pregnancy | Intrapartum | Postpartum and neonatal |
|---|---|---|
| Miscarriage [6] | Preterm delivery [29] | Low Apgar score [30] |
| Multiple gestation [31, 32] | Prolonged pregnancy [33, 34] | Admission to neonatal intensive care unit [35] |
| Congenital anomalies [36–38] | Induction of labour and failure of induction [39, 40] | Thromboembolism [41] |
| Fetal death [6] | Failed attempt of vaginal birth after caesarean section [42] | Infections [39, 43] |
| Chest, genital tract, and urinary infections [44–47] | Obstructed labour [48] | Postpartum hemorrhage [39] |
| Cholecystitis [49] | Shoulder dystocia [50, 51] | Infant death [52] |
| Gestational diabetes [6, 53–55] | Instrumental delivery [39] | Shorter duration of breastfeeding [56, 57] |
| Gestational hypertension [54] and preeclampsia [58, 59] | Caesarean section [60, 61] | Postpartum weight retention [62] |
| Thromboembolism [41] | Uterine scar rupture after previous caesarean section [63] | |
| Macrosomia or large for gestational age [54, 64, 65] | | |
| Mental disorders [66] | | |
| Obstructive sleep apnoea [67] | | |

### 2.2.1 Gestational diabetes (GDM)

GDM is defined as glucose intolerance first detected during pregnancy. The Finnish Current Care Guideline for screening was introduced in 2008, and a 2-hour 75 g oral glucose tolerance test (OGTT) is recommended for all pregnant women except those considered at low risk. Among primiparous women, low risk is defined as age less than 25, normal weight, and no family history of type 2 diabetes, and among multiparous women, as age less than 40, BMI less than 25 kg/m$^2$, and no prior GDM or delivery of a large-for-gestational-age newborn. OGTT thresholds are 5.3, 10.0, and 8.6 mmol/l. The test is performed at 24-28 gestational weeks (GW), but screening is recommended already at trimester 1 for women at high risk (previous GDM; BMI ≥ 30 kg/m$^2$, or weight circumference more than 90 cm; glucosuria in early pregnancy; type 2 diabetes of a parent, a sibling, or a child; oral glucocorticoid treatment; or non-alcoholic fatty live disease). [68]

In Finland, in 2019, OGTT was performed in 66% of pregnancies. The incidence of GDM has increased concomitantly with the increase in obesity, from 8.5% in 2006 to 21% in 2019 (**Figure 3**). Globally, reported incidences of GDM vary between 1 and 28% [69], depending on ethnicity and the diagnostic criteria. Nearly half of GDM cases are attributable to overweight and obesity and could be avoided by prophylaxis of maternal obesity [70].

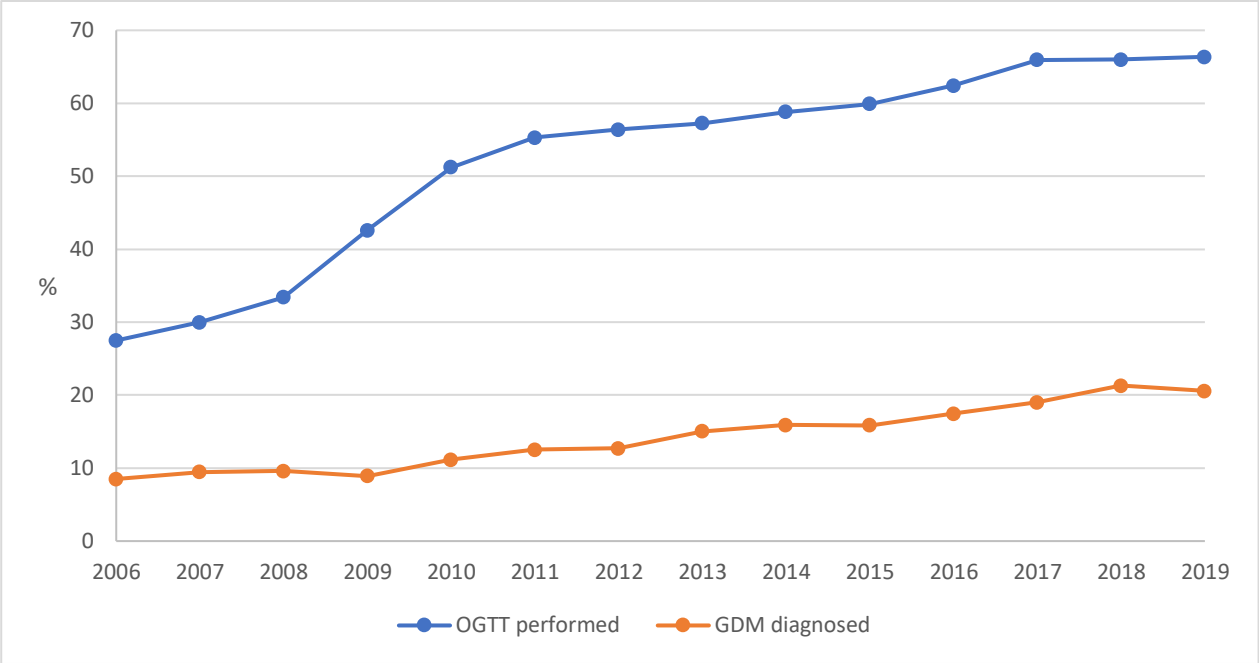



**Figure 3.** *Percentage of pregnancies in which OGTT was performed and prevalence of GDM in Finland in 2006-2022*

Treatment of GDM may improve pregnancy outcomes, and the first-line therapy for GDM is dietary modification. Glucose target levels in the Finnish guideline [68] are fasting blood glucose under 5.5 mmol/l and postprandial (one hour after meal) under 7.8 mmol/l. Medical treatment is initiated if the targets are not met with nutritional therapy and moderate exercise. Insulin has traditionally been the first-line treatment, but the growing body of evidence of metformin [71] during pregnancy has increased its use. Reports of lower birth weight and accelerated postnatal growth resulting in higher mid-childhood BMI after metformin-treated compared to insulin-treated GDM have raised concern about the long-term offspring safety of intrauterine metformin exposure [72]. A recently published Finnish randomised controlled trial detected no differences in anthropometric measures at the age of 9 years between children exposed to metformin vs insulin in utero [73], but the lipid profile of the children was more favourable in the metformin group. Another recent randomised controlled trial among women with obesity (only 16 % with GDM) reported a beneficial cardiovascular effect - lower central hemodynamic and cardiac diastolic indices - in the offspring of women treated with metformin vs placebo [74]. Studies extending to adulthood will be needed to gain more knowledge on long-term consequences for offspring.

GDM increases the risk for several adverse events during pregnancy, many of which, including macrosomia [75], caesarean section [76], and shoulder dystocia [77, 78], complicate pregnancy with maternal obesity even in the absence of GDM. The risk for polyhydramnios [79], fetal cardiomyopathy [80] and neonatal hyperbilirubinemia or hypoglycemia [77, 81] is increased with GDM.

### 2.2.2 Hypertensive disorders in pregnancy

Chronic hypertension (HT) in pregnancy is defined as maternal hypertension (systolic/diastolic blood pressure ≥140/90 mmHg) or the use of antihypertensive medication before GW 20 [82]. Maternal

hypertension developing after GW 20 is considered gestational hypertension (GH), and preeclampsia (PE) is defined as maternal hypertension with proteinuria or, in the absence of proteinuria, with thrombocytopenia, impaired liver function, newly developed renal insufficiency, pulmonary edema, or neurological symptoms [83]. Finnish Current Care Guideline [84] defines also fetal growth restriction in the presence of maternal hypertension as PE. Superimposed PE refers to women with HT developing PE.

In the United States, hypertensive disorders, including GH and PE, are estimated to affect 5–10% of pregnancies [85], and a HT prevalence of 0.9–1.5% in pregnancy has been reported [86]. In Finland, among primiparous women, the incidence of PE in 2006–2013 was 4 %, and the incidence of GH was 6 % [87].

Treatment of PE is limited to symptomatic management and early delivery of the fetus. Isolated or PE-associated hypertension can be treated with beta-blockers (often labetalol) or calcium channel blockers (often nifedipine), and intravenous magnesium sulphate is used for severe PE. Continued fetal and maternal evaluation is needed to balance the fetal and maternal risks and to determine the timing of delivery. Neonatal complications may arise from intrauterine growth restriction (IUGR) [88] or preterm delivery [89], the latter of which may be iatrogenic, needed to halt the progress of the disease, or spontaneous, caused by the underlying disease process.

Finnish Current Care Guideline recommends using acetylsalicylic acid (ASA) 100 mg/day from GW 12 for women with risk factors for PE [84]. The treatment should be initiated with two risk factors of the following: obesity; primiparity or time between pregnancies >10 years; age 40 or over; family history of PE; ovum donation; multiple gestation; or abnormality in one of the biomarkers in the first-trimester combination screening (PAPP-A MoM <0.4). Aspirin treatment should be initiated if one of the following risk factors is present: HT; systemic lupus erythematosus or positive antiphospholipid measurement; chronic kidney disease; type 1 or 2 diabetes; or previous pregnancy complication including PE, placental insufficiency, or placentally induced fetal death.

## 2.3 Long-term complications with maternal obesity, GDM, GH, and PE

Maternal obesity, GDM, GH, and PE are associated with many identical long-term complications for the offspring and the mother; these are listed in **Table 3**. The complications include later obesity, type 2 diabetes, and cardiovascular diseases.

**Table 3.** *Long-term offspring and maternal complications associated with maternal obesity, GDM or hypertensive disorders in pregnancy.*

| **Offspring** | **Maternal** |
|---|---|
| Childhood obesity and adverse body fat distribution [90–92] | Persistent obesity [62, 93, 94] |
| Increased BMI in adolescence and adulthood [95, 96] | Type 2 diabetes [97–101] |
| Glucose intolerance [102] or type 2 diabetes [103] | Metabolic syndrome [104] |
| Metabolic syndrome [104, 105] | Hypertension [97, 106, 107] |
| Hypertension [108] | Dyslipidemia [107] |
| Dyslipidemia [108] | Cardiovascular diseases [107, 109–111] |
| Cardiovascular diseases [112] | |
| Asthma and allergy [8] | |

## 2.4 Metabolism in pregnancy

### 2.4.1 Metabolic changes in pregnancy

Normal development and growth of the fetus depends on the great availability of nutrients, i.e. amino acids (AA), fatty acids (FA), and the primary energy substrate, glucose. Broad changes in maternal metabolism are induced to meet the fetal needs and to prepare the mother for delivery and breastfeeding.

During trimesters 1 and 2, the mother is in an anabolic state, storing nutrients for the increased need of the fetoplacental unit in late gestation and lactation. Hyperphagia increases throughout gestation [113], boosting the availability of exogenous substrates for metabolism. In early pregnancy, the activity of the pancreatic beta cells is increased, and insulin sensitivity of the whole body is unchanged or even augmented [114]. Increased insulin, estrogen and progesterone levels promote fat deposition in adipose tissue [115].

The switch into a catabolic state occurs in trimester 3, when the mother starts utilising the stored nutrients for enhanced transfer through the placenta, enabling rapid fetal growth. As insulin resistance increases, insulin-mediated suppression of hormone-sensitive lipase decreases in adipose tissue (**Figure 4**). This results in increased lipolysis and the release of greater quantities of free fatty acids (FFA) and glycerol into the circulation. Although lipolytic products are released in excess from adipose tissue, their placental transfer is low [116], and the primary fate of these products is the liver, where FFA and glycerol are synthesised into triglycerides that return to the circulation in the form of very low-density lipoproteins (VLDL). Part of the glycerol is used for gluconeogenesis and the FFA for beta-oxidation, releasing ketone bodies (ketogenesis). Both pathways, gluconeogenesis and ketogenesis, become highly accelerated during pregnancy and are also believed to be due to the progressive insulin resistance [117]. The increase in gluconeogenesis, as well as glycogenolysis, is a necessary adaptation to meet the increasing glucose requirements of the fetus and placenta. Maternal use of lipids spares glucose and AAs to the fetus, and ketone bodies can also serve as an alternative fuel for the fetus in the maternal fasting state.

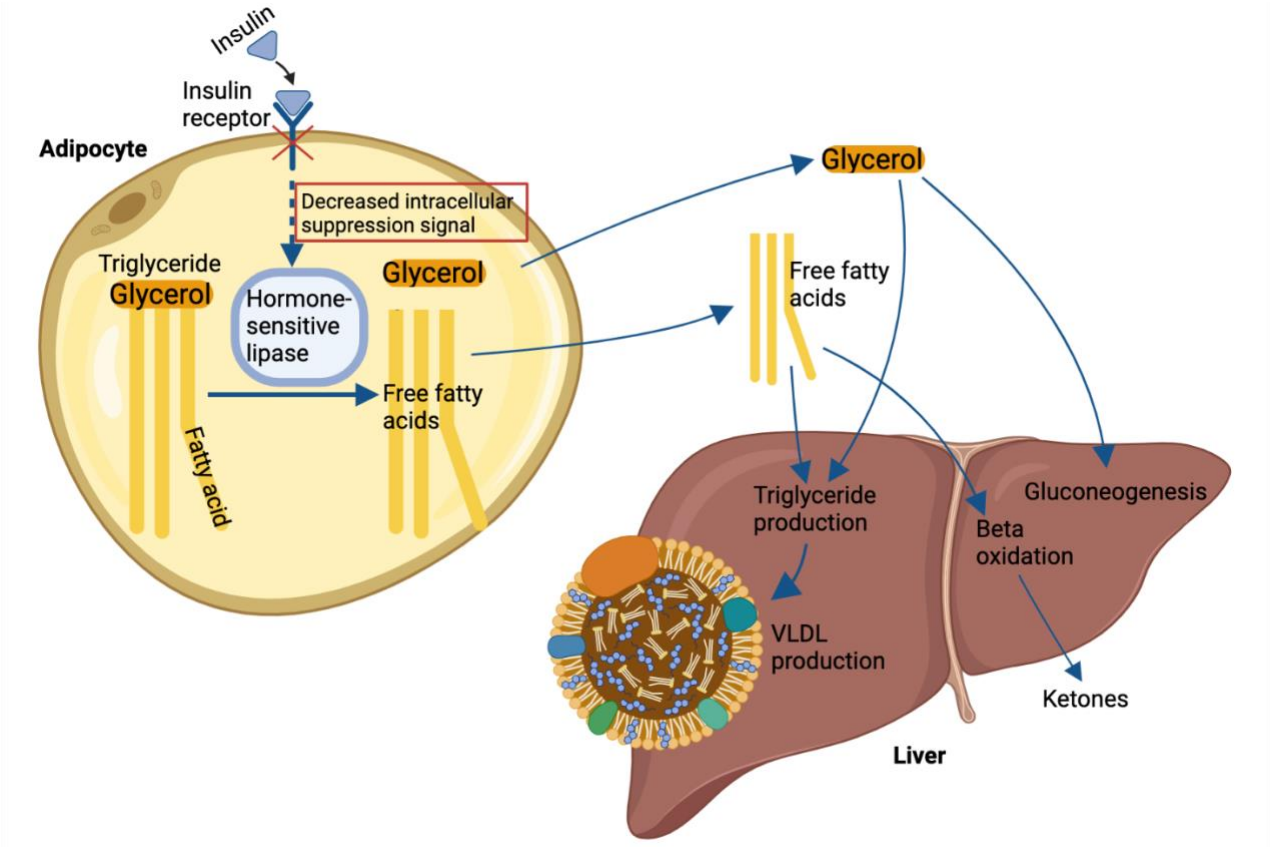



***Figure 4.*** *Insulin-resistance-mediated changes in lipolysis in pregnancy.* *Created with BioRender.com*

Insulin resistance in pregnancy, mainly expressed at the skeletal muscle and adipose tissue level, is physiological and crucial to maintaining the fuel supply to the growing fetus. Potential hormones inducing insulin resistance include estrogen, progesterone, cortisol, human placental lactogen, human placental growth hormone, tumour necrosis factor alpha (TNF-α), and adipose tissue-secreted factors such as leptin and adiponectin [114]. Insulin resistance leads to mild maternal postprandial hyperglycemia which facilitates the flux of glucose to the fetus. Fasting glucose levels decrease in late pregnancy as the fetoplacental unit utilises glucose at a rate corresponding to up to 50% of the total glucose utilised by the mother [118].

In a non-pregnant state, the concentration of triglycerides in low-density lipoproteins (LDL) and high-density lipoproteins (HDL) is lower than their concentration in VLDL. In pregnancy, HDL particles are proportionally enriched with triglycerides and the concentration of both HDL and LDL particles increases throughout pregnancy [119]. Total triglyceride concentrations increase 2- to 4-fold and total cholesterol concentrations 25% to 50% [120]. Near the time of delivery, lipoprotein lipase activity in the mammary gland is induced, circulating triglycerides are directed to this organ, and essential FAs from triglycerides are released for the breastfed infant [116].

Maternal metabolic changes spare AAs for fetal and placental use. The concentration of most AAs in the maternal circulation starts to decrease early in pregnancy. The concentration remains low throughout pregnancy [121] when protein is deposited in maternal and fetal tissues. Of the total protein gain, 40% is represented by the fetus, placenta, and amniotic fluid and the other 60% by the maternal tissues, including the uterus, breast, adipose tissue and increases in blood volume and extracellular fluid [122].

In addition to the significant changes in glucose, lipid, and AA metabolism, pregnancy induces adaptation of endothelial function [123] and generates higher levels of oxidative stress [124]. A shift towards low-grade inflammation [125, 126] is seen as increased levels of pro-inflammatory markers, including interleukin-6, TNF-α [127] and C-reactive protein [128].

### 2.4.2 The placental transport of nutrients

During the entire course of pregnancy, a human fetus is reliant on the placenta. After implantation of the fertilised egg (i.e. blastocyst), the placenta is formed from the trophoblast cells, the outer layer of the blastocyst. In term pregnancy, the placenta is a discoid organ weighing approximately 680 g [129]. The anatomical organisation of the placenta prevents direct contact of maternal and fetal blood, and thus, substrate exchange relies on diffusion channels for metabolite flux along the concentration gradient and on proteins transporting metabolites actively against the concentration gradient [130].

Maternal uterine spiral arteries undergo remodelling beginning very early in pregnancy and form large, dilated vessels in which villi of the placenta are bathed. The surface area of the villi is broad, approximately 12 $m^2$ in normal term pregnancy [131]. Maternal and fetal circulation is separated by three cellular layers: two membranes of syncytiotrophoblast lining the villi – microvillous membrane facing maternal circulation, and the basal membrane on the fetal side – and the endothelium surrounding fetal capillaries [132]. **Figure 5** presents mechanisms of the placental transport of nutrients across these three cellular layers.

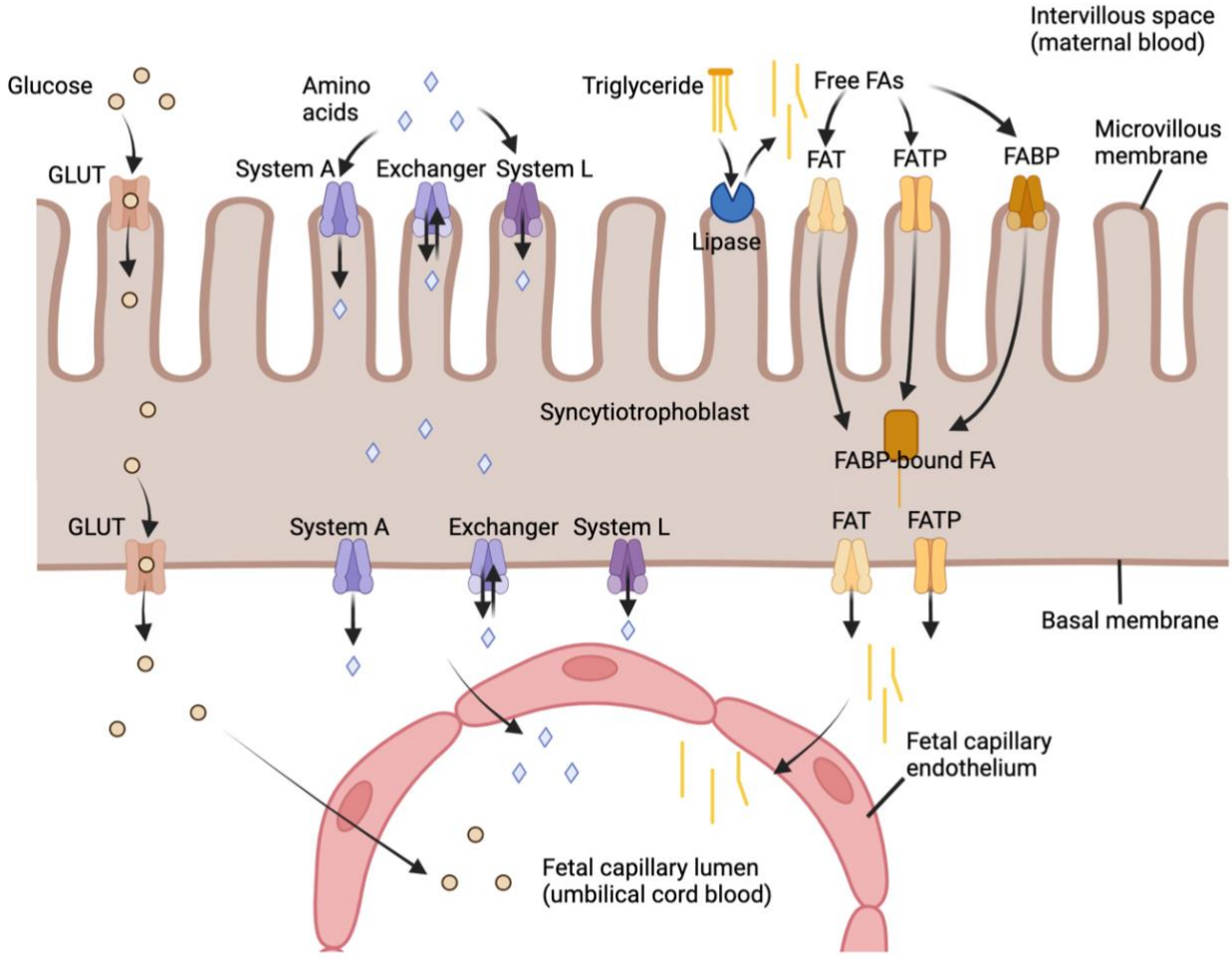



***Figure 5.*** *Nutrient transport across the placenta. GLUT=glucose transporter protein; FA=fatty acid; FAT=fatty acid translocase; FATP=fatty acid transporter protein; FABP=fatty acid binding protein.* *Created with BioRender.com*

Glucose from the maternal side crosses the placenta along the concentration gradient by facilitated diffusion without energy consumption. Glucose transporter protein 1 is the primary placental glucose transporter, as it is the only isoform of the glucose transporter family abundantly expressed throughout pregnancy [132], whereas glucose transporter proteins 3 and 4 play a role in early pregnancy. Of the glucose taken up from maternal blood, 30% is consumed by the placenta to support the active process of AA transport [133]. With advancing gestation, an increase in maternal insulin resistance with increased difference between maternal and fetal glucose concentrations [134] facilitates fetal glucose uptake.

Contrary to glucose concentrations in maternal and fetal circulation, the concentration of AAs is higher in fetal than maternal blood due to the active transport of AAs. The placenta expresses 15 different AA transporters [135], of which the two most studied are System A and System L. System A transports small neutral AAs, such as alanine, glycine and serine, and System L large neutral AAs by exchanging non-essential AAs for essential AAs with branched or large side chains, such as leucine [136]. AA transport activity seems to be regulated to protect the placenta and fetus from under or overnutrition [130]. Smaller babies in otherwise normal pregnancies have higher transport activity

[137], and in a mouse model, similar adaptive regulation has been detected in glucose transport activity [138].

Maternal circulating ketone bodies easily cross the placenta by simple diffusion [139] along the concentration gradient. Ketones are also produced by the placenta, as enzymes linked to ketogenesis have been found in the placenta [140]. Unlike in adults, ketones are an important fuel for the fetal brain and potentially an essential component of brain development [141].

Triglycerides do not cross the placenta but are taken up by the placenta as a function of their concentration [116] and hydrolysed by lipoprotein lipase and endothelial lipase [142–144]. Lipoprotein lipase activity increases with advancing gestation [145]. Endothelial lipase can also hydrolyse HDL, LDL and VLDL lipids [142, 146]. Released FFAs are taken and transported across syncytiotrophoblast with fatty acid transport proteins, fatty acid translocase, plasma membrane fatty acid binding protein, and fatty acid binding proteins [147]. The expression and activity of these transport proteins are regulated by insulin, insulin-like growth factor 1 and leptin [145, 148, 149]. Despite the enhanced release of FFAs from maternal adipose tissue into the circulation, their placental transfer is relatively low. In early gestation, fetal lipids are derived from maternal FFAs and glycerol crossing the placenta, but de novo synthesis in fetal tissues later in pregnancy increases [150].

Cholesterol is a precursor for bile acids and steroid hormones and an essential component of cell membranes, thus playing a pivotal role in fetal development. It is yet to be elucidated whether fetuses use cholesterol from the mother to support their development or whether they entirely rely on their endogenous cholesterol production. Evidence of maternal cholesterol transport comes from Smith-Lemli-Opitz syndrome, a condition with an inability to synthesise cholesterol [151]. Fetuses with the condition develop to term supported by placental transport and are born with low sterol levels.

In addition to the placenta's important role in supplying nutrients to the fetus, the placenta is a central metabolic and endocrine organ in pregnancy. It produces several metabolic proteins (leptin and adiponectin), peptide hormones (human chorionic gonadotropin, placental corticotropin-releasing hormone, ghrelin, human placental lactogen, and human placental growth hormone), cytokines (interleukin 6 and TNF-$\alpha$), and steroid hormones (progesterone and estrogen), all of which regulate glucose and lipid metabolism and adaptation of the metabolism throughout pregnancy [115, 152].

### 2.4.3 Metabolic characteristics of a pregnancy affected by maternal obesity

Obesity in the non-pregnant population is associated with insulin resistance [153], low-grade inflammation [154], and dyslipidemia [155] – conditions observed in normal pregnancy. Thus, women with obesity enter pregnancy with these metabolic derangements normally induced by pregnancy [154]. Adipose tissue is metabolically active with metabolic, endocrine and immune functions [156]. An inflammatory milieu evolves with increasing obesity, and cytokine production by macrophages in the adipose tissue affects post-receptor insulin signalling, resulting in increased insulin resistance [157]. An increase in insulin resistance by 50 to 60% due to pregnancy further increases this metabolic stress in women with obesity [158].

With maternal obesity, increased insulin secretion due to insulin resistance affects early placental growth and gene expression [159]. Moreover, due to insulin resistance, an early pregnancy anabolic state with enhanced lipogenesis is not present, but lipolysis predominates in both early and late gestation [117], elevating circulating FFA levels throughout pregnancy. A decrease in hepatic insulin sensitivity leads to reduced suppression of hepatic glucose production.

Leptin is a satiety signalling peptide that decreases energy storage and increases energy mobilisation in the periphery. Circulating leptin levels gradually increase throughout normal pregnancy, but the increase is greater in maternal obesity [160]. Leptin is produced in adipose tissue, but placental production is a major source of maternal circulating leptin [161]. Central leptin resistance is essential to maintain increased energy intake to support fetal growth in trimesters 2 and 3 [162]. With maternal obesity, leptin resistance appears at the placental level and may alter the expression and signalling of crucial pathways in fetal growth and development [160].

Adiponectin is synthesised almost exclusively by adipocytes but is also expressed in the placenta [163]. Adiponectin enhances insulin sensitivity in the adipose tissues, liver, and skeletal muscle. Circulating adiponectin levels decrease across pregnancy, contributing to the physiological insulin resistance [164]. In pregnancy complicated by maternal obesity and GDM, adiponectin levels are decreased [164–166], and low maternal levels are associated with fetal overgrowth [164, 165].

Human placental lactogen regulates insulin secretion in pancreatic beta-cells by stimulating their proliferation and promoting the expression of anti-apoptotic proteins [167, 168]. Obesity in pregnancy is associated with lower serum concentrations of this lactogen. Disruptions in placental lactogen secretion, both low and high levels, are associated with placental dysfunction and abnormalities in fetal growth, which may be either growth retardation or macrosomia [169].

TNF-α is essential for trophoblast turnover and differentiation and placental development. It seems to play a significant role in the development of insulin resistance [170]. Placental secretion of TNF-α increases throughout pregnancy. Maternal obesity has been associated with variable levels of TNF-α, and this is suggested to contribute to the diversity of obstetrical outcomes with obesity [171].

In normal pregnancy, placental steroidogenesis increases maternal circulating progesterone and estradiol levels 8-fold from six weeks to term. In women with obesity, estradiol and progesterone concentrations of placenta and plasma are lower than in lean women. Lower levels of these hormones are associated with adverse pregnancy events, progesterone deficiency with a higher probability of spontaneous abortion and recurrent miscarriage. [172]

### 2.4.4 Metabolic characteristics of a pregnancy affected by GDM

The pathogenesis of GDM is characterised by the inability of the maternal pancreas to respond to pregnancy's increased insulin requirements. This may be due to the magnitude of insulin resistance, defective function of pancreatic beta cells, or both. As obesity induces insulin resistance, it is a major risk factor for GDM [173]. Increased insulin resistance results in FFA concentrations higher than in normal pregnancy [117].

Despite the major role of maternal obesity in its pathogenesis, GDM is a heterogeneous condition that also affects individuals without obesity [174, 175]. The relative contribution of insulin resistance and insulin secretion defect to the pathophysiology varies according to the maternal phenotype, with the insulin-resistant subtype being more prevalent in women with obesity [176, 177]. In studies of women who develop GDM, the difference in insulin sensitivity between women with obesity or normal weight is most evident in early pregnancy and converges as the acquired insulin resistance of pregnancy increases [178].

Similar to maternal obesity, maternal circulating leptin levels are higher with GDM [179]. Higher levels have been found already in early pregnancy before the diagnosis of GDM, and early hyperleptinemia could be a predictive marker of GDM development [180]. Another predictive marker of GDM development could be a low level of adiponectin, as early pregnancy hypoadiponectinemia in normal-weight women is associated with later GDM diagnosis [181].

Whereas maternal obesity is associated with lower placental lactogen levels than in pregnancy with normal weight, GDM results in increased levels [115]. Levels different from those in normal pregnancy are associated with abnormal fetal growth; lower with growth retardation and higher with macrosomia [169].

Consistent with the association of TNF-α with insulin resistance, higher expression of TNF-α receptors is found in the placenta of women with GDM [182].

### 2.4.5 Metabolic characteristics of a pregnancy affected by hypertensive disorders

Although the cause of PE is not known, the placenta plays a central role in the pathophysiology, which is characterised by abnormalities in trophoblast invasion and remodelling of the spiral arteries. In normal pregnancy, the placenta consumes great amounts of glucose and FAs, and increasing evidence suggests these nutrients regulate placental development [183], optimising pregnancy outcomes. These observations have raised the possibility that glucose and lipid metabolism abnormalities play a role in the etiology and clinical progression of PE. Maternal obesity has a damaging impact on placental integrity and function [184], and PE may be the extreme endpoint of this. Pre-existing maternal diabetes [185] and GDM [186] increase the risk of PE. In the non-pregnant population, substantial overlap of diabetes and hypertension exist, and common pathways are thought to be obesity, inflammation, oxidative stress, and insulin resistance [187].

Similar to obesity and GDM, insulin resistance is associated with PE and is evident far before the clinical manifestations of the disease [118]. A study collecting subcutaneous and visceral adipose tissue biopsies at caesarean section of women with PE or healthy pregnancy [188] demonstrated reduced insulin suppression of lipolysis in adipose tissue with PE. Increased lipolysis results in higher levels of FFAs in preeclamptic than in normal pregnancy [189].

### 2.4.6 Potential mechanisms linking maternal obesity and pregnancy complications to offspring complications

The fetal programming hypothesis, also known as Barker's hypothesis due to the seminal work of David Barker and his group, states that certain environmental factors during embryonic, fetal, and early life development can permanently affect the phenotype of an organism [190]. The first evidence comes from undernutrition programming later disease [191], but animal and clinical studies have later demonstrated that maternal overnutrition can also lead to alterations in physiological regulatory systems. Norbert Freinkel introduced the concept of fuel-mediated teratogenesis, i.e., alterations during the differentiation and proliferation of fetal cells that cause long-term consequences on metabolic and anthropometric functions. Tissues are at their most plastic during organogenesis and maturation, and tissue remodelling may result in irreversible changes and programme lifelong function of the tissues and, thus, offspring health. Although it is not known how the foetal tissues receive signals from the environment, there is little doubt that stimulus is mediated via the placenta [192]. **Figure 6** presents the transgenerational cycle of obesity.

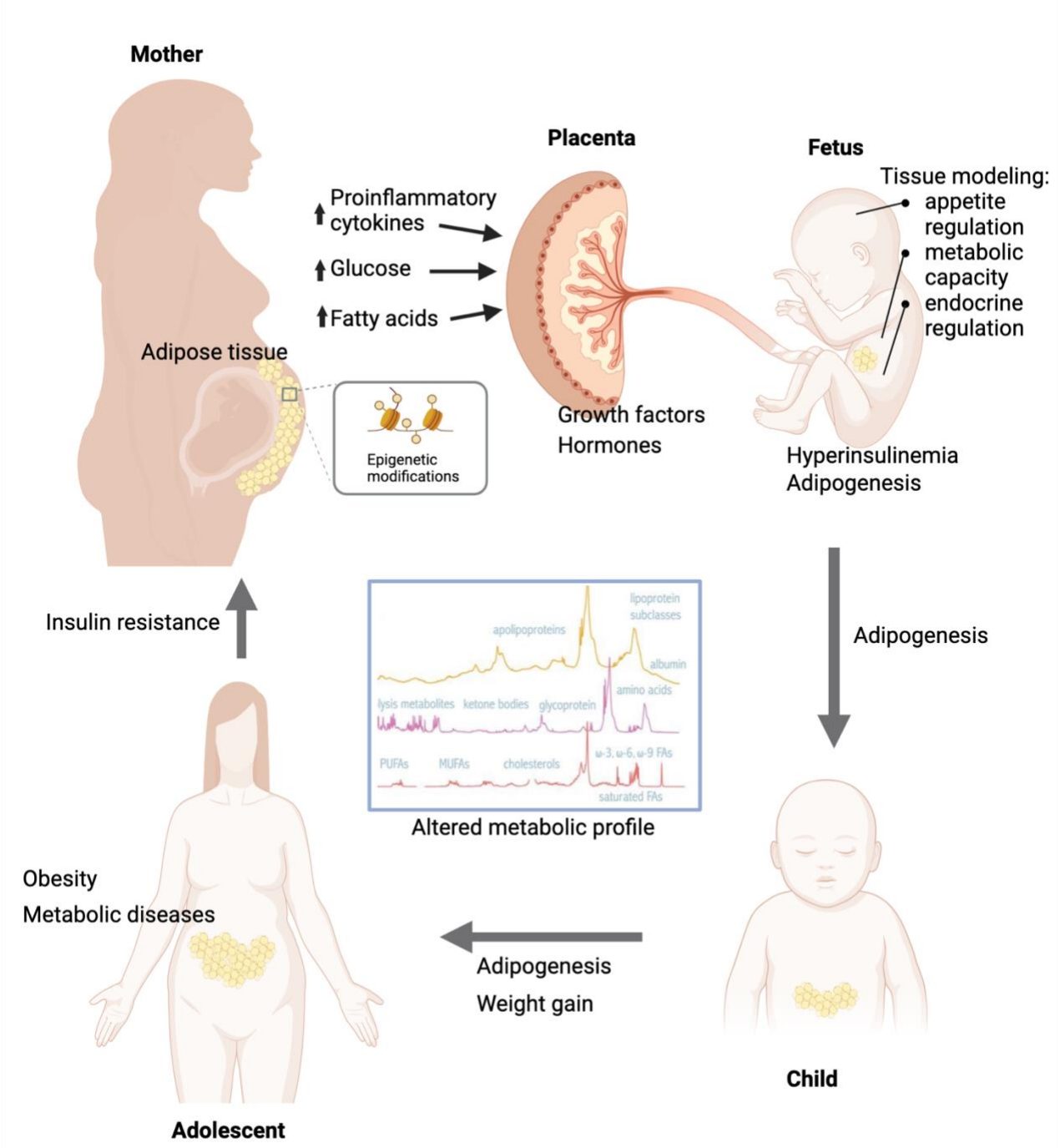



***Figure 6.*** *Effects of maternal obesity and GDM on placenta and fetus, and the transgenerational cycle of obesity. Modified from Corrales* [193]. *Created with BioRender.com*

*The central role of the placenta*

Placentas from mothers with GDM or obesity are frequently larger, with increased placental weight and placental-weight to birth-weight ratio [194]. Glucose transport capacity, seen as a higher number of glucose transporters, seems to be increased in GDM placentas [195, 196]. According to the well-established, over 60 years ago formulated Pedersen hypothesis, increased transplacental transfer of glucose stimulates insulin release by the fetal beta cell and results in fetal macrosomia [197].

Maternal obesity is associated with histopathological changes of inflammation and under-perfusion [184, 198] and greater placental cytokine expression [199]. Maternal GDM and obesity alter placental FA transfer [200]. Differences in FFA transfer rate between women with or without obesity have been demonstrated in an ex vivo model; reduced mobilisation of FFA in the placentas of women with obesity resulted in lower DHA levels in fetal circulation [201]. At term, placentas of women with obesity are characterised by an increase in total lipid content and an accumulation of macrophages and proinflammatory mediators compared to women with normal weight [202, 203]. Some of these placental changes, such as FA storage in lipid droplets by the syncytiotrophoblast, may reflect adaptive responses to protect the fetus in the adverse metabolic environment. The adaptation may, however, be limited in severe metabolic conditions so that the placenta can no longer accumulate lipids, resulting in spillover towards the fetus [196]. Maternal FFA and triglyceride concentrations correlate with fetal birth weight [204, 205].

The fetal liver is vulnerable to excess fuel exposure, and fetal adipose depots in the first half of pregnancy are immature and not available to buffer the excess. Thus, early increased lipids, due to maternal obesity, probably accumulate in the fetal liver, establishing an inflammatory and lipotoxic environment which may increase the risk of non-alcoholic fatty liver disease later in life [206].

Placentally produced adiponectin and leptin influence the development of adipose tissue in the fetus. Maternal obesity is associated not only with maternal but also with cord blood elevated concentrations of leptin [207]. Leptin modulates the formation of the homeostatic endocrine axes. High levels may permanently alter the hypothalamic response to leptin and subsequent regulation of appetite and pancreatic β-cell physiology [208]. Maternal adiponectin levels decrease most in mothers giving birth to large-for-gestational-age infants [164].

*Microbiome*

The maternal intestinal microbiome plays a major role in the development of the infant microbiome and the infant's immune system [209, 210]. Intestinal microbiome of women with obesity differs extensively from that of women with normal weight [211, 212]; accordingly, differences in infant microbiomes have been found [213]. GDM is associated with maternal alterations of the gut, oral and vaginal microbiome and alterations of the gut, oral, and placental microbiome of their offspring [214]. The changes in the microbiome may play a role in the development of long-term complications [215]. In a recent Finnish study, the composition of maternal microbiota predicted maternal prediabetes two years after delivery [216].

*Epigenome*

Increasing evidence points toward a mechanistic connection between the metabolic state of a cell and its epigenome, and a widely hypothesised mechanism that links in utero exposure to health in later life is through epigenetic modifications [217, 218]. Alterations of placental gene expression, epigenetic changes and disturbances in mitochondrial function and homeostasis have been demonstrated in pregnancies complicated by maternal obesity [172, 219–222]. Maternal BMI is associated with placental DNA methylation [223] and offspring cord blood DNA methylation [224–226], and in one study, associations between maternal BMI during pregnancy and leucocyte DNA methylation still existed at the age of 3 years [227]. A recent study following offspring up to the age of 18 years demonstrated cord blood DNA methylation to mediate the effects of maternal BMI on

offspring long-term obesity risk [224]. Still, clear evidence of epigenetic mechanisms comes from animal models, and evidence from clinical studies is limited [217].

*Metabolomics*

Mechanisms linking obesity with adverse maternal and offspring health conditions are probably multifactorial, and multiple metabolic pathways are involved; metabolomics gathers information on all these pathways. Changes in maternal or cord blood metabolome seem to be associated with newborn macrosomia [228] or adiposity [229] and accelerated early growth [230]. Convincing evidence between maternal or fetal metabolome and long-term complications is still lacking. However, examining maternal and cord blood metabolomics enhances understanding the physiopathology of obesity and obesity-related diseases in pregnancy. It may also enable the discovery of novel biomarkers and possible targets for intervention [231].

## 2.5 Metabolomics

### 2.5.1 What is metabolomics?

Metabolomics is the study of metabolites within biofluids, tissues, or organisms. The collection of all these low-molecular-weight molecules constitutes the metabolome, a metabolite profile of a given sample. Metabolomics is a downstream science of other -omic sciences, as it contains all the end-products of genomic, transcriptomic, and proteomic processes (**Figure 7**). The human metabolome consists of the metabolites produced by human cells and by cells of the microbiome. The metabolome can be defined as the phenotype, whereas the genome is the genotype.

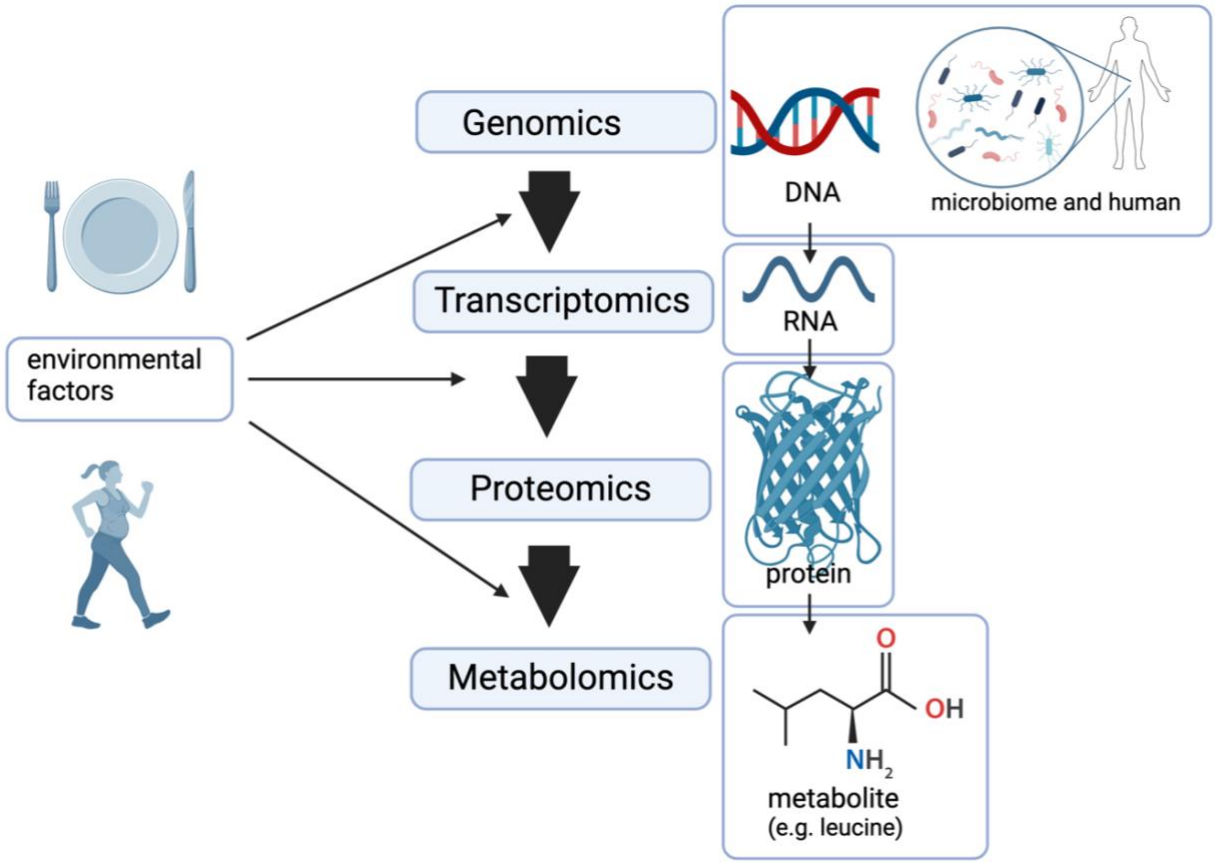



***Figure 7.*** *The relation of metabolomics to other omics sciences and environmental factors.* *Created with BioRender.com*

### 2.5.2 Techniques in metabolomic analysis

The two most frequently utilised analysis methods in metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS), which both have their strengths and weaknesses [232]. The study strategy in metabolomics can be either untargeted or targeted, depending on whether the intention is to detect all the measurable metabolites in a sample or to measure the levels of a defined group of metabolites [233].

NMR is a quantitative method and requires minimal sample preparation [234]. It is a highly reproducible method, and the cost per sample is low. The limitation of the method is low sensitivity, leaving some of the metabolites below the detection level and thus non-visualized. However, the part of the metabolome visualised and quantified is often of crucial importance, and NMR is the preferable method for analysis of large cohorts and quantifying biomarkers [235]. Several of the measures quantified by NMR are comparable to and have been validated against routine laboratory assays. These measures include total cholesterol, triglycerides, and LDL- and HDL-cholesterol, but more detailed stratification of lipoprotein particle size and content obtained by NMR gives profound information [236].

MS is a highly sensitive method capable of detecting compounds present in tissues at very low concentrations. Its reproducibility is low, and it does not offer direct structural information. However, it is the method of choice for tracing environmental agents [237] and is useful in identifying new biomarkers [238].

### 2.5.3 Maternal metabolome in obesity and GDM

**Table 4** presents the studies on maternal metabolome associated with obesity, GDM or both. Many studies have focused on early recognition of GDM or PE; thus, maternal blood samples have frequently been obtained in early pregnancy. Longitudinal sampling across pregnancy is rare. The largest published study, Born in Bradford [239], analysed the metabolomic profile of a cohort of 8774 women with White European or South Asian ethnicity. The NMR panel in the study detected 156 measures at GW 26–28.

***Table 4.*** *Studies on the association of BMI or GDM with maternal metabolome.*

| Reference | n | Analysis method | Sample, GW | Association studied | Main findings |
|---|---|---|---|---|---|
| **Bentley-Lewis 2015 [240]** | 192 | MS | Serum, <12 | GDM | Higher levels of anthranilic acid, alanine, glutamate, allantoin and serin in women who later developed GDM |
| **Lu 2016 [241]** | 817 | MS | Serum, 15 | GDM | Four lipids, TG(51:1), TG(48:1), PC(32:1) and PCae(40:4), predicted later GDM |
| **Hellmuth 2017** [242] | 167 | MS | Plasma, 13, 21 and 31 | BMI | FFA showed a strong positive association with prepregnancy BMI, specifically omega-6 species and MUFA |
| **Jacob 2017** (HAPO study) **[243]** | 1600 | MS | Serum, 28 | BMI, glycemia | Shared and independent pathways associated with prepregnancy BMI and fasting glucose; many of the metabolites associated with prepregnancy BMI lipid-related and those associated with fasting glucose gluconeogenic substrates |
| **Law 2017** [244] | 61 | MS | Plasma, 11-14, 23-27, 29-33 | GDM | Lower levels of several polyunsaturated or chemically modified phospholipids in women with GDM |
| **Hou 2018** [245] | 269 | MS | Serum, 12 | GDM | Significant differences in FFAs, BAs, AAs, organic acids, lipids and organooxygen compounds between women with or without GDM; model consisting of BMI, retinol binding protein 4, n-acetylaspartic acid and C16:1 (cis-7) manifested the best discrimination for GDM |
| **Houttu 2018 [246]** | 99 | NMR | Serum, 13 | Overweight vs obesity | Higher levels of GlycA, three BCAA (isoleucine, leucine and valine), one AA (phenylalanine) and several VLDL-related measures and lower levels in a few HDL-related measures and many fatty acids in women with obesity compared to those with overweight |
| **Mills 2019** (UPBEAT study) [247] | 1158 | NMR | Serum, 17, 28 and 35 | Intervention on obesity | Modest to marked changes across pregnancy in: lipids, an inflammatory marker, glucose, some amino acids, ketone bodies and fluid-balance-related metabolites; with intervention improvements in most VLDL particles and VLDL size in comparison to standard care |
| **Sakurai 2019** [248] | 242 | MS | Serum, 18 | GDM | Glutamine, pyrophosphate and octulose-1,8-bisphosphate differed significantly between GDM and control groups of which glutamine was the best predictive biomarker |
| **Shokry 2019** [249] | 325 | MS | Plasma, at delivery | BMI, GDM | BCAAs leucine and isoleucine, and inflammation markers were positively associated with BMI, sum of hexoses with GDM |
| **Taylor 2019** (Born In Bradford study) [239] | 8774 | NMR | Serum, 26-28 | BMI, GDM | Higher levels of several lipoprotein subclasses and triglycerides associated with GDM and higher BMI |
| **Jiang 2020** [250] | 431 | MS | Serum, 12-16 | GDM | Elevated levels of isoleucine, tyrosine, and alanine levels associated with subsequent incidence of GDM |

| | | | | | |
|---|---|---|---|---|---|
| **Liu 2020** [251] | 486 | MS | Plasma, 10 | GDM | LPC levels (lower LPC14:0 and higher LPC15:0 and LPC18:0) independently associated with increased GDM risk |
| **Mokkala 2020a** [252] | 357 | NMR | Serum, 14 | obesity associated GDM | Higher concentrations of several-sized VLDL particles, medium- and small-sized HDL particles, isoleucine, leucine, and GlycA and lower concentrations of very large-sized HDL particles in women developing GDM; the most predictive marker for GDM was higher concentration of small-sized HDL particles |
| **Mokkala 2020b** [253] | 352 | NMR | Serum, 35 | obesity associated GDM | 137/228 metabolites associated with GDM; higher concentrations of VLDL particles, triglycerides, branched-chain amino acids and GlycA |
| **Raczkowska 2021** [254] | 255 | MS | Serum, 8-14/24-28 | GDM | Alpha-hydroxybutyric acid, beta-hydroxybutyric acid and several fatty acids associated with abnormal glucose tolerance test in the second trimester |
| **Rahman 2021** [255] | 321 | MS (lipidomics) | Plasma, 10-14, 15-26 | GDM | Elevated plasma diglycerides and short, saturated/low unsaturated triglycerides and lower plasma cholesteryl esters, sphingomyelins and phosphatidylcholines associated with higher risk of developing GDM |
| **Zhan 2021** [256] | 103 | MS | Plasma 27, 33 | GDM | Differences in the metabolites related to glycerophospholipid metabolism, linoleic acid metabolism, and D-arginine and D-ornithine metabolism between GDM and non-GDM; monoacylglycerol, dihydrobiopterin, and 13S-hydroxyoctadecadienoic acid, were identified as possible novel biomarkers |
| **Wang 2021** [257] | 1008 | MS (lipidomics) | 6-15 | GDM | Positive associations of phosphatidylinositol 40:6, alkylphosphatidylcholine 36:1, phosphatidylethanolamine plasmalogen 38:6, diacylglyceride 18:0/18:1, and alkylphosphatidylethanolamine 40:5 and negative associations of sphingomyelin 34:1, dihexosyl ceramide 24:0, mono hexosyl ceramide 18:0, dihexosyl ceramide 24:1, and phosphatidylcholine 40:7 with later development of GDM |

BMI, body mass index; GDM, gestational diabetes; MS, mass spectrometry; TG, triglyceride; PC, phosphatidylcholine; PCae, choline ether phospholipid; FFA, free fatty acid; MUFA, monounsaturated fatty acid; BA, bile acid; AA, amino acid; NMR, nuclear magnetic resonance; GlycA, Glycoprotein A; BCAA, branched-chain amino acid; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; LPC, lysophosphatidyl choline

*Lipoproteins and lipids*

Before the era of metabolomic studies, standard laboratory assays demonstrated that the dyslipidemia of pregnancy [119] is more pronounced in women with obesity or GDM [258, 259]. Women with obesity (versus normal weight) or GDM (versus normoglycemia) display higher trimester 3 triglyceride and LDL cholesterol concentrations and lower HDL cholesterol concentrations. Metabolomic studies have further analysed the lipid profiles of women with obesity or pregnancy disorders.

VLDL particle concentration increases across normal pregnancy [260], but women with obesity or GDM – already before the clinical manifestation of the disease – display even higher levels [239, 252, 253, 261]. With obesity or GDM, atherogenic VLDL particles are enriched by triglycerides more than in normal pregnancy [239, 252, 253, 261]. Large HDL particles are antiatherogenic [262]; a tendency toward smaller size of HDL particles has been demonstrated with both obesity and GDM [253, 261]. In a Finnish prospective study among women with overweight or obesity [253], a high concentration of small HDL particles at 14 GW was the most predictive marker for the later development of GDM in women with obesity.

Total cholesterol levels in pregnancy are not altered by GDM [253, 258, 261]. Women with obesity enter pregnancy with higher total cholesterol levels than women with normal weight, but the increase in the levels during pregnancy may be higher among those with normal weight. In a study analysing mid-pregnancy lipid profiles of 225 women [263], normal-weight women had a higher total cholesterol at 24-28 GW than women with obesity. Differences in cholesterol subclasses have been reported between women with or without GDM. In 2015, a systematic review extracting data from 60 studies [258] found lower HDL cholesterol and higher non-HDL cholesterol among women with GDM compared to women without insulin resistance in trimesters 2 and 3, but not in trimester 1. After this review, in the previously mentioned Finnish prospective study among 357 women with overweight and obesity, total cholesterol in small and medium HDL particles in trimester 1 was higher among those who later developed GDM [252]. Higher levels of cholesterol in VLDL in women with obesity, GDM or both have been found in early [252], mid- [239] and late pregnancy [253] samples.

Apolipoproteins play a major role as components of several lipids. Apoliprotein A1 (ApoA-1) is the main protein component of HDL particles, while apoliprotein B (ApoB) is the main component of all atherogenic particles, including VLDL, IDL and LDL particles [264]. Levels of both ApoA-1 and ApoB increase in normal pregnancy [265]. Maternal BMI was associated directly with ApoB and inversely with ApoA-1 in mid-pregnancy in the Born in Bradford study [239]. In trimester 3, in the same Finnish study again [253], ApoA-1 levels of women with obesity and GDM did not differ from those of women with normal pregnancy, possibly reflecting pregnancy-induced 'catch-up' in normal pregnancy.

*Fatty acids (FAs)*

Polyunsaturated fatty acids (PUFA) are classified as omega-3 or omega-6, depending on the location of the first double bond. Docosahexaenoic acid (DHA) belongs to the group of omega-3 PUFAs. DHA is consumed from dietary sources or synthesised – even though not efficiently – from shorter-chain FAs. The effect of omega-3 supplementation on numerous health outcomes [266, 267] and the possible association between higher circulating omega-3 PUFA levels and lower risk of cardiovascular diseases [268, 269] have been extensively studied, but evidence on neither of these links is convincing.

Linoleic acid (LA), an essential FA, is one of the omega-6 PUFAs. Dietary supplementation of LA reduces blood cholesterol, but the reduction may not predict an effect on the prevention of atherosclerosis [270]. In UK Biobank data with 118 461 participants, the level of omega-6 PUFAs was associated with depression but not with myocardial infarction [271].

Although the dietary recommendations to decrease the intake of saturated fatty acids (SFA) to prevent metabolic diseases are consensus, higher circulating levels of SFAs have not been associated with coronary heart disease risk in a meta-analysis [272]. However, higher circulating levels of monounsaturated fatty acids (MUFA) have been associated with coronary heart disease in several recent studies [273, 274].

In normal pregnancy, from trimester 1 to 3, serum concentrations of SFA, MUFA, and omega-6 PUFAs increase, and the concentration of omega-3 PUFAs decreases [275]. One of the few studies with repeated sampling in US women during pregnancy [242] found maternal BMI in trimesters 1 and 2 to be directly associated with most of the FFA metabolites, specifically MUFAs and omega-6 PUFAs. BMI was not associated with omega-3 long-chain PUFAs (including DHA) in any of the trimesters, but in trimester 3, BMI was directly associated with omega-6 PUFAs [242]. In a Finnish study among 99 women [211], early pregnancy ratio of MUFA to total FAs was higher in women with obesity compared to those with overweight. Accordingly, maternal BMI was directly associated with mid-gestation MUFA levels in the multiethnic Born In Bradford study [239]. In this study, in both ethnicities (South Asian and White European), maternal BMI was directly associated with MUFA levels and inversely with ratios of LA to total, omega-3 and omega-6 to total, and PUFA to total FAs. Unlike a smaller US study with repeated sampling [242], Born In Bradford found a direct association between BMI and DHA [239].

Associations between GDM and circulating FFA levels have been partly similar to the association between obesity and FFAs. In the Born In Bradford [239] study, in mid-gestation samples, GDM was associated with higher levels of MUFA, SAFA and total FA in Western European women. DHA levels were higher, and ratios of most PUFAs to total FA were lower with GDM, independent of ethnicity. The Finnish study among 357 women with overweight and obesity [252] found in women who later developed GDM (compared to unaffected), early pregnancy lower ratios of PUFAs, specifically omega-6 long-chain, to total FAs. These same women displayed a higher ratio of MUFAs to total FAs. The same research group [253] found these FA derangements to persist and, additionally, a lower ratio of LA to total FA at a median of 35 GW.

*Amino acids (AAs)*

**Table 5** presents selected AAs and their associations with various health outcomes in a non-pregnant state.

***Table 5.*** *Associations of selected amino acids with various health outcomes.*

| Amino acid | Circulating level | Health outcome |
|---|---|---|
| Alanine | ↑ | Type 1 and 2 diabetes, and myocardial infarction [271] |
| Glutamine | ↓ | Increased mortality and poor clinical outcome [276] |
| Glycine | ↓ | Obesity, type 2 diabetes, metabolic syndrome, and non-alcoholic fatty liver disease [277, 278] |
| Histidine | ↓ | Myocardial infarction [271] |
| ***Branched-chain amino acids*** | | |
| Isoleucine | ↑ | Myocardial infarction with obesity, insulin resistance, type 2 diabetes, metabolic syndrome, and non-alcoholic fatty liver disease [271, 278–280] |
| Leucine | ↑ | Insulin resistance, myocardial infarction, and non-alcoholic fatty liver disease [271, 278, 280] |
| Valine | ↑ | Obesity, insulin resistance, type 2 diabetes, metabolic syndrome, myocardial infarction, and non-alcoholic fatty liver disease [271, 278, 280] |
| ***Aromatic amino acids*** | | |
| Phenylalanine | ↑ | Obesity, insulin resistance, type 2 diabetes, myocardial infarction, and incident heart failure [271, 281, 282] |
| Tyrosine | ↑ | Obesity, insulin resistance, type 2 diabetes [281] |

Derangements in the levels of several AAs are associated with obesity in the non-pregnant population. Accordingly, the Finnish study among 99 women with overweight or obesity [211] found higher levels of isoleucine, leucine, valine and phenylalanine in early pregnancy in women with obesity than those with overweight. The previously mentioned US study with repeated sampling [242] collected maternal samples in all three trimesters in a non-diabetic population of 160 women, of which 31 were with obesity. In this study, the only AAs associated with BMI were glutamic acid and asparagine. Glutamic acid is a precursor of glutamine, and BMI was directly associated with its level in trimester 2. BMI was inversely associated with asparagine in trimester 3. In the Born in Bradford study [239], several associations were found between maternal BMI and AAs. Independent of ethnicity, BMI was directly associated with mid-gestation levels of glycine, phenylalanine, tyrosine, and all three BCAAs and inversely with glutamine. BMI was directly associated with alanine in Western European women and inversely with histidine in South Asian women.

Numerous studies have found early pregnancy (GW ranging from <12 to 18) levels of AAs to predict the later development of GDM. These AAs include alanine [240, 250], glutamine [248], BCAAs isoleucine [250, 252] and leucine [252] and aromatic AA (AAA) tyrosine [250]. In the Born in Bradford study [239], GDM was directly associated with mid-gestation levels of alanine, phenylalanine and all three BCAAs independent of ethnicity. In South Asian women, GDM was directly associated with tyrosine and inversely with glutamine.

*Ketones*

Ketone bodies are produced by the liver and used as an energy source when glucose is not available. Ketone bodies, especially 3-hydroxybutyrate (also known as β-hydroxybutyrate), have been associated with anti-inflammatory and cardiovascularly protective effects [283]. Despite this, high circulating levels of ketone bodies have been associated with acute and chronic heart failure [282, 284].

In pregnancy, the US study with repeated sampling [242] found a positive association between BMI and trimester 3 3-hydroxybutyrate, and in the Born in Bradford study [239], the same observation was made in mid-gestation, independent of ethnicity. In South Asian women, BMI was inversely associated with acetate. A panel of three metabolites (3-hydroxybutyrate, alpha-hydroxybutyrate, and myristic acid) has been proposed as a novel diagnostic method for GDM in a study with a discovery phase of 79 and a validation cohort of 163 women [254]. Higher levels of acetoacetate in trimester 3 among women with overweight or obesity and GDM have been found [253].

*Inflammation marker GlycA*

GlycA (glycoprotein acetyls) is a marker for low-grade inflammation, and it predicts the risk of cardiovascular disease and all-cause mortality [285]. In pregnancy, higher BMI has been associated with higher levels of GlycA [239, 252]. In the Finnish study of 352 women with overweight or obesity, the women with GDM displayed higher levels compared to unaffected women [253].

### 2.5.4 Maternal metabolome in hypertensive disorders

As with GDM, studies on the association between hypertensive disorders and maternal metabolome have focused on early recognition of these diseases, specifically PE. Thus, maternal blood samples have frequently been obtained only in early pregnancy. The exceptions to this are the large Born in Bradford study [239], which obtained mid-gestation samples, and the Vitamin D Antenatal Asthma Reduction Trial (VDAART) study [286] with samples obtained at 10–18 GW and 32–38 GW in a cohort of 109 women.

*Lipoproteins and lipids*

Marked dyslipidemia with alterations similar to those seen with maternal obesity or GDM occurs with PE [118]. Mean circulating levels of triglycerides and FFAs increase about 2-fold in women with PE relative to women with uncomplicated pregnancy [287–289]. As in maternal obesity or GDM, VLDL particles are enriched with triglycerides [260], and cardiovascularly beneficial HDL cholesterol is decreased relative to normal pregnancy [290].

In the Born in Bradford study [239], PE was directly associated with the levels of VLDL lipoprotein subclasses, and the magnitude of the association was greater in South Asian than Western European women. These alterations in lipid profiles were also detected with GH in their study, but the magnitude was similar in both ethnicities. Some studies have found early pregnancy increased lipid levels and atherogenic lipid profile to predict later onset of GH [291] or PE [286, 291].

*Fatty acids (FAs)*

In a subsample of the VDAART study [286], FA biosynthesis was among the top enriched pathways in PE. The Born in Bradford study [239] found PE directly associated with total FAs and inversely associated with FA ratios, including the ratios of DHA, LA, omega-3, omega-6, PUFA, and SFA to total FA.

*Amino acids (AAs)*

In the VDAART study, [286] not only lipid levels, but also AA levels, predicted later PE. Alanine and phenylalanine pathway enrichment was detected at early and late pregnancy sampling points. Both of these AAs predicted the later onset of PE in a discovery phase study [292]. Prediction of PE by phenylalanine was also found in a small study among 11 women with and 11 without PE [293], and high levels of both AAAs, phenylalanine and tyrosine were measured at mid-gestation with both PE and GH in the Born in Bradford study [239]. The levels of all three BCAAs were also higher among women with PE or GH in the Born in Bradford study [239].

*Ketones*

In a validation study with 158 women [294], early pregnancy high circulating acetone levels predicted later development of early-onset PE, but in the Born in Bradford study [239] mid-pregnancy levels of neither of the ketone bodies, 3-hydroxybutyrate or acetone, were associated with PE or GH. In a study among 599 women with high risk for PE [291], high early pregnancy serum levels of 4-deoxythreonic acid, a degradation product of 3-hydroxybutyrate, predicted later PE.

*Inflammation marker GlycA*

Although inflammation is a widely recognised factor in the pathogenesis of PE, GlycA levels have been scarcely measured in women with PE. In the Born in Bradford study, compared to normotensive women, women with PE or GH displayed mid-pregnancy higher levels of GlycA [239].

### 2.5.5 BMI- or obesity-related cord blood metabolome

The cord blood metabolome is a combination of the maternal metabolites transported through the placenta and those synthesised and metabolised by the placenta or the fetus. The degree of correlation between maternal and cord blood metabolome greatly depends on the set of metabolites measured.

*Association between maternal BMI or obesity and cord blood metabolome*

In studies exploring the association of maternal BMI or obesity with the cord blood metabolome, the results have been contradictory. Little or no evidence of an association was found in a study in which 37 mothers were categorised as lean, overweight, and obese [295] or in a study of 912 mother-child dyads [296] after adjustment for birth weight and gestational age. In several other studies, maternal

adiposity status has been associated with various lipids and AAs in cord blood. PREOBE study of 116 mother-child dyads [249] found a positive association between BMI and 3-hydroxybutyric acid, BCAAs leucine and isoleucine, and inflammation markers. Another study among a total of 87 normoglycemic mothers with normal weight or obesity who were scheduled for full-term caesarean section [297] identified 29 metabolites as potential early-life biomarkers of maternal obesity in separate discovery and validation phases. These metabolites belonged to the classes of acylcarnitine, glycerophospholipid, AA, and organic acids. Isoleucine (a BCAA) was among the metabolites that were lower in the obesity-associated cord metabolome in this study. In the secondary analysis of HAPO (Hyperglycemia and Adverse Pregnancy Outcome) study with 1600 mother-child dyads [298], cord blood BCAAs were positively associated with BMI, as was also an AAA, i.e. phenylalanine. Associations were attenuated when adjusted for maternal fasting plasma glucose but remained significant.

*Association between maternal and cord blood metabolome*

A positive correlation of a varying degree has been found in the association between maternal single mid-gestation metabolome and fetal cord blood metabolome at delivery [295, 296, 298, 299]. In the HAPO study [298], nearly all metabolites showed a significant correlation; of the 65 metabolites, the only exceptions were triglycerides, two AAs and an acylcarnitine. The study of 912 mother-child dyads [296] found a modest correlation ($r = 0.11–0.31$) between maternal mid-gestation and cord blood measures at delivery, particularly in AAs and cholesterol subclasses. Significant correlations between maternal and cord blood levels in 83/227 measures were found in a study of 356 mother-child dyads [299]. The measures included glycine, histidine, and all three BCAAs. A study of 24 mother-child dyads with no maternal GDM or hypertension [295] found no correlation between maternal and cord blood metabolome.

## 2.6 Knowledge gaps in the current literature

Maternal obesity is a risk factor for GDM and PE. Compared to women with normal weight, the risk of GDM is approximately 3-fold with class 1 obesity [300] and increases to over 8-fold with class 3 obesity [55]. The risk of PE is 3-fold with class I obesity and approximately 4-fold with class 2-3 obesity [59]. The magnitude of these risks, specifically in second pregnancy, is not known. Further, it is not known how the presence of complications in the first pregnancy or pre-existing conditions like diabetes or HT alter the BMI-associated risk of complications in the second pregnancy.

Pregnancy complications, including maternal obesity, GDM, and hypertensive disorders, greatly alter maternal metabolism, with changes occurring gradually across pregnancy [239, 243, 247, 286]. Despite this, the majority of metabolomic studies are based on a single sampling point in pregnancy. Only a few studies have obtained multiple samples, and no study has focused on the change of the metabolomic profile across pregnancy. Additionally, most studies have focused on obesity, GDM and PE; GH-associated maternal metabolome has been studied very rarely, and no studies exist on maternal metabolome associated with HT.

The risk of pregnancy complications is increased in women with insulin-resistant type of GDM, often with higher pBMI, whereas in GDM with insulin secretion deficiency, the risk of adverse events is not different from normoglycemic women ([176]. It is not known whether this difference in complication rate is reflected in the metabolomic profile of GDM. Further, studies in non-pregnant populations have demonstrated a higher risk for type 2 diabetes and cardiovascular disease in those nonobese adults who display obesity-related metabolomic profile [301, 302]. Women with GDM, even if with nonobesity, have a higher risk for these diseases, but it is not known whether their metabolome resembles that of obesity.

The association between maternal pBMI and newborn umbilical cord blood metabolomic profile has frequently been studied in small cohorts. The results of the three studies with a larger sample size have varied from no associations in a cohort of 321 mother-child dyads [303] to an association between pBMI and BCAAs, AAAs and carnitines in the HAPO study with 1600 dyads from four ancestries [298]. Further, the degree of correlation between the maternal and newborn metabolomes in previous studies has varied from modest to moderate, but no study has explored whether maternal obesity modifies the association between maternal and cord blood metabolomes.

# 3 Aims of the thesis

The aims of this thesis were to explore the association between maternal pBMI and pregnancy complications in a second pregnancy, and to analyse the maternal metabolomic profile in a pregnancy with maternal obesity or pregnancy complications, and analyse the newborn metabolomic profile in association with maternal obesity.

The specific aims were as follows:

1. To study the association between maternal pBMI and GDM, GH, or PE in the second pregnancy, and to explore how first-pregnancy complications, pre-existing diabetes, and HT modify this association. (Study I)

2. To analyse the mean maternal metabolomic profile across pregnancy - with measurement points in early, mid-, and late pregnancy - in a pregnancy complicated by obesity, GDM, PE, GH, or HT, with examination of the change in this profile across pregnancy. (Study II)

3. To examine whether the maternal metabolomic profile differs from that of nonobese non-GDM controls in three study groups: 1. GDM without obesity, 2. GDM with obesity, and 3. obesity with no GDM. Analysis covered both early pregnancy profile and mean profile across pregnancy were analysed. (Study III)

4. To study the association between maternal pBMI and cord blood metabolome at delivery, as well as the association between maternal metabolome across pregnancy and cord blood metabolome, and to explore whether maternal pBMI modifies that association. (Study IV)

# 4 Material and methods

## 4.1 Medical Birth Register-based study (Study I)

### 4.1.1 Study population

The Medical Birth Register (MBR) covers all deliveries in Finland. The register's regular data sources are maternity hospitals, the Population Information System of the Population Register Centre, and Statistics Finland. The register records information on maternal socioeconomic background, healthcare, and interventions during pregnancy and delivery. Maternal pBMI has been recorded since 2006. Women report their prepregnancy weight at the first antenatal clinic visit (mean 9.5 GW), and community health nurses measure the height.

The MBR provided data on all women with their first and second singleton live births in 2006–2013 (n=50 219). Only the women with missing information on weight or height (1256 women, 2.5%) were excluded, after which the study cohort consisted of 48 963 women. Linkage of the data with The Finnish Hospital Discharge Register supplied the inpatient and outpatient data, which responsible physicians recorded as International Classification of Diseases, Tenth Revision (ICD-10) codes.

### 4.1.2 Exposures and outcomes

pBMI was calculated from maternal weight and height in the 2nd pregnancy and classified first according to the six WHO categories presented in **Table 1**. Secondly, a composite variable was created by grouping pBMI into 25 categories (<17kg/m$^2$, 17-39 kg/m$^2$, each BMI point as a single category, and >=40 kg/m$^2$) and determining whether women in each category had the complication. Both the MBR and the Hospital Discharge Register provided information on maternal adverse pregnancy outcomes and prepregnancy conditions. The collected codes included GDM (ICD-10 code O24.4), chronic or preexisting hypertension with PE (O11), mild to moderate PE (O14.0 or O14.9), severe PE (O14.1), GH without significant proteinuria (O13 or O16), type 1 diabetes (E10), type 2 diabetes (E11), preexisting or pregestational type 1 and type 2 diabetes in pregnancy (O24.0 or O24.1), hypertensive diseases (I10-15), and preexisting (chronic) hypertension complicating pregnancy (O10). Information on pathologic result in the glucose tolerance test as a check-box variable resulted in classification as GDM.

### 4.1.3 Statistical methods

The association between maternal prepregnancy conditions, pregnancy outcomes, and maternal pBMI as WHO categories was assessed with a Chi-square test for categorical variables and analysis of variance for continuous variables. Logistic regression was used to analyse the associations between pBMI and GDM, GH, and PE. The possible modifying effect of 1st pregnancy GDM, GH, PE, or pregestational diabetes or hypertension was examined by including an interaction term in the models, and a comparison of the models with and without the interaction term was performed by a likelihood ratio test. Separate analyses were performed with the observation of suggestive evidence (p for interaction <0.1), but only the results considered relevant based on separate analyses or statistically highly significant (p for interaction <0.001) are reported separately. In other cases, the factor was considered a potential confounder.

Models were adjusted for maternal age, smoking during pregnancy, and socioeconomic status. Complications in the 1st pregnancy, pregestational diabetes, and HT were considered as both potential

confounding factors and effect modifiers. Final models were created based on the results of interaction analyses, previous knowledge, and the observed associations between confounding factors and pBMI or outcomes.

Association between pBMI and GDM was analysed separately by 1st pregnancy GDM and was adjusted for maternal age, smoking, 1st pregnancy GH and PE, and HT. Association between pBMI and GH was analysed separately by 1st pregnancy GH or PE and was adjusted for maternal age, smoking, 1st pregnancy GDM and pregestational diabetes. The association between pBMI and PE was not analysed separately by 1st pregnancy PE. This analysis was adjusted for maternal age, smoking, 1st pregnancy GDM, GH and PE, pregestational diabetes and HT. Women with pregestational diabetes were excluded from the analysis on GDM, and women with HT from the analysis on GH.

Logistic regression analyses were performed using a composite variable with the six WHO BMI classes and the first pregnancy complication. Normal weight (BMI 18.5-24.9 kg/m2) and no first pregnancy GDM/hypertensive disorder were used as the reference. Analyses were performed with IBM SPSS for Windows, version 28.0 (IBM, New York, USA).

## 4.2 Metabolomic studies (Studies II–IV)

### 4.2.1 Study population

Metabolomic Studies II–IV are secondary analyses of two large Finnish randomised controlled trials, Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO) and Finnish Gestational Diabetes Prevention (Raskaudiabetes ja elämäntavat, RADIEL). Studies III and IV also utilise data from a Finnish prospective pregnancy cohort study, Intrauterine Sampling in Early Pregnancy (ITU).

*PREDO*

The PREDO study recruited pregnant women between 2005 and 2009 [304]. Women with a singleton pregnancy were recruited at the antenatal clinics of 10 study hospitals during their first ultrasound screening at 12+0 – 13+6 GW. Exclusion criteria were a diagnosis of asthma, allergy to ASA, smoking during pregnancy, previous peptic ulcer, previous placental ablation, inflammatory bowel diseases, rheumatoid arthritis, haemophilia, or thrombophilia.

The PREDO study enrolled 1154 women, of whom 110 had no risk factors and 969 with one (n=737, 68%) to five (n=1, 0.09%) risk factors for PE. The risk factors included earlier PE, IUGR, fetal demise, or GDM, prepregnancy obesity, HT, type 1 diabetes, maternal age below 20 or above 40, systemic lupus erythematosus and Sjögren's syndrome. A subset of 121 women with a second-degree diastolic notch in the uterine artery ultrasound assessment participated in a clinical trial investigating mini-ASA (100 mg; 61 in the ASA and 60 in the placebo group) on preventing PE and IUGR.

Blood samples were obtained at three time points during pregnancy from 425 women at a median of 13.0 (interquartile range, IQR 12.6–13.4), 19.3 (19.0–19.7), and 27.0 (26.6–27.6) GW. Women with blood samples were younger (32.5 vs 33.6 years; p=0.007) and less frequently with obesity (29.1% vs 39.3%, p=0.003) than women without blood samples. Cord blood at delivery was collected from 923 newborns. The background characteristics of the newborns with a cord blood sample did not differ from those of the newborns without.

*RADIEL*

The Finnish Gestational Diabetes Prevention (Raskaudiabetes ja elämäntavat, RADIEL) study recruited women who were at high risk for GDM and who were either planning a pregnancy or pregnant less than 20+0 weeks [305]. High risk was defined as a previous history of GDM or a pBMI $\geq$ 30 kg/m$^2$. Women were recruited between 2008 and 2014 using newspaper and targeted social media notices, personal invitation letters sent out based on hospital registry, through primary health care centres, and in the maternity hospitals of the Helsinki metropolitan area (Helsinki University Central Hospital Department of Obstetrics and Gynecology, Kätilöopisto Maternity Hospital and Jorvi Hospital) and in the South-Karelia Central Hospital in Lappeenranta, in South-Eastern Finland. Exclusion criteria were age less than 18 years, diabetes diagnosed before pregnancy, medications that influence glucose metabolism (e.g., oral corticosteroids and metformin), multiple pregnancies, physical disability, current substance abuse, severe psychiatric disorders, and significant difficulties in cooperating (e.g., inadequate Finnish language skills).

The RADIEL study recruited 720 women who were randomised into an intervention group to prevent GDM and a control group with standard prenatal care. The intervention group received advice on diet and physical activity during the pregnancy. An individual exercise program was planned during their first visit; the women were able to attend exercise groups free of charge, and they had a 2-hour group session with a trained nutritionist. All women attended three visits during which a study nurse took anthropometric measures and performed questionnaires.

Blood samples were obtained at three time points during pregnancy from 339 women at a median of 13.0 (IQR 11.9–14.3), 23.1 (22.6–24.1), and 35.1 (34.4–35.7) GW. Women with blood samples were less frequently with obesity (14.0% vs 20.5%, p=0.04), with GDM (27.9% vs 73.2%, p<0.0001), or with PE (3.3% vs 7.0%, p=0.04) than women without blood samples. Cord blood at delivery was collected from 369 newborns. The background characteristics of the newborns with a cord blood sample did not differ from those of the newborns without.

*ITU (Studies III and IV)*

The Intrauterine Sampling in Early Pregnancy (ITU) recruited women attending the national, voluntary trisomy 21 screening between $9^{+0}$ and $21^{+6}$ GW from April 2012 through December 2017 [306]. The cohort includes 943 women and their children born alive and consists of two arms. The first arm is the 543 women who tested positive in a combination of serum screening (at 9+0–11+6 GW) and ultrasound examination at GW 10+0–13+6. Further on, their chromosomal testing (chorionic villus sampling, amniocentesis, or non-invasive prenatal testing of cell-free DNA in maternal blood) suggested no fetal abnormality. The second arm is the 401 women who were screen-negative and thus did not undergo fetal chromosomal testing. Eligibility criteria included singleton pregnancy, no prenatal diagnosis of fetal chromosomal abnormality, maternal age≥18 years and sufficient Finnish language ability to ensure informed consent.

Blood samples were obtained once during pregnancy from 490 women at a median of 20.6 (IQR 20.1-23.4) GW. Women with blood samples had lower BMI (23.7 vs 24.6 kg/m$^2$, p=0.004) and smoked less often during pregnancy (2.4% vs 5.0%, p=0.001) than women without blood samples. Cord blood at delivery was collected from 410 newborns. The background characteristics of the newborns with a cord blood sample did not differ from those of the newborns without.

The combined cohort of PREDO and RADIEL studies includes 741 women in Study II and 755 women in Study III. Study IV includes 1702 mother-child dyads from PREDO, RADIEL, and ITU studies. The flowchart of the Studies is presented in **Figure 8**, and a further flowchart of Study III is presented in **Figure 9**.

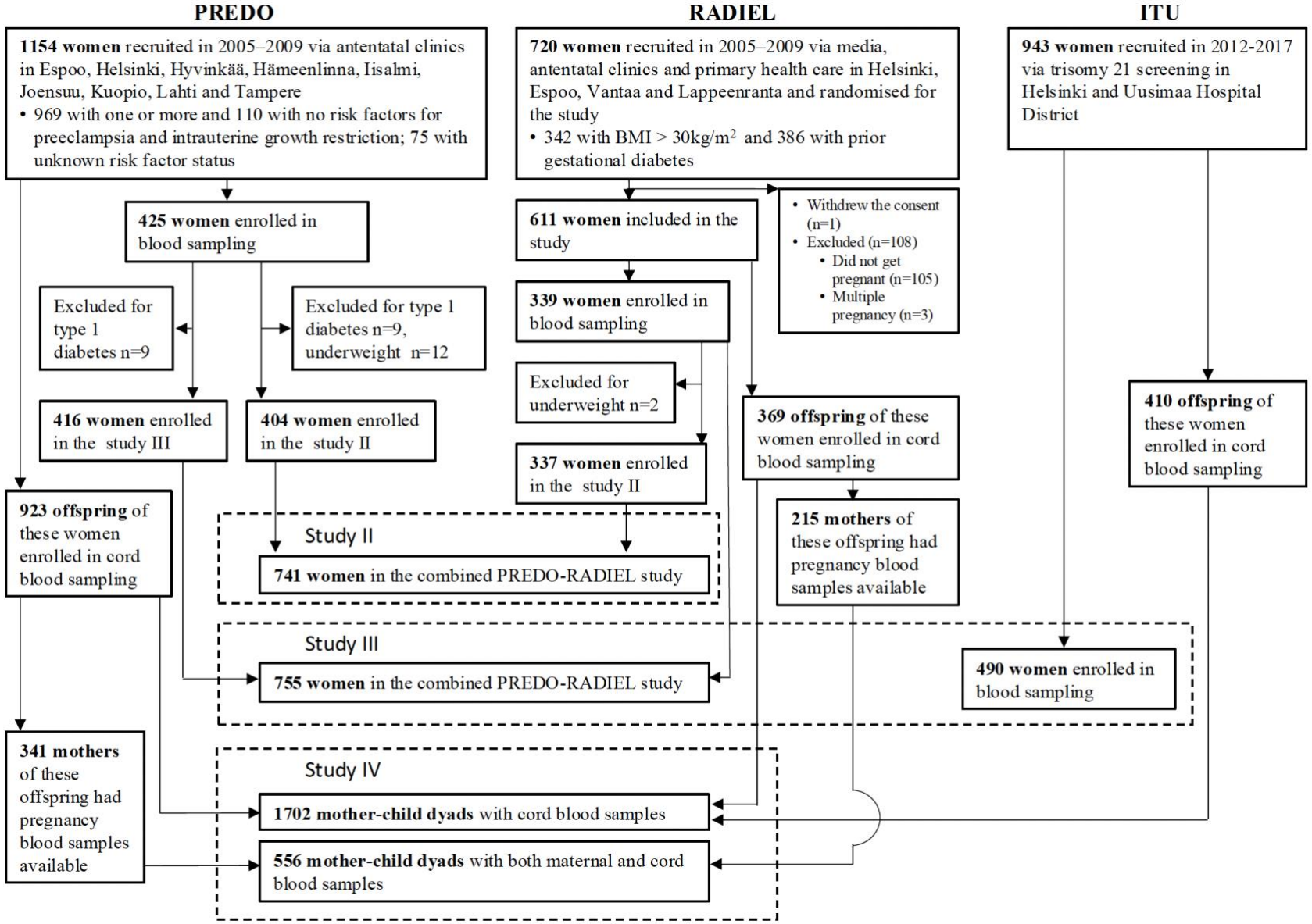



***Figure 8.*** *Flowchart of the Studies II, III and IV.*

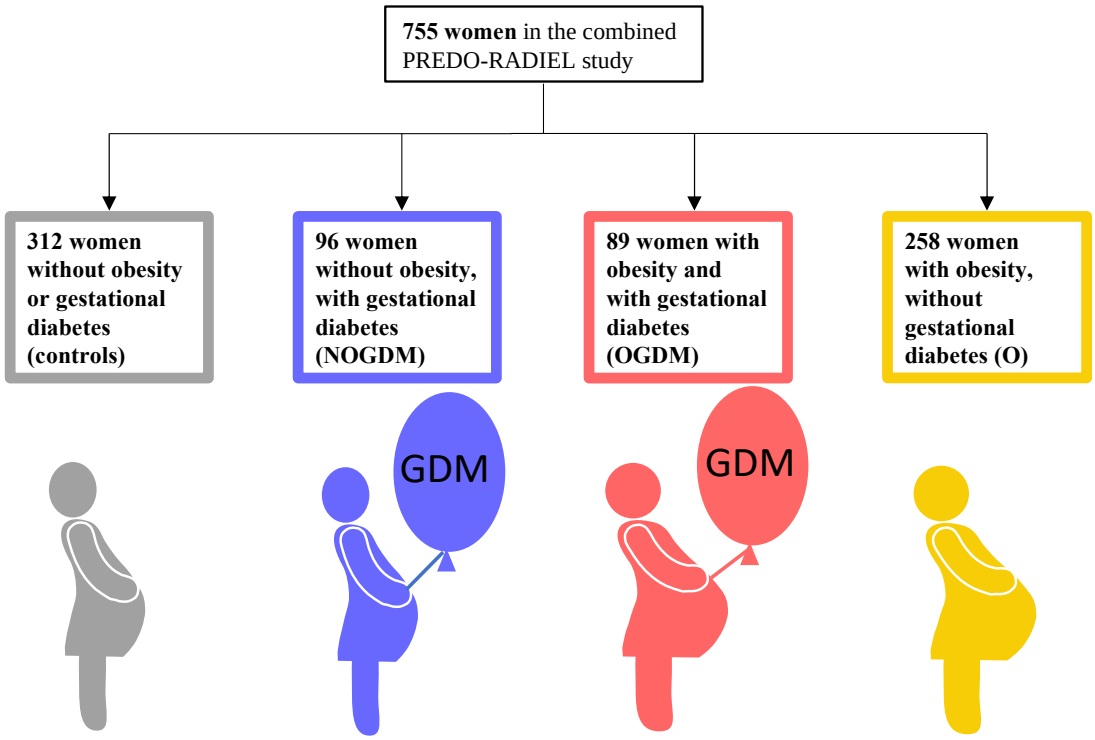



***Figure 9.*** *Further flowchart of the Study III.*

### 4.2.2 Exposures and outcomes

Information on prepregnancy weight and height to calculate pBMI came from MBR and, when available, from antenatal clinic records (in the RADIEL study, the participants recruited before pregnancy). The definition of obesity is according to the previously presented WHO categories (normal weight with BMI 18.5–24.99 kg/m$^2$, overweight with 25–29.99, and obese with >30). Medical records provided the diagnoses of GDM and hypertensive disorders, and a jury of a research nurse and two or more medical doctors verified these.

GDM, PE, GH, and HT were defined as described on pages 17–19. The diagnosis of these complications came from medical records and was verified by a jury composed of a research nurse and two or more medical doctors.

### 4.2.3 Metabolomic profiling using the NMR platform

In all three study cohorts, maternal venous blood samples were drawn from the antecubital vein between 7 and 10 in the morning after at least a 10-h fast. A mixed umbilical cord blood sample was obtained immediately after the child was born. In the RADIEL study, plasma and PREDO and ITU studies, serum was separated and stored at –80°C until analysis.

A high-throughput proton NMR metabolomics platform quantified 225 metabolic measures in maternal samples [307] and 110 in cord blood. The analysis panel captures a range of established and emerging biomarkers of multiple metabolic pathways, including lipid and glucose metabolism, AAs, FAs, ketone bodies, and GlycA as a marker of low-grade inflammation. Of these measures, 37 have been validated against the standard clinical chemistry methods. The number of measures considered

appropriate in each study to form an adequate picture of the systemic metabolism, and thus, used as primary outcomes, varied from 66 to 95 measures. The measures were selected based on previous studies of pregnant populations [296, 308] and studies of the risk of type 2 diabetes and cardiovascular morbidity in nonpregnant populations [309–312]. Studies III and IV utilised the revised Nightingale Health Quantification Library 2020, which is also validated for cord blood samples.

### 4.2.4 Covariates

Covariates were chosen based on previous literature. In Studies II and III, all models were adjusted for maternal age, cohort, and GW at the time of blood sampling (Model 1). The fully adjusted model was additionally adjusted for maternal education (basic/secondary vs tertiary), parity, and substance use (smoking and alcohol, no vs yes) during pregnancy. In Study III, in the fully adjusted model, the analysis of obesity was further adjusted for GDM and hypertensive disorders, analysis of GDM and hypertensive disorders for pBMI, analysis of GDM for hypertensive disorders, and analysis of hypertensive disorders for GDM.

In Study IV, the models analysing the effects of pBMI on cord blood metabolic measures and the effects of maternal measures on cord blood measures were adjusted for cohort, maternal age, parity, smoking and alcohol use during pregnancy, education and child sex. Birth weight, gestational age at delivery, and delivery mode were considered to lie on the same pathway with maternal pBMI and materno-fetal metabolome and were not included as covariates.

### 4.2.5 Statistical methods

The metabolic measures were log-transformed to normalise the distributions, and the values were analysed in cohort-specific standardised units. With the high correlation rate of the metabolic measures, the Bonferroni correction for multiple testing may be too conservative and raise the risk of type II error [313]. The risk can be reduced by applying principal components analysis as a multiple testing correction method for correlated data, thus identifying the effective number of independent tests [314, 315]. In Studies II and III, 25 principal components explained over 99% of the variation in the 68 (Study II) or 66 (Study III) metabolic measures used as the primary outcomes. This resulted in a two-sided p-value <0.002 (0.05/25) to infer statistical significance. In Study IV, 34 principal components explained the same level of variation in the 95 cord blood metabolic measures, resulting in a p-value <0.00147 (0.05/34).

Individual-participant data meta-analytic approach by using mixed model regression analyses was used to study the associations of maternal conditions with the levels of and with the change in the levels of metabolic measures across pregnancy (Studies II and III) and the association of pBMI with the levels of cord blood measures (Study IV). In Study II, the women with overweight or obesity were compared to those with normal weight, women with GDM to those without GDM, and women with PE/GH/HT to those without a hypertensive disorder. The difference in the change of the metabolic measures during pregnancy between the group and the controls was studied by including the interaction effects of maternal conditions vs without the condition x GW at blood sampling in the models. In Study III, women with nonobesity and GDM (NOGDM), obesity and GDM (OGDM), or obesity were compared to the controls without obesity or GDM.

Mixed models analysis allows missing data, and no missing values on metabolic measures were imputed. For the measures below the detection level, a value equivalent to 0.9 multiplied by the non-zero minimum value of the measurement was used. The results of change in the metabolic measures

during pregnancy are reported only among women who provided metabolomic data at all three time points during pregnancy.

In the analysis of the ITU replication cohort in Study III, regression analysis in which the metabolic measures served as dependent variables was used, and women with NOGDM, OGDM, or obesity only were compared to controls in models adjusted for the covariates. Fisher's exact test was used to study whether the differences found in the PREDO-RADIEL cohort were more often in the same direction in the replication cohort than what would be expected by chance alone.

Estimates and their 99.8% CIs (PREDO-RADIEL, Studies II and III) and unstandardised beta coefficients and their 95% CIs (linear regression models in ITU, Study III) are reported as effect size indicators. The estimates and unstandardised regression coefficients represent mean differences [grand mean of the three measurement point values and mean differences in the change (representing the slope)] in the metabolic measures in SD units of women with the condition (overweight/obesity, GDM, hypertensive disorders, NOGDM, OGDM, or obesity only) with women without the condition as the referent in all analyses.

The association between maternal and cord blood metabolic measures (Study IV) was examined using generalised linear regression. As in this analysis, each exposure (maternal measure) is tested for one outcome (cord blood measure); p-value < 0.05 was used to infer statistical significance. Effect size is reported as estimates representing change in cord blood metabolic measures in SD units per each 1 SD unit change in maternal metabolic measures, and their 95% CIs. It was then tested whether the associations between maternal and cord blood metabolic measures in the combined PREDO-RADIEL sample differed according to the level of maternal pBMI. For all interaction terms with p-value<0.05, subanalyses testing associations between maternal and cord blood metabolic measures were conducted separately among women with nonobesity (<30kg/m$^2$) or obesity (≥ 30kg/m$^2$).

The analyses for metabolomic studies were performed with SAS 9.4 (SAS Institute, Inc., Cary, NC).

## 4.3 Ethical aspects

The National Data Protection Authority and the THL Finnish Institute for Health and Welfare, which is the keeper of the two registers, MBR and the Finnish Hospital Discharge Register, approved the register-based study.

All participants in the PREDO, RADIEL, and ITU cohorts provided written informed consent. The study protocols of all three studies were approved by the ethics committees of the Helsinki and Uusimaa Hospital District.

Even though this study is of a quantitative nature and thus assumed to guarantee an objective and non-judgemental assessment of obesity in pregnancy, it must be acknowledged that obesity is a disease carrying huge stigma and prejudices. All efforts must be undertaken to raise public awareness of obesity as a condition that is not voluntary or self-inflicted but a disease with multiple background causative factors, including genetic, hormonal, environmental and psychological aspects. This study adheres to non-stigmatizing language when referring to people living with obesity.

# 5 Results

## 5.1 Association of maternal pBMI with pregnancy complications in second pregnancy (Study I)

### 5.1.1 Background and clinical characteristics in Study I

**Table 6** presents the characteristics of those Finnish women whose both first and second singleton live births were in 2006–2013. Of these 48 963 women, 62% were of normal weight, 26% with overweight and 12% with obesity. The mean pBMI before the 2nd pregnancy was 24 kg/m$^2$ and ranged from 13 to 65 kg/m$^2$.

***Table 6.*** *Background characteristics of the women in Study I by prepregnancy body mass index categories in the second pregnancy in 2006–2013.*

| | **Body mass index category (kg/m$^2$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **<18.5 n=1749** | **18.5–24.9 n=30 380** | **25–29.9 n=11 030** | **30–34.9 n=4003** | **35–39.9 n=1241** | **≥40 n=560** | **Total n=48 963** |
| **Age,** mean in years (SD) | 27.2 (4.8) | 29.2 (4.6) | 29.2 (4.7) | 28.8 (4.9) | 28.6 (4.9) | 28.5 (4.8) | 29.1 (4.7) |
| **Non-smoking**, n (%) | 1433 (82) | 27 252 (90) | 9584 (87) | 3328 (83) | 1012 (81) | 441 (79) | 43 050 (88) |
| **Missing data,** n (%) | 43 (2.5) | 579 (1.9) | 221 (2.0) | 100 (2.5) | 23 (1.9) | 13 (2.3) | 979 (2.0) |
| **Pregestational diabetes**, n (%) | 7 (0.4) | 227 (0.7) | 162 (1.5) | 104 (2.6) | 43 (3.5) | 29 (5.2) | 572 (1.2) |
| **Chronic hypertension**, n (%) | 4 (0.2) | 133 (0.4) | 125 (1.1) | 101 (2.5) | 64 (5.2) | 48 (8.6) | 475 (1.0) |
| **1st pregnancy**, n (%) | | | | | | | |
| **Gestational diabetes** | 56 (3.2) | 1662 (5.5) | 1670 (15) | 1152 (29) | 458 (37) | 242 (43) | 5240 (11) |
| **Gestational hypertension** | 52 (3.0) | 1355 (4.5) | 744 (6.7) | 420 (11) | 164 (13) | 95 (17) | 2830 (5.8) |
| **Preeclampsia** | 37 (2.1) | 961 (3.2) | 508 (4.6) | 253 (6.3) | 115 (9.3) | 54 (9.6) | 1928 (3.9) |
| **2nd pregnancy**, n (%) | | | | | | | |
| **Gestational diabetes** | 52 (3.0) | 1815 (6.0) | 2101 (19) | 1283 (32) | 514 (41) | 301 (54) | 6066 (12) |
| **Gestational hypertension** | 19 (1.1) | 511 (1.7) | 363 (3.3) | 213 (5.3) | 105 (8.5) | 55 (9.8) | 1266 (2.6) |
| **Preeclampsia** | 12 (0.7) | 325 (1.1) | 181 (1.6) | 109 (2.7) | 51 (4.1) | 23 (4.1) | 701 (1.4) |

Gestational hypertension does not include women with further diagnosed preeclampsia

The incidence of 2nd pregnancy GDM was 12%, of GH 2.6%, and of PE 1.4%. The incidences of all these three pregnancy complications increased by pBMI class, but the incidences of GH and PE were lower in the 2nd than in the 1st pregnancy. With HT, the incidence of 2nd pregnancy GDM was 36% and of PE 13%, and with pregestational diabetes, the incidence of 2nd pregnancy GH was 6.0% and of PE 5.8% (not shown in the table). GDM recurred in 60%, GH in 19%, and PE in 17% of the 2nd pregnancies.

### 5.1.2 Gestational diabetes

The unadjusted probability of 2nd pregnancy GDM increased with pBMI (**Figure 10**). The presence of 1st pregnancy GDM modified the association between pBMI and 2nd pregnancy GDM (p for interaction <0.001), and the probability is presented separately for women with or without the condition in the 1st pregnancy. The association was not modified by 1st pregnancy GH (p for interaction 0.806), PE (0.695) or HT (0.190). The adjusted odds ratios (OR) for 2nd pregnancy GDM by prepregnancy body mass index categories and with or without 1st pregnancy GDM are presented in **Table 7**.

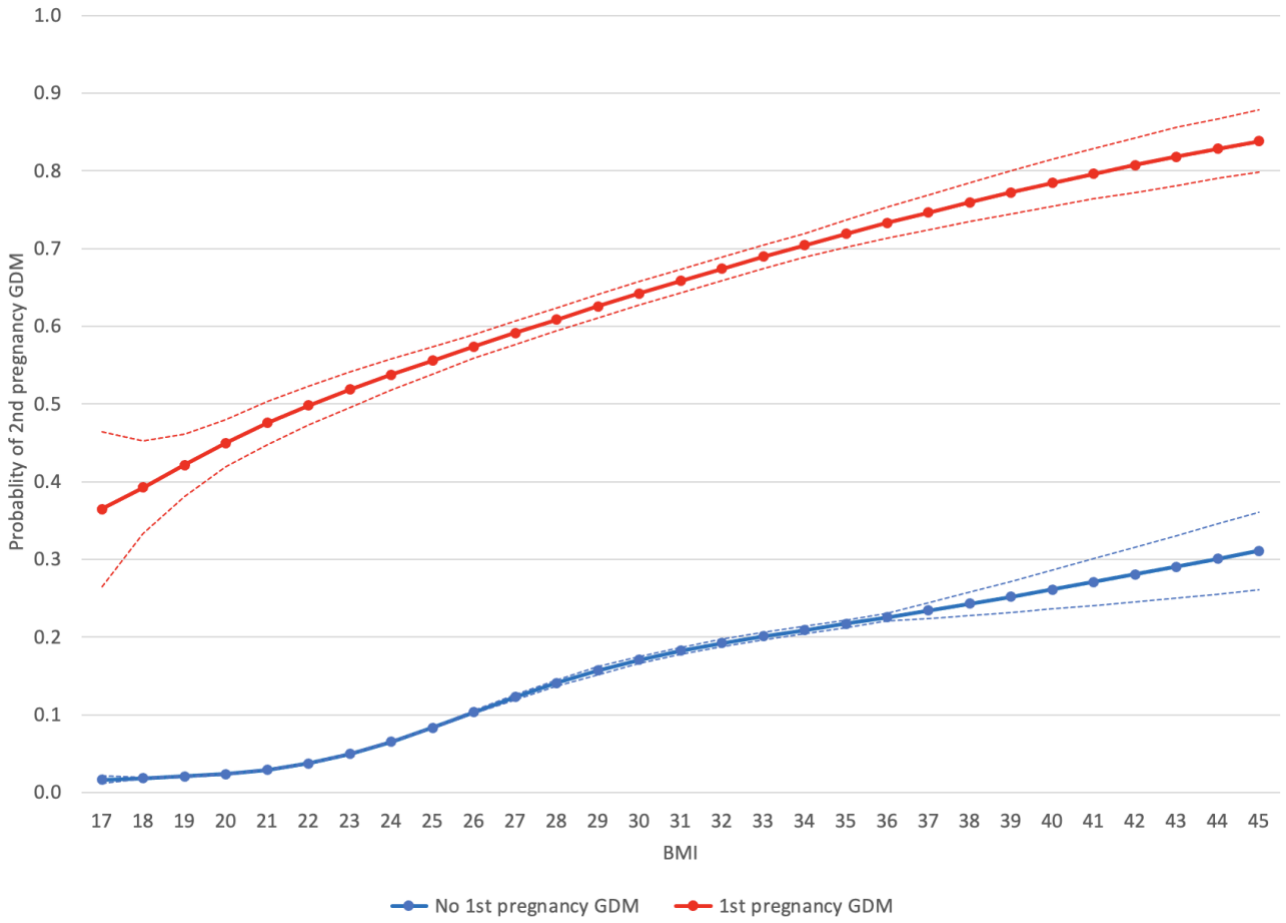



***Figure 10.*** *Unadjusted probability (dashed lines for 95% CI) for 2nd pregnancy gestational diabetes (GDM) by prepregnancy body mass index.*

***Table 7.*** *Adjusted odds ratio (OR) and 95% confidence interval (CI) for 2nd pregnancy gestational diabetes (GDM) by prepregnancy body mass index categories in the 2nd pregnancy.*

| **BMI** | **No 1st pregnancy GDM,** OR (95% CI)* | **1st pregnancy GDM,** OR (95% CI)* |
|---|---|---|
| **<18.5 kg/m²** | 0.52 (0.35–0.77) | 19 (11–33) |
| **18.5–24.9 kg/m²** | reference | 25 (22–28) |
| **25–29.9 kg/m²** | 3.7 (3.4–4.0) | 39 (35–44) |
| **30–34.9 kg/m²** | 6.1 (5.4–6.9) | 56 (48–64) |
| **35–39.9 kg/m²** | 7.6 (6.3–9.2) | 77 (61–97) |
| **>40 kg/m²** | 14 (11–18) | 113 (80–159) |

*adjusted for maternal age, smoking during pregnancy, chronic hypertension, and 1st pregnancy gestational hypertension or preeclampsia

### 5.1.3 Gestational hypertension

The unadjusted probability of 2nd pregnancy GH increased with pBMI (**Figure 11**). The association between pBMI and 2nd pregnancy GH was modified by 1st pregnancy GH and PE (p for interaction <0.001 for both), and these two conditions were combined as 1st pregnancy hypertensive disorder. The results are presented separately for women with and without 1st pregnancy hypertensive disorder. The association was not modified by GDM (p for interaction 0.670) or pregestational diabetes (0.386). The adjusted ORs for 2nd pregnancy GH by prepregnancy body mass index categories and with or without 1st pregnancy hypertensive disorder are presented in **Table 8**.

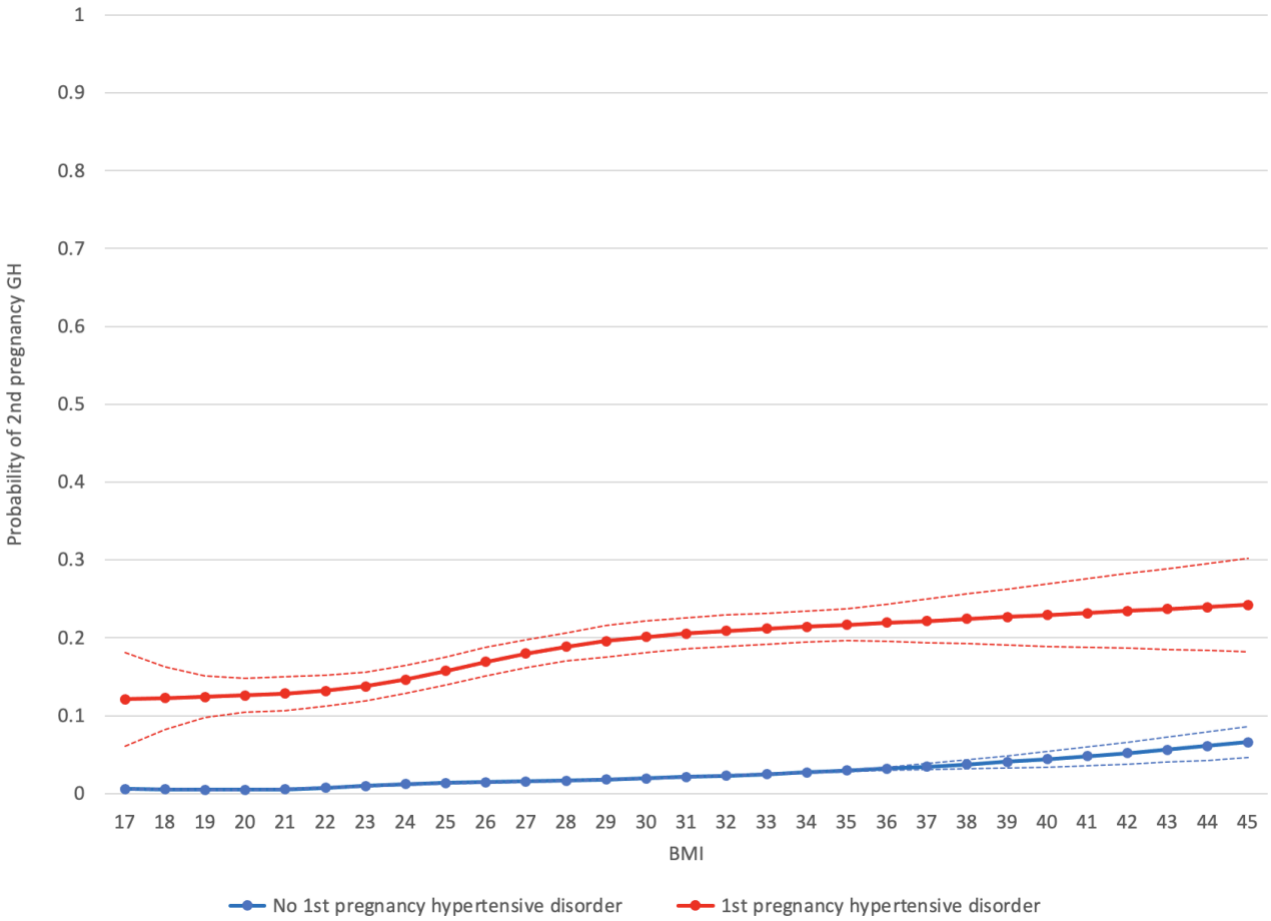



***Figure 11.*** *Unadjusted probability (dashed lines for 95% CI) for 2nd pregnancy gestational hypertension (GH) by prepregnancy body mass index.*

***Table 8.*** *Adjusted odds ratio (OR) and 95% confidence interval (CI) for 2nd pregnancy gestational hypertension by prepregnancy body mass index categories in the 2nd pregnancy.*

| **BMI** | **No 1st pregnancy hypertensive disorder,** OR (95% CI)* | **1st pregnancy hypertensive disorder,** OR (95% CI)* |
|---|---|---|
| **<18.5 kg/m²** | 0.84 (0.43–1.6) | 15 (7.3–32) |
| **18.5–24.9 kg/m²** | reference | 19 (16–23) |
| **25–29.9 kg/m²** | 1.8 (1.4–2.3) | 28 (23–35) |
| **30–34.9 kg/m²** | 2.9 (2.2–3.9) | 35 (27–45) |
| **35–39.9 kg/m²** | 4.9 (3.3–7.2) | 41 (29–58) |
| **>40 kg/m²** | 6.2 (3.7–10.5) | 40 (25–63) |

*adjusted for maternal age, smoking during pregnancy, pregestational diabetes, and 1st pregnancy gestational diabetes

### 5.1.4 Preeclampsia

The unadjusted probability of 2nd pregnancy PE increased from 0.95% with a pBMI of 17 kg/m2 to 5.7% with a pBMI of 45 (data not shown). The association between pBMI and second-pregnancy PE was not modified by pregestational diabetes (p for interaction 0.457) or GDM (0.550). HT (p for interaction 0.047) and 1st pregnancy hypertensive disorder (0.083) modified or tended to modify the association, but separate analyses by the condition status showed no relevance (data not shown). The adjusted ORs for 2nd pregnancy PE by pBMI categories are presented in **Table 9**.

***Table 9.*** *Adjusted odds ratio (OR) and 95% confidence interval (CI) for 2nd pregnancy preeclampsia by prepregnancy body mass index categories in the 2nd pregnancy.*

| **BMI** | **OR (95% CI)*** |
|---|---|
| **<18.5 kg/m²** | 0.81 (0.45-1.45) |
| **18.5–24.9 kg/m²** | reference |
| **25–29.9 kg/m²** | 1.20 (0.99-1.45) |
| **30–34.9 kg/m²** | 1.52 (1.19-1.93) |
| **35–39.9 kg/m²** | 1.81 (1.30-2.52) |
| **>40 kg/m²** | 1.49 (0.93-2.37) |

*adjusted for maternal age, smoking during pregnancy, pregestational diabetes, chronic hypertension, 1st pregnancy gestational diabetes, and 1st pregnancy gestational hypertension or preeclampsia

## 5.2 Metabolome in maternal obesity, GDM and hypertensive disorders (Studies II, III and IV)

### 5.2.1 Background and clinical characteristics of the cohorts in Studies II, III and IV

**Table 10** presents the background characteristics of the 1702 mother-child dyads (Study IV) with umbilical cord blood samples available in PREDO, RADIEL and ITU studies and the comparison to all live births in Finland 2006–2017. Compared to the women in MBR, the women in all three studies were older, less likely to smoke during pregnancy, had more often GDM or hypertension in pregnancy and delivered more often with caesarean section. Compared to the women in MBR, women in the PREDO and RADIEL studies had higher pBMI and, thus, were more often with obesity. Birth weight in the RADIEL study was higher than in MBR.

***Table 10.*** *Characteristics of the 1702 mother-child dyads (Study IV) in PREDO, RADIEL, and ITU studies compared to all live births in the Medical Birth Register (MBR) in Finland 2006–2017.*

| | **PREDO (N=923)** | p* | **RADIEL (N=369)** | p* | **ITU (N=410)** | p* | **MBR (N=670 097)** |
|---|---|---|---|---|---|---|---|
| **Maternal characteristics** | | | | | | | |
| Age, years, mean (SD) | 33.1 (5.6) | <0.001 | 32.9 (4.4) | <0.001 | 34.5 (4.9) | <0.001 | 30.4 (5.3) |
| BMI, kg/m$^2$, mean (SD) | 27.1 (6.4) | <0.001 | 31.5 (5.9) | <0.001 | 23.7 (4.0) | 0.004 | 24.4 (4.9) |
| Obesity, n (%) | 312 (34%) | <0.001 | 243 (66%) | <0.001 | 28 (6.8%) | <0.001 | 83 762 (12.5%) |
| Primiparous, n (%) | 291 (32%) | <0.001 | 121 (33%) | <0.001 | 233 (57%) | <0.001 | 278 639 (41.6%) |
| Caesarean delivery, n (%) | 203 (22%) | <0.001 | 77 (21%) | 0.009 | 85 (21%) | 0.007 | 106 292 (15.9%) |
| Hypertension in pregnancy, n (%) | 175 (19%) | <0.001 | 57 (15%) | <0.001 | 28 (6.8%) | 0.006 | 27732 (4.1%) |
| Gestational diabetes, n (%) | 203 (22%) | <0.001 | 177 (48%) | <0.001 | 81 (19.8%) | <0.001 | 76220 (11.4%) |
| Smoking during pregnancy, n (%) | 46 (5.0%) | <0.001 | 28 (7.6%) | <0.001 | 29 (7.1%) | <0.001 | 98 650 (14.7%) |
| **Offspring characteristics** | | | | | | | |
| Birth weight, g, mean (SD) | 3551 (550) | 0.21 | 3706 (517) | <0.001 | 3523 (481) | 0.82 | 3529 (527) |
| Gestational age at delivery, mean (SD) | 39.8 (1.6) | | 39.9 (1.6) | | 40.0 (1.5) | | - |
| Sex, female, n (%) | 441 (47.8%) | 0.50 | 175 (47.4%) | 0.58 | 203 (49.5%) | 0.80 | 327 535 (48.9%) |

*p values for the difference between the group and MBR were calculated using Pearson $X^2$ tests for categorical variables and t-tests for continuous variables

Study II – comparing the longitudinal metabolomic profile of women with or without obesity, GDM and hypertensive disorders – included 741 women from the PREDO and RADIEL studies, of which 347 (47%) were with obesity, 184 (25%) with GDM, 89 (12%) with HT, 54 (7.3%) with PE, and 52 (7.0%) with GH. Study III – comparing metabolomic profiles of women with nonobesity and GDM (NOGDM), obesity and GDM (OGDM), or obesity only to women without GDM or obesity – included 755 women of PREDO and RADIEL studies, of which 312 (41%) were without GDM or obesity, 96 (23%) with NOGDM, 89 (12%) with OGDM, and 258 (34%) with obesity only.

Mean values of selected metabolic measures in PREDO and RADIEL studies (maternal samples obtained at three time points across pregnancy) are presented in **Table 11**.

***Table 11.*** *Mean values (standard deviations) of selected metabolic measures at three time points in the PREDO-RADIEL cohort.*

| **Metabolic measure** | **1st blood sampling point (n=401; at median 13.0 GW, range 11-16)** | **2nd blood sampling point (n=401; at median 19.3 GW, range 17-23)** | **3rd blood sampling point (n=391; at median 27.0 GW, range 24-31)** |
|---|---|---|---|
| **Total cholesterol (mmol/l)** | 4.64 (0.76) | 5.43 (0.91) | 6.11 (1.1) |
| **LDL cholesterol* (mmol/l)** | 2.5 | 2.9 | 3.4 |
| **HDL cholesterol (mmol/l)** | 1.65 (0.28) | 1.81 (0.32) | 1.83 (0.33) |
| **Total triglycerides (mmol/l)** | 1.17 (0.44) | 1.52 (0.57) | 1.99 (0.69) |
| **Total fatty acids (mmol/l)** | 12.3 (2.11) | 14.5 (2.39) | 16.7 (2.73) |
| **Apolipoprotein B (g/l)** | 0.74 (0.16) | 0.89 (0.20) | 1.06 (0.25) |
| **Apolipoprotein A1 (g/l)** | 1.72 (021) | 1.88 (0.24) | 1.93 (0.25) |
| **Ratio of apolipoprotein B to A1** | 0.44 (0.11) | 0.48 (0.13) | 0.56 (0.15) |
| **Glucose (mmol/l)** | 5.10 (0.93) | 5.03 (0.80) | 5.12 (0.69) |

GW, gestational week; LDL, low-density lipoprotein; HDL, high-density lipoprotein

*estimated with Friedewald equation based on total and HDL cholesterols, and total triglycerides

### 5.2.2 Maternal metabolomic profile (Studies II and III)

*Obesity (Study II)*

Compared to women with normal weight, women with obesity displayed differences in pooled mean across the three measurement points in the fully adjusted model in 42 out of the 68 metabolic measures (the first column in **Figure 12** presents all the significant measures). Women with obesity displayed higher levels of many VLDL-related measures, triglycerides, ApoB and its ratio to Apo A-1, glycolysis-related measures, BCAAs, AAAs, and GlycA. Compared to women with normal weight, women with obesity displayed lower levels of many HDL-related measures and lower ratios of many unsaturated FAs. The results in women with overweight were in the same direction, although less pronounced and did not always reach statistical significance (data not shown).

The change in the levels of 43 metabolic measures across pregnancy in women with obesity significantly differed from that of women with normal weight (**Figure 13**). The increase was smaller in 41 measures, and the decrease was greater in valine and smaller in albumin. The mean diameter of LDL particles decreased in women with obesity while increased in women with normal weight.

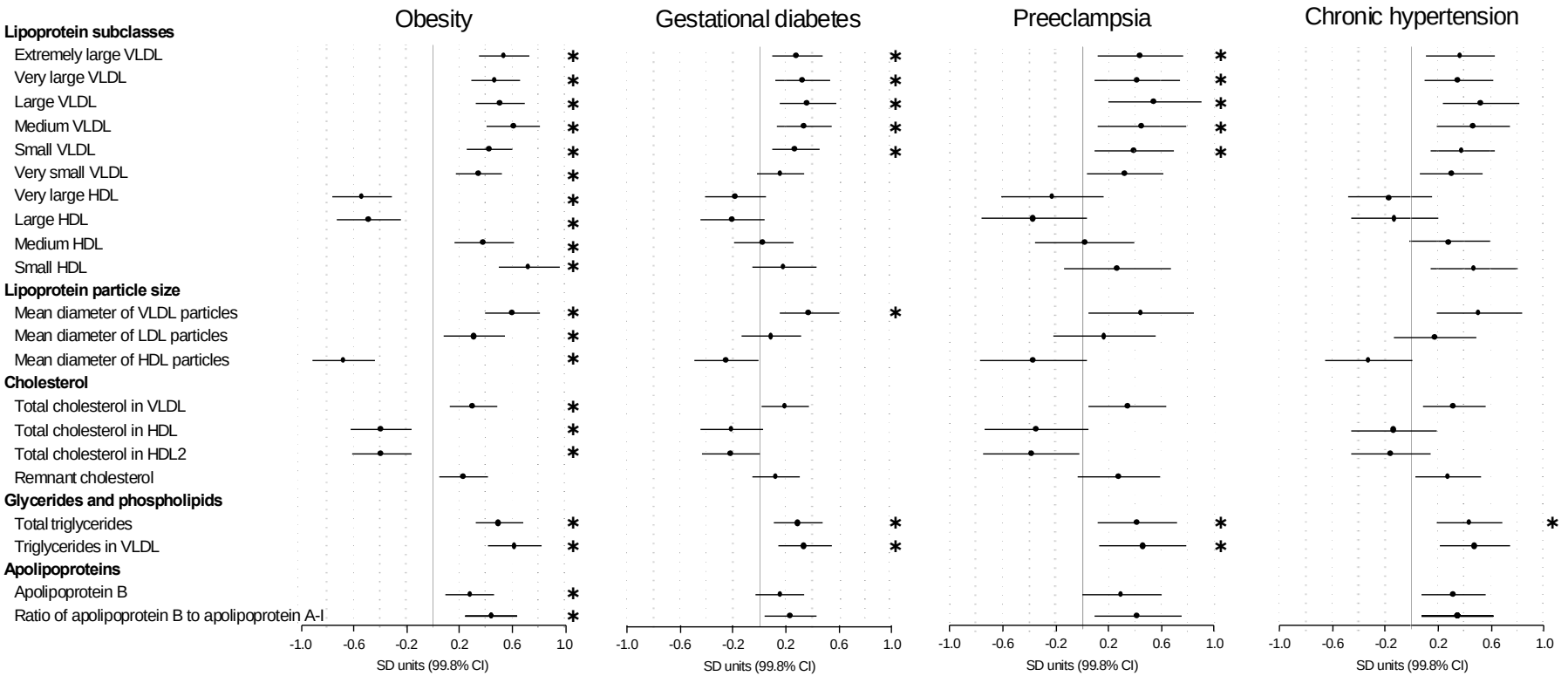



***Figure 12.*** *Pooled mean differences from controls and 99.8% CIs across three measurement points in obesity, gestational diabetes (GDM), preeclampsia (PE), and chronic hypertension (HT). Metabolic measures presented are those 42 in which women with obesity differed from the controls in model 1 (the dots and bars; adjusted for maternal age, cohort, and GW). The asterisks indicate significance in the fully adjusted model (further adjustment for maternal education, parity, smoking and alcohol use during pregnancy, and for GDM, PE, and HT (in obesity), for BMI, PE, and HT (in GDM), for BMI and GDM (in PE and HT). CONTINUES ON THE NEXT PAGE*

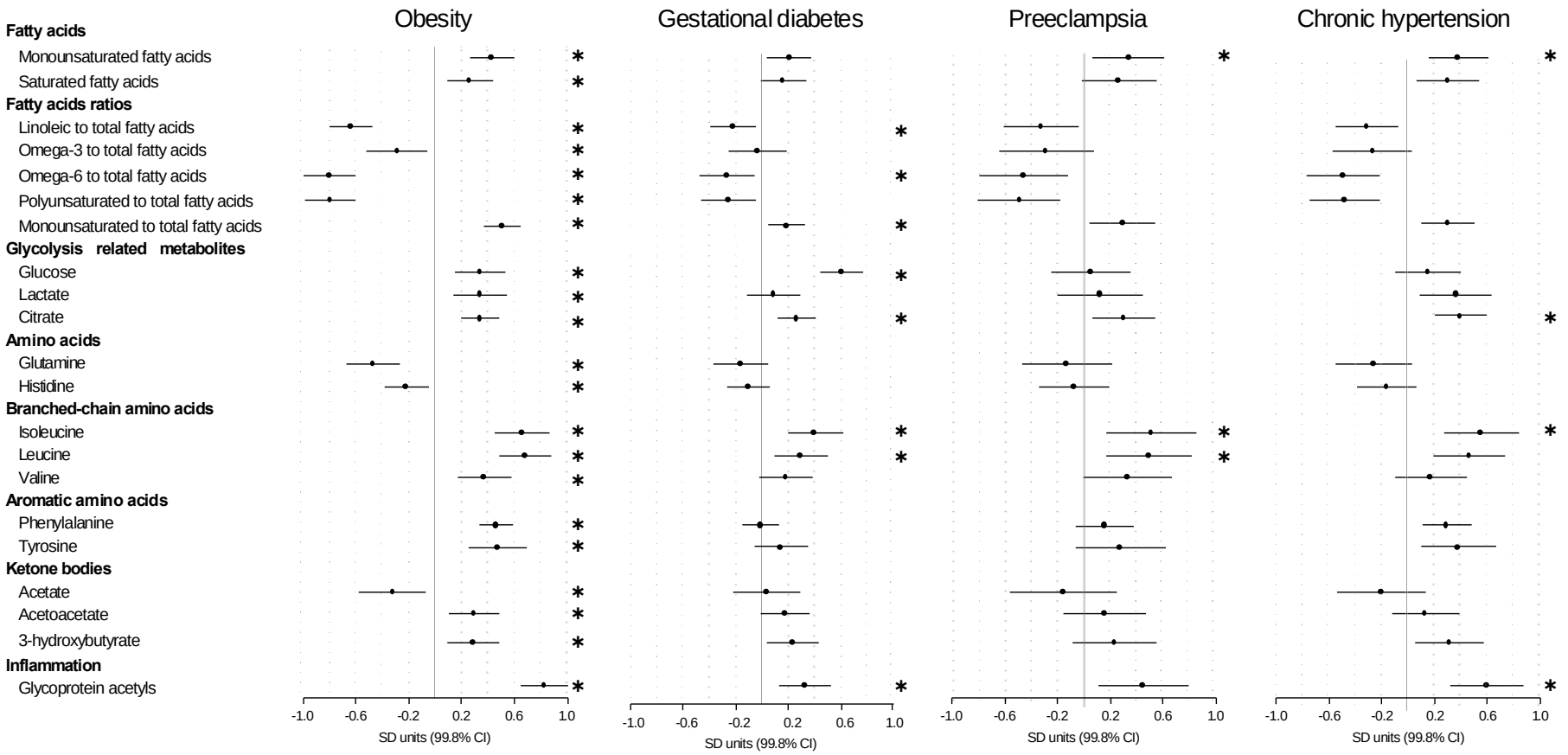



***Figure 12.*** *CONTINUED Pooled mean differences from controls and 99.8% CIs across three measurement points in obesity, gestational diabetes (GDM), preeclampsia (PE), and chronic hypertension (HT). Metabolic measures presented are those 42 in which women with obesity differed from the controls in model 1 (the dots and bars; adjusted for maternal age, cohort, and GW). The asterisks indicate significance in the fully adjusted model (further adjustment for maternal education, parity, smoking and alcohol use during pregnancy, and for GDM, PE, and HT (in obesity), for BMI, PE, and HT (in GDM), for BMI and GDM (in PE and HT).*

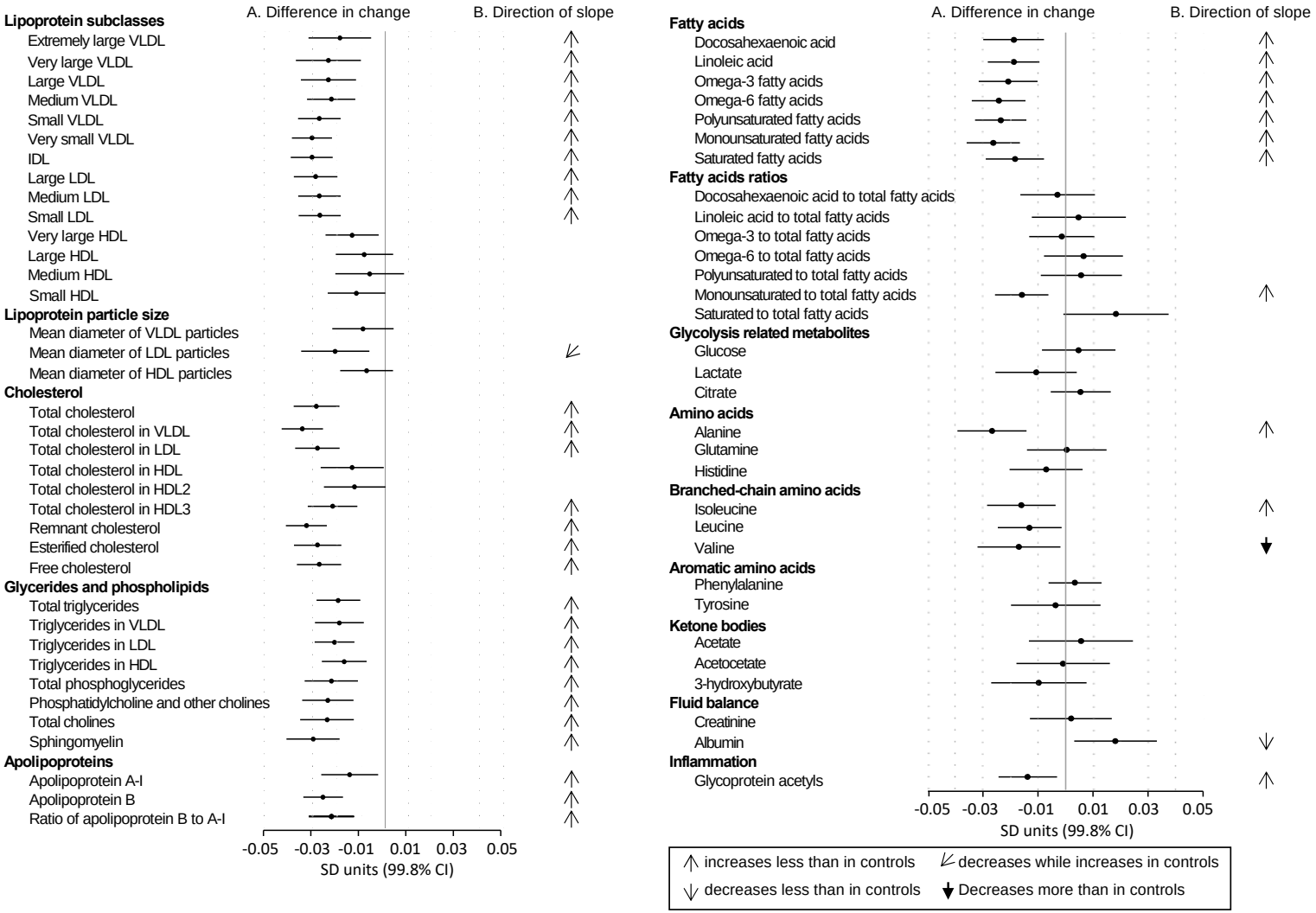



***Figure 13.*** *Difference in change of metabolic measures across pregnancy between women with obesity or with normal weight (Panel A). Dots refer to the mean difference in change per one pregnancy week and bars to their 99.8% CIs in Model 1, adjusted for gestational week at the time of sampling, cohort, maternal age and for the main effects of prepregnancy obesity. Panel B presents the direction of the slope for those metabolic measures in which change is significantly different in the final adjusted model, additionally adjusted for parity, education, substance use during pregnancy, gestational diabetes, and hypertensive disorder.*

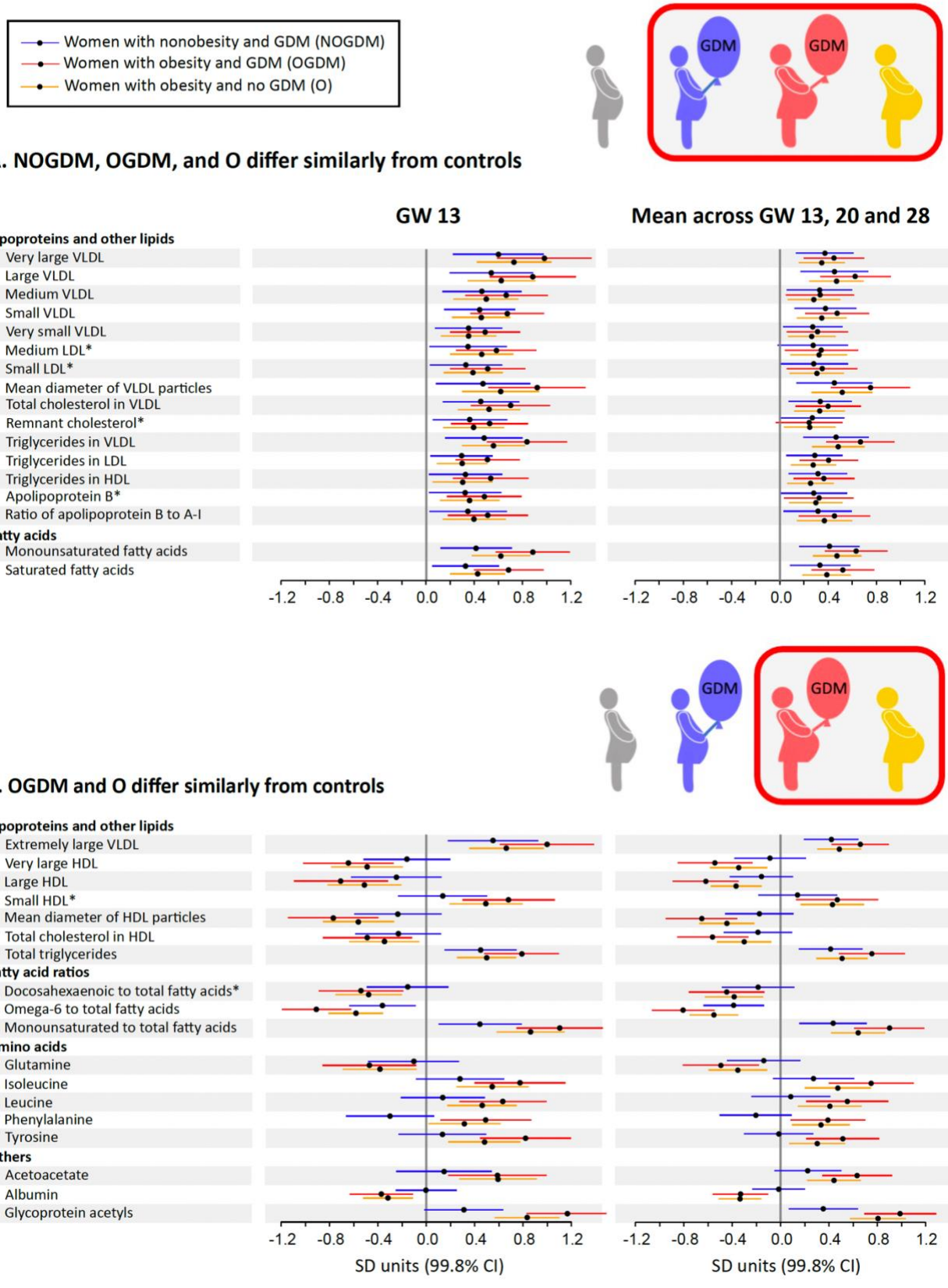
*Gestational diabetes (study II)*

Compared to women without GDM, women with GDM displayed differences in pooled mean across the three measurement points in the fully adjusted model in 23 out of the 68 metabolic measures (second column in **Figure 12**). All these 23 measures were also independently associated with obesity. They included many VLDL-related measures, triglycerides, some FA ratios, glucose, citrate, two BCAAs, and GlycA. The change in the levels of six metabolic measures across pregnancy was significantly different in women with GDM from that of women without.

*Gestational diabetes stratified by obesity status (Study III)*

Compared to the control group (women without GDM or obesity), all three study groups – women with nonobesity and GDM (NOGDM), women with obesity and GDM (OGDM), and women with obesity but no GDM (O) – displayed higher levels in many VLDL-related measures, ApoB, and monounsaturated and saturated FAs (**Figure 14**, Panel A). With the same control group, two of the three study groups, those with obesity – OGDM and O – displayed lower levels in some HDL-related measures and higher levels in total triglycerides, two of the BCAAs, AAAs, and GlycA (**Figure 14**, Panel B). The difference between the control group and OGDM was more pronounced than the difference between the control group and NOGDM or O in some FA ratios and glucose, citrate, valine and 3-hydroxybutyrate (**Figure 14**, Panel C).

In the ITU replication cohort, the differences between the control and three study groups did not reach statistical significance, but 77% were in the same direction. This was more often than would be expected by chance alone ($p<0.001$ from Fisher's exact test).


Women with nonobesity and GDM (NOGDM)
Women with obesity and GDM (OGDM)
Women with obesity and no GDM (O)
GDM
GDM
A. NOGDM, OGDM, and O differ similarly from controls
GW 13
Mean across GW 13, 20 and 28
Lipoproteins and other lipids
Very large VLDL
Large VLDL
Medium VLDL
Small VLDL
Very small VLDL
Medium LDL*
Small LDL*
Mean diameter of VLDL particles
Total cholesterol in VLDL
Remnant cholesterol*
Triglycerides in VLDL
Triglycerides in LDL
Triglycerides in HDL
Apolipoprotein B*
Ratio of apolipoprotein B to A-I
Fatty acids
Monounsaturated fatty acids
Saturated fatty acids
-1.2 -0.8 -0.4 0.0 0.4 0.8 1.2
GDM
GDM
B. OGDM and O differ similarly from controls
Lipoproteins and other lipids
Extremely large VLDL
Very large HDL
Large HDL
Small HDL*
Mean diameter of HDL particles
Total cholesterol in HDL
Total triglycerides
Fatty acid ratios
Docosahexaenoic to total fatty acids*
Omega-6 to total fatty acids
Monounsaturated to total fatty acids
Amino acids
Glutamine
Isoleucine
Leucine
Phenylalanine
Tyrosine
Others
Acetoacetate
Albumin
Glycoprotein acetyls
-1.2 -0.8 -0.4 0.0 0.4 0.8 1.2
SD units (99.8% CI)
SD units (99.8% CI)

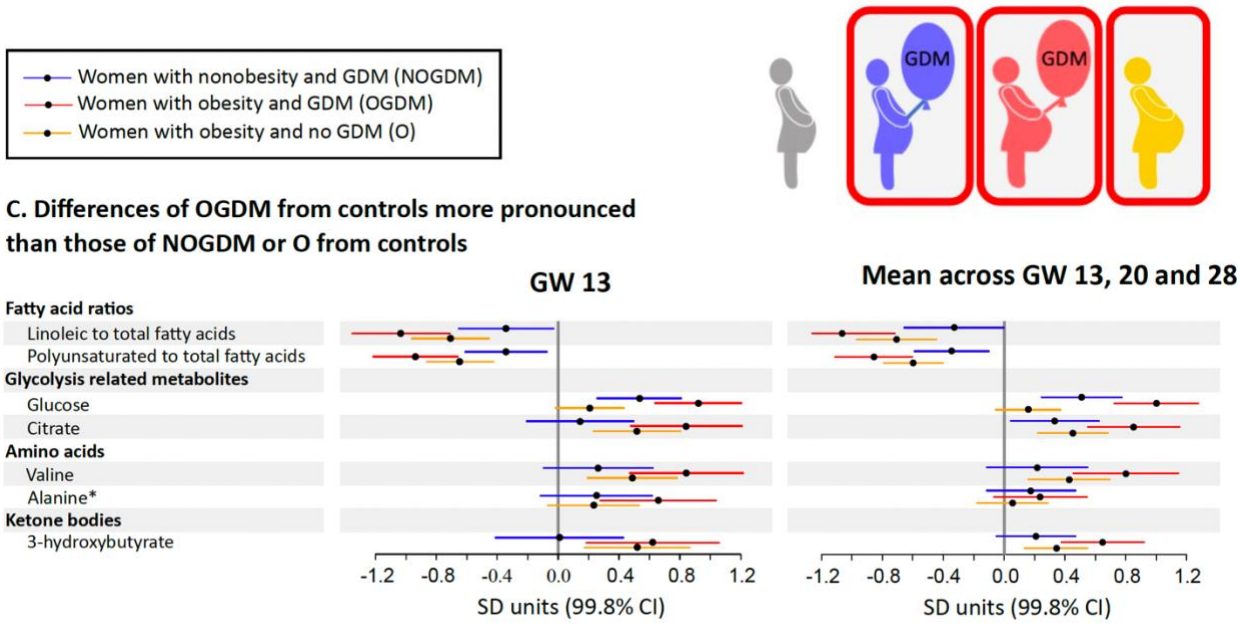



***Figure 14.*** *Mean differences and 99.8% CIs for the three study groups (NOGDM, OGDM, and O) versus the controls at 13 GW (median) and mean levels across median 13, 20, and 28 GW in the metabolites in which the differences of study groups from controls followed a similar pattern. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. *Statistically significant only in early pregnancy*

*Hypertensive disorders (Study II)*

Compared to women without PE, women with PE displayed differences in pooled mean across the three measurement points in 10 out of the 68 metabolic measures in the fully adjusted model (third column in **Figure 12**). All these 10 measures, including VLDL-related measures and triglycerides, were also independently associated with obesity. Change in the levels of two metabolic measures across pregnancy significantly differed in women with PE from those without.

Relative to women without HT, women with HT displayed differences in pooled mean across the three measurement points in six of the 68 metabolic measures in the fully adjusted model (fourth column in **Figure 12**). Five of these measures (not alanine, thus not presented in the figure) were also independently associated with obesity. The change in the levels of three metabolic measures across pregnancy was significantly different in women with HT from that of women without. GH was not significantly associated with any of the measures during pregnancy (data not shown).

### 5.2.3 Cord blood metabolomic profile in maternal obesity (Study IV)

In the meta-analysis of all three study cohorts (PREDO, RADIEL and ITU), in the fully adjusted model, pBMI was significantly associated with the levels of 12/95 metabolic measures (**Figure 15**, Panels A). An inverse association was detected between pBMI and the mean diameter of HDL particles and the concentration of large or very large HDL particles, and between maternal pBMI and ratios of LA to total FAs, PUFA to MUFA, and histidine. pBMI was directly associated with all BCAAs, except valine, phenylalanine, and ketone bodies 3-hydroxybutyrate and acetone.

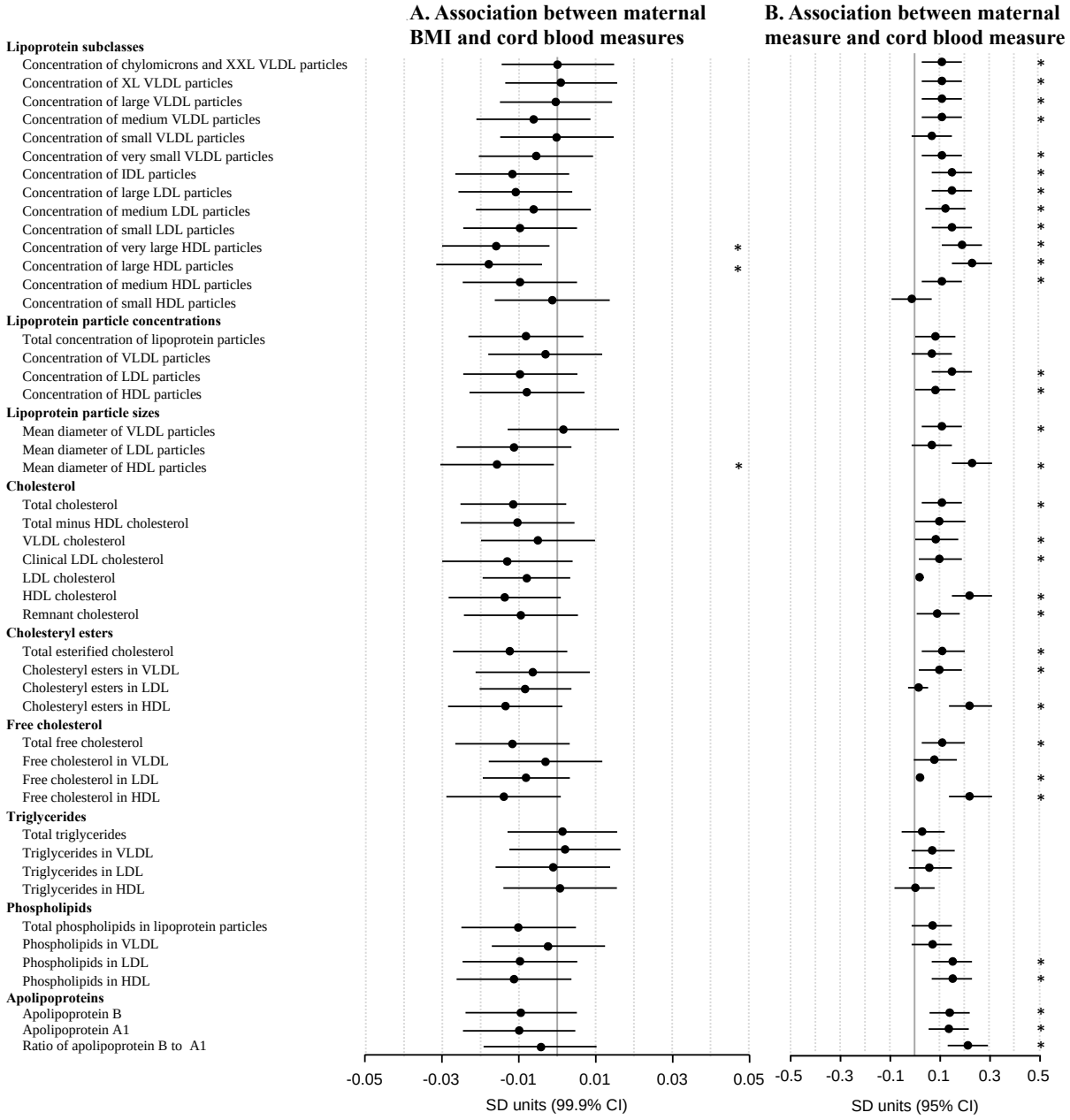



***Figure 15.*** *The association between maternal pBMI and cord blood metabolic measures in the meta-analysis of three studies (PREDO, RADIEL, and ITU) (Panels A), and the association between maternal and cord blood metabolic measures in PREDO-RADIEL (Panels B). Dots represent the mean increase or decrease of cord blood measures in SD units per 1 kg/m$^2$ increase in maternal pBMI (Panel A) or per 1 SD unit increase in maternal measure (Panel B) and bars their 99.9% CIs. The models have been adjusted for cohort, maternal age, education, parity, substance use during pregnancy, and offspring sex. Significant associations are marked with an asterisk.* *CONTINUES ON THE NEXT PAGE*

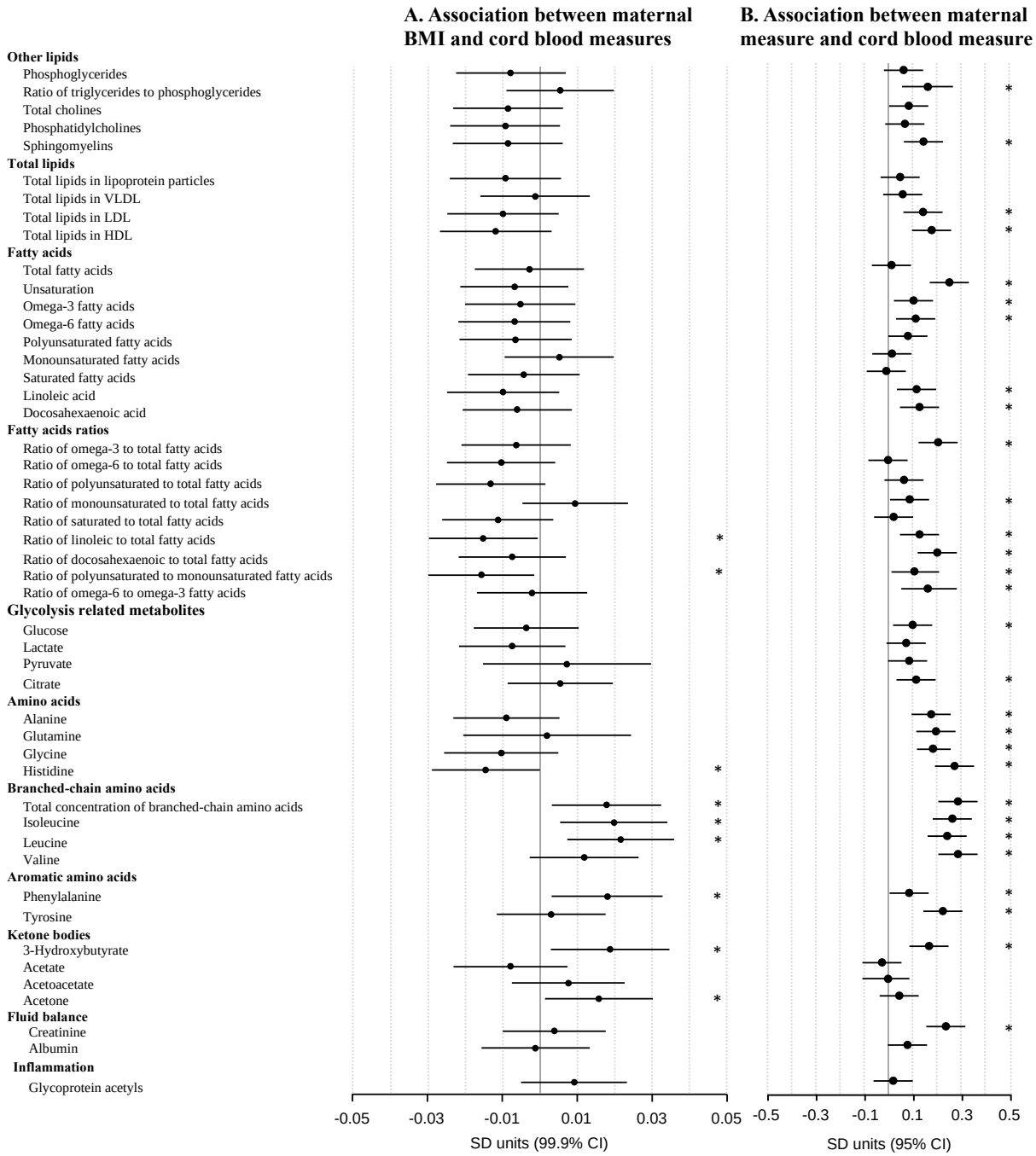



***Figure 15.*** *CONTINUED The association between maternal pBMI and cord blood metabolic measures in the meta-analysis of three studies (PREDO, RADIEL, and ITU) (Panels A), and the association between maternal and cord blood metabolic measures in PREDO-RADIEL (Panels B). Dots represent the mean increase or decrease of cord blood measures in SD units per 1 kg/m$^2$ increase in maternal pBMI (Panel A) or per 1 SD unit increase in maternal measure (Panel B) and bars their 99.9% CIs. The models have been adjusted for cohort, maternal age, education, parity, substance use during pregnancy, and offspring sex. Significant associations are marked with an asterisk.*

Associations between maternal and cord blood levels were significant in the fully adjusted model in 61 of the 95 metabolic measures in the PREDO-RADIEL (mean of three maternal measurements in pregnancy) (**Figure 15**, Panels B). Significant associations between maternal and cord blood levels were found across all other different classes of metabolic measures but not in triglycerides.

Maternal pBMI modified the association between maternal and cord blood levels of 26 metabolic measures, mainly lipids. In women with obesity, maternal levels of these measures were directly associated with cord blood levels. In women without obesity, cord blood levels were associated with maternal levels in only two of these measures (**Figure 16**).

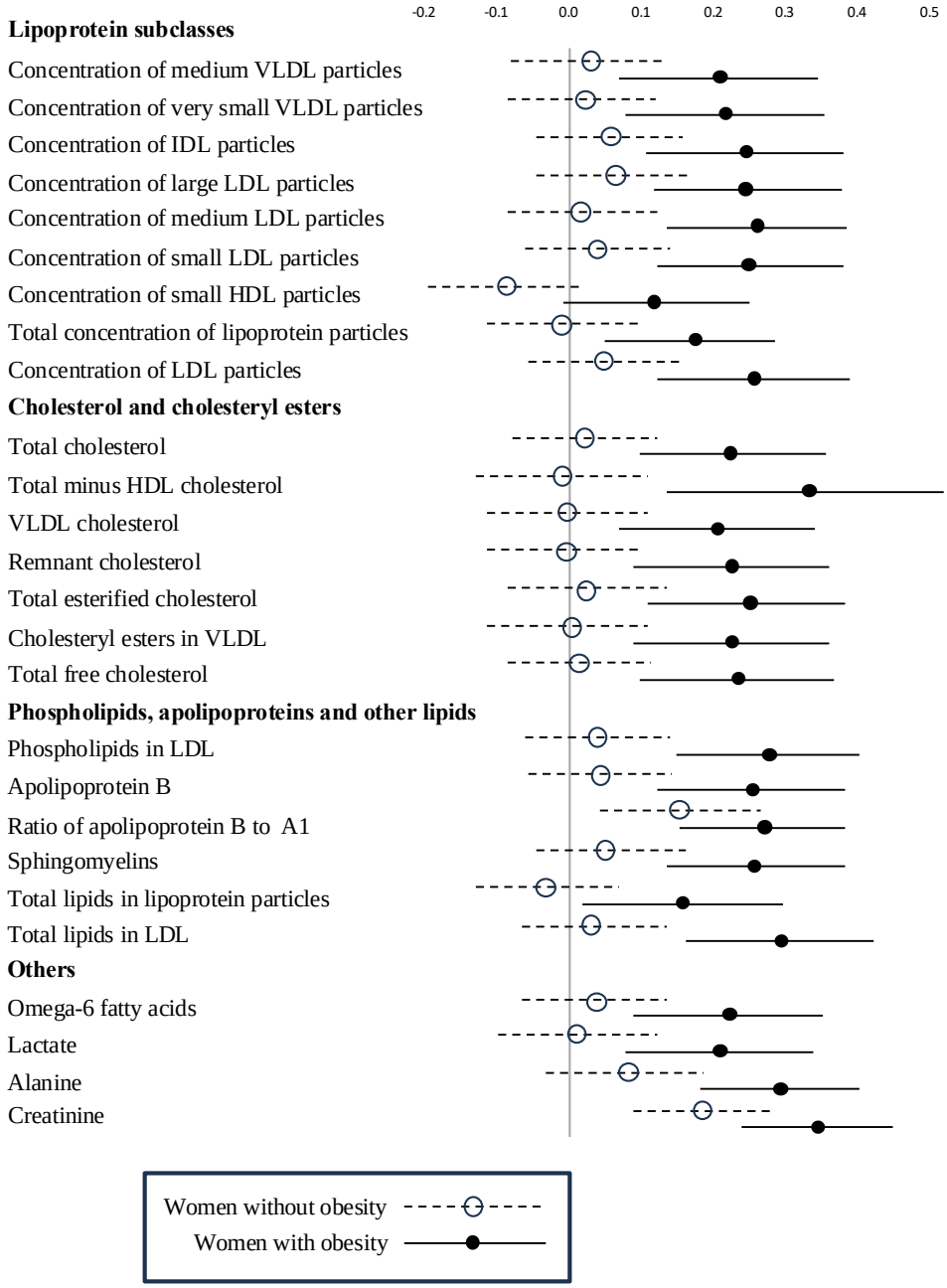



***Figure 16.*** *The association between maternal and cord blood metabolic measures in PREDO-RADIEL in women with or without obesity. Dots represent the mean increase or decrease of cord blood measures in SD units per 1 SD unit increase in maternal measure and bars their 95% CIs. The model has been adjusted for cohort, maternal age, education, parity, substance use during pregnancy, and offspring sex.*

# 6 Discussion

## 6.1 Main findings

Maternal obesity results in increased risk of GDM, GH, and PE, also in the second pregnancy. The outcome of the first pregnancy plays a major role, and the magnitude of the risk for disorders greatly depends on whether the first pregnancy was complicated. The risk for GDM or GH is far higher if the disease has complicated the first pregnancy, but the risk increases with pBMI also in women with an uncomplicated first pregnancy.

Maternal metabolomic perturbations associated with maternal obesity, GDM, and PE, and cord blood metabolomic perturbations associated with maternal obesity are summarised in **Figure 17**. The maternal metabolomic profile of obesity displays atherogenic perturbations, i.e. higher levels of VLDL particles, triglycerides, and ApoB, smaller HDL particles, and a lower degree of FA unsaturation. Perturbations also reflect insulin resistance, meaning higher levels of BCAAs and AAAs, and indicate inflammation, meaning higher GlycA. Metabolomic profiles of GDM and PE partly display these same perturbations, thus suggesting the same underlying pathophysiologic processes.

Cord blood metabolic measures associated with higher maternal pBMI are a lower level of large HDL particles, a lower degree of FA unsaturation, and higher levels of BCAAs, an AAA, and ketone bodies, the same perturbations as in the maternal obesity-related metabolome. The levels of various maternal metabolic measures are associated with the levels of the respective measures in cord blood. The association between maternal and cord blood levels of several metabolic measures, many of which are lipids, is modified by maternal obesity.

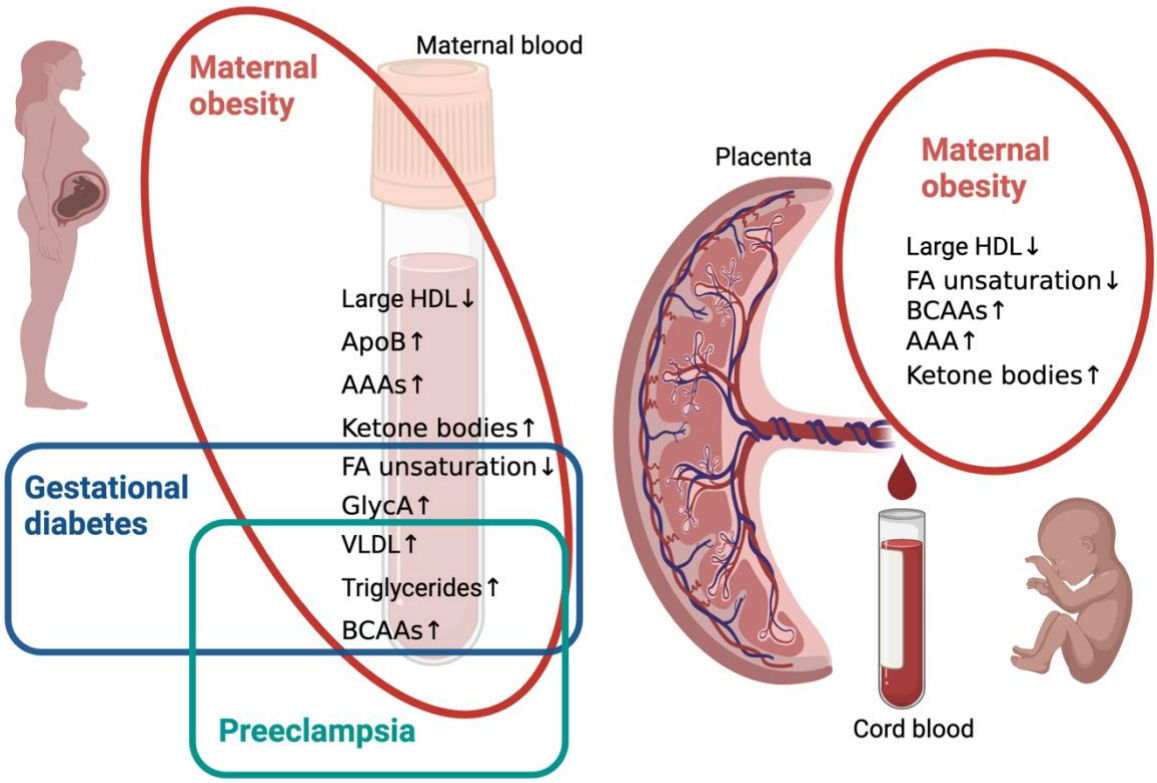



***Figure 17.*** *Summary of metabolomic perturbations associated with maternal obesity, GDM and PE in maternal blood, and perturbations associated with maternal obesity in cord blood.* *Created with BioRender.com*

GDM is associated with metabolomic perturbations even in the absence of obesity. The metabolomic perturbations displayed by women with GDM but no obesity are partly similar to those in women with obesity. The differences in the metabolomic profiles between women with NOGDM or OGDM may be explained by the differences in the underlying pathophysiology of GDM – insulin resistance or insulin-secretion deficit – in these two groups of women.

The metabolomic profile varies along the progression of pregnancy, but with maternal obesity, the change is smaller than in normal-weight pregnancy due to the wide aberrations present already at the beginning of pregnancy. The differences in the metabolomic profiles between women with normoglycemic pregnancy and those later developing GDM can be detected already in early pregnancy.

## 6.2 Strengths and limitations

The association between maternal pBMI and pregnancy complications was studied in a nationwide population-based cohort. The information was obtained from national registers with high coverage and quality [316]. Maternal pBMI in the register is based on prepregnancy weight reported by the expecting mother at the first visit to the antenatal clinic. This may lead to some underestimation but is not assumed to bias associations between pBMI and pregnancy disorders [317]. Underestimation of obesity would bias results towards null.

Information on pBMI has been collected in MBR since 2006, designating the start of this cohort. Despite the large cohort between 2006 and 2013, the number of women with pregestational diabetes or HT was limited, which may have led to an inability to detect some associations. By the publication date of this thesis, a larger cohort from Finnish registers would be available.

The large PREDO and RADIEL studies provide a researcher with a large, well-characterized cohort enriched with pregnancies complicated by obesity, GDM, and hypertensive disorders and thus, high statistical power to detect associations. The number of missing data in both studies is low. Blood samples were available from three time points across the pregnancy, enabling longitudinal analysis. The targeted metabolomic panel has been widely used, and multiple associations between the metabolic measures and health outcomes in several studies, including large Biobank cohorts, have been published [318–320]. The collection of different samples, i.e. plasma and serum, in the two studies was addressed by the statistical methods with SD scaling and adjustment for the cohort. The timing of the samples during pregnancy differed between the cohorts, generating a wide range in gestational weeks, which might diminish some of the findings. Completion of ITU study data enabled replication of the findings in Study III, and the meta-analytic approach with a combination of all three cohorts, PREDO, RADIEL and ITU, provided us with an exceptionally large sample size in study IV.

The metabolomic studies are secondary analyses. In the PREDO study, the original focus was on predicting and preventing PE and IUGR. A small subcohort was randomised to receive either ASA or placebo. The RADIEL study focused on preventing GDM among high-risk women, and the participants were randomised in the intervention group receiving advice on diet and physical activity or in the control group with standard care. The possibility of bias by the intervention was considered, but in Study II, the metabolomic profiles between the ASA/intervention and placebo/standard groups were compared, and no difference was found.

The population in all these four studies came from Finland, an ethnically homogenous country with a high-resource healthcare setting. This may limit the generalisability of the findings.

## 6.3 The risk of second-pregnancy complications by maternal pBMI

As in the previous studies not stratified by parity [321–323], in this study, the risk of GDM and hypertensive disorders increased with pBMI in the second pregnancy. In women with no prior GDM, the risk increased 4-fold already with a shift from normal to overweight, even before reaching the threshold of obesity. Previous studies, including a meta-analysis of nearly a million women [324], have demonstrated a dose-response relationship between pBMI and the risk of GDM across the full range of pBMI. In this study, the risk of GH increased 1.8-fold with the shift from normal to overweight. The findings indicate the necessity to pay attention to not only obesity but also to overweight, both at the population and at an individual level.

The risk for GDM or GH increased with pBMI in the second pregnancy, even if the first pregnancy was not complicated. However, the risk of a complicated pregnancy is far higher if the woman had the same complication in her first pregnancy. Pregnancy is a window to future health, it is said. Likewise, the first pregnancy is a window to health in the second pregnancy.

Finland does not follow a universal GDM screening strategy, but screening is based on risk factors. Women with no prior GDM and no obesity are not referred to OGTT, which results in an inability to

detect the disease in women without these risk factors. This may have affected the results of this study by underestimating the risk in young women with no obesity.

PE typically affects the first pregnancy; the incidence in the second pregnancy is remarkably smaller. In a study based on the Swedish Medical Birth Register, the incidence of PE in the first pregnancy was 4.1% and in the second pregnancy 1.7%; the percentages in this study were alike (3.9% and 1.4%) [325]. Although the risk of PE in this study increased with pBMI, the magnitude of the difference did not differ between women with or without the complication in the first pregnancy. With the low incidence of PE, it is possible that a larger study would be needed to detect the differences.

Etiology of diabetes and hypertension overlap [187]; pre-existing diabetes increases the risk of PE [326], and HT increases the risk of superimposed PE [327] and GDM [328]. In this study, the risk of these pregnancy disorders in women with pre-existing diabetes or HT did not increase with pBMI. The finding should not, however, be interpreted as redundancy of weight management among these women. First, the number of women in this study may not have been enough to detect the effect of adiposity from the underlying greater risk. Second, obesity in pregnancy is associated with many other short- and long-term adverse events to which women with pre-existing diseases and their offspring may be even more prone.

## 6.4 Maternal metabolome

### 6.4.1 The metabolomic profile of obesity and change across pregnancy

The metabolomic profile of maternal obesity is characterised by higher VLDL, triglyceride, and ApoB levels, smaller HDL size, lower level of unsaturation of FAs, higher BCAA levels, and low-grade inflammation, i.e. alterations that are atherogenic and associated with increased risk for various cardiovascular and metabolic diseases [274, 329–332]. These findings have previously been presented in cross-sectional studies, but this study demonstrated the alterations already present in early pregnancy and persist across pregnancy. Pregnancy induces profound changes in maternal metabolism; many of these are comparable to those with obesity in a non-pregnant state, and thus, as women with obesity enter pregnancy with these derangements already present, the change across pregnancy is smaller in women with obesity. A study comparing glucose and insulin metabolism among glucose-tolerant women with severe obesity or with normal weight [333] demonstrated convergence in insulin resistance and similar triglyceride levels between the two groups by the end of pregnancy. Our study demonstrated identical convergence in the metabolic response to pregnancy between women with normal weight or obesity in a more extensive set of metabolites.

High VLDL, triglyceride, and ApoB levels are tightly associated with obesity in a general population [334–336] and constitute a major risk factor for cardiovascular disease [337, 338]. High VLDL levels in early pregnancy demonstrate the absence of an anabolic state of normal early pregnancy in women with obesity; the synthesis rate of triglycerides and VLDL particles by the liver is high with obesity already in early pregnancy. Obesity-associated insulin resistance is seen in this study as higher BCAA and AAA levels, even in the absence of GDM, in line with previous studies [239, 249]. This study demonstrated high levels both in early pregnancy and also in the mean across pregnancy.

Aberrations in several HDL-related measures were associated with obesity in this study. Large HDL particles are antiatherogenic, and in this study, maternal obesity was characterised by a smaller diameter of HDL particles and a lower number of large ones. This is in line with previous studies

demonstrating perturbations in metabolism, composition, and function of maternal HDL with maternal obesity [239, 339]. In an earlier study utilizing RADIEL data, maternal HDL-related measures in trimesters 2 and 3 were associated with offspring early ascending growth [230], a risk factor for childhood obesity [340, 341]. The same study found an association between maternal trimester 3 lower levels of several unsaturated FAs and adverse offspring growth. In this study, we found a lower level of unsaturation, as seen in previous cross-sectional studies [239, 243], to persist throughout pregnancy with maternal obesity.

Low-grade inflammation of obesity was demonstrated in our study by higher levels of GlycA; the difference between women with normal weight or obesity was detected already in early pregnancy. An altered inflammatory profile characterises normal pregnancy, and a balance between pro- and anti-inflammatory cytokines is essential for implantation, placentation, and continuation of a pregnancy [342]. Maternal obesity and associated inflammation skew this balance toward pro-inflammation and have a negative impact on offspring's long-term health, as demonstrated by the association between maternal inflammation and an increased risk for neuropsychiatric disorders and metabolic diseases [342].

### 6.4.2 Similarities in the metabolome of obesity, GDM and hypertensive disorders

GDM and PE are closely interlinked; GDM is an independent risk factor for PE, and treatment of GDM reduces the risk of PE [343]. Pathophysiologic mechanisms of these diseases, including insulin resistance and abnormalities in lipid metabolism, overlap with obesity [343]. Thus, it is not surprising that women with GDM or PE in this study, independent of obesity, displayed many of those metabolomic aberrations detected with maternal obesity, although not in as extensive set of circulating measures. Consistent with the findings of earlier studies [211, 239, 291], high levels of atherogenic VLDL and triglycerides, and BCAAs isoleucine and leucine were displayed in this study by women with either GDM or PE.

In our study, high maternal GlycA levels were displayed with GDM, but contrary to the Born in Bradford study [239], women with PE in our study did not display similar high levels. Inflammation is one of the pathologic processes in PE, and earlier studies have demonstrated inflammation with other biomarkers like proinflammatory cytokines TNF-α or interleukin-6 [343]. Inflammation of PE could have been revealed in our study if a broader panel of inflammation markers had been used, and it is also possible that we could have seen an association with GlycA if the sample size had been larger. Further, PE is a heterogenic disease [344], and differences between the profiles of early- and late-onset disease could have been found with stratification, but this would have also required a larger sample size. HT was associated with high GlycA in our study, demonstrating the inflammatory process in its pathogenesis [345].

In women with obesity, metabolic response to pregnancy, change across three trimesters, was different from that of women without obesity. In women with GDM or hypertensive disorders, change across pregnancy did not remarkably differ from that of women without. Women with GDM or hypertensive disorders develop metabolic perturbations later in pregnancy than women with obesity, and thus, the slope of metabolic measures is closer to normal pregnancy.

Metabolic syndrome is a cluster of metabolic abnormalities, including abdominal obesity, insulin resistance, dyslipidemia, and hypertension [346]. Metabolic syndrome of pregnancy refers to an increased risk of hypertensive complications, metabolic disturbances of nutrient metabolism, and inflammation, and is associated with adverse feto-maternal outcomes [347]. Although these

pregnancy-related conditions often resolve after delivery, women affected in pregnancy may still maintain a subclinical metabolic disorder and carry an increased risk for metabolic syndrome later in life, particularly with post-partum weight gain. Similarities in the metabolomic profiles of obesity, GDM, and PE demonstrated by this study, higher levels of several atherogenic lipids and BCAAS reflect metabolic syndrome of pregnancy in these women.

### 6.4.3 Heterogeneity of GDM and normal weight obesity

The metabolic burden of GDM is evident even in the absence of obesity. All women with GDM, despite their pBMI, display several atherogenic perturbations similar to those in obesity: high VLDL levels, enrichment of the VLDL particles with triglycerides and high ApoB levels. The perturbations are present in early pregnancy, before the diagnosis of GDM, also in those women with NOGDM, and may implicate these women to display metabolic derangements even in a non-pregnant state. According to a Finnish longitudinal cohort study, the risk for type 2 diabetes is increased among normal-weight women with GDM [97]; their hazard ratio for diabetes 20 years after pregnancy was over ten-fold, not far from the corresponding figure for women with overweight but no GDM, which was 12.6. A concept of normal weight obesity, excessive body fat despite normal body weight, associated with increased risk of cardiovascular morbidity and mortality [348], may apply to women with NOGDM. One implication of normal weight obesity may be the finding of an earlier report of the RADIEL study; lean women with GDM display an exceptionally high body fat percentage despite nonobese pBMI [349]. Recent studies in non-pregnant populations have revealed nonobese adults with obesity-like metabolome to have a higher risk for type diabetes and cardiovascular diseases [301, 302].

The heterogeneity of type 2 diabetes is well-established, and increasing evidence is accumulating to point out the heterogeneity of GDM [176, 177]. The difference between the underlying mechanisms in GDM – insulin-resistant, typically obesity-associated, and insulin-deficient, more frequently associated with normal-weight – is manifested as a difference in adverse pregnancy outcomes, including macrosomia, the risk for which is increased only with insulin-resistant subtype [177]. As macrosomia has lately been associated with maternal metabolic derangements of HDL-associated measures [228, 350, 351], it is comprehensible that unlike women with OGDM or obesity only, women with NOGDM did not display derangements in HDL measures in our study. Differences in the degree of insulin resistance between women with NOGDM and OGDM were manifested in this study as the differences in the levels of BCAAs and AAAs. Circulating levels of PUFAs are inversely associated with insulin resistance [352]; lower PUFA to total FAs were among the metabolic derangements in women with OGDM but not in those with NOGDM.

This study was the first to display metabolomic differences between NOGDM and OGDM and to demonstrate women with NOGDM display perturbations similar to obesity. The findings are supported by the replication cohort, in which the results were in the same direction in 77% of the metabolic measures, which is more often than expected by chance alone.

## 6.5 Cord blood metabolome

Consistent with the findings of the HAPO study with 1600 mother-child dyads [298], this study demonstrated pBMI to be associated with several derangements of cord blood metabolome, including HDL-associated measures, and BCAAs isoleucine and leucine. Many previous studies on the association between pBMI and cord metabolome have been characterised by a small sample size or a low number of women with obesity, which may explain the inconsistency of findings in these studies [249, 295, 297, 353]. As far as we know, our study is the largest study examining the association between pBMI and cord metabolome, and the percentage of women with obesity in our study is high (34%).

As previously stated, several studies have found an association between maternal HDL-related measures and risk for macrosomia or offspring accelerated early growth [228, 230]. Cord blood HDL levels have been associated with birth weight [296], but not with later growth or adiposity [354, 355]. Thus, it is likely that the association between pBMI and cord blood HDL measures found in this study does not offer a mechanistic connection between obesity and excessive fetal growth.

Elevated cord blood levels of BCAAs with higher pBMI were demonstrated in this study. As already stated, this finding is supported by the HAPO study [298] and also by a smaller study with 116 newborns [249]. Higher cord blood BCAA levels have been associated with a greater risk for offspring neurodevelopmental impairment, including attention-deficit hyperactivity [356] and IUGR [357]. IUGR could, by accelerated growth, generate the increased risk of later obesity, but instead, low levels of cord blood BCAAs have been associated with childhood overweight [358]. The association between later childhood higher circulating levels of BCAAs and AAAs (tyrosine and phenylalanine) and childhood obesity has been widely and consistently reported, as a systematic review presented [359].

In this study, maternal pBMI was associated with adverse cord blood FA profile, i.e. relatively lower levels of PUFAs. Some studies have found an association of cord blood FA levels with birth weight or neonatal adiposity [360–362] or with neuropsychiatric disorders [363]. As lower cord blood PUFA level was beneficial in terms of blood pressure at the age of 9 [364], the role of early life FA levels may differ from that of the adult population.

In this study, multiple maternal metabolic measures across three trimesters were associated with respective cord blood measures at delivery. The mechanisms behind these associations must be multifactorial, as associations were detected in many of those metabolites not transported across the placenta. Maternal levels of all the eight AAs – transported actively across the placenta – were associated with cord blood levels. FAs are transported selectively with preference towards PUFAs [365]; this study found maternal and cord blood levels of only some FAs to be associated. A Norwegian mother, father, and child cohort study [366] utilised the same NMR panel as ours and demonstrated similar associations between maternal and cord blood levels of several lipids, FAs and AAs. They found minimal associations between paternal and cord blood levels, highlighting the significance of the shared materno-fetal milieu and a minimal contribution of genetic similarities to the associations.

Our study was the first to demonstrate that maternal pBMI modifies the association between levels of several maternal and cord blood metabolic measures. Many of these measures were lipids; association between maternal and cord blood levels of small atherogenic HDL particles and of a PUFA, omega-6 FA was stronger with obesity. The modification effect of maternal obesity on the flux of some nutrients may be an implication of the histopathological findings in the placentas of

mothers with prepregnancy obesity or reflect the regulatory role of the placenta in the transport of nutrients. Notably, the modification effect was present in many lipids, as several studies have suggested maternal lipids to be strong determinants of early and later offspring growth [158, 204, 367–370]. Further studies are needed to explore the influence of these findings on the later growth and development of the offspring.

## 6.6 Clinical implications and future perspectives

Maternal obesity poses short- and long-term consequences for both mother and offspring. Because interventions to reduce gestational weight gain during pregnancy in maternal obesity prove to have limited effect [371], interventions are needed long before pregnancy to control the increasing rate of obesity in children and adolescents - those who are future pregnant mothers. Optimising maternal health and weight even before pregnancy can improve pregnancy-, birth-, and long-term health outcomes associated with obesity. However, this requires long-term approaches that range from behavioural, pharmaceutical, and surgical interventions (treatment of obesity) to population-based public health and economic initiatives (prophylaxis of obesity).

Perturbations in the maternal circulating metabolome associated with obesity are widespread, and the high levels of several of these measures may translate into high cord blood levels. Fuel-mediated teratogenesis refers to the excess flow of nutrients from the maternal to the fetal compartment and affects short- and long-term health in the offspring [372]. Elucidating the mechanisms behind these complications may offer objectives for targeted preventive interventions, and metabolomic studies are needed to provide more insights. Combining metabolomic data with other -omic sciences, i.e. genomics, transcriptomics, and proteomics, can aid in differentiating the effects of the genome from those of the environment and thus in identifying achievable targets for intervention. Dietary supplementation may be one of the keys to improving the outcomes of pregnancies complicated by maternal obesity. Ongoing trials in Clinicaltrials.gov include supplementation with magnesium, vitamin D, probiotics, myo-inositol, fish oil, and omega-3 [373]. However, response to these interventions is limited, as maternal metabolic perturbations are present in early pregnancy and may programme fetal development before initiation of the interventions.

The presence of metabolic aberrations in early pregnancy in women later developing GDM may offer identifiable biomarkers for the detection of those at risk for GDM- and obesity-related health adversities. Nonobese women with GDM frequently remain undetected in the Finnish healthcare system. Although the risk of complications during pregnancy may be low among these women, they carry a long-term risk of later type 2 diabetes, which has already been demonstrated by earlier studies and was in this study manifested as metabolic aberrations similar to those in maternal obesity. The identification of these nonobese women offers substantial potential for prevention, because their maintaining a healthy weight after pregnancy would be crucial, and this could be aided by health counselling.

This study demonstrated that large cohorts are needed to detect metabolic derangements. A meta-analysis of three studies totalling over 1700 mother-child dyads, in which the cord blood metabolomic profile was explored, made this evident. Establishing later growth, adiposity, clinical variables linked to cardiovascular disease, and later circulating metabolomics in a large, well-characterized cohort would provide more insights into intrauterine programming.

# 7 Conclusions

This thesis has explored the association between maternal pBMI and pregnancy disorders in the second pregnancy and has examined the maternal metabolomic profile of obesity and pregnancy complications and the cord blood metabolomic profile of maternal obesity. The following conclusions can be reached:

1. The risk of GDM, GH, and PE increases with pBMI in the second pregnancy, but the magnitude of the risk is far higher if these disorders have complicated the first pregnancy. In the presence of pre-existing diabetes and HT, the risk of pregnancy disorders is independent of pBMI. (Study I)

2. The maternal metabolomic profile displays great derangements in relation to obesity. The metabolomic profile of GDM, PE, and HT in part resembles the profile of obesity. As women with obesity enter pregnancy with adverse metabolic profiles, the change across pregnancy is smaller than in women of normal weight. (Study II)

3. Women with GDM, with or without obesity, display derangements in their metabolomic profile in early pregnancy, and these derangements persist across pregnancy. Many of these derangements are similar to those displayed during obesity only, but some are specific to GDM or to obesity. (Study III)

4. Maternal pBMI is associated with the levels of several metabolites in cord blood at delivery. Maternal and cord blood metabolomes are associated, and those associations vary according to maternal pBMI. (Study IV)

In prepregnancy obesity, the developing fetus is exposed to maternal metabolic derangements early in pregnancy, as shown in Studies II and III. Similar derangements can be found in cord blood along with maternal obesity (Study IV). The finding of maternal obesity's modifying the association between the maternal and the cord blood metabolome (Study IV) is novel.

The metabolomic derangements in the maternal profile across pregnancy and in the cord blood profile at delivery programme the later offspring health at the most vulnerable stage. The presence of early pregnancy derangements explains why interventions to prevent adverse offspring outcomes employing diet or lifestyle changes during pregnancy have for the most part been inadequate. To be effective, interventions must be implemented earlier, before conception.

The findings of this study shed light on the physiological mechanisms leading to adverse short- and long-term outcomes in pregnancies complicated by maternal obesity, GDM, or hypertensive disorder. Some alterations in biochemical markers may prove modifiable by dietary interventions and thus help to prevent future risk for cardiovascular and metabolic diseases.

Understanding the physiological mechanisms leading to adverse short- and long-term outcomes in pregnancies complicated by maternal obesity, GDM, or hypertensive disorders should be the focal point for all strategies to safeguard both maternal and offspring health.

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# The risk of complications in second pregnancy by maternal BMI: The role of first-pregnancy complications, pregestational diabetes and chronic hypertension

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## Abstract

**Introduction:** Maternal obesity is associated with an increased risk of several pregnancy complications. In the second pregnancy, previous pregnancy and other medical history provide additional information about individual morbidity risk. In this study, we assess the risk of pregnancy complications in the second pregnancy by maternal body mass index (BMI) and evaluate how first-pregnancy complications and preexisting conditions modify these associations.

**Material and methods:** We have used nationwide data on all women (n = 48 963) experiencing their first and second pregnancy between 2006 and 2013 in Finland. The associations between the full scale of maternal BMI and pregnancy complications (gestational diabetes, gestational hypertension and preeclampsia) were analyzed using logistic regression and restricted cubic spline regression models and interactions between BMI and first-pregnancy complications, pregestational diabetes or chronic hypertension were tested.

**Results:** The risk of pregnancy complications increased with adiposity. Unadjusted probability of second-pregnancy gestational diabetes with BMI of 25 kg/m$^2$ was 56% and 8.4% among women with and without first-pregnancy gestational diabetes, respectively. The corresponding figures with BMI of 30 kg/m$^2$ were 64% and 17%. Adjusted odds ratio (OR) (95% CI) for second-pregnancy gestational diabetes with BMI of 25 kg/m$^2$ was 45 (34-59) and 3.3 (2.6-4.0) among women with and without first-pregnancy gestational diabetes, respectively, when compared with women with BMI of 20 kg/m$^2$ and no first-pregnancy gestational diabetes. Adjusted OR (95% CI) for second-pregnancy gestational hypertension among women with BMI of 25 kg/m$^2$ was 42 (26-66) and 2.3 (1.4-3.8) among women with and without first-pregnancy hypertensive disorder, respectively, when compared with women with BMI of 20 kg/m$^2$ and no first-pregnancy hypertensive disorder. The risk of preeclampsia increased with adiposity independent of first-pregnancy complications. Pregestational diabetes or chronic hypertension did not modify the association between adiposity and any of the second-pregnancy complications.

**Abbreviations:** BMI, body mass index; CI, confidence interval; GDM, gestational diabetes; GH, gestational hypertension; HDR, Hospital Discharge Register; ICD-10, International Classification of Diseases, Tenth Revision; MBR, Medical Birth Register; OGTT, oral glucose tolerance test; OR, odds ratio; PE, preeclampsia.

**Conclusions:** As maternal BMI increases, the risk of complications increases in the second pregnancy. The risk of gestational diabetes and hypertension is, however, highest among women with complications in the first pregnancy.

**KEYWORDS**
gestational diabetes, obesity, preeclampsia, pregnancy complications, pregnancy-induced hypertension

## 1 | INTRODUCTION

Maternal overweight and obesity are well-known risk factors for several pregnancy-related complications, including gestational diabetes (GDM) and hypertensive disorders.[1,2] The risk of pregnancy complications increases with adiposity in a non-linear way and increased risk can already be seen among women in the normal range for body mass index (BMI).[3-6] Maternal obesity and pregnancy complications have both short-term and long-term adverse effects on maternal and offspring health.[7,8]

In clinical practice, the risk of parous women developing pregnancy complications is evaluated based on prepregnancy BMI and medical history with information about manifestation of complications in the first pregnancy. GDM recurs in 30%-84% of women.[9] Hypertensive disorder recurrence may manifest as preeclampsia (PE) or gestational hypertension (GH) which, according to a meta-analysis of 99 415 women, affect 14% and 9% of the following pregnancies, respectively.[10] With pregestational diabetes or chronic hypertension, the recurrence risk of PE is 15%-20% and 25%, respectively.[11,12] However, little is known about how the combination of adiposity and previous complications affects the risk of complications in the second pregnancy.

In this study we evaluated how first-pregnancy complications, GDM, GH and PE, pregestational diabetes and chronic hypertension modify the association between BMI and second-pregnancy complications. We assessed the shape and the magnitude of the association between full-range of maternal prepregnancy BMI and second-pregnancy complications separately by the conditions found to modify the association in a large, nationwide Finnish cohort.

**Key message**

The risk of second-pregnancy complications increases with higher BMI. Throughout the full range of BMI, the risk of gestational diabetes and hypertension is highest in women with complications in the first pregnancy.

## 2 | MATERIAL AND METHODS

### 2.1 | Study population and data sources

Information on all women regarding their first and second consecutive singleton live births in Finland in 2006-2013 was extracted from the Finnish Medical Birth Register (MBR) (n = 50 219 mothers). The MBR compiles information on maternal prepregnancy weight reported by the mother and height measured by midwives, both at women's first visit to the antenatal clinic usually occurring in the 10th gestational week (mean 9.5 gestational weeks). Women with missing information on weight or height (n = 1256, 2.5%) were excluded, leaving 48 963 women in the study cohort. There were no statistically significant differences in any of the background characteristics or outcomes between the study cohort and the excluded group.

The MBR covers all deliveries in Finland and is routinely linked to the Central Population Register and Cause-of-Death Register to complete the register with missing livebirths, stillbirths and infant deaths. Information on mother's sociodemographic background, maternal healthcare and interventions during pregnancy and delivery are recorded in the register. Information on maternal prepregnancy and pregnancy diagnoses was extracted from the Finnish Hospital Discharge Register (HDR), which includes both inpatient and outpatient data recorded by the responsible physician as International Classification of Diseases, Tenth Revision (ICD-10) codes. HDR and MBR data were linked using the encrypted unique personal identity code available in both data sources.

### 2.2 | Definition of exposure and outcomes

BMI was calculated from maternal weight and height in the second pregnancy and treated as splines and categorical variables. First, BMI was categorized according to the WHO classification:[13] <18.5 kg/m$^2$ (underweight); 18.5-24.9 kg/m$^2$ (normal weight); 25-29.9 kg/m$^2$ (pre-obesity); 30-34.9 kg/m$^2$ (obesity class I); 35-39.9 kg/m$^2$ (obesity class II); and ≥40 kg/m$^2$ (obesity class III). Secondly, we created a composite variable which brought together information on BMI and first pregnancy complication (GDM, GH or PE, one composite variable for each complication). BMI was categorized into 25 categories (<17 kg/m$^2$ or less, 17-39 kg/m$^2$ each BMI point as a single category and ≥40 kg/m$^2$), and within each of these BMI categories, women were further categorized based on whether they had a complication in the first pregnancy or not.

Information on maternal adverse pregnancy outcomes and prepregnancy conditions was extracted from the MBR and the HDR. The following codes were collected: GDM (ICD-10 code O24.4); preexisting (chronic) hypertension with PE (O11); mild to moderate PE (O14.0 or O14.9); severe PE (O14.1); GH without significant proteinuria (O13 or O16); type 1 diabetes (E10); type 2 diabetes (E11); preexisting (pregestational) type 1 and type 2 diabetes in pregnancy (O24.0 or O24.1); hypertensive diseases (I10-15); and preexisting (chronic) hypertension complicating pregnancy (O10). Information on pathologic result in glucose tolerance test was extracted as check-box variables from the MBR. Both the ICD-10 code and the check-box variable were used to define GDM.

Since 2008 the diagnosis of GDM has been made using uniform criteria in Finland.[14] The diagnosis is based on a 2-hour 75-g oral glucose tolerance test (OGTT) with at least one abnormal plasma glucose value determined as ≥5.3 mmol/L (fasting), ≥10.0 (1 hour) and ≥8.6 mmol/L (2 hours) and screening is performed risk-based. OGTT is recommended to be offered to all pregnant women except those who are at low risk. Low-risk parous women are defined as age <40, BMI <25 kg/m$^2$, no previous GDM or a macrosomic newborn. OGTT is performed at 24-28 weeks of gestation, but the high-risk cases (BMI ≥35, GDM in prior pregnancy, glucosuria, family history of diabetes, corticosteroid therapy or polycystic ovary syndrome) were also tested at 12-16 weeks. These criteria were applied in most parts of Finland before 2008, but in some areas the cut-off value of ≥5.1 mmol/L for fasting glucose was used before this.

According to the guidelines generally accepted in Finnish medical care, the following diagnostic criteria for hypertensive disorders in pregnancy apply: GH is defined by a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. PE is defined as hypertension with concurrent new onset proteinuria (≥0.3 g/24 hours) or in the absence of proteinuria, new onset of thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema or cerebral or visual symptoms.[15]

## 2.3 | Statistical analyses

The associations between maternal background factors, pregnancy outcomes and maternal prepregnancy BMI in WHO categories were assessed using Chi-square test (categorical factors) and analysis of variance (continuous factors). The associations between BMI and GDM, GH and PE were analyzed using logistic regression.

To examine whether associations between BMI in WHO categories and the outcomes were modified by GDM, GH or PE in the first pregnancy or pregestational diabetes or chronic hypertension, we included an interaction term in the models and a likelihood ratio test was used to compare the models with and without the interaction term. If suggestive evidence on interaction was observed (*P* for interaction <.1), we performed separate analyses. Only those results where the interaction was considered relevant based on separate analyses or statistically highly significant (*P* for interaction <.001) are reported separately. In other cases, the factor was returned into the pool of potential confounding factors (see below).

Both unadjusted and adjusted models were used. Maternal age, smoking during pregnancy and socioeconomic status were considered as potential confounding factors, and first-pregnancy complications and pregestational diabetes and chronic hypertension were considered as both potential confounding factors and effect modifiers. Factors were chosen for the final adjusted models based on interaction analyses, previous knowledge and the observed associations between confounding factors and both BMI and the outcomes in the present data.

Analyses for GDM were performed separately by first-pregnancy GDM, and the final adjusted model included maternal age, smoking, first-pregnancy GH and PE, and chronic hypertension. Analyses for GH were performed separately by first-pregnancy GH or PE, and the final adjusted model included maternal age, smoking, first-pregnancy GDM and pregestational diabetes. No separate analyses for PE were performed; the final adjusted model thus included maternal age, smoking, first-pregnancy GDM, GH and PE, pregestational diabetes and chronic hypertension. Women with pregestational diabetes or chronic hypertension were excluded from analysis on GDM and GH, respectively.

We examined the shape of the association between BMI and the outcomes by fitting restricted cubic spline regression models. Based on these models, we calculated and plotted predicted probabilities and 95% CI for each outcome. To further quantify and visualize the results of the spline and the interaction analyses, we used the composite BMI-first pregnancy complication-variables in the analysis. Based on visual inspection of spline regression plots, BMI 20 kg/m$^2$ and no first pregnancy complication was set as a reference category for these variables. Results from the spline regression analyses (OR with 95% CI) are presented as the main results. Analyses were performed using STATA, version 12, software (StataCorp LLC, College Station, Texas, USA).

## 2.4 | Ethical approval

This study was approved by the National Data Protection Authority and the THL Finnish Institute for Health and Welfare, which is the keeper of the MBR and the HDR, 25 February 2016 (THL/265/5.05.00/2016).

# 3 | RESULTS

Demographic and clinical characteristics by BMI are outlined in Table 1. In the study population of 48 963 women, 62% were normal weight (BMI 18.5-24.9 kg/m$^2$), 26% overweight (BMI 25-29.9 kg/m$^2$ and 12% obese (BMI ≥30 kg/m$^{2)}$. The mean BMI in the beginning of the second pregnancy was 24 kg/m$^2$ and ranged from 13 to 65 kg/m$^2$. The total incidences of GDM, GH and PE in the second pregnancy were 13%, 2.6% and 1.4%, respectively. The incidence of

GDM increased by BMI class. Although the incidences of GH and PE were lower in the second than in the first pregnancy, the incidences increased by BMI class.

GDM recurred in 60%, GH in 19% and PE in 17% of all pregnancies. Among women with chronic hypertension, the incidence of second-pregnancy GDM was 36% and the incidence of second-pregnancy PE 13%. Of the women with pregestational diabetes, 6.0% developed second-pregnancy GH and 5.8% second-pregnancy PE.

The unadjusted probability of second-pregnancy GDM increased nonlinearly with BMI (Figure 1A). The association was modified by the first-pregnancy GDM (*P* for interaction <.001) and is presented separately for women with and without first-pregnancy GDM. First-pregnancy GH, PE or chronic hypertension did not modify the association between BMI and GDM (*P* for interaction .806, .695 and .190, respectively). Among women with first-pregnancy GDM, within the BMI range 15-50 kg/m$^2$, the probability of second-pregnancy GDM increased from 31% to 88%, whereas in women without first-pregnancy GDM, the probability in the same BMI range increased from 1.3% to 37%. With the shift of BMI from 25 to 30 kg/m$^2$, the unadjusted probability for second-pregnancy GDM increased from 56% to 64% (1.1-fold), and from 8.4% to 17% (2-fold) among women with and without first-pregnancy GDM, respectively. Among women without first-pregnancy GDM, the adjusted OR for second-pregnancy GDM started to increase from BMI 23 kg/m$^2$ (Figure 2A). Adjusted OR (95% CI) for second-pregnancy GDM was 45 (34-59) with BMI of 25 kg/m$^2$ and first-pregnancy GDM, and 3.3 (2.6-4.0) with the same BMI and no first-pregnancy GDM. With BMI of 30 kg/m$^2$, the OR was 55 (41-75) and 7.2 (5.6-9.1) among women with and without first-pregnancy GDM, respectively.

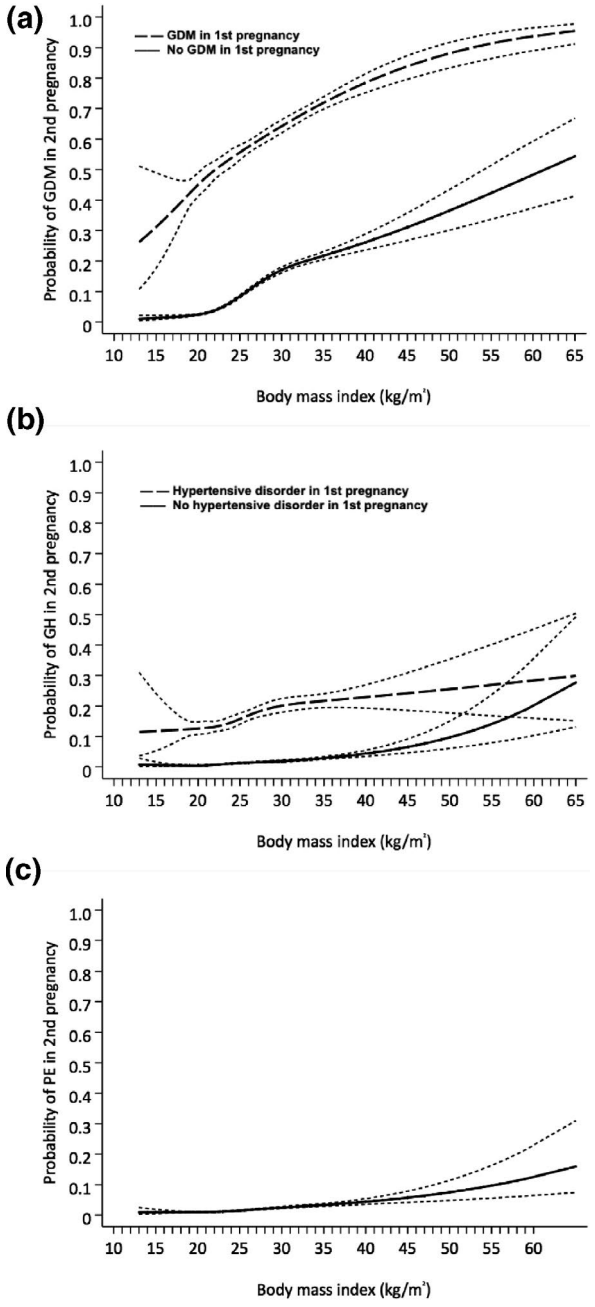


**FIGURE 1** Expected unadjusted probability of (A) gestational diabetes (GDM), (B) gestational hypertension and (C) preeclampsia (PE), in second pregnancy by maternal prepregnancy body mass index. Dashed lines represent 95% confidence intervals

The unadjusted probability of second-pregnancy GH increased with BMI (Figure 1B). The association was modified by first-pregnancy GH and PE (*P* for interaction <.001 for both) and for further analysis we combined these groups as first-pregnancy hypertensive disorders. The association between BMI and GH was not modified by gestational or pregestational diabetes (*P* for interaction .670 and .386, respectively). The probability of second-pregnancy GH increased with BMI range from 15 to 50 kg/m$^2$ from 12% to 26%, and from 0.7% to 9.7% in women with and without first-pregnancy hypertensive disorder. Adjusted OR for GH in second pregnancy increased with BMI (Figure 2B). Adjusted OR (95% CI) for second-pregnancy GH in women with BMI of 25 kg/m$^2$ was 42 (26-66) if first-pregnancy hypertensive disorder was evident and 2.3 (1.4-3.8) if no first-pregnancy hypertensive disorder was evident. With BMI of 30 kg/m$^2$, the OR for second-pregnancy GH was 56 (33-95) and 2.7 (1.4-5.1) for women with and without first-pregnancy hypertensive disorder, respectively.

Neither pregestational nor gestational diabetes modified the association between BMI and PE (*P* for interaction .457 and .550, respectively). Chronic hypertension and first-pregnancy GH or PE modified the association significantly or suggestively (*P* for interaction .047 and .083). Separate analyses showed no relevance (data not shown). The unadjusted probability of second-pregnancy PE increased with adiposity from 0.9% to 7.5% within the BMI range of 15-50 kg/m$^2$ (Figure 1C). Adjusted OR (95% CI) for second-pregnancy PE was 1.4 (0.9-2.1) among women with BMI of 25 kg/m$^2$ and 1.9 (1.2-3.1) among women with BMI of 30 kg/m$^2$ (Figure 2C).

## 4 | DISCUSSION

To our knowledge, this is the first study to evaluate how first-pregnancy complications, pregestational diabetes and chronic

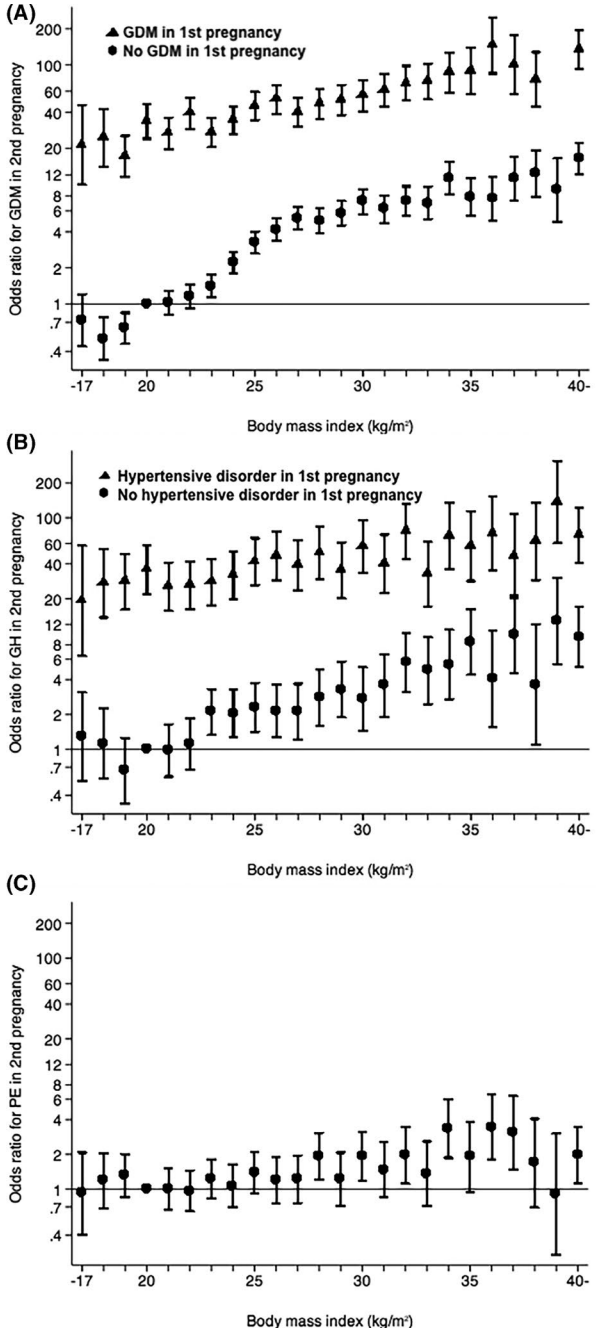


**FIGURE 2** The association between maternal prepregnancy body mass index and the odds ratio for second pregnancy complication. (A) The odds for gestational diabetes (GDM) are adjusted for maternal age, smoking, chronic hypertension and first pregnancy gestational hypertension and preeclampsia. (B) The odds for gestational hypertension are adjusted for maternal age, smoking, pregestational diabetes and first pregnancy gestational diabetes. (C) The odds for preeclampsia (PE) are adjusted for maternal age, smoking, pregestational diabetes and chronic hypertension, and first pregnancy gestational diabetes, gestational hypertension and preeclampsia. Bars represent 95% confidence intervals

hypertension modify the association between maternal prepregnancy BMI and second-pregnancy complications. We showed that although the probability of GDM and GH in the second pregnancy is greater in women with the complication in the first pregnancy, increasing adiposity is a risk factor for GDM and GH among all women. Probability of PE is low in second pregnancy, but obesity still increases the odds for this complication in the second pregnancy. Pregestational diabetes or chronic hypertension did not modify the association between BMI and second-pregnancy complications.

In our study, the OR for GDM in second pregnancy among women without prior GDM started to increase within normal range of BMI. This was also seen in our earlier study of primiparous women.[3] The results are in concordance with a recent meta-analysis of 962 966 women,[16] in which dose-response relationship existed and higher maternal pre-pregnancy BMI was associated with greater risk of GDM across its full range. Previous studies have not, however, analyzed the association separately in the first and second pregnancy. Although concomitant risk factors, obesity and prior GDM, multiply the odds for second-pregnancy GDM, the shift from normal to overweight increases the odds for second-pregnancy GDM, even among women without GDM in first-pregnancy. However, some of the differences in the associations of adiposity and second-pregnancy GDM between the two groups, women with and without prior GDM, may be due to healthcare policies recommending referral, especially of overweight and women with prior GDM, for OGTT. This may affect our results by detecting the condition more often in obese women or in women with prior GDM, and underestimating the risk of GDM in lean women or those without prior GDM.

The etiology of hypertension and diabetes overlap and, in addition to obesity, common pathways are thought to be inflammation, oxidative stress and insulin resistance.[17] Chronic hypertension has been shown to increase the incidence of GDM[18] but the association between BMI and GDM was not modified by prior GH, PE or chronic hypertension in our study.

The unadjusted probability of GH was low in women without first-pregnancy hypertensive disorder, but the OR for second-pregnancy GH increased with BMI in all women. This observation is in line with findings from a recent meta-analysis of 265 270 women.[19] In our study the odds for second-pregnancy GH at any given BMI were higher among women with than those without first-pregnancy GH or PE.

Incidence of PE is low in parous women and a rate of 1.7% was demonstrated in a Swedish cohort.[20] Our study is in concordance with this observation. Although the number of women with PE in our study was low, increasing adiposity was associated with higher OR for PE. No significant or relevant modification in the association between BMI and PE was seen for gestational or pregestational diabetes, or for first-pregnancy or chronic hypertensive disorders. Studies with a larger number of women with PE are needed to confirm or refute these findings. In addition, PE is more than one disease,[21] but we were unable to make a distinction between early and late onset PE. One more factor that we were unable to account for in this study was the use of aspirin for the prevention of PE among women with a history of hypertensive disorders.

**TABLE 1** Maternal characteristics of the cohort by prepregnancy body mass index categories in the second pregnancy

| | Body mass index category | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <18.5 kg/m$^2$ (n = 1749) | | 18.5-24.9 kg/m$^2$ (n = 30 380) | | 25-29.9 kg/m$^2$ (n = 11 030) | | 30-34.9 kg/m$^2$ (n = 4003) | | 35-39.9 kg/m$^2$ (n = 1241) | | ≥40 kg/m$^2$ (n = 560) | | Total (n = 48 963) | | |
| Characteristic | n | % | n | % | n | % | n | % | n | % | n | % | n | % | $P$[b] |
| Age (years); mean (SD) | 27.2 | 4.8 | 29.2 | 4.6 | 29.2 | 4.7 | 28.8 | 4.9 | 28.6 | 4.9 | 28.5 | 4.8 | 29.1 | 4.7 | <.001 |
| Smoking | | | | | | | | | | | | | | | <.001 |
| Smoking during pregnancy | 205 | 11.7 | 1709 | 5.6 | 822 | 7.5 | 391 | 9.8 | 151 | 12.2 | 86 | 15.4 | 3364 | 6.9 | |
| Quit smoking in first trimester | 68 | 3.9 | 840 | 2.8 | 403 | 3.7 | 184 | 4.6 | 55 | 4.4 | 20 | 3.6 | 1570 | 3.2 | |
| Non-smoking | 1433 | 82.0 | 27 252 | 89.7 | 9584 | 86.9 | 3328 | 83.1 | 1012 | 81.2 | 441 | 78.8 | 43 050 | 88.0 | |
| Missing data | 43 | 2.5 | 579 | 1.9 | 221 | 2.0 | 100 | 2.5 | 23 | 1.9 | 13 | 2.3 | 979 | 2.0 | |
| Pregestational diabetes | 7 | 0.4 | 227 | 0.7 | 162 | 1.5 | 104 | 2.6 | 43 | 3.5 | 29 | 5.2 | 572 | 1.2 | <.001 |
| Chronic hypertension | 4 | 0.2 | 133 | 0.4 | 125 | 1.1 | 101 | 2.5 | 64 | 5.2 | 48 | 8.6 | 475 | 1.0 | <.001 |
| 1st pregnancy | | | | | | | | | | | | | | | |
| Gestational diabetes | 56 | 3.2 | 1662 | 5.5 | 1670 | 15.1 | 1152 | 28.8 | 458 | 36.9 | 242 | 43.2 | 5240 | 10.7 | <.001 |
| Gestational hypertension[a] | 52 | 3.0 | 1355 | 4.5 | 744 | 6.7 | 420 | 10.5 | 164 | 13.2 | 95 | 17.0 | 2830 | 5.8 | <.001 |
| Preeclampsia | 37 | 2.1 | 961 | 3.2 | 508 | 4.6 | 253 | 6.3 | 115 | 9.3 | 54 | 9.6 | 1928 | 3.9 | <.001 |
| 2nd pregnancy | | | | | | | | | | | | | | | |
| Gestational diabetes | 52 | 3.0 | 1815 | 6.0 | 2101 | 19.0 | 1283 | 32.1 | 514 | 41.4 | 301 | 53.8 | 6066 | 12.4 | <.001 |
| Gestational hypertension[a] | 19 | 1.1 | 511 | 1.7 | 363 | 3.3 | 213 | 5.3 | 105 | 8.5 | 55 | 9.8 | 1266 | 2.6 | <.001 |
| Preeclampsia | 12 | 0.7 | 325 | 1.1 | 181 | 1.6 | 109 | 2.7 | 51 | 4.1 | 23 | 4.1 | 701 | 1.4 | <.001 |

[a]Excluding women with further diagnosed preeclampsia.

[b]*P* value obtained from analysis of variance (maternal age) or Chi-square test (other factors).

One of the strengths of our study is that it presents a large, nationwide population-based cohort. We have complemented the information obtained from the MBR with data from the HDR. The coverage and quality of Finnish registers is high.[22] The positive predictive value of complications of pregnancy and childbirth in the HDR has been found to be 94%[23] and the proportion of missing data in the registers is low. Maternity and child welfare clinics are free of charge in Finland, resulting in attendance by the entire pregnant population.[24] Nonetheless, prepregnancy BMI was self-reported, which may lead to some underestimation. However, a large systematic review showed that misclassification was moderate and did not bias associations between prepregnancy BMI and pregnancy outcomes.[25] Another limitation of the study is that, despite the large number of women, the number of patients with pregestational diabetes or chronic hypertension was small and some interactions may have been undetected.

Maternal prepregnancy overweight and obesity increase the risk of pregnancy complications. Our study presents new information about how the associations between BMI and complications in second pregnancy are modified by prior conditions. Susceptibility to a pregnancy complication such as GDM, GH or PE, whether caused by genetic predisposition or lifestyle factors, emerges in the first-pregnancy and provides a strong signal for the increased risk of a woman to develop pregnancy complications in the second pregnancy. However, increased adiposity is harmful for all women, even those with no prior complications. Modest changes in prepregnancy BMI may substantially increase the risk of pregnancy complications and more attention needs to be paid to weight gain even before reaching obesity. All women, even when there have been no complications in the first pregnancy, should be advised on the impact of overweight and obesity on future pregnancies and offered lifestyle counseling aimed at attaining a healthy prepregnancy weight. National policy solutions are also needed to tackle the crisis of the continuously increasing epidemic of obesity.

## 5 | CONCLUSION

The risk of second-pregnancy complications increases with BMI, even if the first pregnancy has been uncomplicated. This finding warrants attention from the healthcare personnel counseling and treating pregnant women. Across the full range of BMI, the risk of GDM and GH is, however, highest in women with complications in the first pregnancy.

### CONFLICT OF INTEREST

None.

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Clinical Research Article

Clinical Research Article

# Longitudinal Metabolic Profiling of Maternal Obesity, Gestational Diabetes, and Hypertensive Pregnancy Disorders

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## Abstract

**Context:** Comprehensive assessment of metabolism in maternal obesity and pregnancy disorders can provide information about the shared maternal-fetal milieu and give insight into both maternal long-term health and intergenerational transmission of disease burden.

**Objective:** To assess levels, profiles, and change in the levels of metabolic measures during pregnancies complicated by obesity, gestational diabetes (GDM), or hypertensive disorders.

**Design, Setting and Participants:** A secondary analysis of 2 study cohorts, PREDO and RADIEL, including 741 pregnant women.

**Main Outcome Measures:** We assessed 225 metabolic measures by nuclear magnetic resonance in blood samples collected at median 13 [interquartile range (IQR) 12.4-13.7], 20 (IQR 19.3-23.0), and 28 (27.0-35.0) weeks of gestation.

**Results:** Across all 3 time points women with obesity [body mass index (BMI) ≥ 30kg/m$^2$] in comparison to normal weight (BMI 18.5-24.99 kg/m$^2$) had significantly higher levels of most very-low-density lipoprotein-related measures, many fatty and most amino acids, and more adverse metabolic profiles. The change in the levels of most metabolic measures during pregnancy was smaller in obese than in normal weight women. GDM, preeclampsia, and chronic hypertension were associated with metabolic alterations similar to obesity. The associations of obesity held after adjustment for GDM and hypertensive disorders, but many of the associations with GDM and hypertensive disorders were rendered nonsignificant after adjustment for BMI and the other pregnancy disorders.

**Conclusions:** This study shows that the pregnancy-related metabolic change is smaller in women with obesity, who display metabolic perturbations already in early pregnancy. Metabolic alterations of obesity and pregnancy disorders resembled each other suggesting a shared metabolic origin.

**Key Words:** diabetes, gestational, hypertension, gestational, metabolomics, nuclear magnetic resonance, biomolecular, pre-eclampsia, pregnancy, pregnant women

Maternal obesity complicates an increasing number of pregnancies. In 2016, globally 40% of women were overweight [body mass index (BMI) 25-29.99 kg/m$^2$) and 15% obese (BMI ≥ 30 kg/m$^2$) (1). In less than 5 years, the number of women with obesity is estimated to rise by one third to over 21% (2). Maternal overweight and obesity during pregnancy not only increase the mother's risk for gestational diabetes (GDM), hypertensive disorders, and delivery complications (3), but also the offspring's risk for preterm birth, intrauterine growth restriction, macrosomia, and other perinatal complications, as well as obesity, metabolic disorders, and neurodevelopmental impairment in childhood and later life (4).

While the underlying mechanisms mediating the adverse effects of maternal obesity on the offspring still remain unknown, recent studies have implicated that perturbations in the maternal metabolome during pregnancy may play a role (5,6). A series of studies have shown that higher prepregnancy BMI, GDM, and preeclampsia (PE) are associated with alterations in blood or urinary metabolome, including several lipoprotein-related variables, triglycerides, specific amino acids (AA), fatty acids (FA), and inflammatory markers (7-10). These studies are, however, limited by having measured maternal metabolic profile at only 1 time point during pregnancy or having pooled metabolome data across trimesters. Normal pregnancy is associated with profound changes in the maternal metabolism to meet the physiological demands imposed by the pregnancy and to ensure adequate growth and development of the fetus (11). Yet, it remains unknown if maternal overweight and obesity, GDM, and hypertensive disorders induce changes in the maternal metabolic signatures above and beyond to that induced by the pregnancy in itself. Studying changes in the maternal metabolome profiles during pregnancy may help to identify novel biomarkers for therapeutic targets and critical time windows for preventive measures, and potential pathways that underpin the intergenerational transmission of metabolic adversities.

Against this background, the aim of this study was to assess if maternal prepregnancy overweight and obesity, GDM, and hypertensive disorders were associated with alterations in the levels and profiles of metabolic measures and in change in the levels across 3 serial time points during pregnancy in 2 Finnish studies comprising 741 pregnant women. We used targeted high-throughput proton nuclear magnetic resonance (NMR)-based metabolomics interrogating 225 metabolic measures.

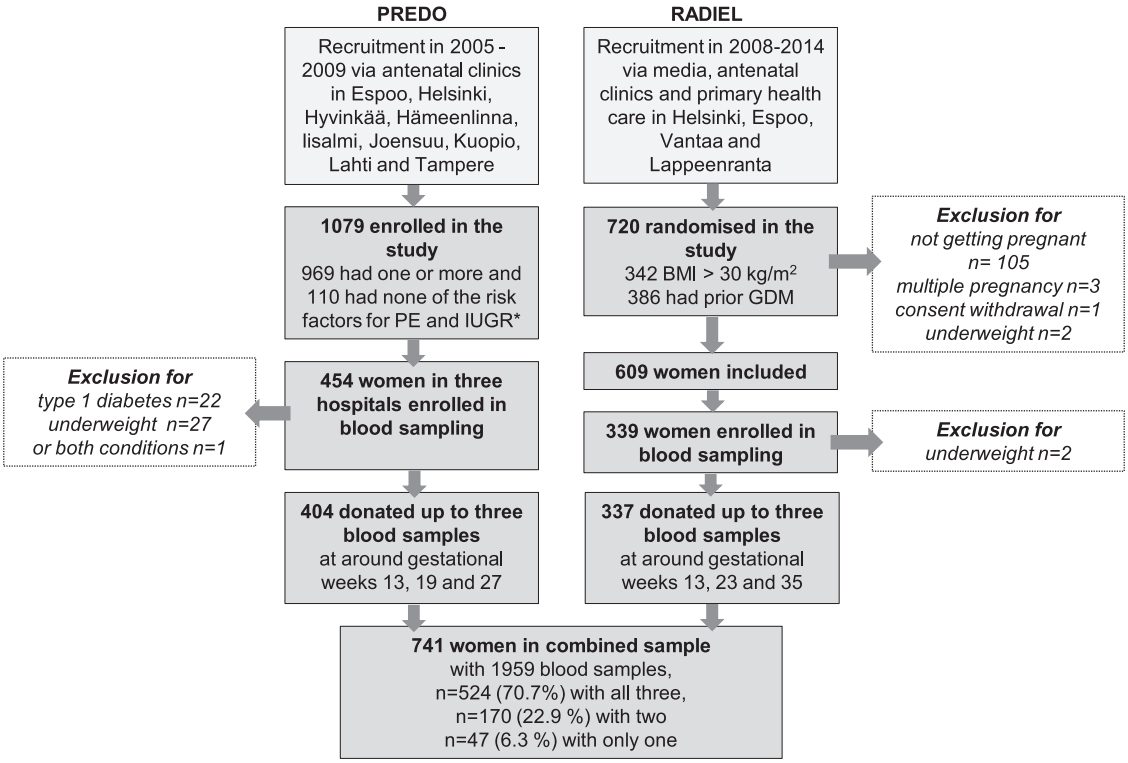
## Subjects

The study population came from 2 Finnish studies: the Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction (PREDO) study (12) and the Finnish Gestational Diabetes Prevention (RADIEL) study (13). The flowchart is presented as Figure 1.

The PREDO study enrolled 1079 pregnant women between 12 and 14 weeks of gestation from 10 hospitals. Details of the enrollment are presented in Figure 1. Of the 404 women giving blood samples, a subgroup with second-degree diastolic notch in the uterine blood flow were randomized to receive low-dose aspirin (n = 61) or placebo (n = 60) for preventing PE. Women providing blood samples in the PREDO cohort were younger (32.5 *vs* 33.6 years; $P = 0.007$) and less likely to be obese (29.1% *vs* 39.3%; $P = 0.003$) than women who did not.

The RADIEL study enrolled 720 women in a randomized, controlled trial to prevent GDM by lifestyle intervention among high-risk women (prior GDM and/or prepregnancy obesity) planning a pregnancy or in the first half of pregnancy (before 20 weeks of gestation). Of the 337 women giving blood samples, 177 were randomized in the intervention group receiving advice on diet and physical activity and 160 in the control group (standard care). In the RADIEL cohort the women providing blood samples were less likely to be obese (14.0% *vs* 20.5%; $P = 0.04$) and have GDM (27.9% *vs* 73.2%; $P < 0.0001$) or PE (7.0% *vs* 3.3%; $P = 0.04$) than women who did not.

All study participants signed informed consent and the study protocols were approved by ethics committees of the Helsinki and Uusimaa Hospital District.




*Risk factors for PE and IUGR: prepregnancy BMI≥30kg/m2, previous pregnancy complication like PE, intrauterine growth restriction, GDM or fetal demise; prepregnancy obesity; chronic hypertension; type 1 diabetes; age below 20 or above 40 years; systemic lupus erythematosus; or Sjögren's syndrome.

PE preeclampsia; GDM gestational diabetes; IUGR intrauterine growth restriction

**Figure 1.** Flowchart of the participants.

## Methods

### Metabolic Profiling Using the NMR Platform

In both cohorts, venous blood samples were drawn from the antecubital vein between 7 and 10 AM after at least a 10-h overnight fast. In the PREDO study plasma and in the RADIEL study serum was separated immediately and stored at −80°C until analysis, in which 225 metabolic markers were quantified by using a high-throughput proton NMR metabolomics platform (Nightingale Health Ltd, Helsinki, Finland). These metabolic measures cover multiple metabolic pathways, including 186 lipoprotein lipids and their subclasses, 9 FA and 7 ratios of FA, 5 other lipids, 8 AA, 3 ketone bodies, and 2 metabolites related to fluid balance and three to gluconeogenesis and 1 to inflammation. Following the lead of earlier studies using this metabolomics platform, we used 68 of these metabolic measures as our primary outcomes (9,14). However, we show the results also for the entire metabolomics platform. Details of the experimentation and applications of the NMR metabolomics platform have been described previously (15). In brief, the thawed samples (260 µL) were carefully mixed with sodium phosphate buffer (260 µL) and moved to NMR tubes. The setup is a combination of Bruker AVANCE III 500 MHz (a selective inverse room temperature probe head) and Bruker AVANCE III HD 600 MHz spectrometers (a cryogenically cooled triple resonance probe head; CryoProbe Prodigy TCI), both with the SampleJet robotic sample changer. The lipid extraction procedure was done manually (Integra Biosciences VIAFLO 96 channel electronic pipette) based on multiple extraction steps containing saturated sodium chloride solution, methanol, dichloromethane, and deuterochloroform and data were collected in full automation with the 600 MHz instrument. Computers that controlled the spectrometers do the Fourier transformations to NMR spectra and automated phasing. A centralized server performs various automated spectral processing steps, including overall signal check for missing/extra peaks, background control, baseline removal, and spectral area-specific signal alignments, and the spectral information was compared to 2 quality control samples. This NMR platform has been used in studies of pregnant and nonpregnant populations (9,14,16). Of all the metabolites 37 have been validated against the standard clinical chemistry methods.

### Prepregnancy Overweight/Obesity, Gestational Diabetes, and Hypertensive Disorders

Prepregnancy BMI was calculated from prepregnancy weight and height recorded in antenatal clinic records and the Medical Birth Register and, when available, from prepregnancy weight and height measurements (the participants recruited before pregnancy) in the RADIEL study. In both cohorts, diagnoses of GDM and hypertensive disorders were extracted from medical records and verified by a jury comprising of a research nurse and 2 or more medical doctors.

Normal weight (BMI 18.5-24.99 kg/m$^2$), overweight (BMI 25-30 kg/m$^2$), and obesity (BMI ≥ 30 kg/m$^2$) were defined according to World Health Organization guidelines (17). The diagnostic thresholds for GDM were, according to the Finnish guidelines, 5.3, 10.0, and 8.6 mmol/L in a 2-h 75-g oral glucose tolerance test (18). Hypertensive disorders were assessed according to the criteria of the American College of Obstetricians and Gynecologists recommendations (19). Definition for chronic hypertension (HT) was systolic/diastolic blood pressure ≥ 140/90 mmHg present prepregnancy or diagnosed before 20 weeks of gestation or medication for HT before 20 weeks of gestation. Definition for gestational HT was systolic/diastolic blood pressure ≥ 140/90 mmHg occurring after 20 weeks of gestation in a previously normotensive woman, and definition for PE was systolic/diastolic blood pressure ≥ 140/90 mmHg with proteinuria ≥ 300 mg/24 h or equivalent with dipstick in two consecutive measurements.

### Covariates

We chose the covariates included in the models based on previous literature. In all models we first adjusted for maternal age (9), cohort, and gestational week at the time of blood sampling (Model 1). Next, we adjusted for level of maternal education (basic/secondary *vs* tertiary) (9), parity (9,14), and substance (tobacco and alcohol no *vs* yes) use during pregnancy (14) (Model 2). In additional models (Model 3), overweight and obesity were further adjusted for GDM and hypertensive disorders, and analyses of GDM and hypertensive disorders were additionally adjusted for BMI (9), and GDM further for hypertensive disorders, and hypertensive disorders for GDM. We also assessed the potential confounding of the intervention trials in the PREDO and RADIEL studies. Supplementary Figures 1 and 2 (20) show that interventions were not associated with the metabolic markers during pregnancy, thus, intervention was not accounted for in the analyses. The effect of different samples, serum, and plasma was accounted by the adjustment for cohort.

### Statistical Analysis

To study associations of maternal overweight/obesity, GDM, and hypertensive disorders with the levels of and with change in the levels of metabolic measures during

pregnancy, we applied individual-participant data meta-analytic approach by using mixed model regression analyses. In these analyses, the repeated metabolic measures represented the within-person outcome variables, and gestational week at the blood sampling the time-varying within-person predictor variable. Normal weight *vs* overweight/ obesity, normoglycemia *vs* GDM, normoglycemia *vs* insulin/ diet treated GDM, and normotension *vs* HT/gestational HT/PE were included into these models as between-person fixed effects to test if the levels of maternal metabolic measures differed according to these pregnancy conditions. Interaction between normal weight *vs* overweight/obesity, normoglycemia *vs* GDM, and normotension *vs* HT/gestational HT/PE × gestational week at blood sampling tested if the within-person change in the levels of the metabolic measures during pregnancy differed between these pregnancy conditions. We defined unstructured covariance and first-order autoregressive error covariance matrices, used the cohort as a fixed effect, and allowed random effects to account for individual differences in the intercept and in the time-varying gestational week-related slopes.

To identify women with different metabolic profiles during pregnancy we applied latent class analysis (LCA). For these analyses we pooled data for each metabolic measure from the 3 sampling points into a grand average. We compared solutions with 2 to 6 latent classes. Based on criteria for the optimal number of classes described by Kongsted and Nielsen (21), the optimal solution was based on (1) goodness-of-fit criteria (Akaike information criterion, Bayesian information criterion), (2) reasonable distribution of participants across subgroups (at least 10% of the sample), (3) high certainty of classification identified by posterior probabilities, and (4) clear clinical characteristics of the participants within each of the identified groups. We applied logistic regression analysis to examine if the odds to belong to latent classes, identified by the LCA as the optimal, varied according to the pregnancy conditions.

The associations were adjusted for all covariates. Data were missing for substance use and education level (Table 1) and missing values in these variables were coded into a separate category.

The metabolic measures were log-transformed to normalize their distributions. We analyzed the values in standardized units with the SDs summarized in the combined sample so that they had the same value in both cohorts. Due to significant amount of collinearity in the metabolomics data, standard Bonferroni correction for multiple testing may be overly conservative and increase the risk of type II error (22). To overcome this risk, we applied principal components analysis approach, which is one of the most commonly used methods to reduce multidimensionality in metabolomics data and determine the number of independent tests (14,16,23-25) and is suggested as the first step in approaching metabolomics data analysis (22). This approach is analogous to multiple comparison correction routinely applied in genome-wide association studies, where the significance level is set up based on the assumption of the number of independent loci in the genome (26-29). Hence, by using the principal components analysis approach, we identified 25 principal components, which explained over 99% of the variation in the 68 metabolic measures that we used as the primary outcomes. Therefore, 2-sided $P < 0.002$ (0.05/25) was used to infer statistical significance.

As effect size indicators we present estimates and their 99.8% CIs (mixed model) and odds ratios and their 95% CIs (logistic regression models). Estimates represent mean differences (pooling data from the 3 sampling points into a grand average) and differences in the change (estimate of slope) of the metabolic measures across the three sampling points between women with and without the pregnancy condition. If the estimate reflecting differences in the level of change is negative, the metabolic measure increases less or decreases more, and if the estimate is positive, the metabolic measure increases more or decreases less during pregnancy in women with the disorder compared to women without the disorder.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The circular diagrams were created using R (R Core Team 2020) EpiViz package (30-32).

## Results

Women in the PREDO study were younger, had higher education, were less often obese, and had more often chronic or gestational HT or PE than women in the RADIEL study (Table 1). The second and third sampling points in the PREDO study were at an earlier gestational stage than in the RADIEL study. Of the study population, 524 (70.7%) women provided all 3 blood samples, 169 (22.8%) 2 samples, and 48 (6.5%) 1 sample (Table 1) and the number of samples at first time point was 625; at second, 666; and at third, 667.

The results for all the 225 metabolic measures are presented as circular diagrams in the supplementary material [see Supplemental Figures 3-5 in (20)], and results of the 68 metabolic measures used as the primary outcomes are presented in Figures 2 to 5.

### Prepregnancy Overweight and Obesity

Compared to normal-weight women, women with obesity had higher mean levels (pooled across the 3 measurement

**Table 1.** Characteristics of the study participants by cohort

| | PREDO (N = 404) | RADIEL (N = 337) |
|---|---|---|
| Gestational age, mean (range) | | |
| At the first blood sampling point | 13.0 (11.1-16.7) | 13.0 (6.0-17.7) |
| At the second blood sapling point | 19.4 (17.1-22.9) | 23.1 (20.1-27.6) |
| At the third blood sampling point | 27.0 (24.1-31.1) | 35.1 (30.6-38.9) |
| Maternal age, years, mean (SD) | 32.6 (5.2) | 33.4 (4.5) |
| Data not available | 0 | 0 |
| Education level, n (%) | | |
| Secondary or lower | 196 (49.5) | 232 (69.0) |
| Tertiary | 200 (51.5) | 104 (31.0) |
| Data not available | 8 (1.1) | 1 (0.3) |
| Parity, n (%) | | |
| Primiparous | 128 (31.7) | 114 (33.8) |
| Multiparous | 276 (68.3) | 223 (66.2) |
| Data not available | 0 | 0 |
| Smoking during pregnancy, n (%) | | |
| No | 374 (93.3) | 323 (96.1) |
| Smoked at any time during pregnancy | 27 (6.7) | 13 (3.9) |
| Data not available | 3 (0.7) | 1 (0.3) |
| Alcohol use during pregnancy, n (%) | | |
| No | 308 (86.5) | 315 (95.2) |
| Yes | 48 (13.5) | 16 (4.8) |
| Data not available | 48 (11.9) | 6 (1.8) |
| Body mass index category, n (%) | | |
| Normal weight (18.5-24.99 kg/m$^2$) | 195 (48.3) | 69 (20.7) |
| Overweight (25-29.99 kg/m$^2$) | 85 (21.0) | 45 (13.4) |
| Obese (≥30 kg/m$^2$) | 124 (30.7) | 223 (66.2) |
| Data not available | 0 | 0 |
| Hypertensive disorders, n (%) | | |
| Normotension | 254 (62.9) | 292 (86.7) |
| Gestational hypertension | 36 (8.9) | 16 (4.8) |
| Preeclampsia | 43 (10.6) | 11 (3.3) |
| Chronic hypertension | 71 (17.6) | 18 (5.4) |
| Data not available | 0 | 0 |
| Gestational diabetes mellitus, n (%) | | |
| Normoglycemia | 314 (77.7) | 243 (71.22) |
| Gestational diabetes mellitus | 90 (22.3) | 94 (27.9) |
| Data not available | 0 | 0 |

points) of many lipoprotein lipids including all very-low-density lipoprotein (VLDL) subclasses and mean diameter of VLDL particles, small high-density (HDL) particles, cholesterol and triglycerides in VLDL, and total triglycerides; monounsaturated FAs (MUFA), saturated fatty acids (SFA), and MUFA to total FA ratio; branched-chain AAs (BCAA) and aromatic AAs; and inflammation marker glycoprotein acetyls (GlycA) in the fully adjusted model, including adjustment for GDM and hypertensive disorders (Fig. 2A). Women with obesity had lower mean levels of very large and large HDL lipoprotein subclasses and mean diameter for HDL particles, and some FA ratios, including polyunsaturated fatty acids (PUFA) to total FA ratio. Out of the 68 metabolic measures, the change in the levels of 43 measures across the 3 sampling points was significantly different (smaller increase in 41 measures, greater decrease in valine and smaller decrease in albumin) between obese and normal weight women in the fully adjusted model [Fig. 2B; also see Supplementary Figure 6 in (20)]. The results were similar when comparing overweight women with normal-weight women, although the levels of metabolic measures and their change were less pronounced and not always statistically significant.

## Gestational Diabetes

Compared to normoglycemic women, women with GDM had higher/lower mean levels of many of the same metabolites as obesity (Fig. 3A). Of the 68 metabolic measures, 23 associations were significant in the Model 1, but when fully adjusted, including adjustment for BMI and hypertensive disorders, 9 of the associations were rendered nonsignificant (Fig. 3A). The associations that remained significant after full adjustment included all VLDL subclasses (except for very small size), mean diameter for VLDL, VLDL and total triglycerides, BCAA isoleucine and leucine, linoleic to total FA ratio, and the inflammation marker GlycA. Out of the 68 metabolic measures, the change in the levels of 6 measures across the 3 sampling points differed between GDM and normoglycemic women in the fully adjusted model [Fig. 3B; also see Supplemental Figure 7 in (20)]. The differences between normoglycemic and GDM women were more pronounced in insulin-treated than in diet-treated group [see Supplementary Figure 8 in (20)].

## Hypertensive Pregnancy Disorders

PE was associated with higher/lower mean levels of many of the same metabolites as obesity. Of the 68 metabolic measures, 19 association were significant in Model 1, but when fully adjusted, including adjustment for BMI and GDM, 9 were rendered nonsignificant (Fig. 4A). The associations that remained significant after full adjustment were 5 lipoprotein subclasses (from extremely large to small VLDL), total triglycerides and triglycerides in VLDL, MUFA, isoleucine, and leucine. Out of the 68 metabolic measures, the change in the levels of 2 measures across the 3 sampling

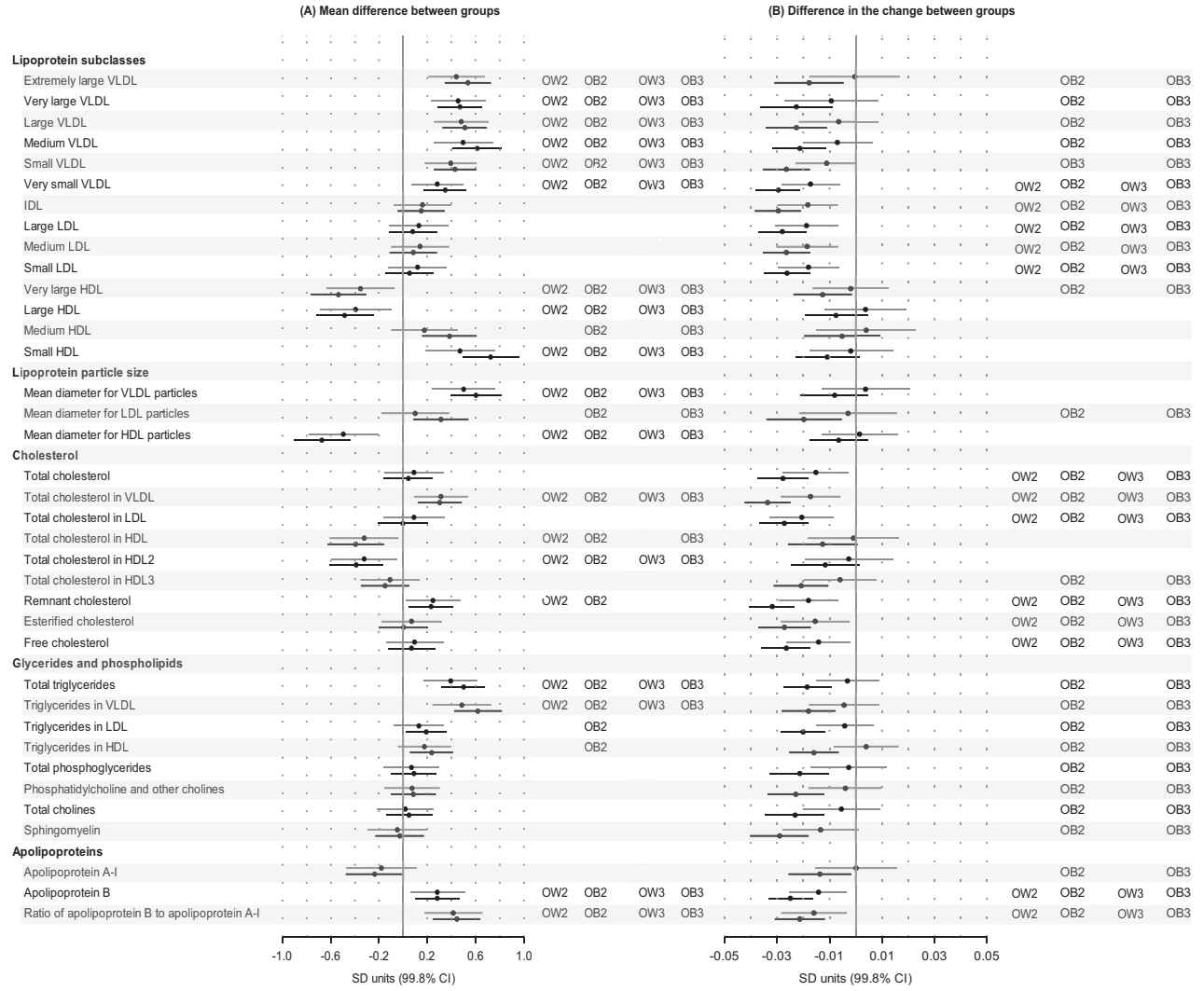



**Figure 2.** Mean differences [pooled mean across the 3 consecutive measurement points (A)] and differences in the change [slopes (A)] of metabolic measures during pregnancy between women with prepregnancy overweight or obesity in comparison to women with normal weight. Dots refer to mean differences and change per 1 pregnancy week in the metabolic measures in SD units and error bars, to their 99.8% CIs between overweight (gray) and normal weight women and between obese (black) and normal weight women. In the analyses of mean differences (main effect models), the associations were adjusted for gestational week at the time of blood sampling, cohort, and maternal age and the analyses of change (interaction models) additionally for the main effects of prepregnancy overweight/obesity (Model 1; dots and bars); further adjustments included parity, education, and substance use during pregnancy (significance is indicated with OW2 for overweight and OB2 for women with obesity), and gestational diabetes and hypertensive disorders (significance is indicated with OW3 for overweight and OB3 for women with obesity).

points differed between women with PE and normotension in the fully adjusted model [Fig. 4B; also see Supplementary Figure 9 in (20)].

HT was also associated with higher/lower mean levels of many of the same metabolites as obesity, but many of them were rendered nonsignificant after adjustment for BMI and GDM. Out of the 68 metabolic measures, 24 of the 29 significant associations (in Model 1) became nonsignificant (Fig. 5A). The associations that remained significant were total triglycerides, MUFA, citrate, isoleucine, and GlycA. Out of the 68 metabolic measures, change in the levels of 3 measures across the 3 sampling points differed between women with HT and normotension in models adjusted for all covariates [Fig. 5B; also see Supplementary Figure 9 in (20)].

Gestational HT was not associated significantly with any of the metabolic measures during pregnancy [see Supplementary Figure 10 in (20)].

## Metabolic Profiles: Latent Class Analysis

The optimal LCA solution identified 3 classes of women who differed significantly for 52 out of 68 metabolic measures; in addition, 9 metabolic measures differed significantly between 2 classes [see Supplementary Tables 1 and 2 in (20)]. Supplemental Table 3 (20) shows the number of

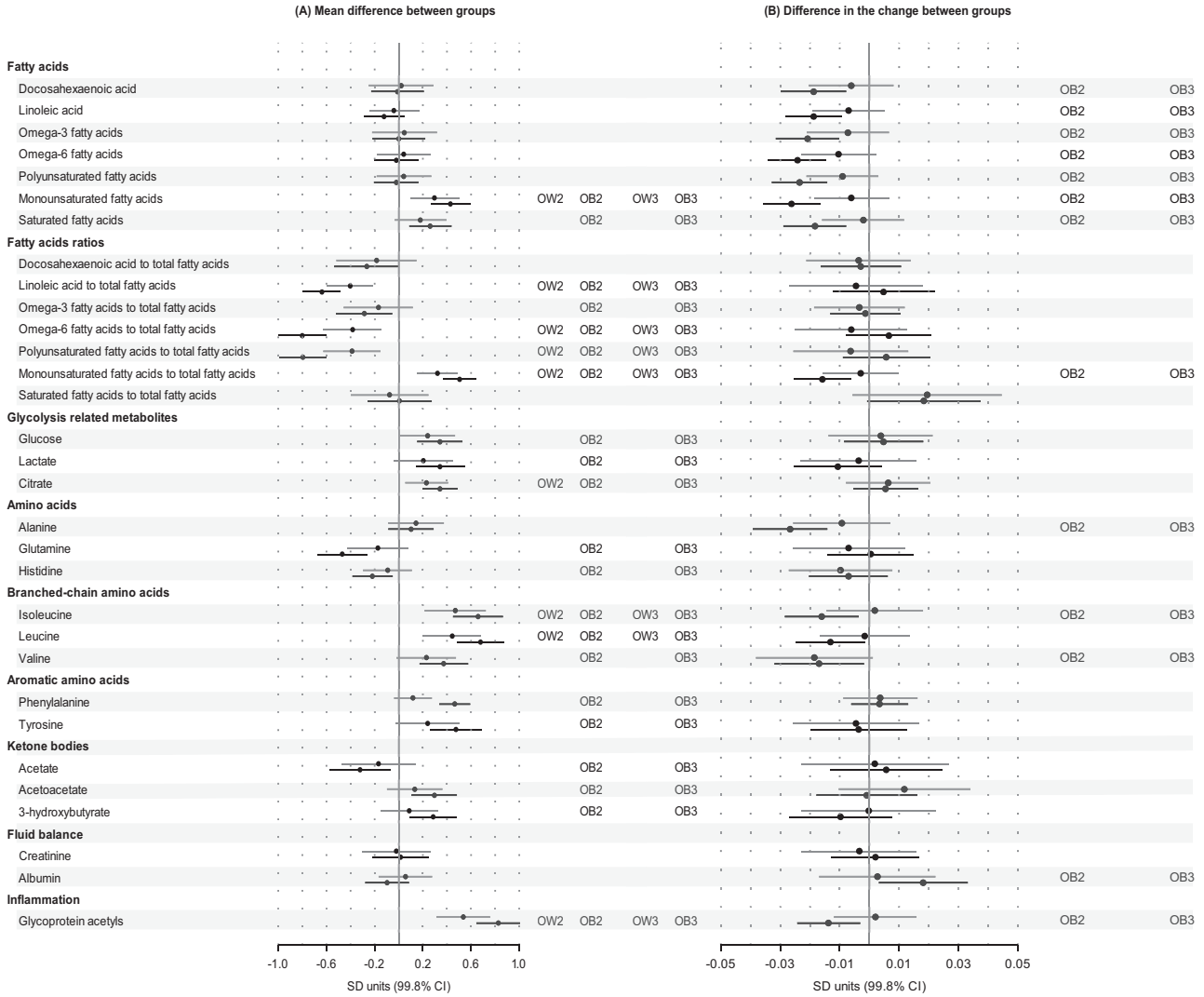



**Figure 2.** Continued.

women in the 3 latent classes according to different pregnancy conditions. Metabolic profile of women in the class 3 was characterized by higher levels of lipoproteins, cholesterol, triglycerides, AA, and GlycA and a lower ratio of PUFA to total FA. With the exception of acetate and some FA ratios, the levels of most metabolites gradually increased from classes 1 to classes 2 and 3 [see Supplementary Table 2 in (20)]. Across all adjustment models women with obesity compared to women with normal weight had significantly higher odds to belong to class 3 than class 1, and women with PE compared with those with normotension had a significantly higher odds to belong to class 2 than class 1 (Table 2).

## Discussion

Our study shows that women with prepregnancy obesity have adverse levels of metabolic measures throughout 3 time points during pregnancy and smaller pregnancy-induced changes in the levels compared to normal weight women. Women with obesity displayed higher lipoprotein levels during pregnancy, their fatty acid levels were characterized by higher MUFA and SFA and lower relative levels of PUFA to total FA, their amino acid levels were characterized by higher BCAA and aromatic AA, and they displayed higher levels of GlycA when compared to normal-weight women. The metabolic profile of women with prepregnancy obesity was characterized by a pattern that recapitulated the bivariate associations and pointed to profound and broad metabolic perturbations. Metabolic alterations related with GDM, PE, and HT resembled the alterations related with obesity.

Our study clearly highlights the broad attenuated metabolic response to pregnancy among women with obesity. Most metabolic markers demonstrated smaller changes across pregnancy in obese than in normal-weight women.

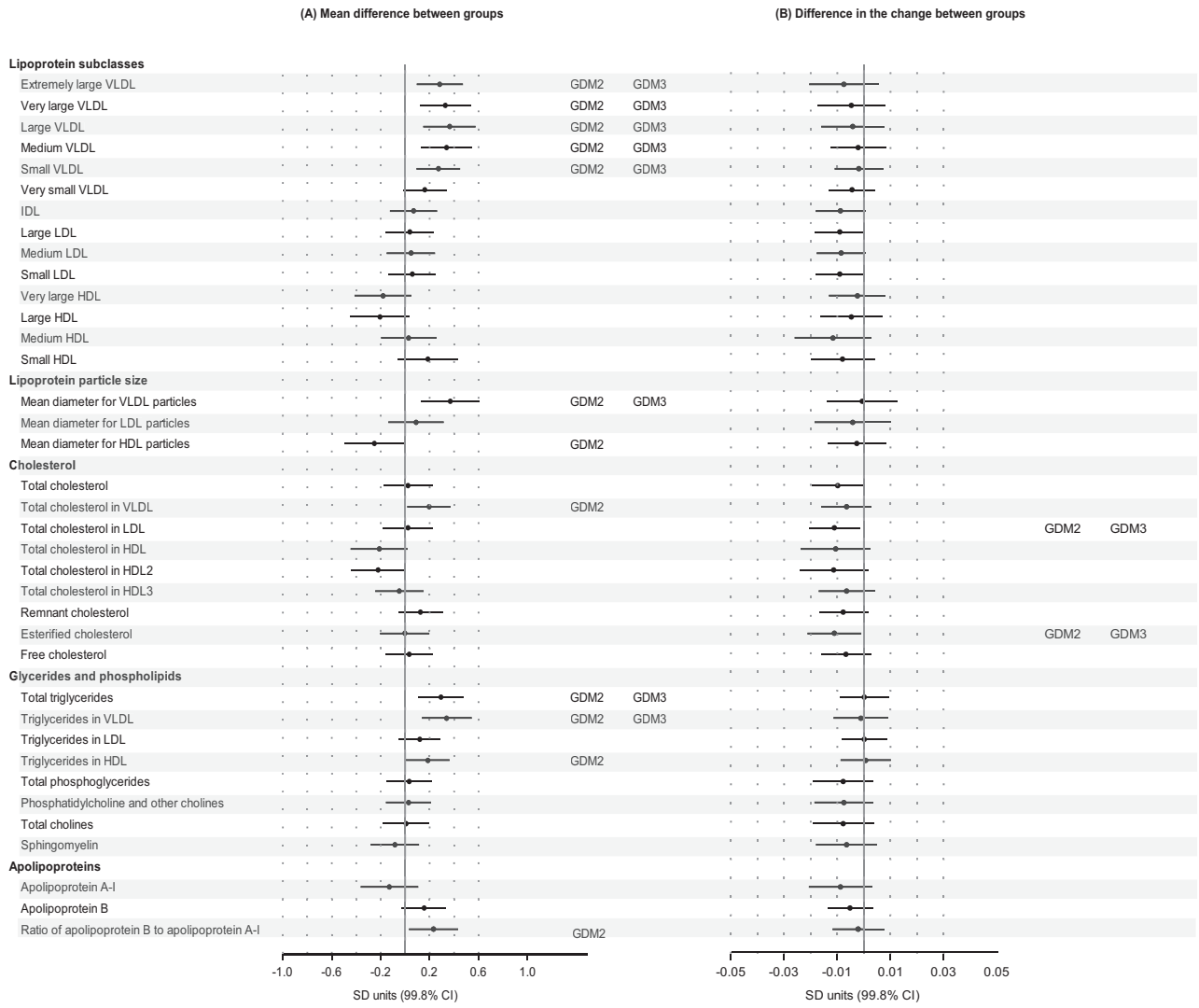



**Figure 3.** Mean differences [pooled mean across the 3 consecutive measurement points (A)] and differences in the change [slopes (B)] of metabolic measures during pregnancy between women with gestational diabetes in comparison to normoglycemic women. Dots refer to mean differences and change per 1 pregnancy week in the metabolic measures in SD units and error bars, to their 99.8% CIs. In the analyses of mean differences (main effect models) the associations were adjusted for gestational week at the time of blood sampling, cohort, and maternal age, and the analyses of change (interaction models) additionally for the main effects of gestational diabetes (model 1; dots and bars); further adjustments included parity, education and substance use during pregnancy (significance is indicated with GDM2), and body mass index and hypertensive disorders (significance is indicated with GDM3).

Metabolic response to pregnancy, evaluated by insulin resistance, converges by the end of pregnancy between women with severe obesity and normal weight according to a study by Forbes et al (33). We have now shown the same kind of convergence in a broader set of metabolic markers. In another study the ability of pregnant women with obesity to adapt to changes in energy fuel demands (eg, from fasting to a postabsorptive state) was less flexible, and they displayed higher inflammation marker levels after test meal (34). Obesity, metabolic inflexibility, and inflammation may enhance each other resulting in adverse long-term effects, such as increased triglycerides and impaired glucose metabolism and insulin resistance (35). Interestingly, in our study, adaptability to pregnancy in women with GDM, PE, or HT seemed, in turn, to be quite similar to women without these complications.

We showed that prepregnancy obesity was associated with atherogenic alterations in lipoproteins consisting of higher levels and larger VLDL particles, smaller HDL particles, and higher levels of triglycerides as well as with high levels of MUFA and SFA and low relative levels of PUFA across pregnancy. Similar adverse lipoprotein levels have been previously presented in cross-sectional studies (9,36). Women with obesity demonstrate net lipolysis (eg, release of free FA mainly from adipose tissue) throughout pregnancy, in contrast with normal-weight

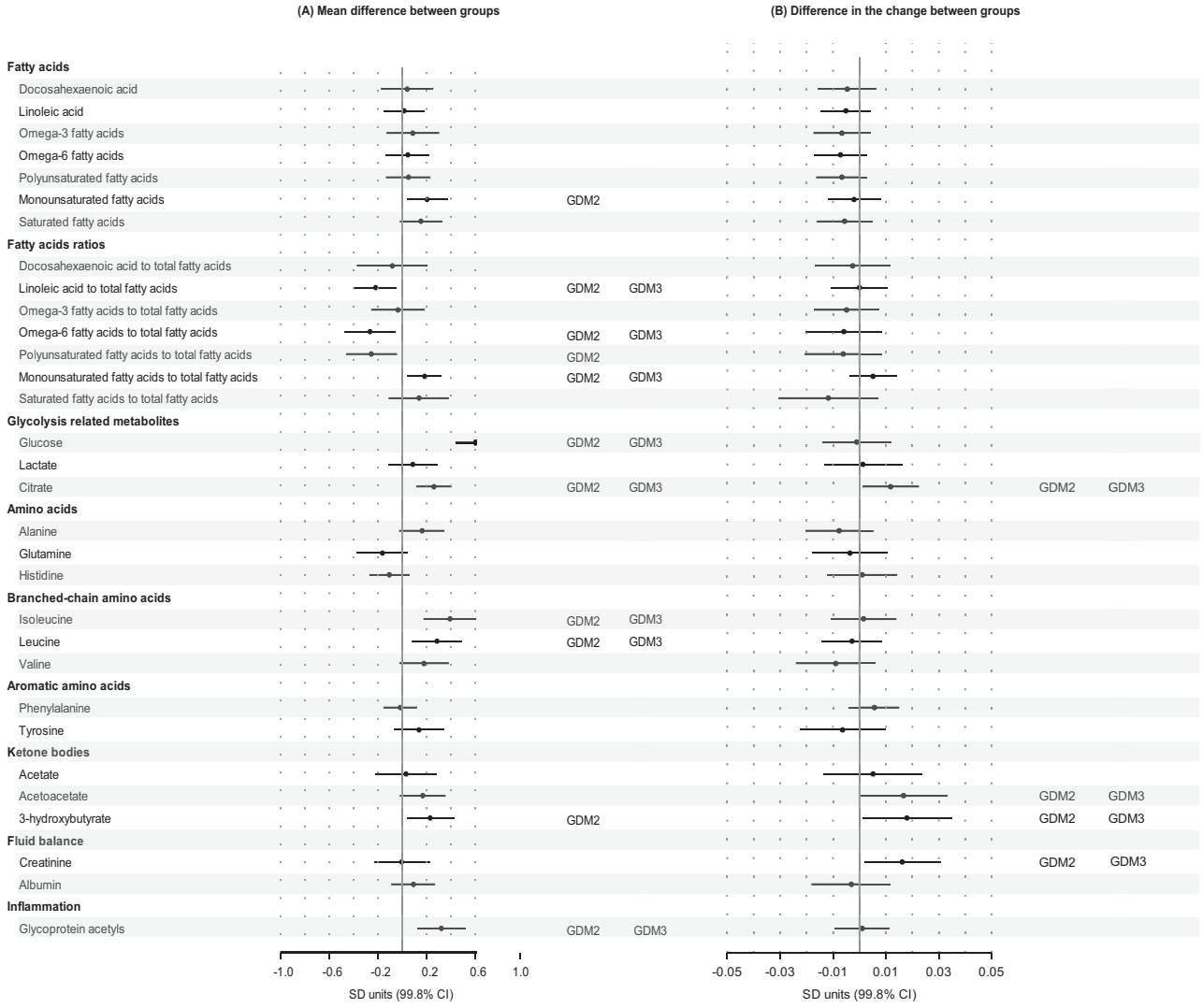



**Figure 3.** Continued.

women who demonstrate anabolic lipogenesis in early gestation and lipolysis in late gestation (11). Accordingly, the levels of FA in women with obesity in our study were unfavorable already in early pregnancy and stayed at a perturbed level across pregnancy. Obesity-enhanced lipolysis, insulin resistance, and increased inflammation induce hypertriglyceridemia and VLDL secretion from liver (37). Also, reduced activity of lipoprotein lipase results in higher levels of circulating VLDL lipoproteins and triglycerides (37). Excess VLDL may provoke endothelial and placental dysfunction, which have been suggested to explain the associations between maternal hyperlipidemia, obesity, PE, and GDM (38). The high MUFA levels in obesity and pregnancy disorders are probably a consequence of increased lipolysis, lack of fatty acid oxidation, and increased de novo lipogenesis (39). In our study, obesity was associated with a lower ratio of PUFA to total FA that is mainly a consequence of higher total levels of MUFA and SFA. The impact of low relative levels of PUFA on the fetal development should be studied further.

Our longitudinal study strengthens the findings of cross-sectional studies showing prepregnancy obesity to be associated with high levels of BCAA and aromatic AA (9,36). Reduced utilization of BCAAs in liver and adipose tissue, and de novo synthesis of BCAAs by gut microbiota contribute to accumulation of BCAAs in plasma, and obesity is tightly related to reduced activity of BCAA catabolism enzymes and to the changes in the microbiota (40). BCAAs have also been causally linked with insulin resistance (40). In contrast to leucine and isoleucine, we found valine levels decreasing during pregnancy, as seen before (14). Additionally, we demonstrated a greater decrease in obese compared to normal-weight women. It has been hypothesized that valine might have different metabolic effects depending on the adiposity status (40).

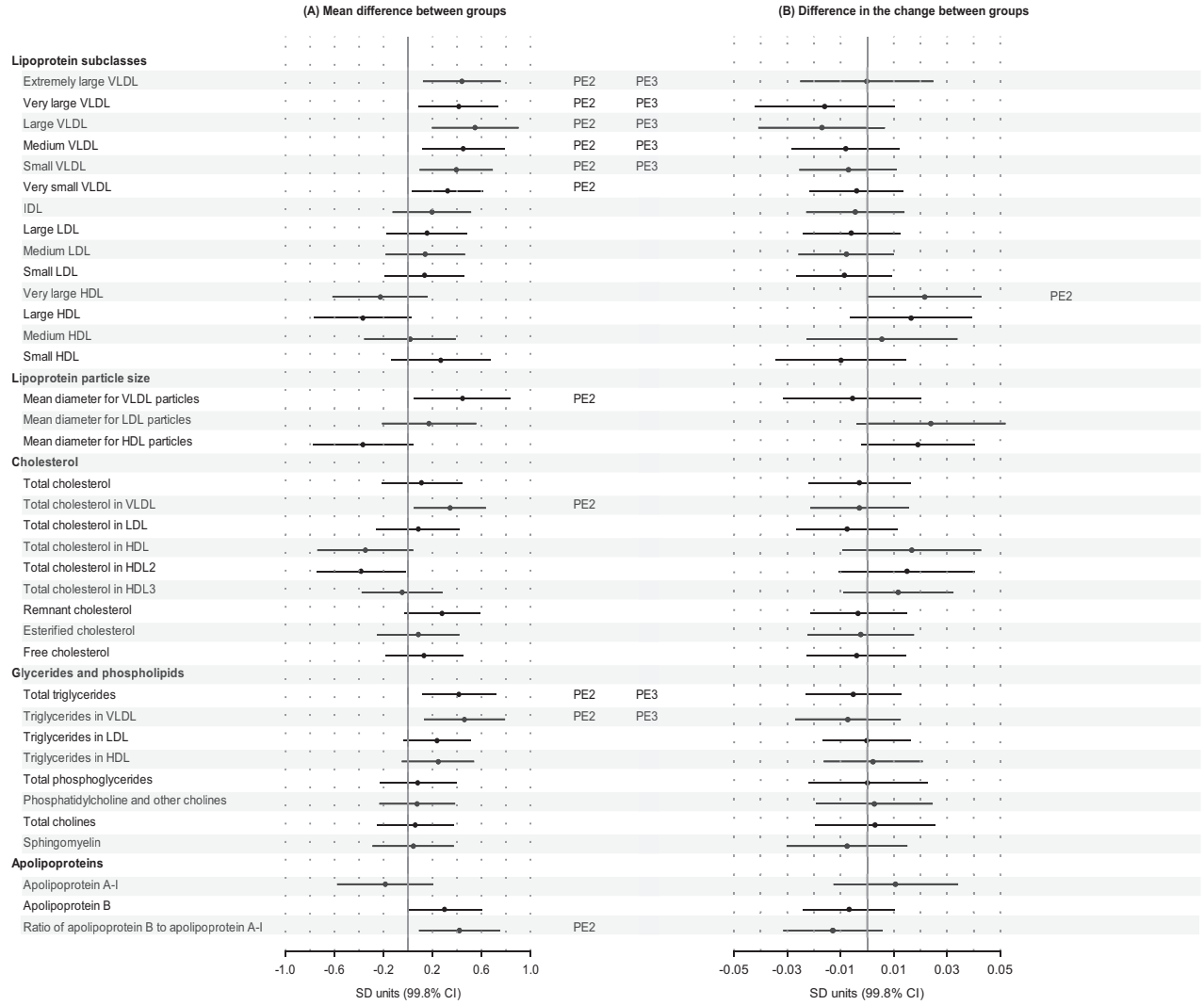



**Figure 4.** Mean differences [pooled mean across the 3 consecutive measurement points (A)] and differences in the change [slopes (B)] of metabolic measures during pregnancy between women with preeclampsia in comparison to normotensive women. Dots refer to mean differences and change per one pregnancy week in the metabolic measures in SD units and error bars, to their 99.8% CIs. In the analyses of mean differences (main effect models) the associations were adjusted for gestational week at the time of blood sampling, cohort, and maternal age, and the analyses of change (interaction models) additionally for the main effects of preeclampsia (Model 1; dots and bars); further adjustments included parity, education, and substance use during pregnancy (significance is indicated with PE2) and body mass index and gestational diabetes (significance is indicated with PE3).

Underlying pathophysiologic processes, insulin resistance, low-grade inflammation, oxidative stress, and endothelial dysfunction (41), along with coexistence of obesity and pregnancy disorders, may explain the similarities in metabolic profiles of obesity, GDM, PE, and HT. The origins of GDM, PE, and HT are, however, complex and multifactorial, related to genetic predisposition or lifestyle factors (42). In our study, metabolic measures, which remained significantly associated with GDM and PE in fully adjusted models, were many VLDL measures, triglycerides, some FAs and BCAAs, isoleucine, and leucine, as seen also in the previous cross-sectional studies (9,43-45). In nonpregnant populations HT has also been associated with increased concentrations of many lipids like VLDL and triglycerides (46) which was also seen in our study but rendered nonsignificant after adjustment for BMI and GDM.

We demonstrated persistently higher levels of inflammation marker GlycA across pregnancy complicated by obesity, GDM, and HT. GlycA is a marker of inflammation associated with multiple metabolic aberrations including type 2 diabetes and cardiovascular disease (47). GlycA levels elevate during normal pregnancy (14) and are higher in obese than in overweight pregnant women (36). In our study PE was not independently associated with GlycA levels, but inflammation of PE could have

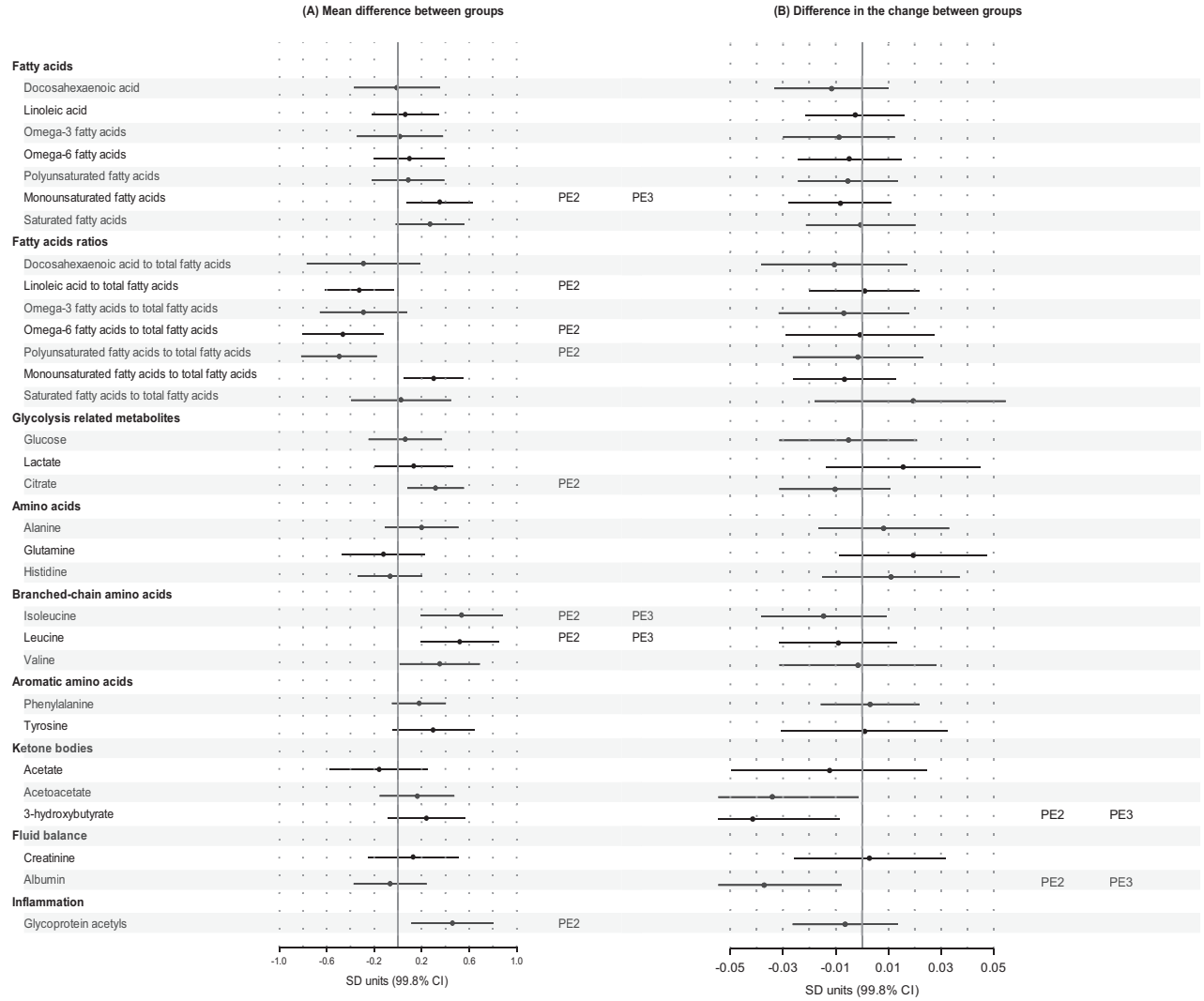



**Figure 4.** Continued.

been demonstrated by using a broader panel of inflammation markers.

The strength of our study lies in its longitudinal study design, which allowed us not only to study mean levels of the metabolic markers but change in their levels across 3 serial time points during pregnancy. The targeted panel of metabolic measures we used has been widely studied previously in pregnant and nonpregnant populations, and some of the metabolites have been proved to give quantitative results comparable to conventional laboratory techniques (15). Furthermore, our sample included women at risk for GDM and PE. This resulted in higher number of women with overweight/obesity, GDM and hypertensive disorders in our sample than seen in a general population of pregnant women, which provided higher statistical power to detect associations. Despite the large sample size, in latent class analyses using categorical rather than continuous outcome, the power was still limited as our predictor variables were dichotomous. The targeted metabolomics panel precludes discovery of novel molecules and high-risk sample limits generalizations to all pregnant women. Generalizability may also be limited by the fact that both study populations came from a Nordic high-income country. The studies collected different samples, plasma and serum, but to our knowledge, the plausible bias due to different samples is minimal (48), and we have addressed the issue by applying the statistical methods with SD scaling and adjustment for cohort. Combining 2 cohorts generates a challenge of a wide time range in blood sampling points, which might diminish some of the findings.

In conclusion, our findings indicate that, when compared to normal weight, women with prepregnancy obesity have profoundly perturbed metabolic levels and profiles during pregnancy and display smaller pregnancy-induced change in the levels of the metabolic measures. The metabolic perturbations in pregnancies complicated

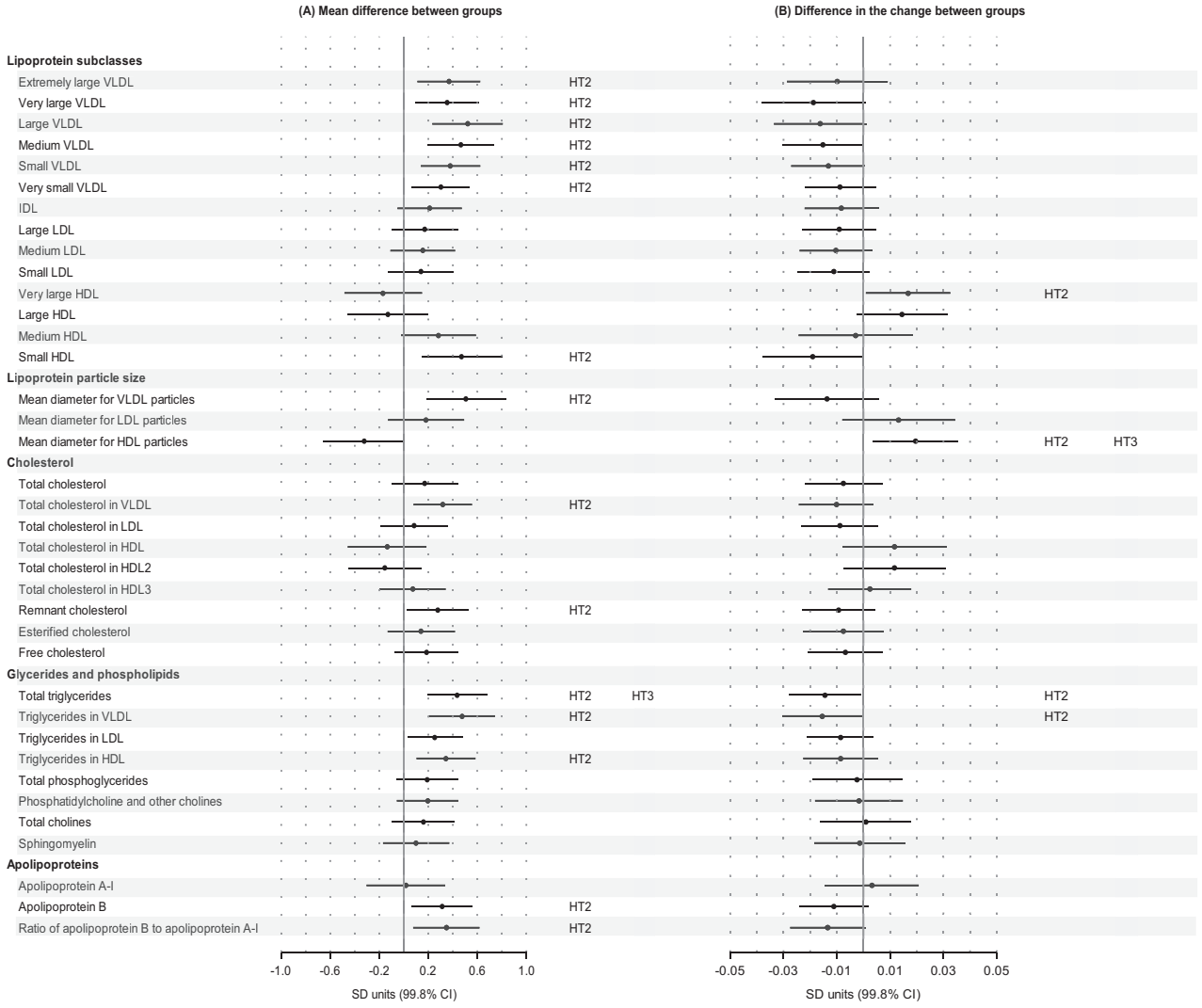



**Figure 5.** Mean differences [pooled mean across the 3 consecutive measurement points (A)] and differences in the change [slopes (B)] of metabolic measures during pregnancy between women with chronic hypertension in comparison to normotensive women. Dots refer to mean differences and change per 1 pregnancy week in the metabolic measures in SD units and error bars, to their 99.8% CIs. In the analyses of mean differences (main effect models) the associations were adjusted for gestational week at the time of blood sampling, cohort, and maternal age, and the analyses of change (interaction models) additionally for the main effects of chronic hypertension (Model 1; dots and bars); further adjustments included parity, education, and substance use during pregnancy (significance is indicated with HT2), and body mass index and gestational diabetes (significance is indicated with HT3).

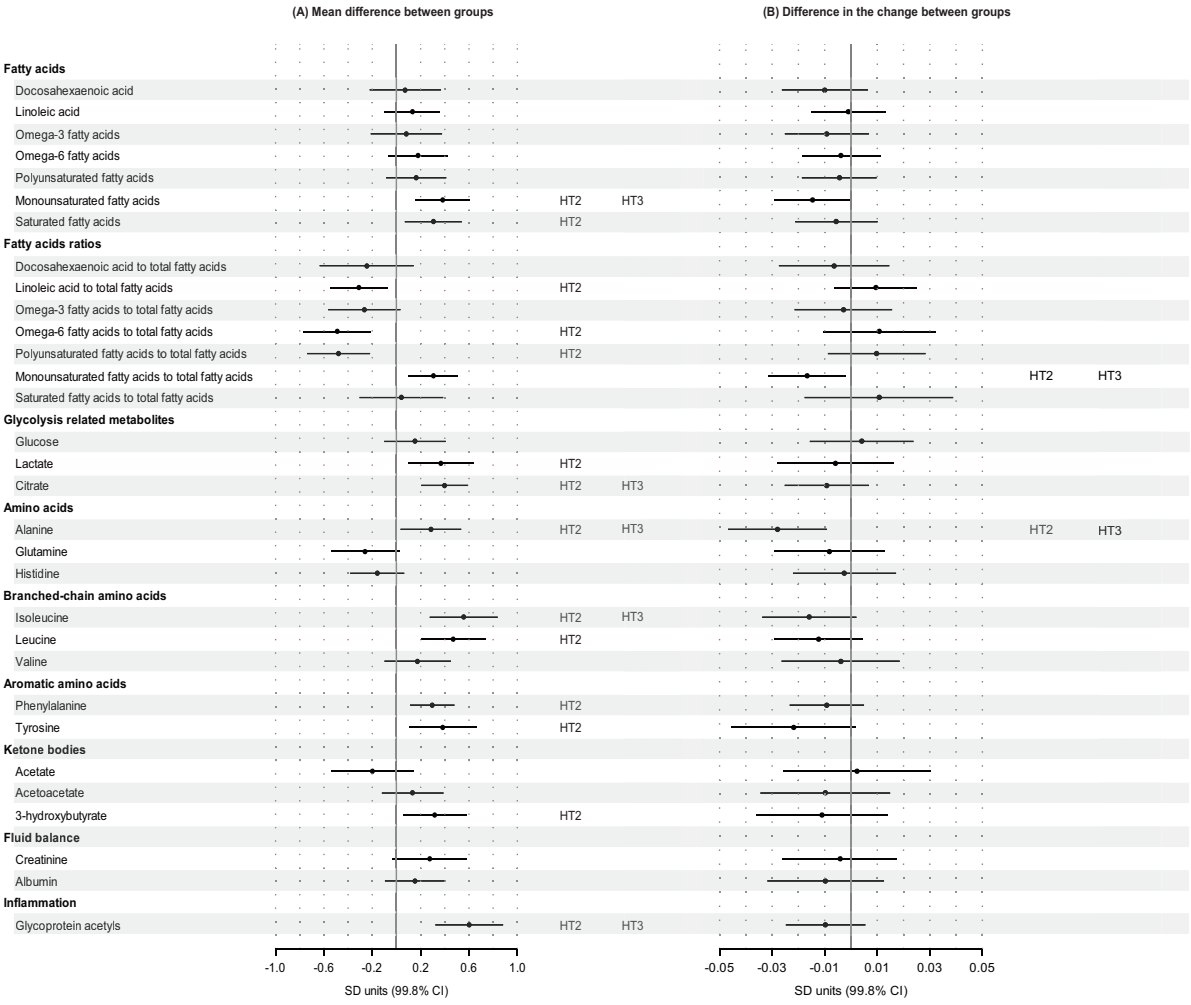



**Figure 5.** Continued.

**Table 2.** Odds ratio with 95% CI for women with overweight, obesity, gestational diabetes, and hypertensive disorders to belong to latent classes with different metabolic profiles during pregnancy

| | Latent class 2 *vs* latent class 1 | | | Latent class 3 *vs* latent class 1 | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* | OR | 95% CI | *P* |
| Overweight versus normal weight | | | | | | |
| Model 1 | 1.32 | 0.77, 2.26 | 0.31 | 1.75 | 0.90, 3.43 | 0.10 |
| Model 2 | 1.46 | 0.83, 2.56 | 0.19 | 1.90 | 0.96, 3.78 | 0.07 |
| Model 3 | 1.29 | 0.73, 2.30 | 0.38 | 1.74 | 0.87, 3.51 | 0.12 |
| Obesity versus normal weight | | | | | | |
| Model 1 | 1.74 | 1.10, 3.43 | 0.02 | 2.02 | 1.16, 3.35 | 0.01 |
| Model 2 | 1.64 | 1.01, 2.64 | 0.04 | 2.12 | 1.19, 3.80 | 0.01 |
| Model 3 | 1.46 | 0.89, 2.40 | 0.13 | 1.95 | 1.08, 3.52 | 0.03 |
| Gestational diabetes versus no diabetes | | | | | | |
| Model 1 | 1.51 | 0.92, 2.47 | 0.11 | 1.39 | 0.78, 2.47 | 0.26 |
| Model 2 | 1.53 | 0.92, 2.55 | 0.10 | 1.34 | 0.75, 2.41 | 0.33 |
| Model 3 | 1.41 | 0.84, 2.36 | 0.19 | 1.23 | 0.68, 2.22 | 0.49 |
| Gestational hypertension versus normotension | | | | | | |
| Model 1 | 1.11 | 0.53, 2.29 | 0.79 | 1.20 | 0.48, 3.00 | 0.69 |
| Model 2 | 1.07 | 0.51, 2.26 | 0.86 | 1.18 | 0.47, 2.98 | 0.72 |
| Model 3 | 1.03 | 0.49, 2.19 | 0.94 | 1.10 | 0.43, 2.79 | 0.84 |
| Preeclampsia versus normotension | | | | | | |
| Model 1 | 2.34 | 1.04, 5.27 | 0.04 | 2.32 | 0.85, 6.32 | 0.10 |
| Model 2 | 2.80 | 1.17, 6.72 | 0.02 | 2.73 | 0.95, 7.82 | 0.06 |
| Model 3 | 2.58 | 1.06, 6.23 | 0.04 | 2.36 | 0.81, 6.84 | 0.11 |
| Chronic hypertension versus normotension | | | | | | |
| Model 1 | 2.63 | 1.31, 5.29 | 0.007 | 3.06 | 1.33, 7.01 | 0.008 |
| Model 2 | 2.25 | 1.11, 4,59 | 0.03 | 2.81 | 1.21, 6.49 | 0.02 |
| Model 3 | 2.04 | 0.99, 4.21 | 0.054 | 2.37 | 1.01, 5.58 | 0.05 |

Model 1 is adjusted for maternal age and cohort, Model 2 is additionally adjusted for maternal education, parity, and substance use during pregnancy, and Model 3 is additionally adjusted for gestational diabetes and hypertensive disorders (in analyses of overweight and obesity), body mass index and hypertensive disorders (in analyses of gestational diabetes), or body mass index and gestational diabetes (in analyses of hypertensive disorders).
Abbreviation: OR, odds ratio.

by GDM, PE, and HT resembled the perturbations seen in obesity, but some of these associations were explained by BMI. Future studies are warranted to explore the influence of disturbed maternal metabolome on long-term maternal health as well as newborn metabolic health and growth.

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***Disclosure Summary:*** The authors report no conflicts of interest.

***Data Availability:*** Data sets generated during the current study are not publicly available but will be made available upon reasonable request. Requests are subject to further review by the national register authority and by the ethical committees.

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# Metabolomic Profiles of Nonobese and Obese Women With Gestational Diabetes

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## Abstract

**Context:** In non-pregnant population, nonobese individuals with obesity-related metabolome have increased risk for type 2 diabetes and cardiovascular diseases. The risk of these diseases is also increased after gestational diabetes.

**Objective:** This work aimed to examine whether nonobese (body mass index [BMI] < 30) and obese (BMI ≥ 30) women with gestational diabetes mellitus (GDM) and obese non-GDM women differ in metabolomic profiles from nonobese non-GDM controls.

**Methods:** Levels of 66 metabolic measures were assessed in early (median 13, IQR 12.4-13.7 gestation weeks), and across early, mid (20, 19.3-23.0), and late (28, 27.0-35.0) pregnancy blood samples in 755 pregnant women from the PREDO and RADIEL studies. The independent replication cohort comprised 490 pregnant women.

**Results:** Nonobese and obese GDM, and obese non-GDM women differed similarly from the controls across early, mid, and late pregnancy in 13 measures, including very low-density lipoprotein-related measures, and fatty acids. In 6 measures, including fatty acid (FA) ratios, glycolysis-related measures, valine, and 3-hydroxybutyrate, the differences between obese GDM women and controls were more pronounced than the differences between nonobese GDM or obese non-GDM women and controls. In 16 measures, including HDL-related measures, FA ratios, amino acids, and inflammation, differences between obese GDM or obese non-GDM women and controls were more pronounced than the differences between nonobese GDM women and controls. Most differences were evident in early pregnancy, and in the replication cohort were more often in the same direction than would be expected by chance alone.

**Conclusion:** Differences between nonobese and obese GDM, or obese non-GDM women and controls in metabolomic profiles may allow detection of high-risk women for timely targeted preventive interventions.

**Key Words:** gestational diabetes, metabolomics, obesity

**Abbreviations:** AA, amino acid; BMI, body mass index; FA, fatty acid; GDM, gestational diabetes mellitus; HDL, high-density lipoprotein; IQR, interquartile range; ITU, InTraUterine Sampling in Early Pregnancy Study; LA, linoleic acid; NOGDM, nonobese with gestational diabetes mellitus; O, obese without gestational diabetes mellitus; OGDM, obese with gestational diabetes mellitus; PREDO, Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction study; PUFA, polyunsaturated fatty acid; RADIEL, Finnish Gestational Diabetes Prevention study; VLDL, very low-density lipoprotein.

Hyperglycemia in pregnancy affects globally 17% of pregnancies, with 80% being due to gestational diabetes mellitus (GDM) (1). GDM may lead to increased risk for several adverse perinatal outcomes, including cesarean delivery, macrosomia, shoulder dystocia, and neonatal hypoglycemia (2). GDM also poses long-term consequences both for mother and offspring. Not only are women with GDM at higher risk for developing subsequent type 2 diabetes, metabolic

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syndrome, and cardiovascular disorders (3), but also their offspring have an increased risk for obesity, type 2 diabetes, and neurodevelopmental and behavioral disorders (4).

Like type 2 diabetes (5, 6), GDM is a heterogeneous condition (7-10). Although body mass index (BMI) is a major risk factor for GDM, 20% to 66% of women with GDM are nonobese (BMI < 30) (11, 12). Nonobesity- and obesity-related GDM likely reflect differences in underlying pathophysiology, with insulin-secretion deficit characterizing more often those with nonobese GDM (NOGDM) (13) and insulin resistance obese (BMI ≥ 30) GDM (OGDM) (9). Even though obesity during pregnancy is associated with perturbations in the metabolome (14, 15), what remains unknown is whether women with NOGDM and OGDM differ in their metabolomic characteristics. Two recent studies in general populations have demonstrated that nonobese adults have a higher risk for type 2 diabetes and cardiovascular disease if their metabolomic profiles are unhealthy, and hence characteristic of obesity (16, 17). This suggests that the metabolomic profiles of women with NOGDM may instead resemble the profiles of women with OGDM, and obese without GDM (O), than differ from them.

Against this background we examined whether women with NOGDM, OGDM or O differed from the nonobese non-GDM controls in their metabolomic profiles in early, and across early, mid, and late pregnancy.

## Materials and Methods

### Participants

The study population consisted of 2 Finnish studies: the Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction (PREDO; ISRCTN.com registration no. ISRCTN14030412) study (18) and the Finnish Gestational Diabetes Prevention (RADIEL; ClinicalTrials.gov registration no. NCT01698385) study (19). A flowchart is presented in Supplementary Fig. S1 (20).

The PREDO study recruited 1079 pregnant women with known risk factors for preeclampsia and intrauterine growth restriction between 12 and 14 weeks of gestation from 10 hospitals. A subgroup with a bilateral second-degree diastolic notch in the uterine blood flow were randomly assigned to receive low-dose aspirin (n = 61) or placebo (n = 60) to prevent preeclampsia. Blood samples were obtained at a median 13.0 (interquartile range [IQR] 12.6-13.4), 19.3 (19.0-19.7), and 27.0 (26.6-27.6) weeks of gestation from 425 women. In the PREDO cohort, those women who provided blood samples were younger (aged 32.5 vs 33.6 years; $P = .007$) and less likely to be obese (29.1% vs 39.3%; $P = .003$) than women who did not.

The RADIEL study recruited 720 women at high risk for GDM (BMI ≥ 30 or prior GDM or both) into a randomized clinical trial, for prevention of GDM by lifestyle intervention. These women were either planning a pregnancy or were at less than 20 weeks of gestation at enrollment. Blood samples were obtained from 339 women at a median 13.0 (IQR 11.9-14.3), 23.1 (22.6-24.1), and 35.1 (34.4-35.7) weeks of gestation. Of them, 177 were randomly assigned to the intervention group receiving advice on diet and physical activity, and 160 were in the control group (standard care). In the RADIEL cohort, those women who provided blood samples were less likely to be obese (14.0% vs 20.5%; $P = .04$) or to have GDM (27.9% vs 73.2%; $P < .0001$) or preeclampsia (3.3% vs 7.0%; $P = .04$) than women who did not.

In the combined PREDO-RADIEL cohort, no women eligible for data analyses had type 1 diabetes. The timing of blood samples in the combined cohort was at a median 13.0 (IQR 12.4-13.7), 20.0 (19.3-23.0), and 28.0 (27.0-35.0) weeks of gestation. Of the combined cohort of 755 women, 535 (70.9%) provided all 3 blood samples, 171 (22.7%) 2 samples, and 49 (6.5%) 1 sample, resulting in a sample size of 639, 679, and 678 women at the first, second and third time point (Supplementary Table S1) (20).

The replication cohort comprised a subsample of 490 pregnant women of the 943 pregnant women of the InTraUterine Sampling in Early Pregnancy Study (ITU) (21). These women provided one blood sample at a median 20.6 (IQR 20.1-23.4) gestational weeks. Women who provided the blood sample were less likely to be obese (3.4% vs 6.2%; $P = .006$) and more likely to have a tertiary education (83.7% vs 70.4%; $P < .0001$) than the women who did not.

All study participants signed an informed consent, and the study protocols were approved by the ethics committee of the Helsinki and Uusimaa Hospital District.

### Methods

#### Metabolomic profiling

Venous blood was collected in all 3 cohorts between 7 and 10 AM after at least a 10-hour overnight fast. Plasma (in PREDO and ITU) and serum (in RADIEL) were separated immediately and stored at −80 °C until analysis. A high-throughput proton nuclear magnetic resonance metabolomics platform quantified 225 metabolic measures using the Nightingale Health Quantification Library 2020 (Nightingale Health Ltd). The analysis panel includes biomarkers of lipid and glucose metabolism, amino acids (AAs), fatty acids (FAs), ketone bodies, and a marker of low-grade inflammation. This method has been widely used in studies of pregnant and nonpregnant populations (22-25). Of all the metabolic measures, 37 have been validated against the standard clinical chemistry methods. Details of the experimentation are described elsewhere (26). Following the lead of earlier studies using the same metabolomics platform, 66 of these metabolic measures were considered appropriate to form an adequate picture of the systemic metabolism, and served as the primary outcomes (22, 23). Those measures not included in the analysis were composition within the various lipoprotein subclasses and relative lipoprotein lipid concentrations.

### Gestational Diabetes Mellitus and Prepregnancy Nonobesity and Obesity

In all cohorts, the diagnosis of GDM came from medical records and in RADIEL and in PREDO was verified additionally by a jury composed of a research nurse and 2 or more medical doctors. Exceeding or equaling one or more of the plasma glucose thresholds (5.3, 10.0, and 8.6 mmol/L) in a 2-hour 75-g oral glucose tolerance test led to a diagnosis of GDM (27).

The Finnish Medical Birth Register, collecting data on prepregnancy weight and height, verified at the first visit to the antenatal clinics, provided information for calculating BMI. Among the participants recruited before pregnancy in the RADIEL study, we used prepregnancy weight and height measured at the last study visit before pregnancy. Obesity

was defined according to World Health Organization guidelines (28) as prepregnancy BMI of 30 or greater. Nonobesity was defined as prepregnancy BMI less than 30.

### Covariates

We adjusted for cohort (PREDO, RADIEL) and weeks of gestation at time of blood sampling (all cohorts). The other covariates were chosen based on the literature, and included maternal age (years) (22), parity (primiparous vs multiparous) (22, 23), and smoking (no vs yes) (23), which were drawn from the Medical Birth Register; education (basic/secondary vs tertiary) (22) and alcohol use (no vs yes) (23) were reported in early pregnancy (all cohorts).

### Statistical Analysis

To study whether women with NOGDM, OGDM, or O differed from the controls in the metabolic measures during pregnancy, we applied an individual-participant data meta-analytic approach by using mixed-model regression analyses (PREDO, RADIEL). The repeated metabolic measures represented the within-person outcome variables, and gestational week at blood sampling the time-varying within-person predictor variables in these analyses. The groups of women—NOGDM, OGDM, O, and the controls (referent)—and the other covariates were included in these models as between-person fixed effects. We included interaction effects of NOGDM, OGDM, and O (with controls as the referent) × gestational week at blood sampling into the models to study whether women with NOGDM, OGDM, or O differed from controls in the change in the metabolic measures during pregnancy.

We defined unstructured covariance and first-order autoregressive error covariance matrices and allowed random effects to account for individual differences in the intercept. As the mixed models allow missing data, we did not impute missing values on metabolic measures (missingness per metabolic measure is shown in Supplementary Table S1) (20). However, if the measure was below detection level, we used a value equivalent to 0.9 multiplied by the nonzero minimum value of that measurement. For the between-groups fixed factors, missingness was minimal, and we conducted complete case analyses, except for smoking and alcohol consumption during pregnancy for which missing values were coded in a separate category.

We log-transformed the metabolic measures to normalize their distributions and analyzed the values in cohort-specific standardized units. As the metabolic measures are highly correlated, the Bonferroni-correction for multiple testing may be overly conservative and raise the risk of type II error (29). To reduce this risk, principal components analysis has been applied as a multiple testing correction method for correlated data to identify the effective number of independent tests (30, 31). We identified 25 principal components, which explained more than 99% of the variation in the 66 metabolic measures that we used as the primary outcomes. Therefore, 2-sided $P$ less than .002 (.05/25) was used to infer statistical significance.

In the analysis of the replication cohort, we used linear regression analysis in which the metabolic measures served as dependent variables, and women with NOGDM, OGDM, or O were compared to controls in models adjusted for the covariates. We used the Fisher's exact test to study whether the group differences found in the combined PREDO-RADIEL cohort were more often in the same direction in the ITU replication cohort than what would be expected by chance alone.

As effect size indicators we report estimates and their 99.8% CIs (mixed models in PREDO-RADIEL) and unstandardized β coefficients and their 95% CIs (linear regression models in ITU). The estimates and unstandardized regression coefficients represent mean differences (grand mean of the early, mid, and late pregnancy values; and mean differences in the change [estimate representing slope]) in the metabolic measures in SD units of women with NOGDM, OGDM, or O, with controls as the referent in all analyses. To ensure that missing data would not influence the findings on change, we report the results of change also among women who provided metabolomic data at all 3 time points during pregnancy.

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc).

## Results

### Background Characteristics

Table 1 presents the characteristics of the control group (n = 312) and of the 3 study groups: women with NOGDM (n = 96), with OGDM (n = 89), and with O (n = 258). The results for all 66 metabolic measures across pregnancy and in early pregnancy are presented in Supplementary Fig. S2 and S3 (20).

### Metabolic Measures in Which Differences of Nonobese With Gestational Diabetes Mellitus, Obese With Gestational Diabetes Mellitus, and Obese Without Gestational Diabetes Mellitus From Controls are Similar

Among these 66 metabolic measures, there were 13 in which all study groups—women with NOGDM, OGDM, or O—differed significantly from the controls and in which, in effect size, the mean differences of these groups from controls were similar (Fig. 1; numeric values in Supplementary Table S2, Panel A (20)). These 13 metabolic measures included very small to large, and mean diameter of very low-density lipoprotein (VLDL), total cholesterol in VLDL, triglycerides in VLDL, LDL, and high-density lipoprotein (HDL), ratio of apolipoprotein B to apolipoprotein A-I, and monounsaturated and saturated FAs. The differences in all these measures were statistically significant also at the early pregnancy measurement point (see Fig. 1B). In small and medium LDL, remnant cholesterol, and apolipoprotein B, women with NOGDM, OGDM, or O also differed from controls in a similar manner, but all the differences were statistically significant only at the early pregnancy measurement point (see Fig 1B). In the analysis of the change, during pregnancy there were no metabolic measures in which the 3 study groups differed from the controls significantly, and in which, in effect size, the differences in change were similar (Supplementary Fig. S4 (20)).

### Metabolic Measures in Which Differences of Obese With Gestational Diabetes Mellitus From Controls Were More Pronounced Than Were the Differences of Nonobese With Gestational Diabetes Mellitus and Obese Without Gestational Diabetes Mellitus From Controls

Among these 66 metabolic measures, there were 6 in which women with OGDM differed significantly from the controls

**Table 1. Characteristics of the 755 women with either nonobesity and no gestational diabetes mellitus, nonobesity and gestational diabetes mellitus, obesity and gestational diabetes mellitus, or obesity and no gestational diabetes mellitus**

| Variable | Nonobese non-GDM (controls) (n = 312) | Nonobese GDM (NOGDM) (n = 96) | $P^b$ | Obese GDM (OGDM) (n = 89) | $P^b$ | Obese non-GDM (O) (n = 258) | $P^b$ |
|---|---|---|---|---|---|---|---|
| Cohort, n (%) | | | <.0001 | | <.0001 | | <.0001 |
| PREDO | 248 (79%) | 44 (46%) | | 45 (51%) | | 79 (31%) | |
| RADIEL | 64 (21%) | 52 (54%) | | 44 (49%) | | 179 (69%) | |
| Maternal age (mean, SD), y | 32.6 (5.3) | 33.9 (4.4) | .02 | 34.1 (4.6) | .009 | 32.5 (4.8) | .99 |
| BMI (mean, SD) | 23.4 (3.1) | 25.0 (2.7) | <.0001 | 35.8 (4.3) | <.0001 | 34.3 (3.8) | <.0001 |
| Parity, n (%) | | | .002 | | .67 | | <.0001 |
| Primiparous | 83 (27%) | 11 (11%) | | 26 (29%) | | 126 (49%) | |
| Multiparous | 228 (73%) | 86 (89%) | | 63 (71%) | | 132 (51%) | |
| Education level, n (%) | | | .34 | | .001 | | <.0001 |
| Secondary or lower | 143 (46%) | 51 (53%) | | 59 (66%) | | 178 (69%) | |
| Tertiary | 161 (52.0%) | 46 (47%) | | 28 (31%) | | 79 (31%) | |
| Data not available[a] | 7 (2.3%) | 0 | | 2 (2.2%) | | 1 (0.4%) | |
| Smoking or alcohol use during pregnancy, n (%) | | | .60 | | .83 | | .38 |
| No | 236 (84.0%) | 80 (87.0%) | | 65 (83.3%) | | 218 (87.2%) | |
| Smoked/used alcohol at any time during pregnancy | 45 (16.0%) | 12 (13.0%) | | 13 (16.7%) | | 32 (12.8%) | |
| Data not available | 30 (9.7%) | 5 (5.2%) | | 11 (12.4%) | | 8 (3.1%) | |

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; PREDO, Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction; RADIEL, Finnish Gestational Diabetes Prevention.
[a]Unless indicated otherwise, no missing data.
[b]Calculated for the difference between study group and controls.

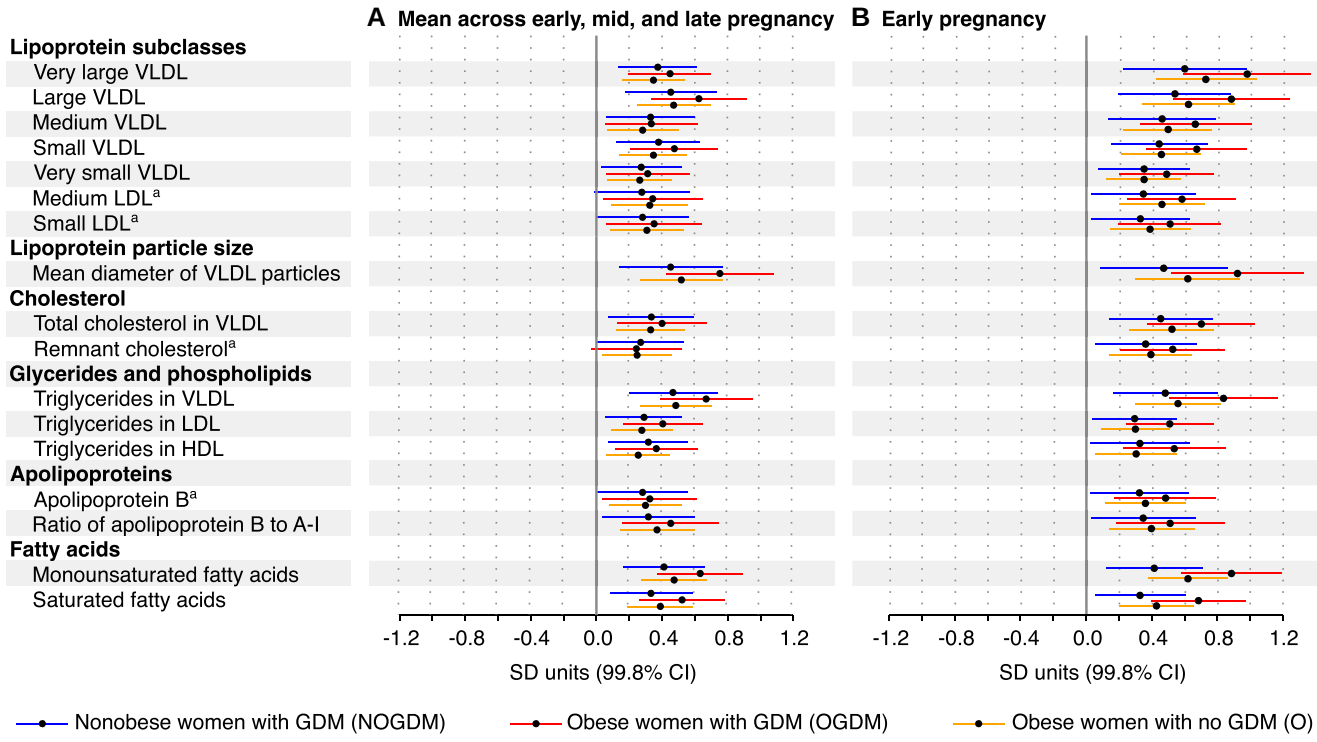


**Figure 1.** Mean differences and 99.8% CIs in those metabolic measures in which nonobese women with GDM (NOGDM), obese women with GDM (OGDM), and obese women with no GDM (O) differed in a similar manner from nonobese controls with no GDM across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

and in which, in effect size, the differences from controls were more pronounced in women with OGDM than in women with NOGDM or O (Fig. 2, numeric values in Supplementary Table S2, Panel B (20)). These 6 measures included linoleic acid (LA) to total FAs, and polyunsaturated fatty acids (PUFA) to total FA ratios, glucose, citrate, valine, and 3-hydroxybutyrate. At the early pregnancy measurement point, differences between women with OGDM and controls were more pronounced in both of the FA ratios, and glucose, and additionally, in alanine (Fig. 2B).

In the analysis of change during pregnancy, there were 7 metabolic measures in which women with OGDM differed significantly from controls, and in which, in effect size, the differences in change from controls were more pronounced in women with OGDM than in women with NOGDM or O (Supplementary Fig. S4 and S5(20)). These measures included intermediate-density lipoprotein, small HDL, several cholesterol-related measures, total cholines, and sphingomyelin (all increased less during pregnancy, except for sphingomyelin, which decreased more in women with OGDM).

### Metabolic Measures in Which Differences of Obese With Gestational Diabetes Mellitus and Obese Without Gestational Diabetes Mellitus From Controls Were More Pronounced Than Differences of Nonobese With Gestational Diabetes Mellitus From Controls

Among these 66 metabolic measures, there were 16 in which obese groups—women with OGDM, or O—differed significantly from the controls and in which, in effect size, the mean differences of these obese groups from controls were similar (see Fig. 3; numeric values in Supplementary Table S2C (20)). In these metabolic measures, women with NOGDM differed less (on 5 of these measures) or did not differ significantly (on 11 of these measures) from controls (see Fig. 3). These measures included several HDL-related measures, different FA ratios, some AAs, and glycoprotein acetylation. Differences in 13 of these measures, and additionally in small HDL particles and in the docosahexaenoic to total FA ratio, were statistically significant also at the early pregnancy measurement point (see Fig 3B).

In the analysis of change during pregnancy, there were 24 metabolic measures in which women with OGDM or O differed significantly from controls, and in which, in effect size, the differences in change from controls were similar in women with OGDM or O, whereas women with NOGDM differed less in change from controls or did not differ significantly (Supplementary Fig. S4 and S5 (20)). These measures included most sizes of VLDL and LDL, total cholesterol in VLDL, remnant cholesterol, triglycerides, apolipoprotein B, many FAs, some AAs, and glycoprotein acetylation (all increased less during pregnancy, except for tyrosine, which decreased more in women with OGDM or O).

### Replication of the Results in the InTraUterine Sampling in Early Pregnancy Study Cohort

In ITU, findings concerning the differences of NOGDM, OGDM, and O from the controls were tested for replication. Even though not all the differences replicated or reached statistical significance in ITU, they were in the same direction more often than would be expected by chance alone ($P < .001$ from Fisher's exact test) (Supplementary Table S2 (20)). Of the metabolic measures in which mean differences (across early, mid, and late pregnancy or in early pregnancy) of NOGDM, OGDM, and O from controls were similar, 80% were in the same direction in ITU; in which mean differences of OGDM from controls were more pronounced than differences of NOGDM or O from controls, 71% were in the same direction in ITU; and in which mean differences of OGDM and O from controls were more pronounced than differences of NOGDM from controls, 76% were in the same direction in ITU (see Supplementary Table S2 (20)).

## Discussion

The main findings of our study are 3-fold. First, our findings demonstrated that—when compared to controls (nonobese non-GDM)—women with NOGDM, OGDM, or O differed significantly in 13 metabolomic measures. These differences were similar in effect size, suggesting that women with NOGDM, OGDM, or O display similar metabolomic profiles. Second, in 6 of the metabolomic measures, differences from the controls of women with OGDM were more pronounced in effect size than were the differences from the controls of women with NOGDM or O. These differences suggest that across these metabolic measures, such differences reflect the nonobesity- and obesity-related pathophysiology of GDM. Finally, we identified 16 additional metabolomic measures in which the differences of women with OGDM or O from the controls were more pronounced in effect size than were the differences of women with NOGDM from controls, the latter differences being mostly not statistically significant. These differences suggest that across these 16 metabolomic measures, such differences reflect the pathophysiology of nonobesity and obesity, rather than nonobesity- and obesity-related pathophysiology of GDM. Our findings are supported by the replication in an independent sample of pregnant women, in which the results were in the same direction in 77%, more often than would be expected by chance alone.

Our first finding, similar adverse metabolomic profiles with perturbations in VLDL-related measures, triglycerides in VLDL, LDL, and HDL, ratio of apolipoprotein B to A-I, and some FAs among women with NOGDM, OGDM, or O is in parallel with others' study findings demonstrating similar perturbations in obesity and GDM (15, 22). The metabolic perturbations in women with NOGDM highlights the metabolic burden of GDM even in the absence of obesity. In our earlier study (7), lean women with GDM carried an increased risk for subsequent diabetes and had a surprisingly high body fat percentage 5 years post partum despite their seemingly nonobese BMI. Another longitudinal Finnish birth cohort study (32) demonstrated risk for type 2 diabetes to be markedly increased also among normal-weight women with GDM; the hazard ratio for diabetes 20 years after pregnancy was more than 10-fold. Among women with prepregnancy overweight, or with concomitant overweight and GDM, the corresponding figures exceeded 12 and 47. That many of the metabolic aberrations we detected were already evident in early pregnancy may reflect that the underlying disease process was already present weeks or months before GDM diagnosis, and may offer identifiable biomarkers for detecting women at high risk. This would be crucial especially for nonobese women, as they frequently remain undetected, and their identification is of great clinical relevance and offers substantial potential for prevention.

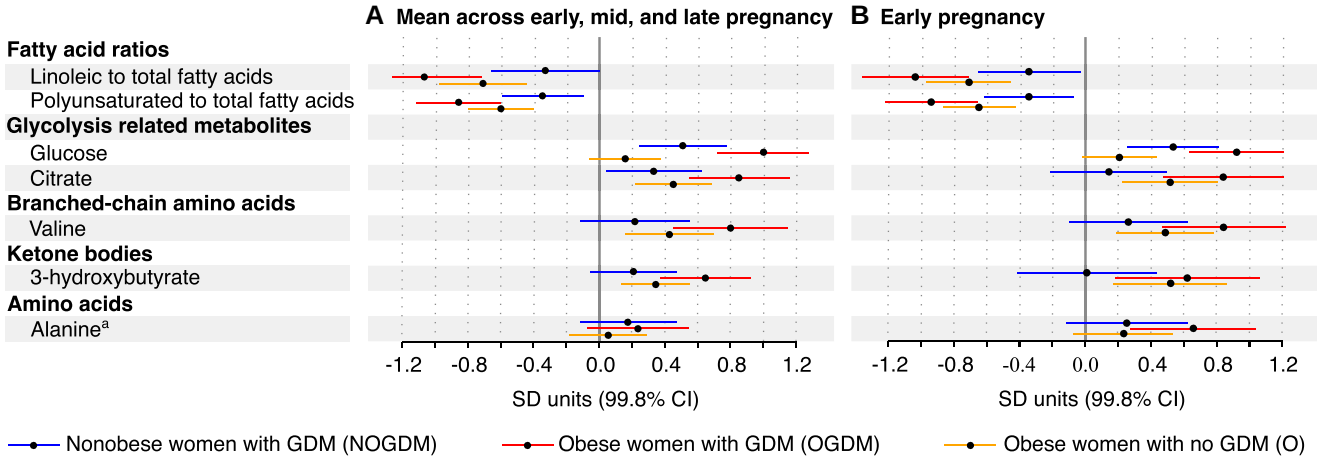


**Figure 2.** Mean differences and 99.8% CIs in those metabolic measures in which differences of obese women with GDM (OGDM) from controls (nonobese with no GDM) are more pronounced than are the differences of nonobese women with GDM (NOGDM), and of obese women with no GDM (O) from controls across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

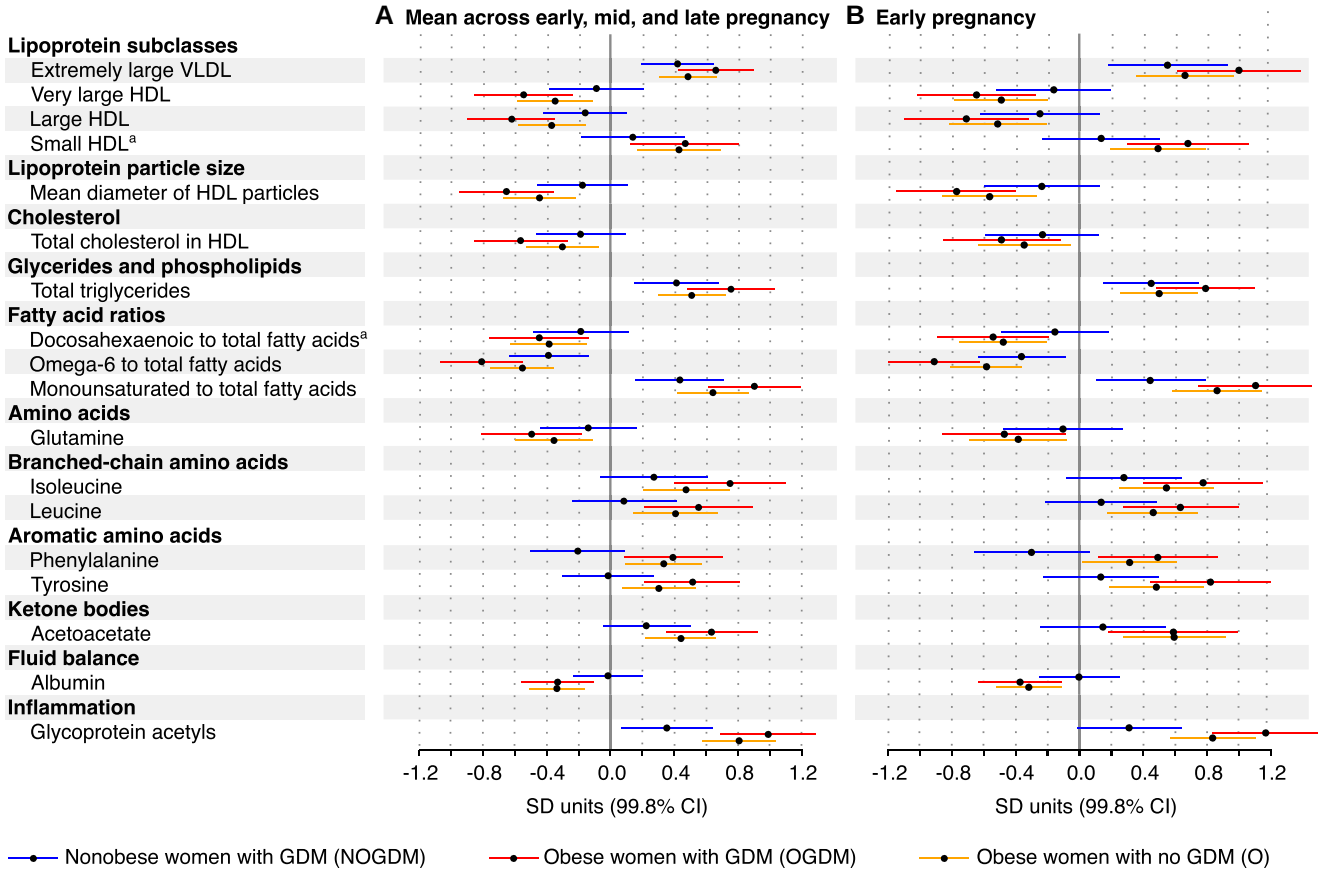


**Figure 3.** Mean differences and 99.8% CIs in those metabolic measures in which differences of obese women with GDM (OGDM), and of obese women with no GDM (O) from controls (nonobese with no GDM) are more pronounced than are the differences of nonobese women with GDM (NOGDM) from controls across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

Second, we identified 6 metabolic measures distinguishing GDM subtypes: NOGDM and OGDM, across pregnancy, with differences not reflecting nonobesity and obesity. The first ones were LA to total FAs, and PUFA to total FAs, and previous studies, without separately studying the GDM subtypes, have reported lower ratios (22) among women with

GDM. Our finding of lower ratios of these FAs among women with OGDM, presumably insulin resistant, than among women with NOGDM is consistent with findings of an inverse association between circulating PUFAs, LA, and insulin resistance (33). The level of valine, a branched-chain amino acid with a well-established association with insulin resistance (34), was lower in women with NOGDM than in women with OGDM (9). Two of the measures distinguishing women with NOGDM and OGDM were glycolysis related, that is, glucose and citrate, and higher levels may reflect a poorer balance of glycemic control among women with obesity. The sixth metabolic measure distinguishing NOGDM from OGDM was a ketone body, 3-hydroxybutyrate, suggested in one study as a prognostic metabolic biomarker for GDM (35). In the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (36), elevated levels of 3-hydroxybutyrate were observable in women with GDM at 28 weeks of gestation after adjustment for BMI. In our study, only women with OGDM, not NOGDM, had significantly higher 3-hydroxybutyrate levels, both in early and across pregnancy, than did controls. Separate analyses of nonobese and obese women in earlier studies could have possibly revealed new information about the usefulness of 3-hydroxybutyrate as a biomarker for GDM across the full spectrum of BMI.

The obesity-related perturbations we detected among women with O or OGDM, but not NOGDM, included lower levels of many HDL-related measures, higher levels of total triglycerides, relatively higher levels of saturation of FAs, higher levels of many insulin resistance–associated (34, 37) measures like branched-chain and aromatic amino acids, and higher level of inflammation. For women with NOGDM, preventing excessive weight gain in pregnancy and maintaining normal BMI afterward may be important for sustaining levels comparable to controls in these obesity-related measures, and thus avoiding morbidity associated with many of these metabolic perturbations. Moreover, these perturbations may be important in explaining differences in risk for macrosomia or cesarean delivery between GDM subtypes (9). Low HDL cholesterol has already been associated with accelerated epigenetic aging of the placenta (38) and higher offspring birth weight (39, 40). The obesity-related metabolic perturbations in our study may be one mechanism connecting the intrauterine environment and offspring outcomes and warrant further studies.

We have earlier reported smaller change across pregnancy in many of the metabolic measures among obese compared to normal-weight, and among GDM compared to non-GDM women (15). The findings in this study indicate that the difference in change from controls is evident only among women with OGDM, not with NOGDM, and thus, may be associated with the differences in the pathophysiology of GDM among obese and nonobese women.

Among the strengths of our study is its longitudinal design, allowing us to identify both the mean levels of metabolic measures across pregnancy as well as early pregnancy levels. The metabolic panel was targeted and has been widely used in previous studies. Many of the metabolic measures have been validated against conventional laboratory techniques. Our sample included a large number of nonobese and obese women, with and without GDM, thus providing ample statistical power. In addition, we performed a replication study in another independent cohort, providing robust evidence of perturbations in the lipid profiles of the study groups. The relatively small sample size in the replication cohort may explain our inability to replicate all results. One limitation of our study is the possible effect of the original study interventions. We have, however, already shown (15) that interventions were not associated with differences in metabolomic profiles. Plausible bias due to different samples, plasma and serum, in 2 cohorts, seems minimal (41) and our statistical methods, with SD scaling and adjustment for cohort, were designed to address this issue. An additional limitation is that our study included women from an ethnically homogeneous, high-resource Nordic setting, which limits the generalizability of our results.

In conclusion, our study highlights the fact that women with NOGDM, OGDM, or O differ from controls by displaying similar metabolomic profiles, by displaying differences that reflect nonobesity- and obesity-related pathophysiology of GDM, and by displaying differences that are obesity driven. These findings may allow identification of women at risk for GDM, and at long-term risk for GDM- and obesity-related health adversities and allow tailoring of timely targeted preventive interventions.

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## Author Contributions

K.R., S.B.K., P.V.G., H.S.H., and E.H. designed the research. K.R., S.B.K., P.V.G., H.S.H., and E.H. conducted the study. P.V.G. and H.S.H. performed the statistical analyses. H.S.H., E.H., K.R., and S.B.K. drafted the manuscript and had primary responsibility for the final content. All authors contributed to the interpretation of the findings and critically reviewed, commented on, read, and approved the final manuscript. K.R., S.B.K., and P.V.G. are the guarantors of this work.

## Disclosures

The authors have nothing to disclose.

## Data Availability

Data sets generated during the presents study are not publicly available but will be made available on reasonable request. Requests are subject to further review by the national register authority and by the ethical committees.

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