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Disclosure summary:

The authors report no conflict of interest.

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 two 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, 12.4-13.7), 20 (19.3-23.0) and 28 (27.0-35.0) weeks of gestation.

Results

Across all three 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 non-significant after adjustment for BMI and the other pregnancy disorder.

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.

Keywords: Diabetes, Gestational; Hypertension, Gestational; Metabolomics; Nuclear Magnetic Resonance, Biomolecular; Pre-Eclampsia; Pregnancy; Pregnant women

Introduction

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 five 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 one time-point during pregnancy or they have 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 three serial time points during pregnancy in two 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 two 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 1,079 pregnant women between 12-14 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.

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.

Methods

Metabolic profiling using the NMR platform

In both cohorts, venous blood samples were drawn from the antecubital vein between 7-10 AM after at least a 10-hour 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, nine FA and seven ratios of FA, five other lipids, eight AA, three ketone bodies, and two metabolites related to fluid balance and three to gluconeogenesis and one 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 non-pregnant 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 two 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 WHO 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-hour 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 hypertension before 20 weeks of gestation. Definition for gestational hypertension 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. Supplemental figures 1 and 2 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 hypertension / 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 hypertension/PE x 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 three sampling points into a grand average. We compared solutions with two to six latent classes. Based on criteria for the optimal number of classes described by Kongsted and Nielsen [20], the optimal solution was based on (1) goodness-of-fit criteria (Akaike Information Criterion [AIC], Bayesian Information Criterion [BIC]), (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 [21]. To overcome this risk, we applied principal components analysis (PCA) 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, 22-24] and is suggested as the first step in approaching metabolomics data analysis [21]. 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 [25-28]. Hence, by using the PCA approach, we identified twenty-five principal components, which explained over 99% of the variation in the 68 metabolic measures that we used as the primary outcomes. Therefore, two-sided P<0.002 (0.05/25) was used to infer statistical significance.

As effect size indicators we present estimates and their 99.8% confidence intervals (CI) (mixed model) and odds ratios and their 95% CIs (OR, logistic regression models). Estimates represent mean differences (pooling data from the three 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). The circular diagrams were created using R (R Core Team 2020) EpiViz package [29-31].

Results

Women in the PREDO study were younger, had higher education, were less often obese and had more often chronic or gestational hypertension 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 three blood samples, 169 (22.8%) two samples, and 48 (6.5%) one 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 (Supplemental figures 3, 4 and 5) [32] and results of the 68 metabolic measures used as the primary outcomes are presented in Figures 2-5.

Prepregnancy overweight and obesity

Compared to normal-weight women, women with obesity had higher mean levels (pooled across the three measurement 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 fatty acids (MUFA), saturated fatty acids (SFA), and MUFA to total FA ratio; branched-chain amino acids (BCAA) and aromatic amino acids (AAA); and inflammation marker glycoprotein acetyls (GlycA) in the fully adjusted model, including adjustment for GDM and hypertensive disorders (Figure 2, left panel). 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 three 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 (Figure 2, right panel; Supplemental figure 6). 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 (Figure 3, left panel). Of the 68 metabolic measures, 23 associations were significant in the model 1, but when fully adjusted, including adjustment for BMI and hypertensive disorders, nine of the associations were rendered non-significant (Figure 3, left panel). 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 six measures across the three sampling points differed between GDM and normoglycemic women in the fully adjusted model (Figure 3, right panel; Supplemental figure 7). The differences between normoglycemic and GDM women were more pronounced in insulin-treated than in diet-treated group (Supplemental figure 8).

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, nine were rendered non-significant (Figure 4, left panel). The associations that remained significant after full adjustment were five 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 two measures across the three sampling points differed between women with PE and normotension in the fully adjusted model (Figure 4, right panel; Supplemental figure 9).

HT was also associated with higher / lower mean levels of many of the same metabolites as obesity, but many of them were rendered non-significant after adjustment for BMI and GDM. Out of the 68 metabolic measures, 24 of the 29 significant associations (in model 1) became non-significant (Figure 5, left panel). The associations that remained significant were total triglycerides, MUFA, citrate, isoleucine and GlycA. Out of the 68 metabolic measures, change in the levels of three measures across the three sampling points differed between women with HT and normotension in models adjusted for all covariates (Figure 5, right panel; Supplemental figure 9).

Gestational hypertension was not associated significantly with any of the metabolic measures during pregnancy (Supplemental figure 10).

Metabolic profiles: Latent class analysis

The optimal LCA solution identified three classes of women who differed significantly for 52 out of 68 metabolic measures, and additionally 9 metabolic measures differed significantly between two classes (Supplemental tables 1 and 2). Supplemental table 3 shows the number of women in the three 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, GlycA and lower ratio of PUFA to total FA. With the exception of acetate and some fatty acid ratios, the levels of most metabolites gradually increased from classes 1 to classes 2 and 3 (Supplemental table 2). Across all adjustment models women with obesity compared to women with normal weight had significantly higher odds to belong to class 3 than 1 and women with PE compared with those with normotension had a significantly higher odds to belong to class 2 than 1 (Table 2).

Discussion

Our study shows that women with prepregnancy obesity have adverse levels of metabolic measures throughout three 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 AAA, and they displayed higher level 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. 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 (e.g. 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, 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, e.g. release of free FA mainly from adipose tissue, throughout pregnancy, in contrast with normal weight 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 AAA [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].

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 FA and BCAAs isoleucine and leucine, as seen also in the previous cross-sectional studies [9, 43-45]. In non-pregnant 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 non-significant 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 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 three serial time points during pregnancy. The targeted panel of metabolic measures we used has been widely studied previously in pregnant and non-pregnant 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 two cohorts generates a challenge of 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 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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Data Availability

Datasets 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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Figure 1 Flowchart of the participants.

Figure 2 Mean differences (pooled mean across the three consecutive measurement points; left panel) and differences in the change (slopes; right panel) 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 one pregnancy week in the metabolic measures in SD units and error bars to their 99.8% confidence intervals 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).

Figure 3 Mean differences (pooled mean across the three consecutive measurement points; left panel) and differences in the change (slopes; right panel) of metabolic measures during pregnancy between women with gestational diabetes in comparison to normoglycemic 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% confidence intervals. 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).

Figure 4 Mean differences (pooled mean across the three consecutive measurement points; left panel) and differences in the change (slopes; right panel) 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% confidence intervals. 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).

Figure 5 Mean differences (pooled mean across the three consecutive measurement points; left panel) and differences in the change (slopes; right panel) of metabolic measures during pregnancy between women with chronic hypertension 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% confidence intervals. 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).

Table 1. Characteristics of the study participants by cohort

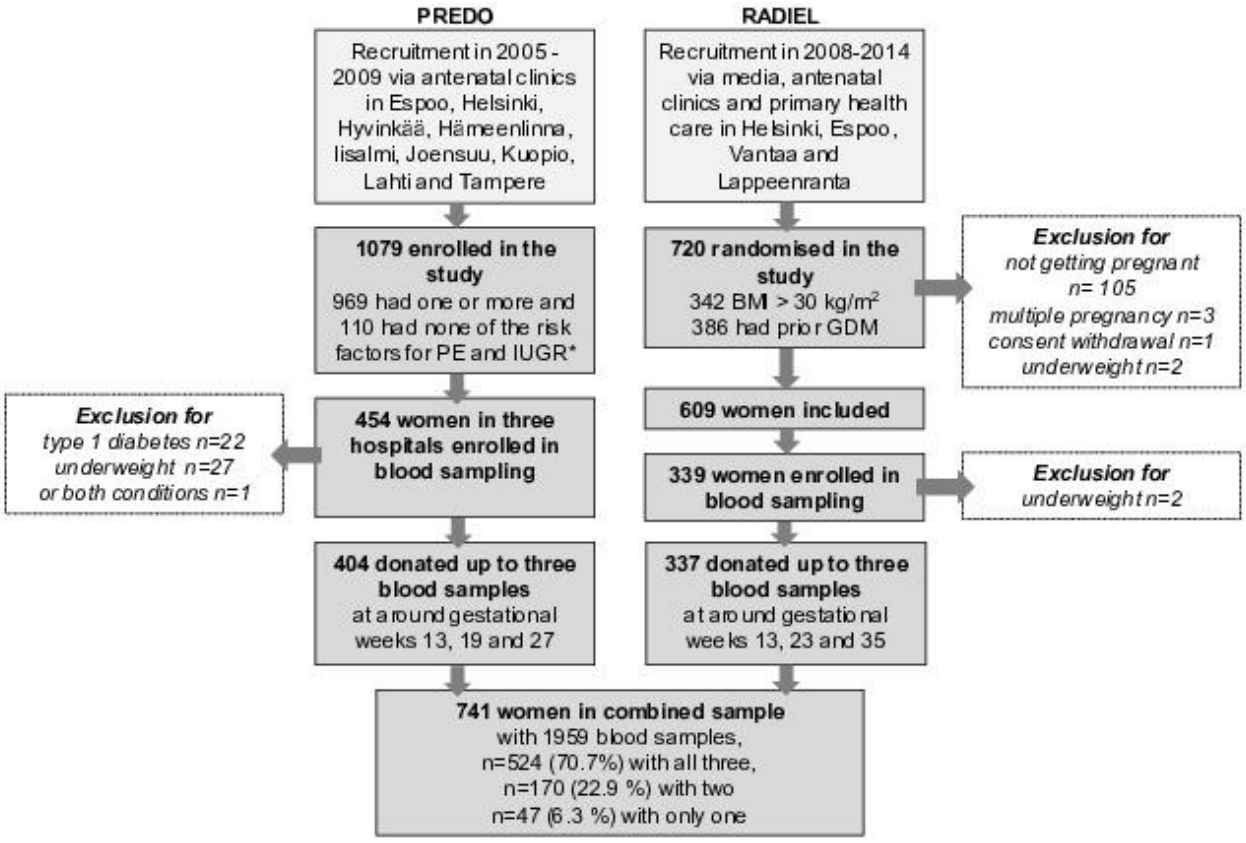
	Mean (SD) or N (%)	
	PREDO (N=404)	RADIEL (N=337)
Gestational age at the 1st blood sampling point (mean, range)	13.0 (11.1-16.7)	13.0 (6.0-17.7)
Gestational age at the 2nd blood sapling point (mean, range)	19.4 (17.1-22.9)	23.1 (20.1-27.6)
Gestational age at the 3rd blood sampling point (mean, range)	27.0 (24.1-31.1)	35.1 (30.6-38.9)
Maternal age, years	32.6 (5.2)	33.4 (4.5)
Data not available	0	0
Education level		
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		
Primiparous	128 (31.7%)	114 (33.8%)
Multiparous	276 (68.3%)	223 (66.2%)
Data not available	0	0
Smoking during pregnancy		
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		
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		
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		
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		
Normoglycemia	314 (77.7%)	243 (71.22%)
Gestational diabetes mellitus	90 (22.3%)	94 (27.9%)
Data not available	0	0

Table 2. Odds ratio (OR) with 95% confidence intervals (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 versus latent class 1			**Latent class 3 versus 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 additionally for maternal education, parity and substance use during pregnancy and model 3 additionally for gestational diabetes and hypertensive disorders (in analyses of overweight and obesity), or for body mass index and hypertensive disorders (in analyses of gestational diabetes), or for body mass index and gestational diabetes (in analyses of hypertensive disorders)

Figure 1



*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 2A

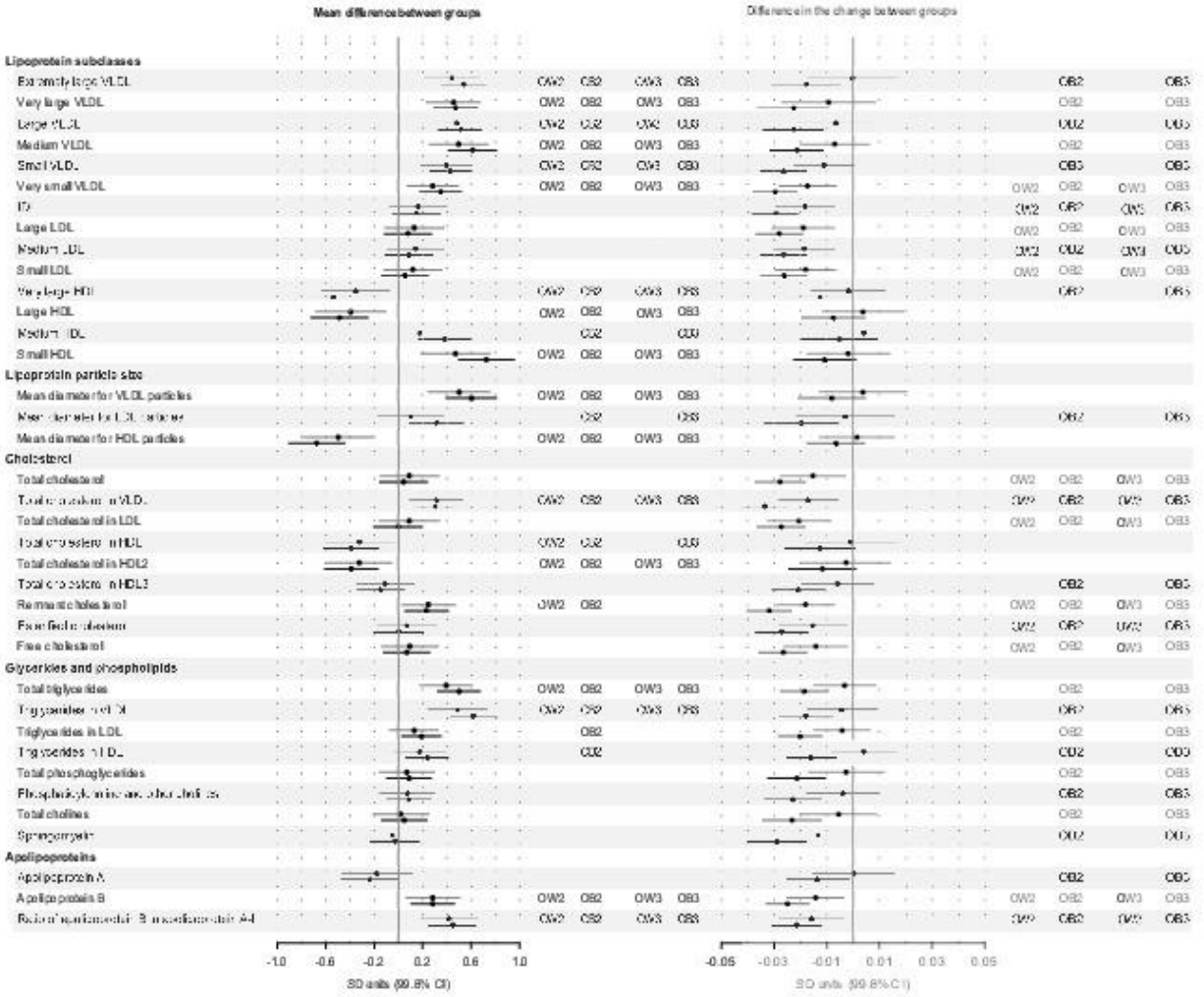


Figure 2B

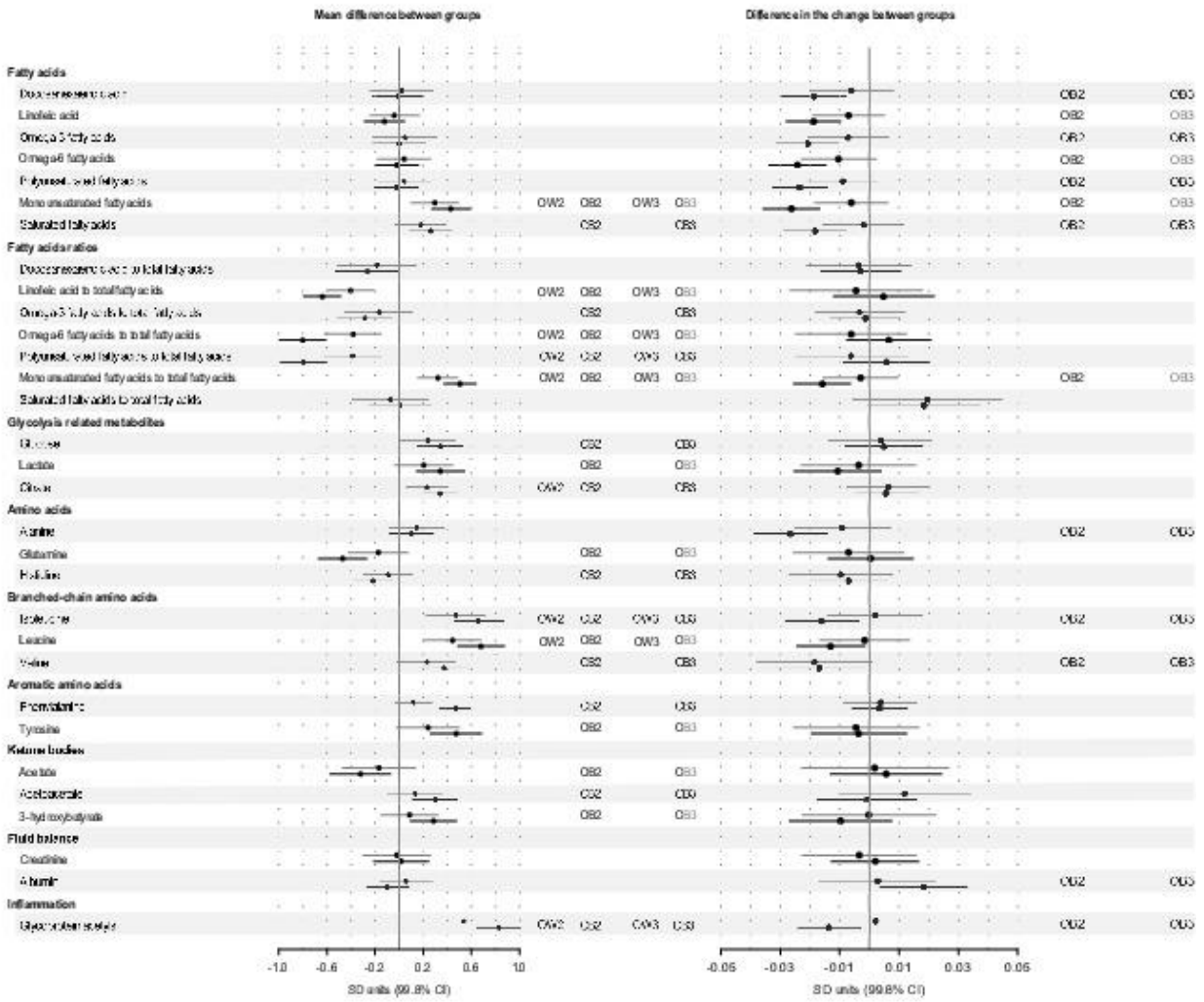


Figure 3A

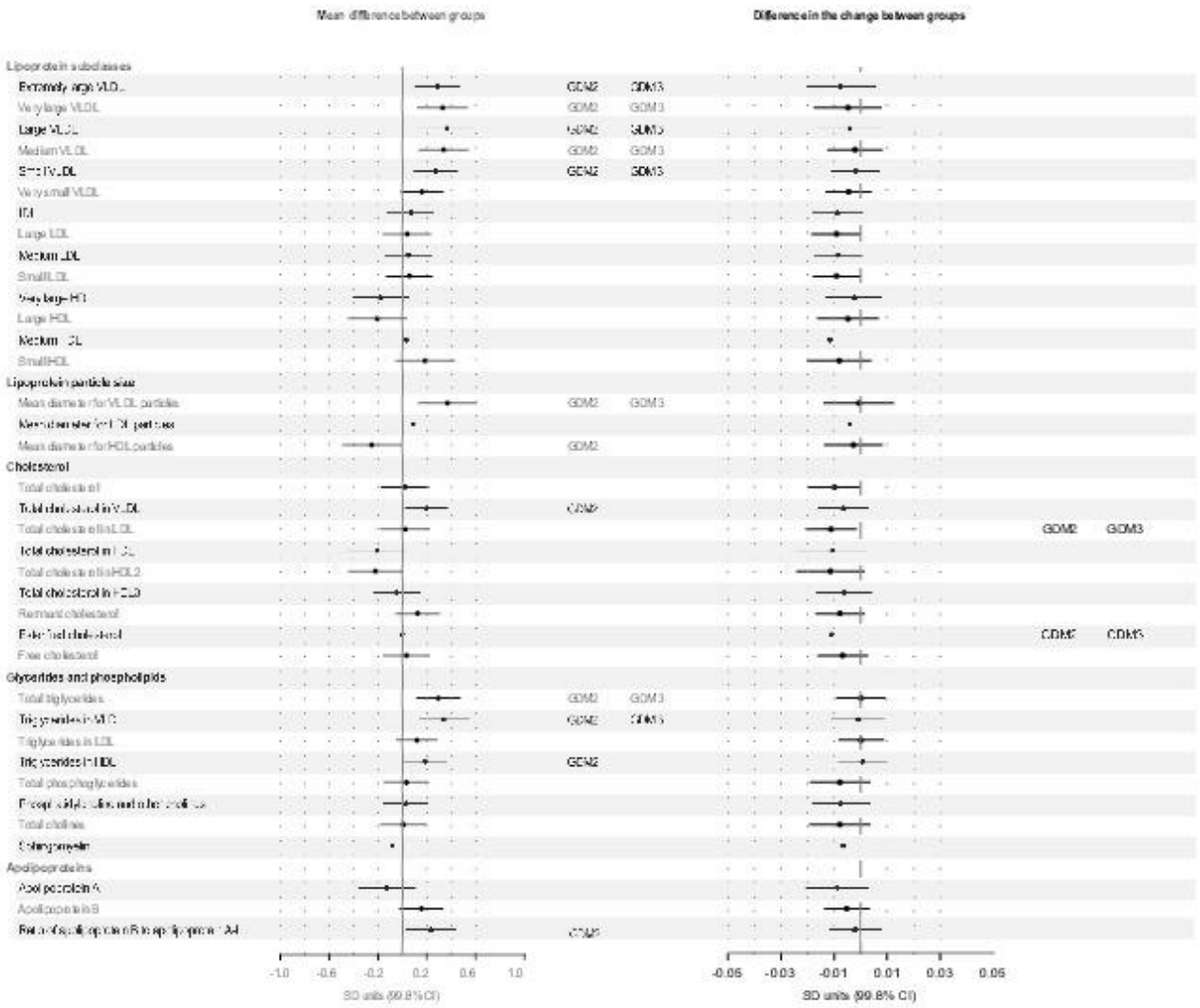


Figure 3B

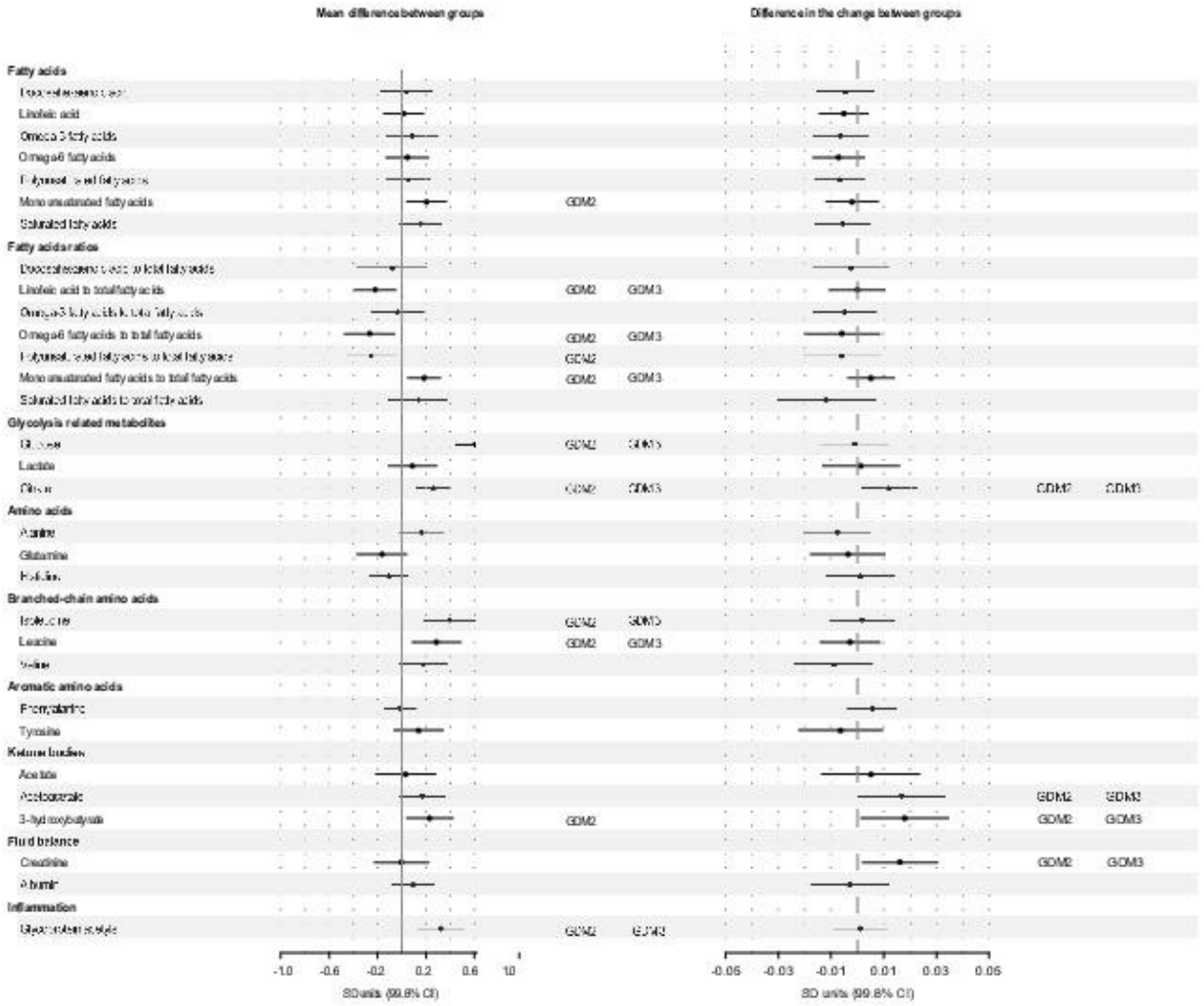


Figure 4A

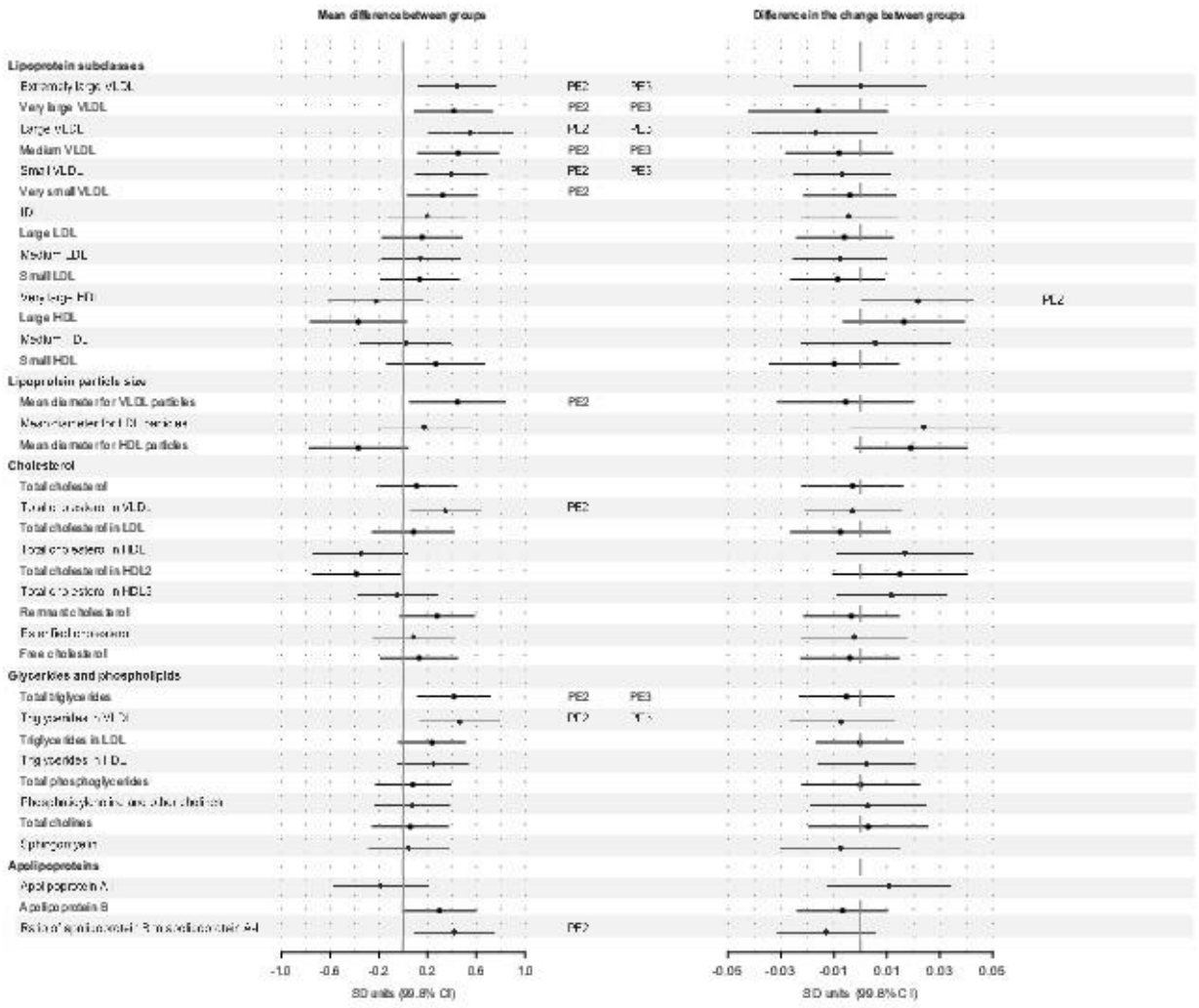


Figure 4B

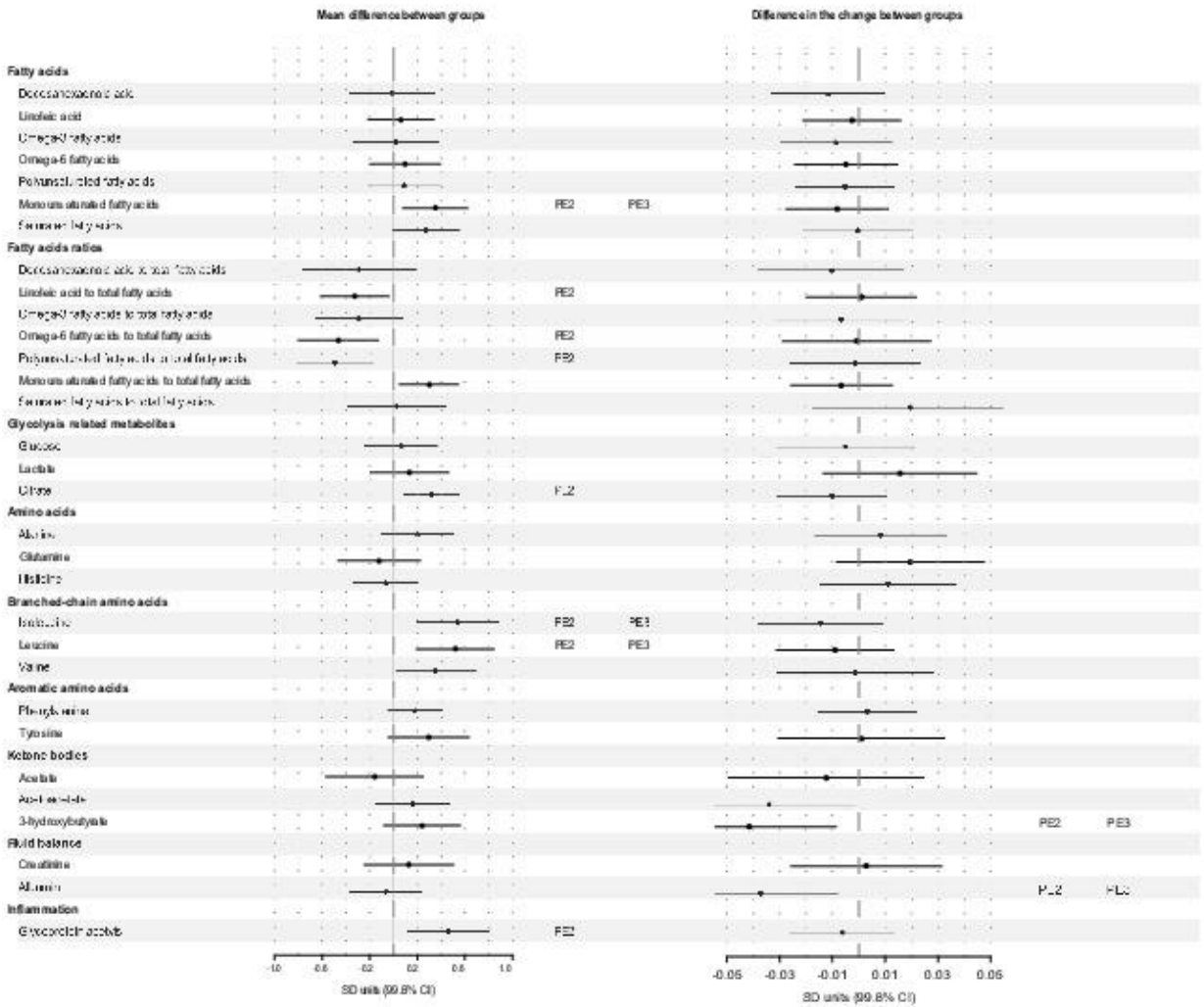


Figure 5A

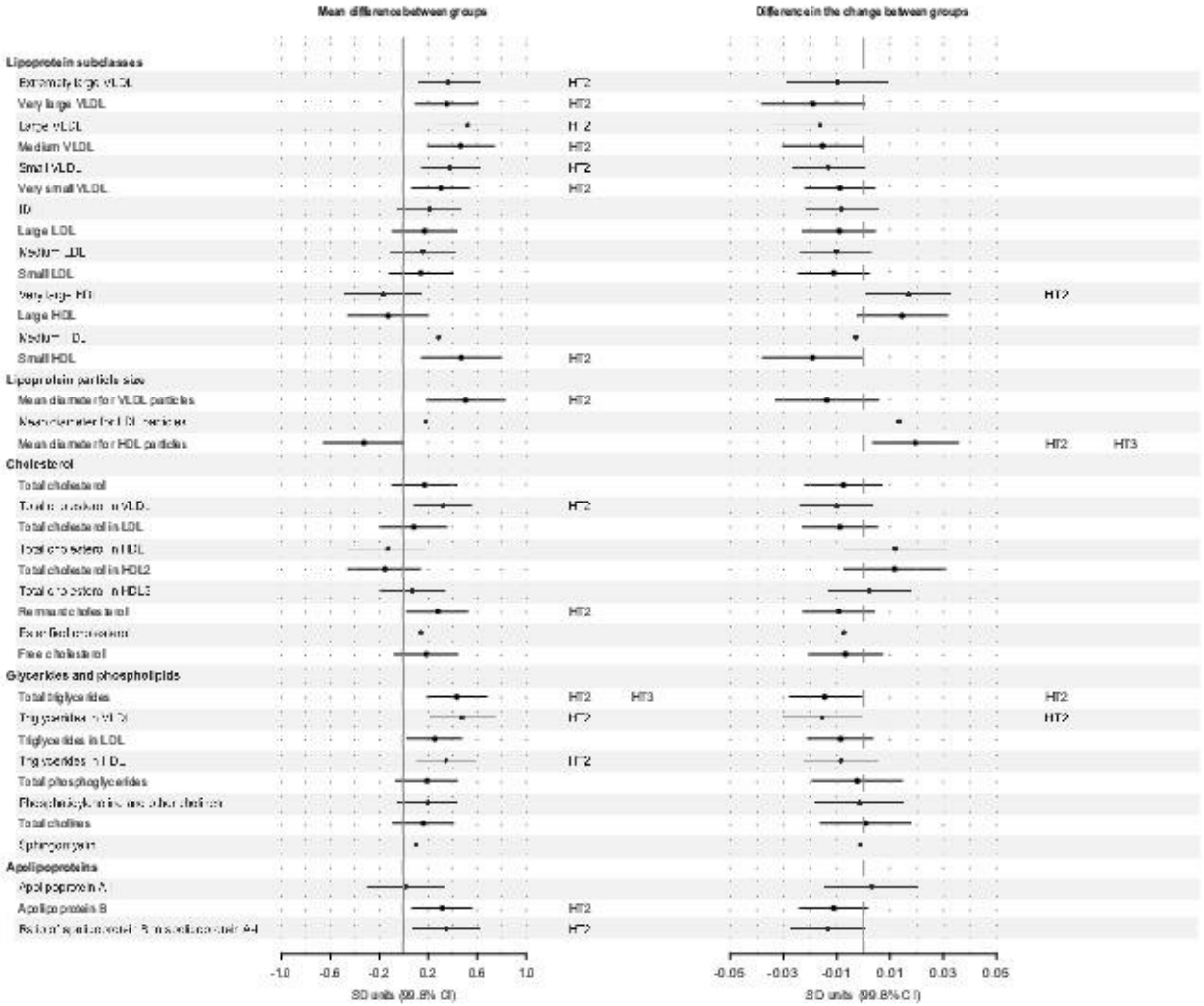


Figure 5B