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Report on the relationship of early-life exposures with differences in metabolic profiles and the extent to which these mediate associations of early-life exposures with cardiometabolic risk factors and disease

LifeCycle report D4.4

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List of Abbreviations

AGA: Appropriate for Gestational Age
BMI: Body Mass Index
CHD: Coronary Heart Disease
CI: Confidence Interval
CIMT: Carotid Intima-Media Thickness
GD : Gestational Diabetes
1H-NMR: Proton Nuclear Magnetic Resonance
HDL: High Density Lipoprotein
HDP: Hypertensive Disorders of Pregnancy
IVW: Inverse Vriance Weighting
LDL: Low-Density Lipoprotein
LGA: Large for Gestational Age
MR: Mendelian Randomization
MV: MultiVariable
PE: Pre-Elcampsia
PTB: Preterm Birth
PUFA: Poly Unsaturated Fatty Acids
PWV: Pulse wave velocity
RCT: Randomised Controlled Trial
SD: Standard Deviation
SGA: Small for Gestational Age
SNP: Single Nucleotide Polymorphism
STA: Saturated Fatty Acids
UK: United Kingdom
UPBEAT: UK Pregnancies Better Eating and Activity Trial
VLDL: Very Low Density Lipoprotein
WP: work package

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Executive summary

The aim of this deliverable was to explore whether variation in maternal pregnancy or offspring blood levels of multiple metabolites mediated effects of early life stressors on future cardiometabolic health, such as high blood pressure, subclinical markers of atherosclerosis or high levels of glucose and cholesterol. For metabolites to mediate early-life stressors on later health outcomes, they must be causally influenced by the early life stressors and in turn cause cardiometabolic outcomes. Therefore, we looked at both of these possibilities. We found evidence that that maternal obesity was related to more marked changes in multiple metabolites during pregnancy, and that a lifestyle intervention could improve these obesity related changes. We also found that being born large for gestational age, which is more likely in those whose mother is obese during pregnancy is associated with lower levels of cholesterol, fatty acids, several lipoprotein subclasses, three branched-chain amino acids, and an inflammatory marker in adolescence and early adulthood. These differences would be expected to related to lower risk of adverse cardiometabolic outcomes in later life based on several existing published studies, including Mendelian randomization and randomised controlled trials of lipid lowering by statins. By contrast we did not find robust evidence that exposure to hypertensive disorders of pregnancy, gestational diabetes, preterm birth or small for gestational age influenced offspring metabolism beyond early infancy, nor did we find younger age at menarche influenced subsequent variation in multiple metabolites, once possible confounding by body mass index was adjusted for. Importantly, we did not find robust evidence that variation in multiple metabolites influenced sub-clinical atherosclerosis in children or adults. Taken together these findings suggest that multiple metabolites are unlikely to be key mediators of potential effects of the early life stressors we have explored on subsequent cardiometabolic health. The lack of association of multiple metabolites with sub-clinical atherosclerosis would argue against early life stressors affecting atherosclerosis via mechanisms involving multiple metabolites. These findings should be considered in light of current metabolomic platforms only partially covering the metabolome, though they do provide detailed coverage of lipids, lipoproteins, fatty acids and glycolysis metabolites that are relevant to cardiometabolic health.

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1. Introduction

The aim of WP4 is to examine the associations of early-life stressors during pregnancy, infancy and early childhood with cardiometabolic outcomes across the life course. The objective of task 4.3 is to explore the extent to which systemic differences in multiple metabolites mediate the relationships between early-life stressors, for example exposure to maternal gestational diabetes or early age at menarche, and later life cardiometabolic outcomes, such as higher blood pressure or higher blood glucose levels. The related deliverable (4.4) is to report on the relationship of early-life stressors with differences in multiple metabolite lives in maternal pregnancy blood and offspring early life blood, and the extent to which any differences in maternal or offspring blood metabolites mediate associations of early-life stressors with cardiometabolic outcomes. Central to LifeCycle and this delivery is taking a life-course approach, and in this deliverable we have done that by assessing associations of early-life stressor with multiple metabolites assessed at different ages from infancy to early adulthood. This deliverable has been met through five studies that are described below in the main body of this report. We started this deliverable by determining the potential impact of maternal pregnancy obesity on change in maternal metabolite levels in pregnancy and the extent to which a lifestyle intervention in obese pregnant women could mitigate against more adverse metabolic change. The second study examined the relationship between multiple metabolites and sub-clinical atherosclerosis in children and adults. This is highly relevant to this deliverable because for multiple metabolites to mediate effects they must affect the outcomes (here cardiometabolic outcomes, such as subclinical atherosclerosis) as well as be influenced by early life stressors. The remaining three papers focus on early life stressors beyond maternal pregnancy obesity, and their impacts on multiple metabolites across the life course. This included examining biological sex as an exposure and its relation to multiple metabolites changes across the life course. We also assessed the relation of early menarche on multiple metabolites in as a second early life exposure. Finally, in a substantial paper we explored the impact of all common adverse pregnancy and perinatal outcomes (hypertensive disorders of pregnancy (HDP), gestational diabetes (GD), preterm birth (PTB), small and large for gestational age (SGA, LGA) on multiple metabolites across the life course.

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2. Description of progress and results

2.1 The effect of a lifestyle intervention in obese pregnant women on change in gestational metabolic profiles: findings from the UK Pregnancies Better Eating and Activity Trial (UPBEAT) RCT (1)

Pregnancy is associated with widespread change in metabolism, which may be more marked in obese women. Whether lifestyle interventions in obese pregnant women improve pregnancy metabolic profiles remains unknown. Our objectives were to determine the magnitude of change in metabolic measures during obese pregnancy, to indirectly compare these to similar profiles in a general pregnant population, and to determine the impact of a lifestyle intervention on change in metabolic measures in obese pregnant women. Data from a randomised controlled trial of 1158 obese (BMI ≥ 30 kg/m^2) pregnant women recruited from six UK inner-city obstetric departments were used. Women were randomised to either the UPBEAT intervention, a tailored complex lifestyle intervention focused on improving diet and physical activity, or standard antenatal care (control group). UPBEAT has been shown to improve diet and physical activity during pregnancy and up to 6-months postnatally in obese women and to reduce offspring adiposity at 6-months; it did not affect risk of gestational diabetes (the primary outcome). Change in the concentrations of 158 metabolic measures (129 lipids, 9 glycerides and phospholipids, and 20 low-molecular weight metabolites), quantified three times during pregnancy, were compared using multilevel models. The role of chance was assessed with a false discovery rate of 5% adjusted p values.

All very low-density lipoprotein (VLDL) particles increased by 1.5-3 standard deviation units (SD) whereas intermediate density lipoprotein and specific (large, medium and small) LDL particles increased by 1-2 SD, between 16 and 36 weeks' gestation. Triglycerides increased by 2-3 SD, with more modest changes in other metabolites. Indirect comparisons suggest that the magnitudes of change across pregnancy in these obese women were 2- to 3-fold larger than in unselected women (n = 4260 in cross-sectional and 583 in longitudinal analyses) from an independent, previously published, study. The intervention reduced the rate of increase in extremely large, very large, large and medium VLDL particles, particularly those containing triglycerides.

In conclusion, there are marked changes in lipids and lipoproteins and more modest changes in other metabolites across pregnancy in obese women, with some evidence that this is more marked than in unselected pregnant women. The UPBEAT lifestyle intervention may contribute to a healthier metabolic profile in obese pregnant women, but our results require replication.

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Selected Results

In work undertaken prior to starting LifeCycle we had shown that women underwent widespread metabolic change on becoming pregnant, which then returned to pre-pregnancy levels. This deliverable is concerned with the extent to which variation in maternal gestational and offspring (cord-blood and postnatal) metabolic traits mediate early life (intrauterine, infancy and early childhood) stressors on subsequent offspring cardiometabolic health. Maternal gestational blood metabolites are intimately related offspring growth and development and plausibly offspring subsequent cardiometabolic health. In this paper we were interested in whether maternal gestational obesity resulted in a more pronounced metabolic response to pregnancy, and if so, whether an lifestyle intervention could reduce that response.

By indirect comparison between our previous work and results in this study of obese women we showed that obese women have a much more marked change in all of the classes of metabolites explored (lipids, lipoproteins, fatty acids, glycolysis metabolites , ketone bodies and amino acids) across pregnancy than seen in the general population (not selected for obesity) do on becoming pregnant.

Importantly, we showed that a lifestyle intervention that improved dietary intake, increased light (walking) physical activity and reduced gestational weight gain, reduced the rate of increase in extremely large, very large, large and medium VLDL particles, particularly those containing triglycerides (**Figure 1**). By contrast small VLDL did not differ between the groups and there was a tendency to concentrations being higher in the intervention arm for very small VLDL. Other metabolic traits did not notably differ between the two randomised arms.

2.2 A cross-cohort study examining the associations of metabolomic profile and subclinical atherosclerosis in children and their parents: The Child Health CheckPoint Study and Avon Longitudinal Study of Parents and Children (ALSPAC) (2)

High-throughput nuclear magnetic resonance profiling of circulating metabolites is suggested as an adjunct for cardiovascular risk evaluation. The relationship between metabolites and subclinical atherosclerosis remains unclear, particularly among children. Therefore, we examined the associations of metabolites with carotid intima-media thickness (cIMT) and arterial pulse wave velocity (PWV). Data from two independent population-based studies was examined; (1) cross-sectional associations with cIMT and PWV in 1178 children (age 11–12 years, 51% female) and 1316 parents (mean age 45 years, 87% female) from the CheckPoint study (Australia); and (2) longitudinal associations

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in 4249 children (metabolites at 7–8 years, PWV at 10–11 years, 52% female), and cross-sectional associations in 4171 of their mothers (mean age 48 years, cIMT data) from ALSPAC (The Avon Longitudinal Study of Parents and Children; UK). Metabolites were measured by the same nuclear magnetic resonance platform in both studies, comprising of 69 biomarkers. Biophysical assessments included body mass index, blood pressure, cIMT and PWV. In linear regression analyses adjusted for age, sex, body mass index, and blood pressure, there was no evidence of metabolite associations in either children or adults for cIMT at a 10% false discovery threshold.

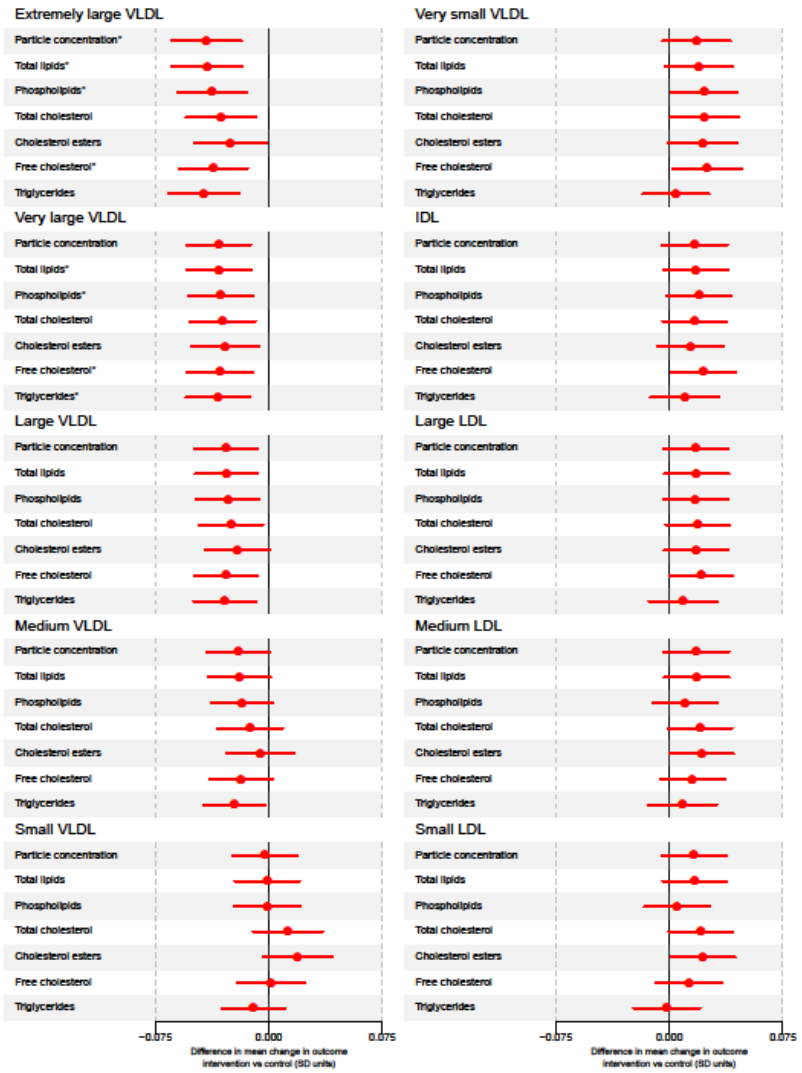

Figure 1: Differences in VLDL particles comparing lifestyle intervention to standard care randomised arms of the UPBEAT RCT. *Results show differences in mean concentrations (SD units) comparing obese pregnant women randomised to a lifestyle intervention to those randomised to standard care.*

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In CheckPoint adults, glucose was positively, and some high-density lipoprotein-cholesterol derived measures and amino acids (glutamine, histidine, tyrosine) inversely associated with PWV. In conclusion, these data suggest that in children circulating metabolites have no consistent association with cIMT and PWV once adjusted for body mass index and blood pressure. In their middle-aged parents, some evidence of metabolite associations with PWV were identified that warrant further investigation.

Selected results

There were no replicated associations across two independent cohorts for any associations with PWV or CIMT in children. Specifically cross-sectional associations in CheckPoint were not seen in longitudinal analyses in ALSPAC. For adults (the children's parents and mostly mothers: 87% of Checkpoint and 100% ALSPAC) there was no evidence of any associations with CIMT in cross-sectional analyses. It was not possible to independently replicate, the positive associations of glucose, and inverse associations of some high-density lipoprotein-cholesterol derived measures and amino acids (glutamine, histidine, tyrosine) with PWV seen in the adult Checkpoint participants because PVW has not been measured in ALSPAC adults.

2.3 Sex differences in systematic metabolites at four life stages: cohort study with repeated metabolomics (3)

Males experience higher rates of coronary heart disease (CHD) than females, but the circulating traits underpinning this difference are poorly understood. We examined sex differences in systemic metabolites measured at four life stages, spanning childhood to middle adulthood. Data were from the Avon Longitudinal Study of Parents and Children (7727 offspring, 49% male; and 6500 parents, 29% male). Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy from a targeted metabolomics platform was performed on plasma or serum samples to quantify 229 systemic metabolites (including lipoprotein-subclass-specific lipids, pre-glycaemic factors, and inflammatory glycoprotein acetyls). Metabolites were measured in the same offspring once in childhood (mean age 8 years), twice in adolescence (16 years and 18 years) and once in early adulthood (25 years), and in their parents once in middle adulthood (50 years). Linear regression models estimated differences in metabolites for males versus females on each occasion (serial cross-sectional associations).

At 8 years, total lipids in very-low-density lipoproteins (VLDL) were lower in males; levels were higher in males at 16 years and higher still by 18 years and

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50 years (among parents) for medium-or-larger subclasses. Larger sex differences at older ages were most pronounced for VLDL triglycerides—males had 0.19 standard deviations (SD) (95% CI = 0.12, 0.26) higher at 18 years, 0.50 SD (95% CI = 0.42, 0.57) higher at 25 years, and 0.62 SD (95% CI = 0.55, 0.68) higher at 50 years. Low-density lipoprotein (LDL) cholesterol, apolipoprotein-B, and glycoprotein acetyls were generally lower in males across ages. The direction and magnitude of effects were largely unchanged when adjusting for body mass index measured at the time of metabolite assessment on each occasion. In conclusion, our results suggest that males begin to have higher VLDL triglyceride levels in adolescence, with larger sex differences at older ages. Sex differences in other CHD-relevant metabolites, including LDL cholesterol, show the opposite pattern with age, with higher levels among females. Such life course trends may inform causal analyses with clinical endpoints in specifying traits which underpin higher age-adjusted CHD rates commonly seen among males.

Selected results

At 8 years, total lipids were lower among males than females in all lipoprotein subclasses, except for HDL subclasses in which total lipids were higher among males. At 16 years, levels of total lipids in (medium and larger) VLDL subclasses were similar between the sexes, but sex differences in these emerged at 18 years and were evident at 50 years for subclasses that were medium or larger—e.g. total lipids in large VLDL were higher among males than females by 0.21 SD (95% CI = 0.14, 0.28), by 0.45 SD (95% CI = 0.37, 0.52), and by 0.72 SD (95% CI = 0.65, 0.79) at 18 years, 25 years, and 50 years, respectively. The higher levels of VLDL lipids seen among males at older ages were most pronounced for triglycerides in VLD. Cholesterol was higher among males in large VLDL particles, but lower among males in other particles including LDL, with inconsistent sex differences at 25 years apart from cholesterol in HDL. Sex differences in lipoprotein particle sizes themselves were larger at older ages—appearing higher among males for VLDL and lower among males for HDL (**Figure 2**). Results were largely unchanged with adjustment for body mass index (BMI). Fatty acid levels tended to be lower among males than females, with some evidence that this difference was larger at older ages (e.g. males had lower polyunsaturated fatty acids by -0.26 SD (95% CI:-0.32, -0.21) at 8 years -1.10 SD (95% CI: -1.16, -1.05) at 50. Glucose was consistently higher among males between childhood and early adulthood, whereas by 50 years it was -0.24 SD (95% CI: -0.30, -0.17) lower among males, with higher levels of lactate and citrate at 50 years consistent with the lower glucose levels. Amino acids were consistently higher among males after 8 years, particularly branched chain amino acids (**Figure 3**).

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2.4 From menarche to menopause: the impact of reproductive factors on the metabolome of 65,699 women (4)

In this project we combined exploring the potential impact of variation in age at menarche with metabolic traits alongside associations of parity and natural age at menarche with the same traits. Here we focus on results for age at menarche (early life stressor).

We explored the relation between age at menarche with 249 metabolic measures in 65,699 UK Biobank women using multivariable regression (MV), Mendelian randomization (MR) and a male negative control (parity only). Older age of menarche was related to a less atherogenic metabolic profile in MV and MR, which was largely attenuated when accounting for adult body mass index. In MV higher parity related to complex changes in lipoprotein-related traits and the lack of associations for the majority of these in the male negative control supports these findings; MR analyses had substantial uncertainty making comparison with MR unreliable. Our findings indicate that older age at menarche is related to a more healthy metabolic profiles and provides insights into the possible mechanisms through which younger age at menarche might increase future adverse cardiometabolic outcomes.

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Figure 2: Sex differences in lipoprotein triglycerides by age

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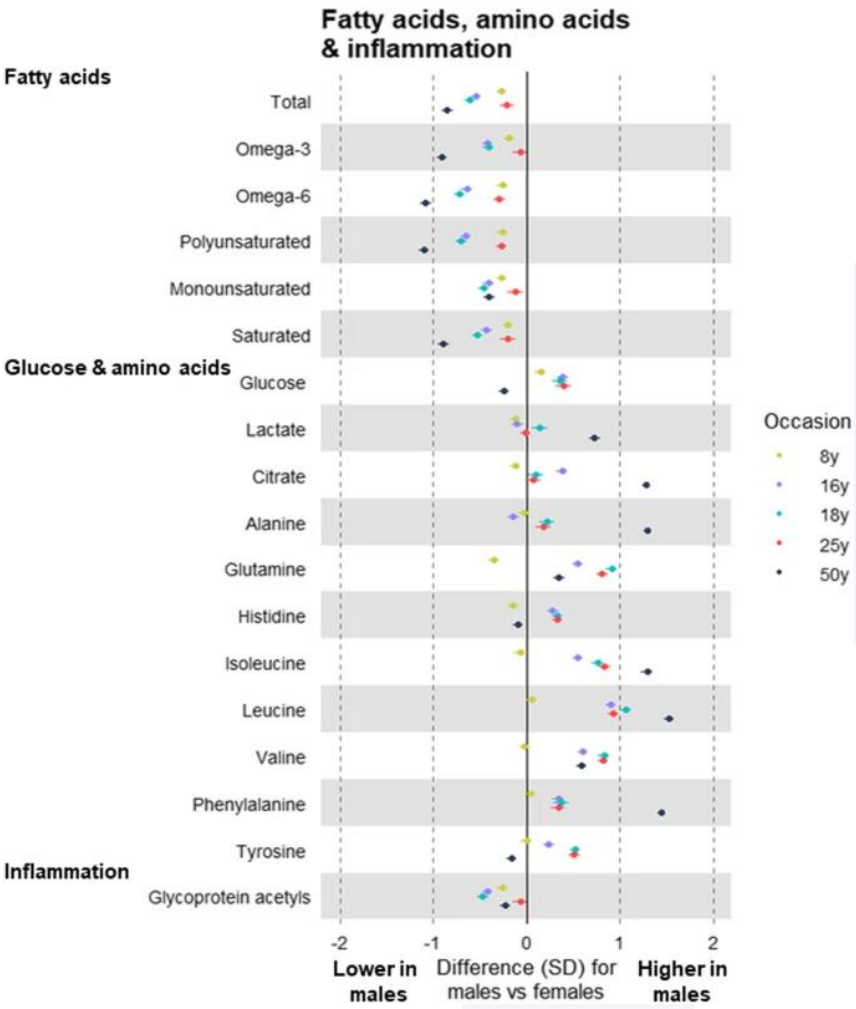

Figure 3: Sex differences in Fatty acids, glucose, amino acids and an inflammatory marker by age

Preliminary results

Our *a priori* hypothesis was that younger age at menarche would related to a more adverse metabolic profiles that might mediate the increased risk of adverse cardiometabolic health in women who have experienced a younger age at menarche. As the genome-wide data that we used for Mendelian randomization (MR) analyses reflected older age at menarche, all results are presented for that but are interpreted in relation to younger age.

Figure 4 provides results for associations of older age at menarche with metabolites from confounder adjusted MV and MR. In the main multivariable regression analyses (adjusting for age at baseline, education and body

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composition at age 10), older age at menarche was related to higher concentration of glutamine, glycine, albumin, apolipoprotein A1, phosphatidylcholines, and sphingomyelins, and lower concentration of alanine, branched-chain amino acids (isoleucine, leucine and valine), aromatic amino acids (phenylalanine and tyrosine), fatty acids (monounsaturated fatty acids (MUFA), omega-3 polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA)), glycolysis-related metabolites (glucose, lactate, pyruvate), acetoacetate, and glycoprotein acetyls (GlycA). Older age at menarche was also associated with numerous lipoprotein-related traits, particularly with higher number of particles, size, and lipid content in high-density lipoprotein (HDL) particles and lower number of particles, size, and lipid content in very low-density lipoprotein (VLDL) particles. The associations of age at menarche with HDL-related traits was mostly due to larger HDL subclasses (i.e. medium, large and very large particles), while associations with VLDL-related traits were observed across VLDL subclasses. In sensitivity analyses with further adjustments for potential mediation by BMI, smoking ad alcohol status in adulthood (mean (SD) age: 56 (8) years) , findings attenuated towards the null for most metabolic measures. with few exceptions, such as glutamine, glycine, omega-3 PUFA, pyruvate, lactate, and acetoacetate.

For the MR analyses, we selected 389 SNPs as instruments for age at menarche, which explained 7.4% of its phenotypic variance with a corresponding mean F statistics of 63. Overall, main MR estimates using inverse probability weighting (IVW) were in agreement with MV estimates in direction and magnitude; however, as expected, there was a higher degree of uncertainty for MR estimates. Given the *a priori* evidence of bidirectional effects between age at menarche and BMI, we also performed multivariable MR accounting for adult BMI to estimate the direct effects of age at menarche on metabolic measures, which resulted in estimates partly or completely attenuating to the null for most metabolic measures with similar exceptions to those seen for MV. MR Sensitivity analyses to explore instrument validity (between SNP heterogeneity, leave-one-out analyses, MR-Egger and weighted median) were all consistent with the main findings.

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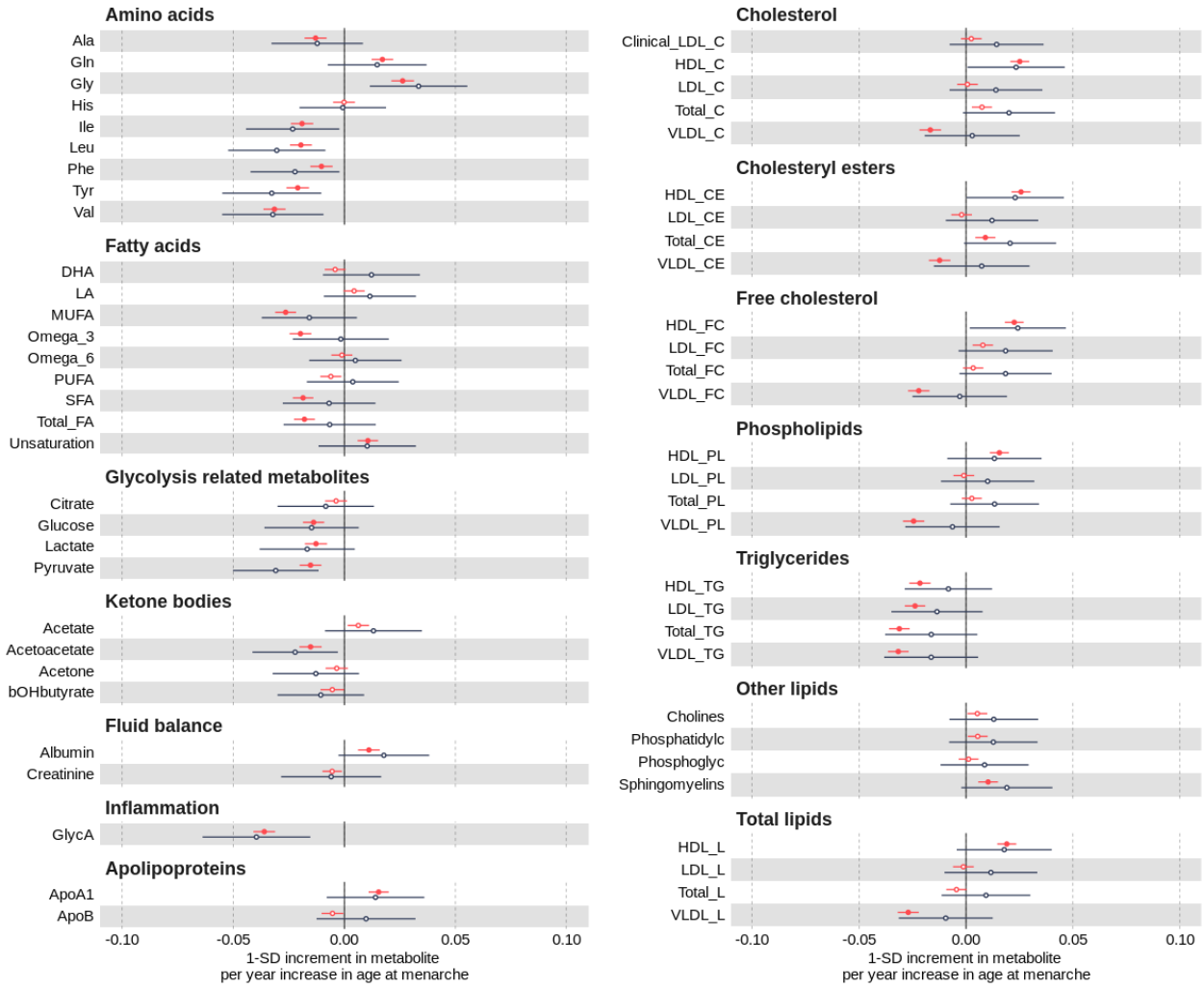

Figure 4: Multivariable regression and mendelian randomisation analyses of older age at menarche with multiple metabolic traits. *Figure shows confounder adjusted multivariable regression (red results) and mendelian randomisation (black results) differences in mean metabolic trait values by 1 year older age at menarche in SD units. Filled dots indicate associations that pass the multiple testing p-value threshold and empty dots indicate those that did not pass this threshold.*

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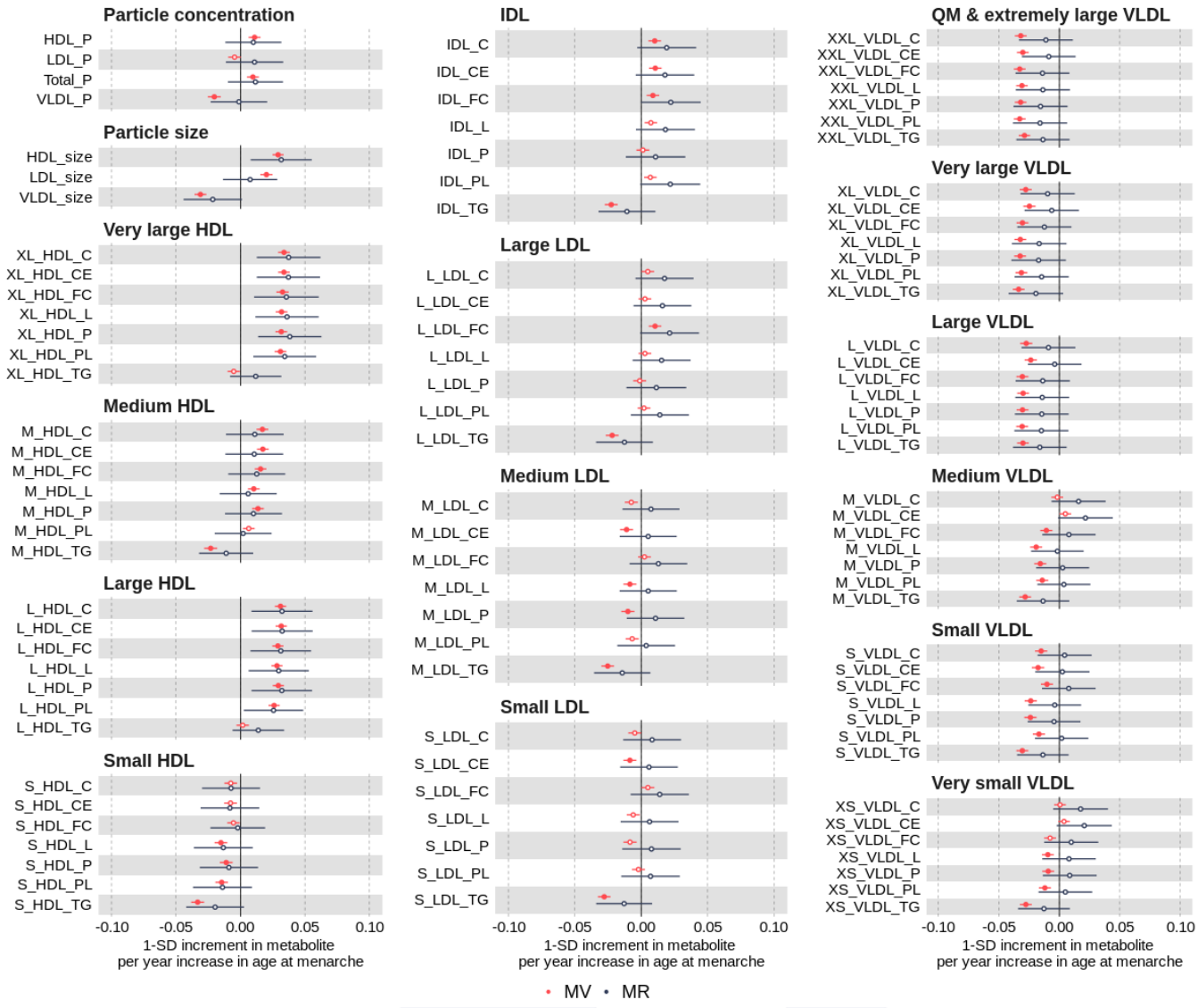

Figure 4: Multivariable regression and mendelian randomisation analyses of older age at menarche with multiple metabolic traits **Continued**

2.5 Effect of common pregnancy and perinatal complications on offspring metabolic traits across the life course: a multi-cohort study (5)

Common pregnancy and perinatal complications are associated with postnatal offspring cardiometabolic risk factors. It is plausible they will influence multiple metabolic traits in offspring and these associations might differ with offspring age. We used data from eight population-based cohort studies to systematically assess and compare associations of pre-eclampsia (PE), gestational hypertension

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(GH), gestational diabetes (GD), preterm birth (PTB), small (SGA) and large (LGA) for gestational age (vs. appropriate size for gestational age (AGA)) with 162 plasma/serum-based nuclear magnetic resonance-derived metabolic traits representing lipids, lipoproteins, fatty acids, amino acids, glycerides/phospholipids, glycolysis, ketones, fluid balance, and inflammation. Confounder-adjusted regression models were used, and results were combined using meta-analysis by age categories for neonates (cord blood), infancy (mean ages: 1.1-1.6y), childhood (4.2-7.5y), adolescence (12.0-16.0y), and adulthood (22.0-67.8y).
Offspring numbers for each age category/analysis varied from 8,925 adults (441 PTB) to 1,181 infants (135 GD); 48.4% to 60.0% were females. Pregnancy complications (PE, GH, GD) were each associated with up to three metabolic traits in neonates (P≤0.001) with some limited evidence of persistence to older ages. PTB and SGA were associated with 32 and 12 predominantly neonates' metabolic traits respectively which included an adjusted mean difference in standard deviation (SD) units of -0.89SD albumin for PTB (95%CI: -1.10 to -0.69, $P=1.3x10^{-17}$) and -0.41SD total lipids in medium HDL for SGA (95%CI: -0.56 to -0.25, $P=2.6x10^{-7}$), with mostly little evidence of persistence to older ages. LGA was inversely associated with 19 wide-ranging metabolic traits, with these emerging in adolescence (e.g., -0.11SD total fatty acids, 95%CI: -0.18 to -0.05, P=0.0009), and with persistence for several traits to younger but not older adulthood. There was some overlap in the metabolic traits that each pregnancy and perinatal complications were associated with, including positive associations with glucose for GD/PTB, and inverse associations with total lipids in small HDL for PTB/LGA. In conclusion, these reassuring findings indicate little evidence of wide-spread and long-term impact of common pregnancy/perinatal complications on offspring metabolism, with most associations observed in new-borns only, and for perinatal but not pregnancy complications. Longer-term effects of LGA on metabolism require replication and assessment of causality e.g., with Mendelian randomization.

Selected results

PE, GH, PTB, and SGA were associated with 1, 1, 32, and 12 metabolic traits, respectively, with all but 3 of these associations observed for neonates only. PE was associated with lower levels of aromatic amino acid phenylalanine in infancy (mean difference: -0.44SD, 95%CI: -0.67 to -0.22, P=0.0002), with differences close to zero in neonates, children, and adults, but there was some evidence in favour of lower levels in adolescents (mean difference: -0.17SD, 95%CI: -0.34 to -0.01, P=0.03). GH was inversely associated with the ketone acetate in infants

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(mean difference: -0.43SD, 95%CI: -0.59 to -0.28, P=3.7x10^{-8}), although equivalent differences for other age groups were either close to zero or were imprecisely estimated (**Figure 5**).

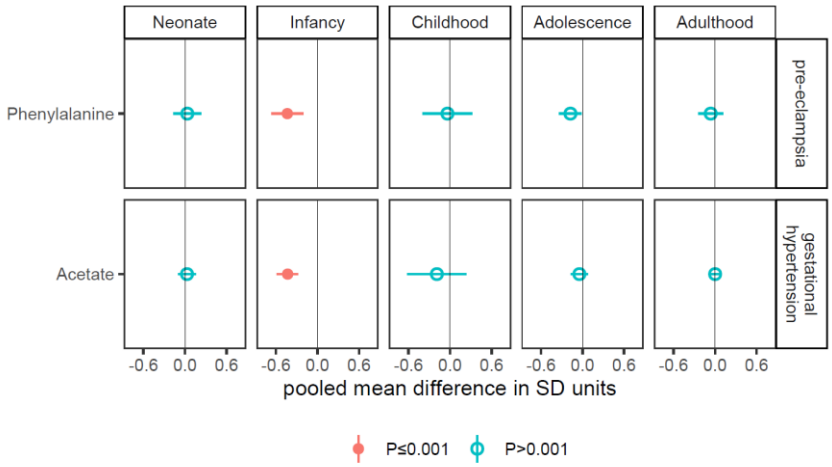

Figure 5: Associations of pre-eclampsia and gestational hypertension with offspring NMR-derived metabolic traits. *Figure shows the pooled adjusted mean differences in standard deviation (SD) units in offspring NMR-derived metabolic traits for pre-eclampsia (minus no pre-eclampsia) and gestational hypertension (minus no gestational hypertension), for associations reaching the P<0.001 threshold in any one of the five age categories, and equivalent associations in all other age categories (to explore differences by age). Results are adjusted for offspring sex age, and confounders. Horizontal bars represent the 95% confidence intervals.*

SGA (vs. AGA) in neonates was inversely associated with total cholesterol in HDL, total cholesterol in HDL2, total cholesterol in HDL3, total lipids in medium HDL, concentration of medium HDL particles, apolipoprotein A-I, and histidine, and positively associated with total cholesterol in VLDL, total lipids in very small VLDL, concentration of very small VLDL particles, and omega-3 fatty acids (Figure 5). Differences ranged in magnitude from -0.21SD (95%CI: -0.33 to -0.08, P=0.001) for histidine to -0.41SD (95%CI: -0.56 to -0.25, P=2.6x10^{-7}) for total lipids in medium HDL. Most were reduced at older ages but there was some evidence for higher levels of total cholesterol in VLDL, total lipids in very small VLDL, concentration of very small VLDL particles, and omega-3 fatty acids during adolescence e.g., mean differences in total lipids in very small VLDL in neonates and adolescence were 0.34SD (95%CI: 0.20 to 0.47, P=1.4x10^{-6}), and 0.17SD (95%CI: 0.06 to 0.29, P=0.003), respectively. SGA was also inversely associated with amino acid alanine in childhood (-0.25SD, 95%CI: -0.38 to -0.11, P=0.0003), with no clear differences in alanine at other age groups (**Figure 6**).

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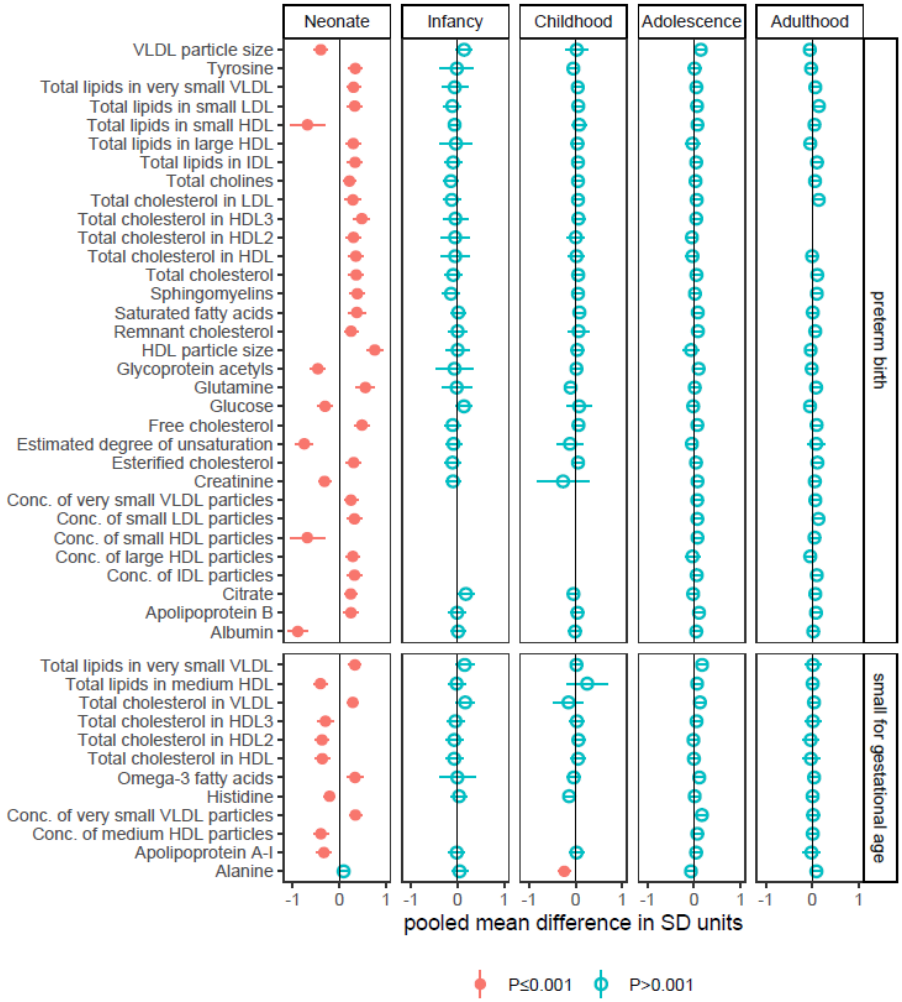

Figure 6: Associations of preterm birth and small for gestational age with offspring NMR-derived metabolic traits *Figure shows the pooled adjusted mean differences in standard deviation (SD) units in offspring NMR-derived metabolic traits for preterm birth (minus not preterm birth), and small for gestational age (minus appropriate size for gestational age), for associations reaching the P<0.001 threshold in any one of the five age categories, and equivalent associations in all other age categories (to explore differences by age). Results are adjusted for offspring sex age, and confounders. Horizontal bars represent the 95% confidence intervals.*

Of the NMR-derived metabolic traits that PE, GH, PTB and SGA were associated with, four were found among the mass spectroscopy measures in the Generation R Study and included for replication (PTB: *tyrosine*, *sphingomyelins, glutamine*; SGA: *histidine*). Consistent with the pooled difference for PTB neonates in NMR-derived *tyrosine* (0.34SD, 95%CI: 0.19 to 0.49, P=7.0x10^{-6}) and *sphingomyelins* (0.39SD, 95%CI: 0.23 to 0.54, P=6.8x10^{-7}), PTB was also associated with higher *tyrosine* (0.82SD, 95%CI: 0.40 to 1.24, p=0.0001, n=725 (29 PTB)) and

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sphingomyelins (0.49SD, 95%CI: 0.07 to 0.90, *P*=0.02) in neonates in Generation R. In contrast, the meta-analysis association of PTB with *glutamine* (0.56SD, 95%CI: 0.36 to 0.76, $P=2.4x10^{-8}$) did not replicate in Generation R (-0.09, 95%CI: -0.51 to 0.34, *P*=0.7). Lastly, mean difference in *histidine* for SGA (vs. AGA) neonates was similar in the meta-analysis (-0.21SD, 95%CI: -0.33 to -0.08, *P*=0.001) and Generation R but this result was imprecisely estimated (-0.25SD, 95%CI: -0.61 to 0.11, *P*=0.2, n=651 (35 SGA)).

GD and LGA were associated with 3 and 19 metabolic traits, respectively. GD was associated with smaller *LDL particle size* (mean difference: -0.25SD, 95%CI: -0.39 to -0.10, *P*=0.0007) and with lower *isoleucine* (mean difference: -0.27SD, 95%CI: -0.41 to -0.14, *P*=0.00008) in neonates, with differences in both metabolic traits close to zero for older ages (**Figure 7**). GD was positively associated with *glucose* in infants (mean difference: 0.35SD, 95%CI: 0.18 to 0.52, *P*=0.00005), with no difference in *glucose* found for other age categories (**Figure 7**).

Of the nineteen associations of LGA with offspring metabolic traits, none were observed in neonates or infants, two were observed in children, sixteen in adolescents, and one in adults (**Figure 7**). All were inverse associations and represented lower levels of cholesterol, fatty acids, lipoprotein subclasses, three branched-chain amino acids, and the inflammatory marker *glycoprotein acetyls* for LGA (vs. AGA). Associations ranged in magnitude from -0.10SD (95%CI: -0.16 to -0.04, *P*=0.001) for *leucine* in adolescents to -0.19SD (95%CI: -0.29 to -0.09, *P*=0.0003) for *valine* in adulthood. For differences observed in adolescence, equivalent differences in adults were slightly attenuated and had wider confidence intervals, and for some traits there also evidence for lower levels in childhood, e.g., difference in *total lipids in small HDL* in childhood, adolescence and adulthood was -0.14SD (95%CI: -0.24 to -0.04, *P*=0.004), -0.13SD (95%CI: -0.19 to -0.07, *P*=.00006), and -0.08SD (95%CI: -0.16 to 0.01, *P*=0.1), respectively.

Of the 22 associations identified for GD and LGA, only one metabolic trait overlapped with mass spectroscopy measures in the Generation R Study (LGA and child *total triglycerides*). The inverse association in our meta-analysis (difference in child *total triglycerides* for LGA vs. AGA (-0.15SD, 95%CI: -0.24 to -0.06, *P*=0.001) was weaker and imprecisely estimated in Generation R (-0.04SD, 95%CI: -0.40 to 0.32, *P*=0.8, n=339 total with n=75 LGA),

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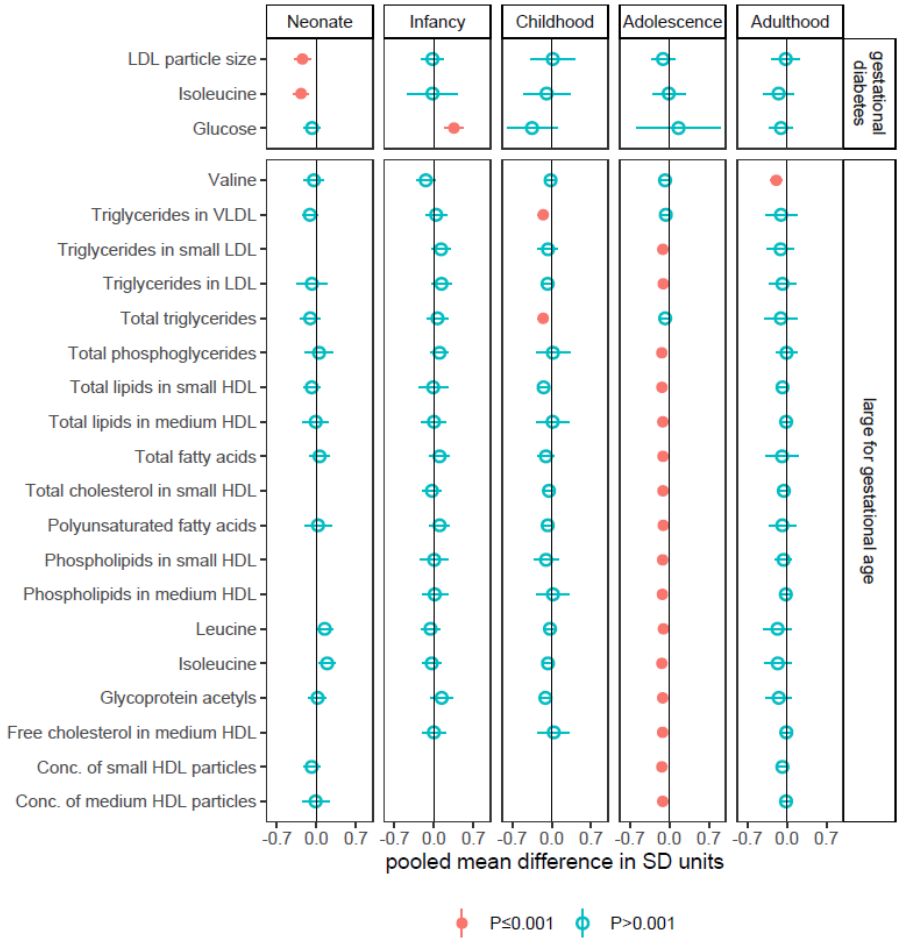

Figure 7: Association of gestational diabetes and large for gestational age with offspring NMR-derived metabolic traits. *Figure shows the pooled adjusted mean differences in standard deviation (SD) units in offspring NMR-derived metabolic traits for gestational diabetes (minus no gestational diabetes), and for large gestational age (minus appropriate size for gestational age), for associations reaching the P<0.001 threshold in any one of the five age categories, and equivalent associations in all other age categories (to explore differences by age). Results are adjusted for offspring sex age, and confounders. Horizontal bars represent the 95% confidence intervals.*

Comparing identified pregnancy/perinatal complication–metabolic trait associations showed that PE/GH were each associated with a different metabolic trait, PTB/SGA were associated with *total HDL cholesterol* in neonates (higher with PTB and lower with SGA), and both GD (neonate) and LGA (adolescent) were inversely associated with *isoleucine*. Additionally, SGA (neonates) and LGA (adolescents) were both inversely associated with *concentration of medium HDL particles*, PTB (neonate) and LGA (adolescence) were both inversely associated

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with *GlycA* and *total lipids in small HDL*, and both PTB (inverse association: neonates) and GD (positive association: infants) were associated with *glucose*

For most (72%) of the total 3,787 results there was little to no between-cohort heterogeneity ($I^2 \leq 25\%$, with $I^2 = 0\%$ for 86% of these), and 8% of the results showed evidence of substantial or high heterogeneity between cohorts ($I^2 \geq 75\%$). Four of these were results that met the $P \leq 0.001$ threshold and all were for PTB neonates (total lipids in small and very large HDL, and concentrations of small and very large HDL particles). Inspecting results from the two cohorts contributing to this age (BiB and BIS) uncovered a consistent direction of association in both but mean differences in BiB double those in BIS (e.g., mean difference in *total lipids in small HDL* was -0.86SD in BiB and -0.48SD in BIS. Among other results with substantial or high heterogeneity, 97 were for LGA–adult metabolic traits. Further investigation revealed that this was because LGA was inversely associated with adult biomarkers in ALSPAC (age 24.5 years), with smaller positive associations seen in NFBC1966 (age 46.6 years). Notably, results for NFBC1966 at age 31.2 years were between the ALSPAC age 24.5 years results and the NFBC1966 age 46.6 years results, suggesting a possible age effect (**Figure 8**).

Of the twenty associations identified with metabolic traits beyond infancy, one was for SGA, and nineteen were for LGA, and all except one for LGA were included in trajectory analysis from childhood to adulthood in the ALSPAC cohort. Consistent with the meta-analysis, SGA (vs. AGA) was associated with lower alanine at baseline though this difference was reduced with increasing child age, with alanine slightly higher in SGA from mid adolescence. Similarly, most associations between LGA and metabolic traits appeared to change with age from childhood to adulthood e.g., the inverse association observed in our meta-analysis between LGA (vs. AGA) and triglycerides in VLDL was attenuated with older age (**Figure 9**).

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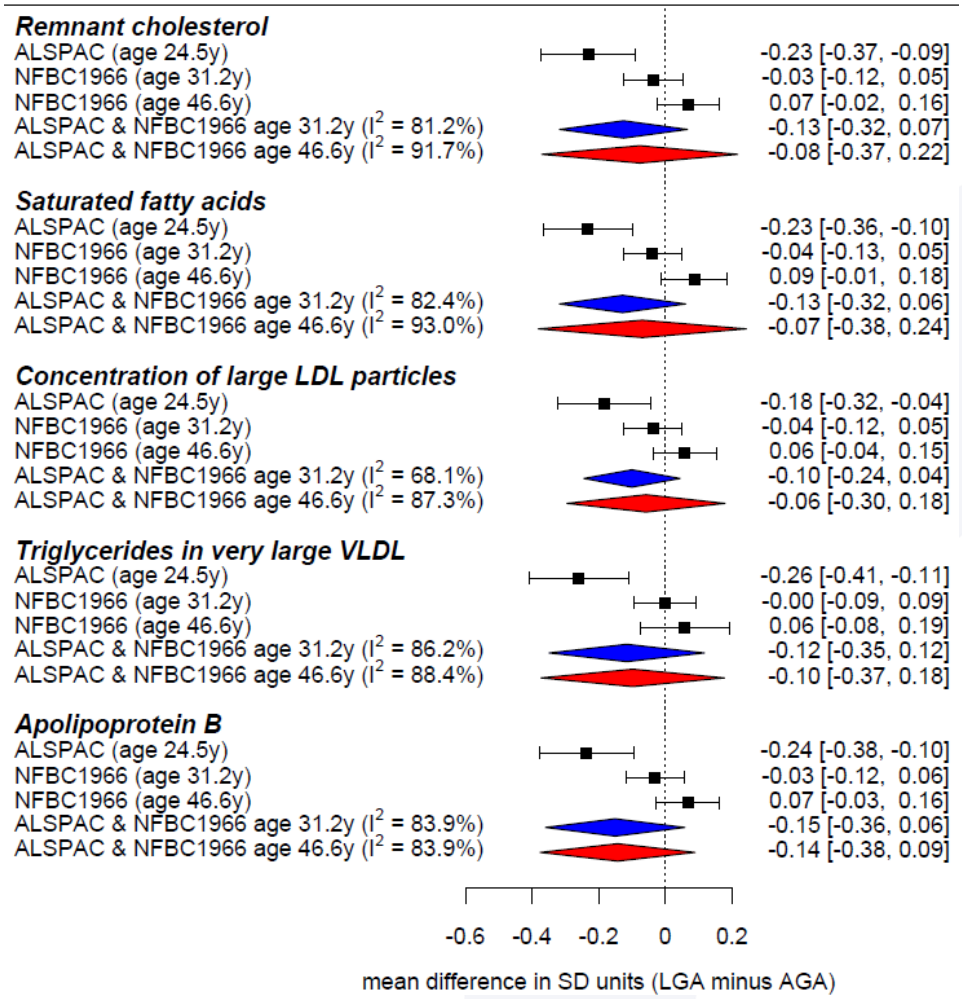

Figure 8: Between-cohort differences in associations of large for gestational age with selected NMR-derived metabolic traits in adults. *Figure shows the cohort-specific and pooled adjusted mean differences in standard deviation (SD) units in five NMR-derived metabolic traits between adults born large gestational age (LGA) and appropriate size for gestational age (AGA). The pooled results from ALSPAC and NFBC19666 (age 46.6y) from the meta-analysis are presented, with the pooled result for ALSPAC and age 31.2y NFBC19666 also presented to highlight differences with age.*

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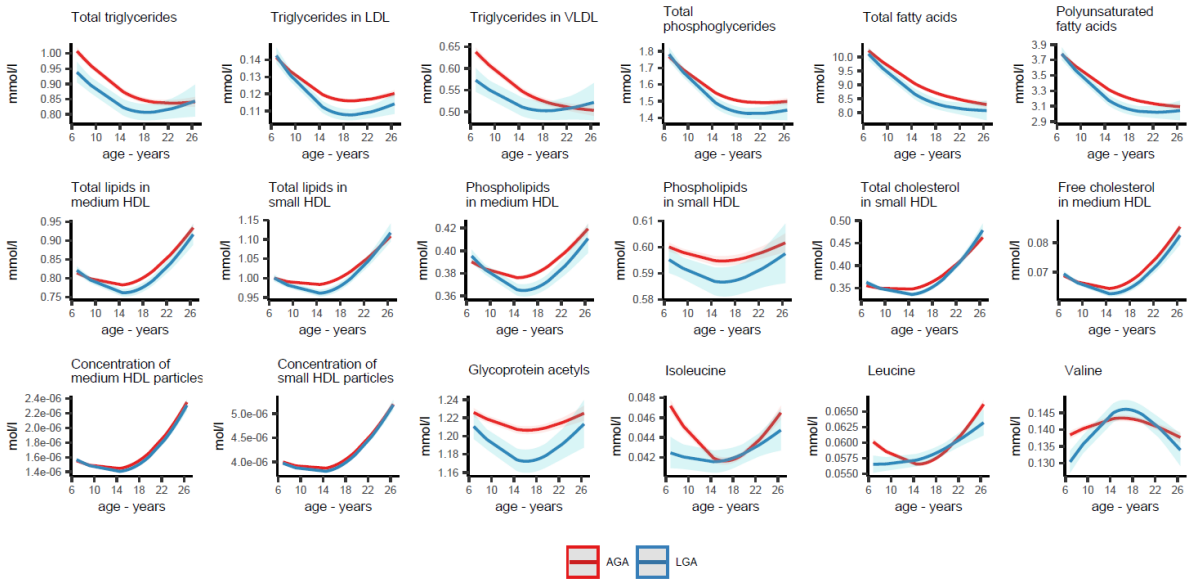

Figure 9: Predicted mean trajectories of MR-derived metabolic traits from age 7-26 years for offspring born large for gestational age and appropriate size for gestational age from the ALSPAC cohort. *Figure shows the predicted mean NMR-derived metabolic trait trajectories from age 7-26 years for ALSPAC offspring born large for gestational age (LGA, N=500) and appropriate size for gestational age (AGA, N=4,480), for 18 metabolic traits that were identified in the meta-analysis. Predicted values were obtained from adjusted (for sex and confounders) natural cubic spline mixed effects models that included an interaction term with age to allow both LGA/AGA to have different metabolic trait trajectories.*

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3. Conclusion

We found evidence that that maternal obesity was related to more marked changes in multiple metabolites during pregnancy, and that a lifestyle intervention could improve these obesity related changes. We also found that being born large for gestational age is associated with lower levels of cholesterol, fatty acids, several lipoprotein subclasses, three branched-chain amino acids, and an inflammatory marker in adolescence and early adulthood. These differences would be expected to related to lower risk of adverse cardiometabolic outcomes in later life. By contrast we did not find robust evidence that exposure to hypertensive disorders of pregnancy, gestational diabetes, preterm birth or small for gestational age influenced offspring metabolism beyond early infancy, nor did we find younger age at menarche influenced subsequent variation in multiple metabolites, once possible confounding by body mass index was adjusted for. Importantly, we did not find robust evidence that variation in multiple metabolites influenced sub-clinical atherosclerosis in children or adults. Taken together these findings suggest that multiple metabolites are unlikely to be key mediators of potential effects of the early-life stressors we have explored on subsequent cardiometabolic health. The lack of association of multiple metabolites with sub-clinical atherosclerosis would argue against early life stressors affecting atherosclerosis via mechanisms involving multiple metabolites.

Based on the above, **future plans** beyond LifeCycle include attempting to replicate results for the association of LGA with multiple metabolites arising in adolescence through observational analyses using the subset of UK Biobank with gestational age and from maternity record linkage, and the large Norwegian cohort HUNT, which will have NMR metabolite data in the next 12 months. We further intend to undertaken exploratory two-sample MR analyses using genetic instruments derived in the MR-PREG consortia and for LGA and outcome data from UK Biobank NMR GWAS (that we have contributed to) and the original Kettunen NMR GWAS. Much of this will continue with PhD students from University of Bristol, British Heart Foundation and Wellcome Trust doctoral training programmes that we have access to. With a recently awarded ERC – Advanced grant and separately awarded UK Medical Research Council programme grant, we will further explore the interplay between age at menarche, body mass index and multiple metabolites in their relation to future cardiometabolic outcomes, as well as exploring methods for undertaking MR with high dimension and highly correlated multiple metabolites and extending

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the early age at menarche associations to a wider range of measures of age at puberty in females and males.

4. Contribution of partners

Researchers from all partners have indirectly contributed to this deliverable through discussions of project and analysis plans and interpretation of results at LifeCycle General Assembly and WP meetings.

- **UNIVBRIS**: has led this deliverable and has had a leading role in all reported projects.
- **BTHFT**: contributed to published paper 4 and the main multicohort analysis for the on-going project on pregnancy/perinatal complications and offspring metabolites. For both of these their contribution was via BiB cohort data and commenting on the analysis plan and drafts of papers/conference abstracts.
- **ERASMUS**: contributed replication analysis with mass spectroscopy metabolites in the Generation R Study for the project on pregnancy/perinatal complications and offspring metabolites, and commenting on drafts of the paper.
- **UOULU**: contributed to the main multicohort analysis with the NFBC1966 and NFBC1986 cohorts for the project on pregnancy/perinatal complications and offspring metabolites, as well providing comments on current drafts of the paper.

5. Deviations from original plan

This deliverable has been fulfilled fully in line with the original plan as state in the Grant Agreement.

6. Dissemination activities

All of the studies described in this deliverable have been presented at the LifeCycle General Assembly with extended partners, they have been placed on preprint servers at the same time as submission to peer reviewed journals.

7. References

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