Report on the relationships of early-life exposures with trajectories of cardiovascular and metabolic risk factors from birth to adulthood

Work package 4 - Task 4.2 - Deliverable 4.2

Authors:

Deborah A Lawlor, Ahmed Elhakeem, Tim Cadman, Johan Lerbech Vinther, Anne-Marie Nybo Anderson.

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1. Introduction

The aim of WP4 is to examine the associations of early-life stressors during preconception, pregnancy, infancy and early childhood with cardiovascular and metabolic outcomes during fetal life, childhood, adolescence and adulthood. The objective of task 4.2 is to identify early-life stressors related to cardio-vascular and cardio-metabolic outcomes across the life-course, including whether associations vary with age. This related deliverable (4.2) is to report on the relationships of early-life stressors with trajectories of cardio-vascular and -metabolic risk factors from birth to adulthood. We have done this through assessment of associations at different ages (using repeat cross sectional analyses) and with trajectories of change over age. We have further explored the extent to which these associations might be causal by using genetic instrumental variables. A considerable amount of work has been undertaken for this deliverable. Further work, delayed due to effects of the COVID pandemic on access to servers and data continues.

2. Work performed

2.1.1 Summary of selected published papers

Section 2.1.3 provides a list of publications relevant to this deliverable. Below we describe some of these published projects, chosen to illustrate the range of exposures, methods of analyses and studies with relevant data that have contributed to research for this task that has been already published.

Intrauterine exposures and fetal growth

Two published studies have identified factors related to fetal growth (using unique repeat ultrasound scan data on four parameters – head circumference, abdominal circumference, femur length, and estimated fetal weight).[References 1 and 2 in Section 2.1.3] These provide key understanding of impacts on fetal growth that provide a reference for better understanding the relationship of intrauterine exposures that influence fetal growth (and hence birth weight) and potentially also future offspring cardio-vascular/ metabolic health. One of these two papers explored the relationship of maternal smoking and quitting smoking on fetal growth and used the two LifeCycle cohorts, the only ones with detailed repeat fetal ultrasound scan data:

Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Ellis Voerman E, Felix JF, Tilling K, Lawlor DA. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. *PLoS Medicine* **2019, 16(11): e1002972. doi: 10.1371/journal.pmwd.1002972.**

That paper was done in collaboration with WP7 and was reported in detail in deliverable 7.2 that has been submitted. We have not further described it hear.

The second published paper explored fetal growth trajectories in relation to ethnicity, gestational diabetes, circulating fasting and postload glucose. This included one LifeCycle cohort, the only one which has both detailed fetal growth oral glucose tolerance test data on all participants.

Brand JS, West J, Tuffnell S, Bird PK, Wright J, Tilling K, Lawlor DA. Gestational diabetes an ultrasound assessed fetal growth in South Asian and White European women: findings from a prospective cohort. *BMC Medicine* **2018; 16:203.**

Abstract
Background: Maternal gestational diabetes (GDM) is an established risk factor for large size at birth, but its influence on intrauterine fetal growth in different ethnic populations is less well understood. Here, we examine the joint associations of GDM and ethnicity with longitudinal fetal growth in South Asian and White European origin women.

Methods: This study included 10,705 singletons (4,747 White European and 5,958 South Asian) from a prospective cohort of women attending an antenatal clinic in Bradford, in the North of England. All women completed a 75-g oral glucose tolerance test at 26-28 weeks' gestation. Ultrasound measurements of fetal head circumference (HC), femur length (FL) abdominal circumference (AC) and estimated fetal weight (EFW), and corresponding anthropometric measurements at birth were used to derive fetal growth trajectories. Associations of GDM and ethnicity with these trajectories were assessed using multilevel fractional polynomial models.

Results: 832 pregnancies (7.8%) were affected by GDM; 10.4% of South Asians and 4.4% of White Europeans. GDM was associated with a smaller fetal size in early pregnancy [differences (95% CI) in mean HC at 12 weeks and mean AC and EFW at 16 weeks comparing fetuses exposed to GDM to fetuses unexposed (reference) = -1.8 mm (-2.6; -1.0), -1.7 mm (-2.5; -0.9) and -6 gram (-10; -2)] and a greater fetal size from 24 weeks' gestation [differences (95% CI) in mean HC, AC and EFW comparing fetuses exposed to GDM to those unexposed = 0.9 mm (0.3; 1.4), 0.9 mm (0.2; 1.7) and 7 g (0; 13) at 24 weeks]. Associations of GDM with fetal growth were of similar magnitude in both ethnic groups. Growth trajectories, however, differed by ethnicity with South Asians being smaller than White Europeans irrespective of GDM status. Consequently, South Asian fetuses exposed to GDM were smaller across gestation than fetuses of White Europeans without GDM.

Conclusions: In both ethnic groups, GDM is associated with early fetal size deviations prior to GDM diagnosis, highlighting the need for novel strategies to diagnose pregnancy hyperglycemia earlier than current methods. Our findings also suggest that ethnic specific fetal growth criteria are important in identifying hyperglycemia-associated pathological effects.

Selected results

Ethnicity and fetal growth
Except for femur length (FL), South Asian fetuses were smaller than White European fetuses from 20 weeks gestation through to birth, with the largest difference observed for abdominal circumference (AC) and estimated fetal weight (EFW). Absolute and proportional differences in fetal size increased with increasing gestational age, and the difference in means (95% CI) comparing South Asians to White Europeans for AC and EFW were -3.3 mm (-4.0; -2.5) and -17 g (- 24; -11) at 24 weeks, and -12.4 mm (-13.9; -10.9) and -206 g (-232; -180) at 40 weeks, respectively. Mean differences in head circumference (HC) by ethnicity were smaller [-1.0 mm (-1.5; -0.5) at 24 weeks and –5.4 mm (-6.1; -4.6) at 40 weeks comparing South Asians to White Europeans]. There was also some evidence of South Asian fetuses having a greater FL between 20 and 28 weeks' gestation but not towards the end of the third trimester.

Gestational diabetes, fasting and postload glucose and fetal growth
Fetuses of women who were later diagnosed with GDM were smaller in early pregnancy [difference in mean HC at 12 weeks and mean AC and EFW at 16 weeks comparing fetuses exposed to GDM to

fetuses not exposed to GDM (reference) = -1.8 mm (-2.6; 1.0), -1.7 mm (-2.5; -0.9) and -6 gram (-10; -2) respectively]. This pattern of early growth restriction was followed by enhanced growth with fetuses of GDM complicated pregnancies being larger at 24 weeks' gestation [difference in means comparing fetuses exposed to GDM vs. those unexposed were 0.9 mm (0.3; 1.4) for HC, 0.9 mm (0.2; 1.7) for AC and 7 g (0; 13) for EFW respectively]. From 24 weeks to delivery, absolute and proportional differences in AC and EFW increased with increasing gestational age [mean differences associated with GDM at 40 weeks gestation were 5.1 mm (3.5-6.8) and 171 g (140; 202) respectively], while the larger HC observed at 24 weeks attenuated towards the end of pregnancy and was no longer detectable at term (mean difference = 0.1 mm (-0.8; 1.1) at 40 weeks). The FL growth trajectory did not notably differ by GDM status across pregnancy.

To evaluate whether associations of GDM with fetal growth are continuous across the glucose distribution, we also assessed associations with gestational fasting and 2-hour postload glucose levels in women who were not diagnosed with GDM. Gestational glucose levels were positively and linearly associated with fetal AC and EFW starting from 20-24 weeks' gestation and also with fetal HC from 28 weeks' gestation. Overall, associations with HC, AC and EFW were somewhat weaker for 2-hour postload glucose than fasting glucose levels. While the mean difference in fetal HC observed with GDM did not persist to term, gestational glucose levels below the diagnostic threshold were positively associated with fetal HC until birth. As with GDM, there was no clear evidence of gestational glucose being associated with FL.

Joint ethnicity and gestational diabetes associations with fetal growth
Figure 1 shows the observed mean differences in fetal size across gestation by GDM in each ethnic group. There was no evidence of effect modification by ethnicity; the direction and magnitude of associations of GDM with fetal growth were similar in South Asians and White Europeans.
As the association of GDM with fetal growth did not differ by ethnicity, and South Asians were consistently smaller in size than White Europeans across pregnancy for most growth measures (independently of GDM), South Asian fetuses exposed to GDM were smaller across gestation than White European fetuses not exposed to GDM **(Figure 2).** This difference was largest for AC and EFW and detectable from early pregnancy [difference in mean AC and EFW at 24 weeks comparing South Asians exposed to GDM to White Europeans **not** exposed to GDM = -2.3 mm (-3.4; -1.2) and -10 g (-10; -1) respectively] and increased as pregnancy progressed, such that by term (40 weeks) it was -7.1 mm (-9.4; -4.8) for AC and -45 g (-86; -4) for EFW.

Conclusions and future research building on these studies of fetal growth
In both South Asian and White European populations, GDM is associated with early fetal size deviations prior to GDM diagnosis. GDM is diagnosed at 26-28 week to coincide with pregnancy related increases in insulin resistance. Our findings highlight the need for novel strategies to diagnose pregnancy hyperglycemia earlier than current methods. Our findings also suggest that ethnic specific fetal growth criteria are important in identifying hyperglycemia-associated pathological effects.

On the back of the two LifeCycle studies that have explore maternal exposures with fetal growth we have established a wider collaboration of cohorts with repeat ultrasound fetal growth measurements. In that collaboration, which also includes the two LifeCycle cohorts, we are (a) extending the growth trajectories to include infant and childhood weight and height (proxied by femur length lin fetuses) so that we can then explore intrauterine exposures with growth throughout pregnancy, infancy and early childhood and (b) undertaking a genome-wide association analysis of fetal growth trajectories to

explore the possibility of undertaking two-sample mendelian randomization to determine whether intrauterine exposures (including maternal smoking and glucose, as well as others) causally impact fetal growth, and whether those that do are also causally influencing subsequent offspring cardiometabolic health.

Figure 1: Associations of gestational diabetes with fetal growth across gestation in White Europeans and South Asians.
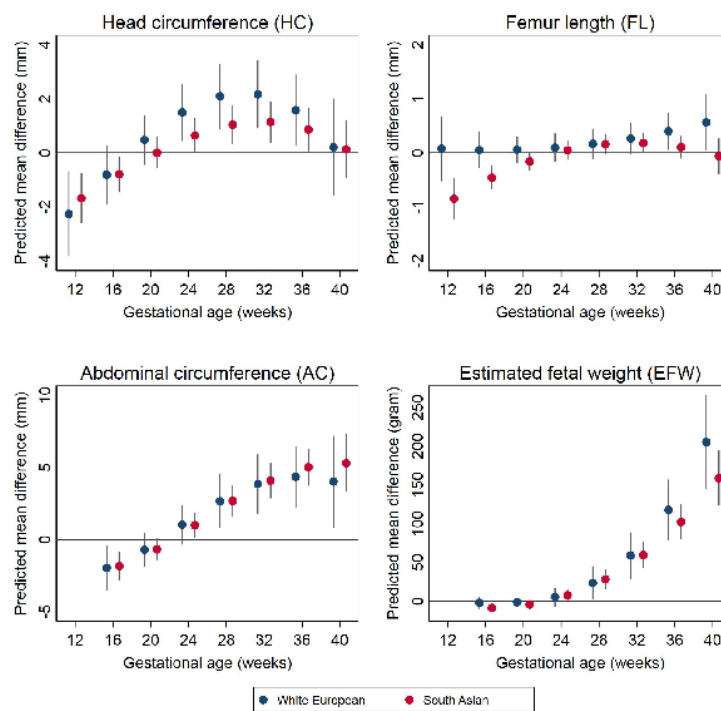
Figure 2: Joint associations of ethnicity and gestational diabetes with fetal growth.
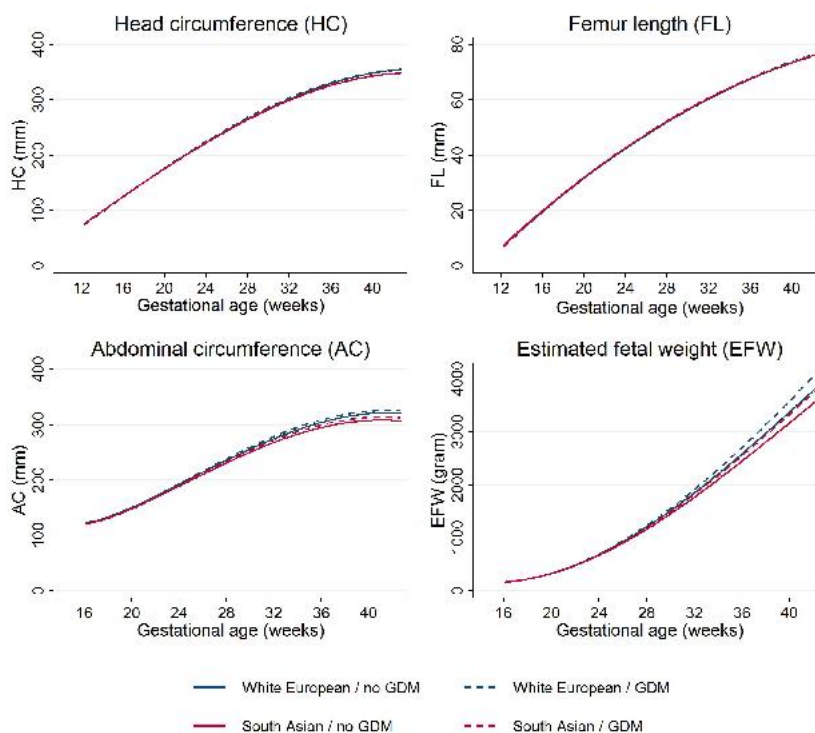
In utero exposure to higher maternal BMI and gestational weight gain, and subsequent offspring cardio-vascular/-metabolic health.

In a series of papers including LifeCycle and the ERC funded MOCO collaboration we have examined associations of exposure to maternal higher early/pre-pregnancy BMI and higher gestational weight gain on pregnancy complications and subsequent offspring health. [References 3-4 in section 2.1.3] Two of these are summarised below.

Voerman E. and the LifeCycle Project-Maternal Obesity and Childhood Outcomes Study Group. Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *JAMA* 2019; 321: 1702-1715.

Abstract

Importance Both low and high gestational weight gain have been associated with adverse maternal and infant outcomes, but optimal gestational weight gain remains uncertain and not well defined for all pre-pregnancy weight ranges.

Objectives To examine the association of ranges of gestational weight gain with risk of adverse maternal and infant outcomes and estimate optimal gestational weight gain ranges across pre-pregnancy body mass index categories.

Design, Setting, and Participants Individual participant-level meta-analysis using data from 196 670 participants within 25 cohort studies from Europe and North America (main study sample). Optimal gestational weight gain ranges were estimated for each pre-pregnancy body mass index (BMI) category by selecting the range of gestational weight gain that was associated with lower risk for any adverse outcome. Individual participant-level data from 3505 participants within 4 separate hospital-based cohorts were used as a validation sample. Data were collected between 1989 and 2015. The final date of follow-up was December 2015.

Exposures Gestational weight gain and early pregnancy BMI.

Main Outcomes and Measures The main outcome termed *any adverse outcome* was defined as the presence of 1 or more of the following outcomes: preeclampsia, gestational hypertension, gestational diabetes, cesarean delivery, preterm birth, and small or large size for gestational age at birth.

Results Of the 196,670 women (median age, 30.0 years [quartile 1 and 3, 27.0 and 33.0 years] and 40,937 were white) included in the main sample, 7809 (4.0%) were categorized at baseline as underweight (BMI <18.5); 133 788 (68.0%), normal weight (BMI, 18.5-24.9); 38 828 (19.7%), overweight (BMI, 25.0-29.9); 11 992 (6.1%), obesity grade 1 (BMI, 30.0-34.9); 3284 (1.7%), obesity grade 2 (BMI, 35.0-39.9); and 969 (0.5%), obesity grade 3 (BMI, ≥40.0). Overall, any adverse outcome occurred in 37.2% (n = 73 161) of women, ranging from 34.7% (2706 of 7809) among women categorized as underweight to 61.1% (592 of 969) among women categorized as obesity grade 3. Optimal gestational weight gain ranges were 14.0 kg to less than 16.0 kg for women categorized as underweight; 10.0 kg to less than 18.0 kg for normal weight; 2.0 kg to less than 16.0 kg for overweight; 2.0 kg to less than 6.0 kg for obesity grade 1; weight loss or gain of 0 kg to less than 4.0 kg for obesity grade 2; and weight gain of 0 kg to less than 6.0 kg for obesity grade 3. These gestational weight gain ranges were associated with low to moderate discrimination between those with and those without adverse outcomes (range for area under the receiver operating characteristic curve, 0.55-0.76). Results for discriminative

performance in the validation sample were similar to the corresponding results in the main study sample (range for area under the receiver operating characteristic curve, 0.51-0.79).

Conclusions and Relevance In this meta-analysis of pooled individual participant data from 25 cohort studies, the risk for adverse maternal and infant outcomes varied by gestational weight gain and across the range of pre-pregnancy BMI. Optimal gestational weight gain ranges had limited predictive value for the outcomes assessed, and results suggest that pre-pregnancy BMI might be a more important target for interventions to reduce adverse pregnancy and infant cardiovascular/ cardiometabolic related outcomes than gestational weight gain.

Selected results

The absolute risk for any adverse pregnancy or perinatal outcome increased across the full range of maternal pre-pregnancy BMI and was largely independent of gestational weight gain (**Figure 3**). The lowest absolute risks were observed among women with low to normal BMI and a moderate to high total gestational weight gain. The lowest risk was 26.7% (16 of 60) for women with a BMI of less than 18.0 and gestational weight gain of 26.0 kg to 27.9 kg. The highest absolute risks were observed among women with a high BMI and a high gestational weight gain. The highest risk was 94.4% (17 of 18) for women with a BMI of 40.0 or greater and gestational weight gain of 20.0 kg to 21.9 kg.

Figure 4 shows unadjusted risk for any outcome and for each outcomes separately by categories of maternal BMI and gestational weight gain.

Among women categorized as underweight, the absolute risk for any adverse outcome ranged from 29.2% (387 of 1326) for gestational weight gain of 14.0 kg to 15.9 kg to 50.2% (203 of 404) for gestational weight gain of less than 8.0 kg. Of all outcomes separately, the absolute risk in women categorised as underweight, was highest for small size for gestational age (highest risk: 32.1% [125 of 390] for gestational weight gain <8 kg). Among women categorized as normal weight, the absolute risk for any adverse outcome ranged from 31.7% (7314 of 23 073) for gestational weight gain of 14.0 kg to 15.9 kg to 46.9% (1256 of 2679) for gestational weight gain of 28.0 kg or greater and was highest at both extremes of gestational weight gain.

 Among women categorized as overweight, the absolute risk for any adverse outcome increased from 37.3% (249 of 667) for gestational weight gain of 2.0 kg to 3.9 kg to 56.4% (624 of 1107) for gestational weight gain of 28.0 kg or greater. Of all outcomes separately the absolute risk was highest, in those categorised as overweight, for caesarean delivery (highest risk: 25.1% [272 of 1084] for gestational weight gain of ≥28.0 kg).

Among women categorized as obese at grades 1, 2, or 3, the absolute risk for any adverse outcome increased across the range of gestational weight gain. The highest absolute risks were 63.7% (160 of 251) for gestational weight gain of 28.0 kg or greater in women categorized as obesity grade 1, 67.7% (384 of 567) for gestational weight gain of 16.0 kg or greater in women categorized as obesity grade 2, and 78.8% (93 of 118) for gestational weight gain of 16.0 kg or greater in women categorized as obesity grade 3.

The association of maternal pre-pregnancy BMI with the risk for any adverse outcomes was stronger than the association of gestational weight gain. The ORs for the risk of any adverse outcome were 1.28 (95% CI, 1.27-1.29) and 1.04 (95% CI, 1.03-1.05) per 1-SD increase in maternal pre-pregnancy BMI and gestational weight gain, respectively (*P*<.001 for difference between the two exposures).

The optimal gestational weight gain ranges associated with the lowest risks for any adverse outcome appear in **Figure 5**.

Figure 3: Heatmaps showing associations of joint maternal BMI and gestational weight gain with a composite of any maternal or infant adverse outcome.
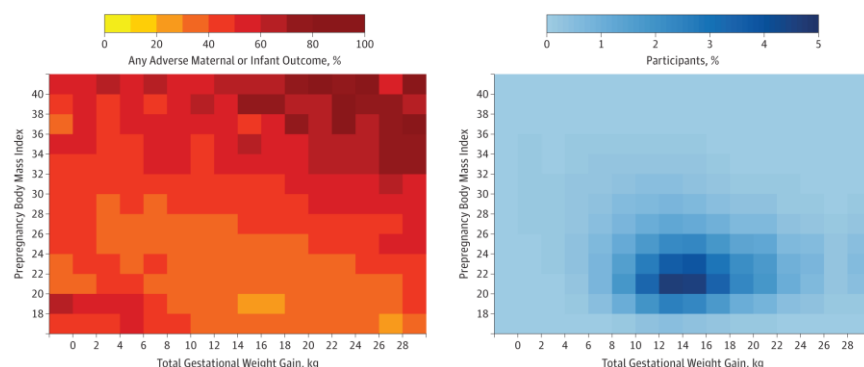
Left panel heatmap shows absolute risks of any adverse maternal, and infant outcome and right panel heat map shows percentages of participants for each combination of BMI and gestational weight gain. Absolute risk was calculated as number of participants (any adverse outcome)/No. of participants BMI & gestational weight gain category) × 100. The percentages of participants were calculated as the number of participants with each combination of body mass index and gestational weight gain as a percentage of the total study sample. Any adverse outcome includes preeclampsia, gestational hypertension, gestational diabetes, cesarean delivery, preterm birth and small or large size for gestational.

Figure 4: Association of gestational weight gain in BMI categories with each outcome.
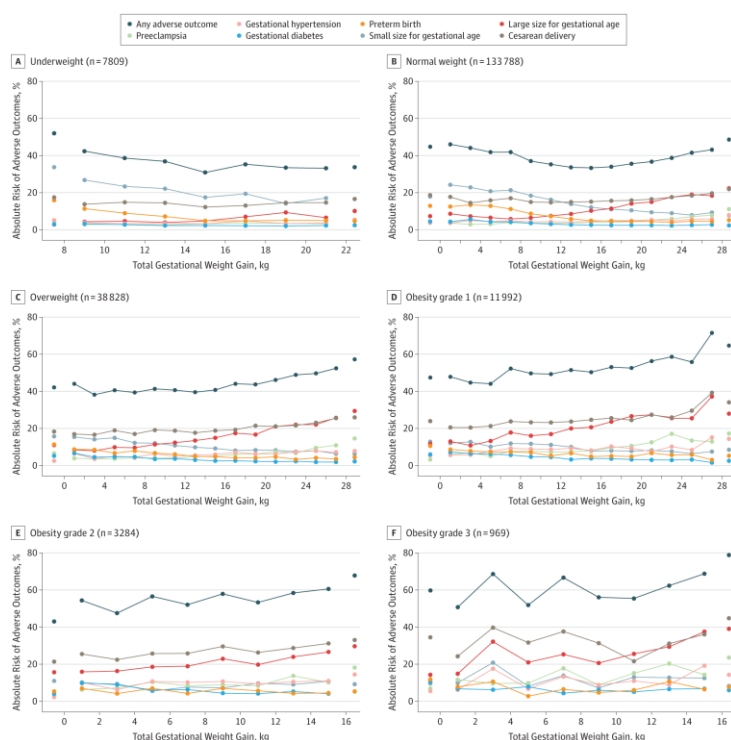
Figure 5: Optimal gestational weight gain ranges within BMI categories for minimsing any adverse maternal or infant outcome.
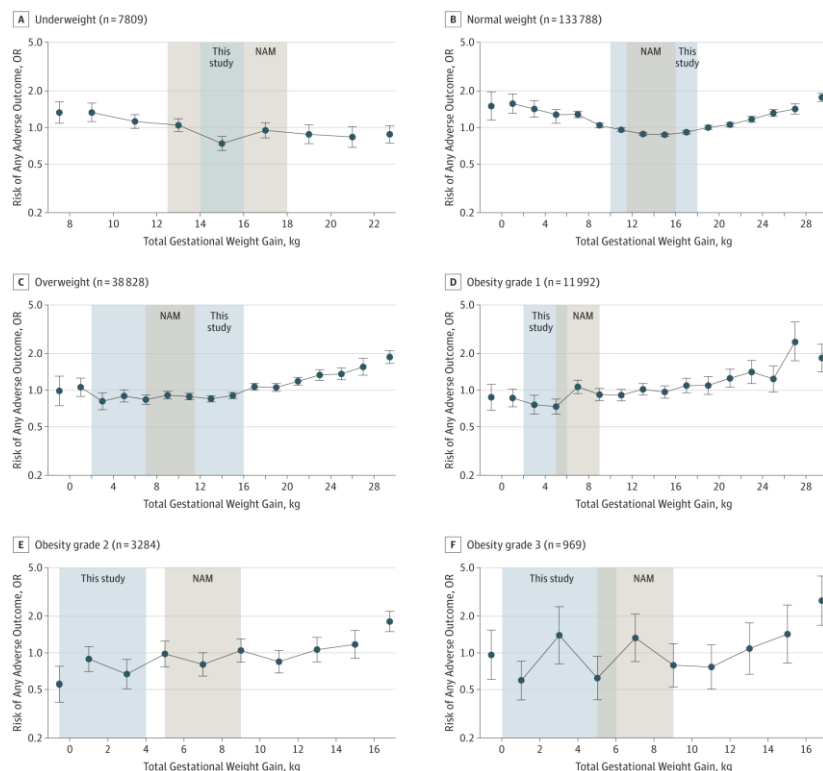
Conclusion

The findings from this study suggest that pre-pregnancy weight might be a more important target for interventions to reduce adverse pregnancy and infant cardiovascular/ cardiometabolic related outcomes than gestational weight gain. This is further reenforced by randomized controlled trials of lifestyle (diet, physical activity or both) in overweight and obese women that result in lower gestational weight gain but do reduce risk of adverse maternal pregnancy or infant perinatal outcomes. Based on current evidence, future clinical trials designed to reduce weight-related maternal and infant adverse outcomes should focus on maternal weight before or at the start of pregnancy.

Voerman E, Santos S, Patro Golab B, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLOS Medicine.* 2019; 16: e1002744

Abstract

Background: Maternal obesity and excessive gestational weight gain may have persistent effects on offspring fat development. However, it remains unclear whether these associations differ by severity of obesity, and whether they are restricted to the extremes of maternal body mass index (BMI) and gestational weight gain. We aimed to assess the separate and combined associations of maternal BMI and gestational weight gain with the risk of overweight/obesity throughout childhood.

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Methods and findings: We conducted an individual participant data meta-analysis of data from 162,129 mothers and their children from 37 pregnancy and birth cohort studies from Europe, North America, and Australia. We assessed the individual and combined associations of maternal pre-pregnancy BMI and gestational weight gain, both in clinical categories and across their full ranges, with the risks of overweight/obesity in early (2.0–5.0 years), mid (5.0–10.0 years) and late childhood (10.0–18.0 years), using multilevel binary logistic regression models with a random intercept at cohort level adjusted for maternal sociodemographic and lifestyle-related characteristics. We observed that higher maternal pre-pregnancy BMI and gestational weight gain both in clinical categories and across their full ranges were associated with higher risks of childhood overweight/obesity, with the strongest associatons in late childhood (odds ratios [ORs] for overweight/obesity in early, mid, and late childhood, respectively: OR 1.66 [95% CI: 1.56, 1.78], OR 1.91 [95% CI: 1.85, 1.98], and OR 2.28 [95% CI: 2.08, 2.50] for maternal overweight; OR 2.43 [95% CI: 2.24, 2.64], OR 3.12 [95% CI: 2.98, 3.27], and OR 4.47 [95% CI: 3.99, 5.23] for maternal obesity; and OR 1.39 [95% CI: 1.30, 1.49], OR 1.55 [95% CI: 1.49, 1.60], and OR 1.72 [95% CI: 1.56, 1.91] for excessive gestational weight gain). The proportions of childhood overweight/obesity prevalence attributable to maternal overweight, maternal obesity, and excessive gestational weight gain ranged from 10.2% to 21.6%. Relative to the effect of maternal BMI, excessive gestational weight gain only slightly increased the risk of childhood overweight/obesity within each clinical BMI category (*p*-values for interactions of maternal BMI with gestational weight gain: *p* = 0.038, *p* < 0.001, and *p* = 0.637 in early, mid, and late childhood, respectively). Limitations of this study include the self-report of maternal BMI and gestational weight gain for some of the cohorts, and the potential of residual confounding. Also, as this study only included participants from Europe, North America, and Australia, results need to be interpreted with caution with respect to other populations.

Conclusions In this study, higher maternal pre-pregnancy BMI and gestational weight gain were associated with a higher risk of childhood overweight/obesity, with the strongest associations at later childhood ages. The additional association of gestational weight gain in women who are overweight or obese before pregnancy is small. Given the large population impact, future intervention trials aiming to reduce the prevalence of childhood overweight and obesity should focus on maternal weight status before pregnancy, in addition to weight gain during pregnancy.

Selected results

Compared to maternal normal weight, maternal underweight was associated with lower risks of overweight/obesity throughout childhood and maternal overweight and obesity were associated with higher risks of overweight/obesity throughout childhood, with stronger associations at later ages (ORs for overweight/obesity in late childhood: 2.28 [95% CI: 2.08, 2.50] and 4.47 [95% CI: 3.99, 5.23] for maternal overweight and obesity, respectively). Among women with obesity, the risk of offspring overweight/obesity increased further for higher classes of maternal obesity (ORs for overweight/obesity in late childhood: 4.16 [95% CI: 3.56, 4·87], 5.98 [95% CI: 4.50, 7.94], and 5.55 [95% CI: 3.25, 9.45] for obesity class I, class II, and class III, respectively, as compared to normal weight). These associations were not explained by gestational diabetes or gestational hypertensive disorders and additional adjustment for gestational-age-adjusted birth weight attenuated the associations only slightly.

Compared to adequate gestational weight gain, inadequate gestational weight gain was associated with a lower risk of overweight/obesity in early and mid-childhood, but not in late childhood. Compared to adequate gestational weight gain, excessive gestational weight gain was associated with a higher risk of childhood overweight/obesity in early, mid, and late childhood (ORs 1.39 [95% CI: 1.30, 1.49], 1.55 [95% CI: 1.49, 1.60], and 1.72 [95% CI: 1.56, 1.91], respectively). Compared to maternal normal weight,

maternal underweight was associated with a higher risk of childhood underweight, whereas maternal overweight and obesity were associated with a lower risk of childhood underweight in early, mid, and late childhood. Similarly, compared to adequate gestational weight gain, inadequate gestational weight gain was associated with higher risks of childhood underweight, and excessive gestational weight gain with lower risks.

Figure 6 shows that higher maternal pre-pregnancy BMI was across the full range associated with higher risk of offspring overweight/obesity and higher offspring BMI SDS throughout childhood. The ORs for childhood overweight/obesity per kg/m^2 increase in maternal pre-pregnancy BMI were 1.08 (95% CI: 1.07, 1.09), 1.12 (95% CI: 1.11, 1.12), and 1.16 (95% CI: 1.15, 1.17), in early, mid, and late childhood, respectively. Similarly, higher maternal gestational weight gain across its full range was associated with a higher risk of overweight/obesity and higher childhood BMI in early, mid, and late childhood (**Figure 6**). The ORs for childhood overweight/obesity per SD increase in gestational weight gain were 1.14 (95% CI: 1.11, 1.17), 1.16 (95% CI: 1.14, 1.18), and 1.14 (95% CI: 1.09, 1.20), in early, mid, and late childhood, respectively.

Regardless of their mothers' gestational weight gain, children of mothers with underweight tended to have a lower risk of overweight/obesity, whereas children of mothers with overweight or obesity had a higher risk of overweight/obesity, as compared to children whose mothers had normal weight and adequate gestational weight gain. Within each maternal BMI category, excessive gestational weight gain tended to increase the risk of overweight/obesity in early and mid-childhood only slightly.

Figure 6: Risk of overweight or obesity across childhood by categories of maternal pre-pregnancy BMI and gestational weight.
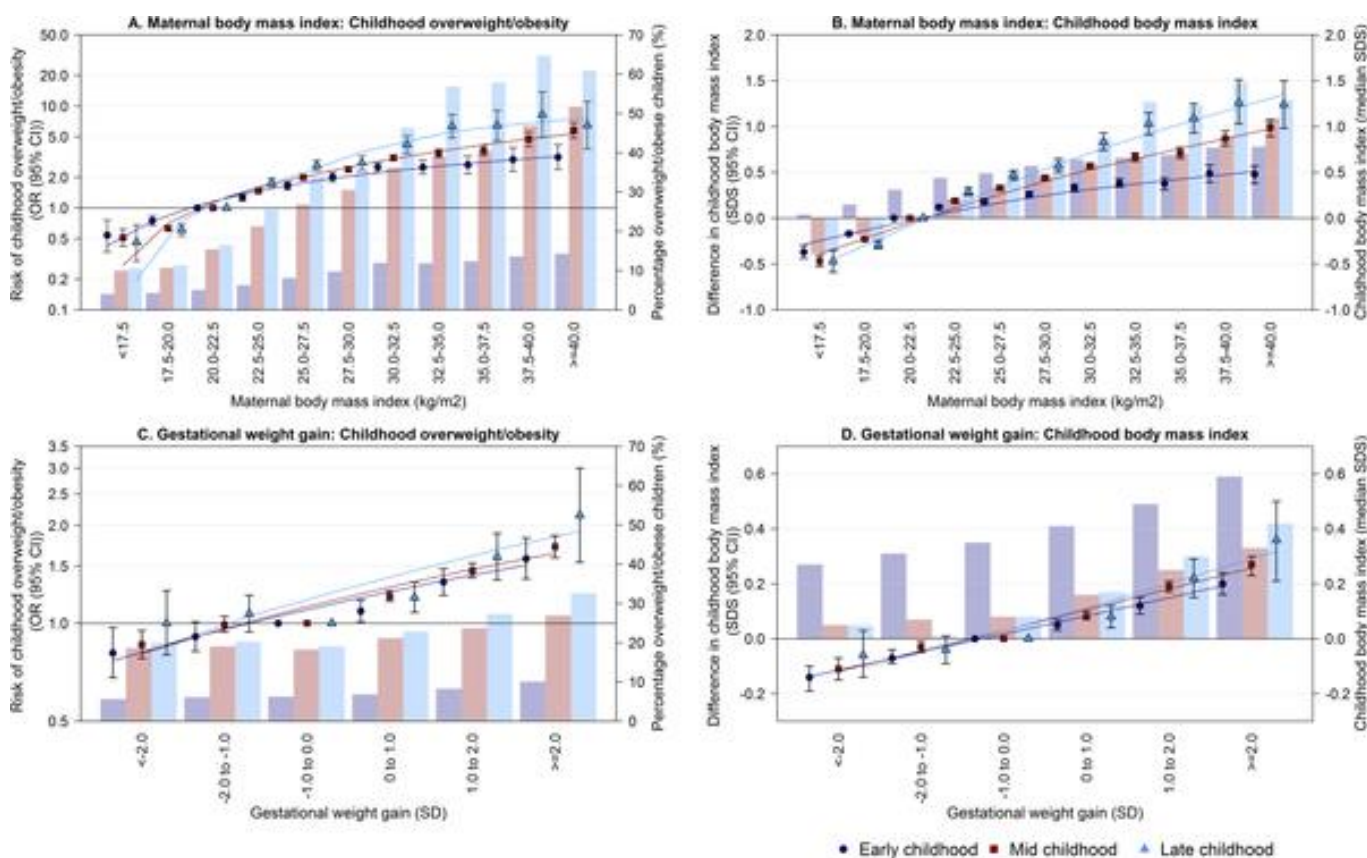
Conclusion

This study extends the previous study described above by looking beyond pregnancy and perinatal outcomes to later offspring health. It shows that maternal pre-/early-pregnancy BMI has a considerably stronger association with subsequent offspring overweight/obesity in early-, mid- and late-childhood. If these associations are causal they would suggest interventions that effectively sustain healthy weight prior to becoming pregnancy may be more important for offspring health than those that promote limiting gestational weight gain.

Mendelian randomization analyses to explore causal effects of intrauterine exposures on offspring cardiovascular/cardiometabolic health.

Using genetic variants as instrumental variables (known as Mendelian randomization (MR) analyses) can produce causal effect estimates that are less likely to be confounded by socioeconomic, environmental or behavioural characteristics or influenced by reverse causality than conventional multivariable analyses. We have used this approach, with sensitivity analyses that can bias MR, and compared results to multivariable regression in order to determine whether intrauterine exposures that are associated with subsequent offspring are likely to reflect causal effects.[references 6 to 10 in section 2.1.3]. In the first of these a genome-wide association study that uses a novel statistical method to enable the effects of maternal and fetal genetic variants on birth weight to be separated, is then extended to MR analyses of the effects of intrauterine exposures influencing birth weight on later life offspring cardiovascular/metabolic health.[6] This paper is described below.

Warrington NM, Beaumont RM, et al. et al. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. *Nature Genetics* 2019; 51: 804-814.
Abstract

Birth weight variation is influenced by fetal and maternal genetic and non-genetic factors, and has been reproducibly associated with future cardio-metabolic health outcomes. In expanded genome-wide association analyses of own birth weight (n = 321,223) and offspring birth weight (n = 230,069 mothers), we identified 190 independent association signals (129 of which are novel). We used structural equation modelling to decompose the contributions of direct fetal and indirect maternal genetic effects, then applied Mendelian randomization to illuminate causal pathways. For example, both indirect maternal and direct fetal genetic effects drive the observational relationship between lower birth weight and higher later blood pressure: maternal blood pressure-raising alleles reduce offspring birth weight, but only direct fetal effects of these alleles, once inherited, increase later offspring blood pressure. Using maternal birth weight-lowering genotypes to proxy for an adverse intrauterine environment provided no evidence that it causally raises offspring blood pressure, indicating that the inverse birth weight– blood pressure association is attributable to genetic effects, and not to intrauterine programming.

Selected results

Genome-wide association results
The GWAS meta-analysis of own birth weight (n = 321,223) identified 146 independent SNPs at genome-wide significance ($P < 6.6 \times 10^{-9}$). The GWAS meta-analysis of offspring birth weight (n = 230,069 mothers) identified 72 independent SNPs ($P < 6.6 \times 10^{-9}$). Applying the more lenient significance threshold used previously ($P < 5 \times 10^{-8}$), 211 and 105 SNPs reached significance for own and offspring birth weight, respectively. Taking account of SNPs that were identified in both the maternal and fetal

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(own) there were 209 lead SNPs reaching $P < 6.6 \times 10^{-9}$. Further analyses sought to explore how the maternal and fetal genotypes combined to influence birth weight at each of these variants.

Structural equation modelling (SEM), which accounts for the correlation between fetal and maternal genotypes and thereby provides unbiased estimates of the maternal and fetal genetic effects on birth weight, was used to separate the 209 lead SNPs into 5 categories. Using the confidence intervals (CIs) around the SEM-adjusted maternal and fetal effect estimates, we identified: (i) 64 SNPs with fetal-only effects; (ii) 32 SNPs with maternal-only effects; (iii) 27 SNPs with directionally concordant fetal and maternal effects and (iv) 15 SNPs with directionally opposing fetal and maternal effects. For example, rs10830963 at *MTNR1B* was identified in both the own-birth-weight ($P = 2.8 \times 10^{-11}$) and offspring-birth-weight GWAS ($P = 9.1 \times 10^{-39}$), but the SEM analysis revealed that its effect was exclusively maternal ($P_{\text{SEMfetal}} = 0.7$; $P_{\text{SEMmaternal}} = 4.6 \times 10^{-19}$). In contrast, rs560887 at *G6PC2* was identified only in the GWAS of offspring birth weight ($P = 1.2 \times 10^{-14}$), but was found to have directionally opposing maternal and fetal effects ($\beta_{\text{SEMfetal}} = -0.03$; $P_{\text{SEMfetal}} = 2.8 \times 10^{-8}$; $\beta_{\text{SEMmaternal}} = 0.04$; $P_{\text{SEMmaternal}} = 5.4 \times 10^{-14}$).

To extend the estimates of adjusted maternal and fetal effects genome wide, we developed a weighted linear model (WLM), which yields a good approximation to the SEM with equivalent estimates for the 209 lead SNPs. This was necessary because the SEM is too computationally intensive to fit across the genome. The adjusted fetal and maternal genotype effect estimates on birth weight from the WLM are hereafter referred to as WLM-adjusted estimates and are used in subsequent Mendelian randomization analyses.

Genetic correlation and MR results

The 209 lead birth-weight-associated SNPs were genetically correlated with other traits (**Figure 7**). For many traits (for example, adult height), the fetal-specific genetic correlation was similar to the maternal-specific genetic correlation, but for some traits, the fetal- and maternal-specific genetic correlations were different in magnitude (for example, systolic blood pressure (SBP)) or even in direction (for example, type 2 diabetes (T2D)). For several glycaemic traits (for example, fasting glucose), the genetic correlations estimated using the WLM-adjusted effects were substantially different from those estimated using the unadjusted effects, showing the importance of accounting for the maternal–fetal genotype correlation.

The separation of direct fetal genotype effects from indirect maternal genotype effects on birth weight offers the novel opportunity to estimate unconfounded causal influences of intrauterine exposures by using MR analyses. We applied two-sample MR to estimate causal effects of maternal exposures on offspring birth weight, focusing on height, glycemic traits and blood pressure. We also used the WLM-adjusted fetal effects to estimate the casual effect of the offspring's genetic potential on their own birth weight, and compared the results with the estimated maternal causal effects. The assumptions of these analyses are show in **Figure 8a**.

Our two-sample MR using 693 height-associated SNPs estimated that a 1 SD (6 cm) higher maternal height is causally associated with a 0.11 SD. (95% CI: 0.10 to 0.13) higher offspring birth weight (**Figure 8b**), independent of the direct fetal effects.

Maternal glucose is a key determinant of fetal growth: it crosses the placenta, stimulating the production of fetal insulin, which promotes growth. As a consequence, strong positive associations are seen between maternal fasting glucose, fetal insulin levels and offspring birth weight. In a randomized

clinical trial of women with gestational diabetes mellitus, glucose control was shown to reduce offspring birth weight and a previous (smaller) MR analyses supported a causal effect of higher maternal circulating glucose on birth weight. Therefore, we anticipated detecting a positive causal effect of maternal glucose on offspring birth weight. Our two-sample MR supported our expectation: using 33 fasting glucose-associated SNPs we estimated causal effect of a 0.18 SD. (95% CI: 0.13 to 0.23) higher offspring birth weight due to 1 SD. (0.4 mmol l^{-1}) higher maternal fasting glucose, independent of the direct fetal effects **Figure 8c**). A large part of the genetic variation underlying fasting glucose levels is implicated in pancreatic β-cell function and thus overlaps with the genetics of insulin secretion. To estimate the causal effect of insulin secretion on birth weight, we used 18 SNPs associated with the disposition index—a measure of insulin's response to glucose, adjusted for insulin sensitivity. Alleles that increase insulin secretion in the mother tend to decrease her glucose levels, which consequently reduces insulin-mediated growth of the fetus. This was reflected in the negative causal estimate from the MR analysis of the effect of the maternal disposition index on offspring birth weight (−0.17SD per 1 SD higher maternal disposition index (95% CI: −0.26 to −0.08).

Observational studies of the relationship between birth weight and later-life blood pressure have produced mixed findings. Some studies indicate that lower birth weight is associated with higher later-life blood pressure and related comorbidities, whereas others have shown that this relationship could be driven by a statistical artifact due to adjusting for current weight. We have previously shown that genetic factors account for a large proportion of an association between lower birth weight and higher blood pressure, but it was not clear whether this was due to direct fetal genotype effects or indirect maternal effects, or a combination of the two. We explored this association further using several complementary analyses. The estimate of the birth weight–SBP covariance explained was higher when using the maternal genotyped SNP associations with offspring birth weight (65% (95% CI: 57 to 74%)) than when using the fetal genotype associations with own birth weight (56% (95% CI: 48 to 64%). A similar pattern was seen with the birth weight–diastolic blood pressure (DBP) covariance (maternal: 72% (95% CI: 58 to 85%); fetal: 56% (95% CI: 46 to 67%)). Together with the larger maternal than fetal genetic correlation for SBP (**Figure 7**), these results point to the importance of indirect maternal effects of blood pressure on offspring birth weight. In line with this, MR analyses indicated that a 1 SD (10 mmHg) higher maternal SBP is causally associated with a 0.15 SD (95% CI: −0.19 to −0.11) lower offspring birth weight, independent of the direct fetal effects (**Figure 8d**). In contrast, there was no fetal effect of SBP on own birth weight, after adjusting for the indirect maternal effect (−0.01 SD per 1 SD (10 mmHg), 95% CI: −0.05 to 0.03; **Figure 8d**).

A key question is whether maternal SNPs that reduce offspring birth weight through intrauterine effects are also associated with higher SBP in their adult offspring. Such an association would suggest that the maternal intrauterine effects cause the later SBP effect (that is, through developmental adaptations) (**Figure 9**). To investigate this possibility, we tested the conditional association between maternal and offspring genetic scores for birth weight and offspring SBP, as measured in 3,886 mother–offspring pairs in the UK Biobank, with sensitivity analyses in 1,749 father–offspring pairs. The fetal genetic score for lower birth weight was associated with higher offspring SBP, even after adjustment for maternal (or paternal) birth weight genotypes. However, when adjusted for fetal genotypes, the maternal genetic score for lower birth weight was associated with lower (not higher) offspring SBP.

Conclusions
The MR results show that taller women have on average heavier infants via an intrauterine mechanism and that fetal own genetic predisposition to be taller increases birth weight. MR results also underline

the importance of maternal circulating pregnancy glucose and fetal insulin in fetal growth, and show that fetal genetic effects link lower birth weight with reduced insulin secretion and higher T2D risk in later life. In relation to blood pressure, our results show that the observed negative correlation between birth weight and later SBP is driven by (1) the causal effect of higher maternal SBP on lower offspring birth weight, in combination with (2) the subsequent transmission of SBP-associated alleles to offspring, which then increase offspring SBP (**Figure 9**), rather than by long-term developmental responses to adverse in utero conditions.

Figure 7: Correlations of maternal and fetal birth-weight influencing SNPs with other traits.
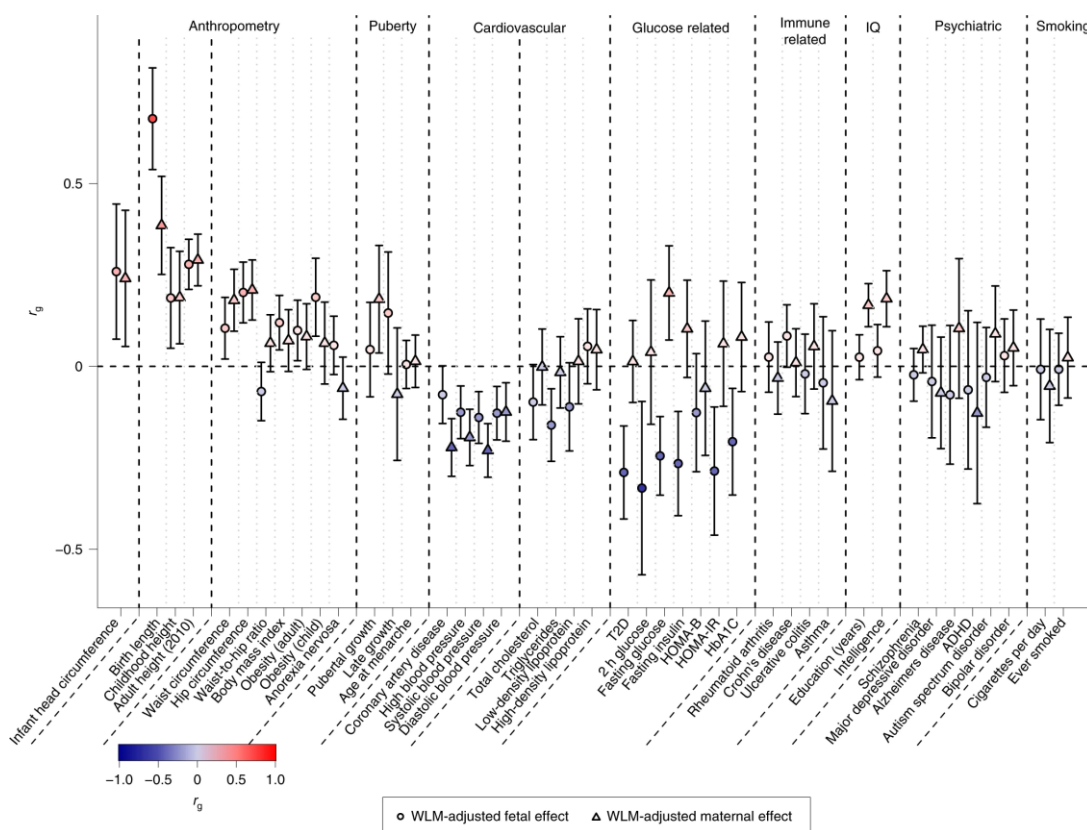
Figure 8: Mendelian randomization results of effects of maternal and fetal traits on birth weight
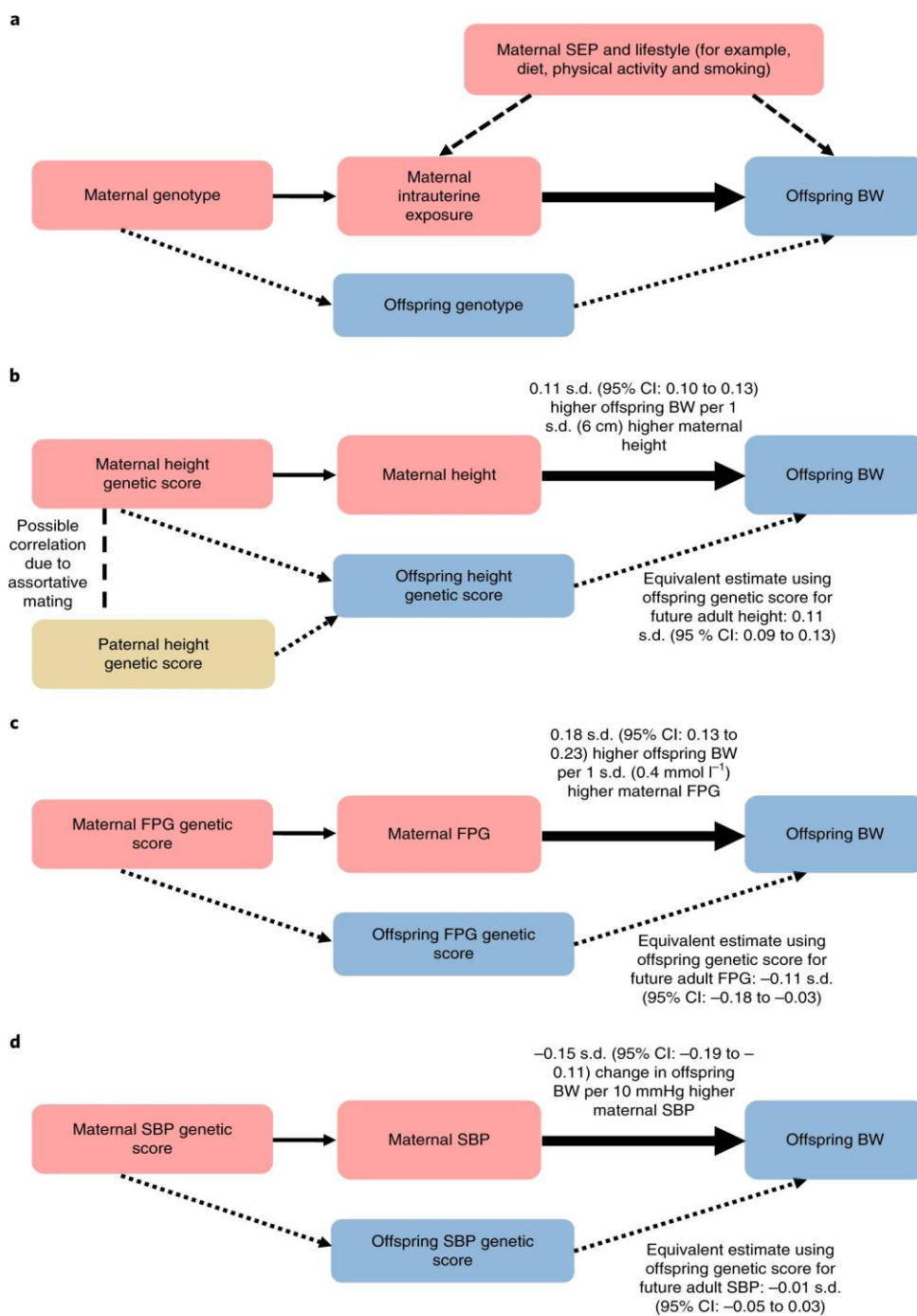
a, Since maternal and fetal genotypes are correlated, it is essential to account for offspring genotype in these analysis. The continuous, thin arrow represents the relationship between the genetic instrument and intrauterine exposure. The dashed arrows represent potential confounding via maternal characteristics, which, under MR assumptions, are not associated with the genetic instrument. The dotted arrows represent potential violation of MR assumptions via offspring genotype. The thick arrow represents the causal effect of interest. **b**, Higher offspring birth weight is caused by direct fetal genetic effects of height-raising alleles and indirect effects of maternal height-raising alleles. **c**, Higher maternal fasting glucose levels increase offspring birth weight. Conversely, direct fetal genetic effects of glucose-raising alleles reduce birth weight. **d**, Higher maternal SBP is causally associated with lower offspring birth weight. After adjusting for maternal effects, there was no evidence of an effect of offspring's own SBP genetic score on own birth weight.

Figure 9: Mendelian randomization analyses exploring the observational association of lower birth weight with future offspring higher systolic blood pressure
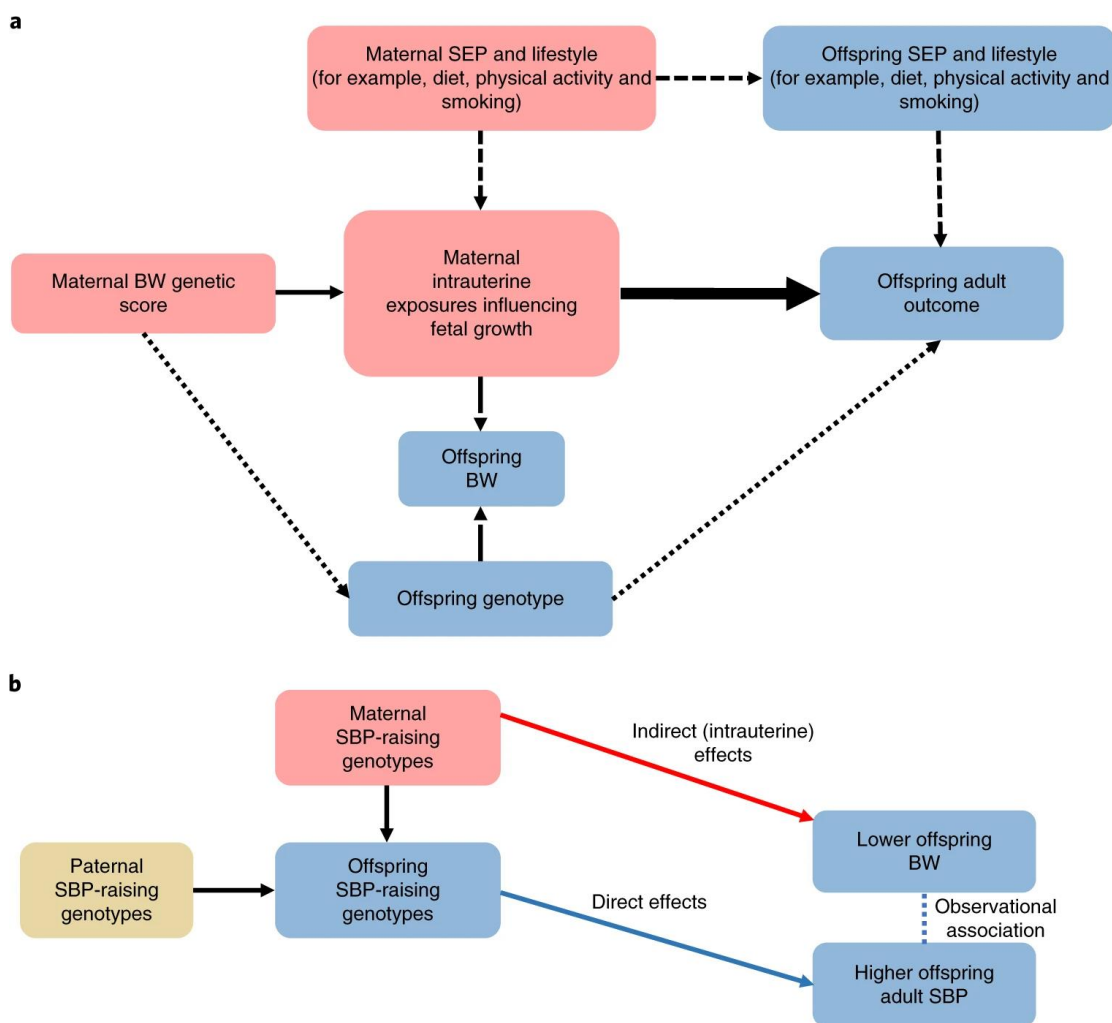
a, Maternal genotype should be associated with offspring birth weight independent of offspring genotype, so it is essential to adjust the analysis for offspring genotype. The continuous, thin arrow represents the relationship between the genetic instrument and intrauterine exposure. The long-dashed arrows denote the (maternal and possibly fetal) genotype associations with birth weight; these arrows highlight the assumption that genetic variation influences offspring adult outcome via intrauterine growth, not birth weight. The short-dashed arrows represent potential confounding via maternal and offspring characteristics. The dotted arrow represents potential violation of assumptions of the MR analysis via offspring genotype. The thick arrow represents the causal effect of interest. **b**, Our results show that the observed negative correlation between birth weight and later SBP may be driven by the causal effect of higher maternal SBP on lower offspring birth weight (red arrow), in combination with the subsequent transmission of SBP-associated alleles to offspring (blue arrow), which then increase offspring SBP.

A further three publications that have used different datasets and some different MR analyses to explore whether exposure to higher maternal pre-/early pregnancy BMI increases the risk of higher offspring BMI.[7-9 in the reference list in section 2.1.3] These all suggest that this is not a causal relationship but rather previous observational associations were likely confounded. Below we describe the most recent of these, which is the largest, has most statistical power and covers the greatest range of ages from birth to early adulthood.[9] This study included two of the LifeCycle cohorts with relevant data and is published as a pre-print and currently under peer review with *BMC Medicine*.

Bond TA, Richmond RC, Karhunen V, et al. Exploring the causal effect of maternal pregnancy adiposity on offspring adiposity: Mendelian randomization using polygenic risk scores. *MedRxiv* 2021; doi: 10.1101/2021.04.01.21251414

Abstract

Background It has been hypothesised that greater maternal adiposity before or during pregnancy causes greater offspring adiposity in childhood and adulthood, via causal intrauterine or periconceptional mechanisms. Previous Mendelian randomization (MR) estimates were imprecise, with wide confidence intervals that included potentially important protective or adverse effects, and may have been biased by collider effects or imperfect adjustment for genetic inheritance. Here we use an improved MR approach to investigate whether associations between maternal pre-/early pregnancy body mass index (BMI) and offspring adiposity from birth to adolescence are causal, or due to confounding.

Methods and findings We undertook multivariable (MV) regression and MR analyses using mother-offspring pairs from two UK cohorts: Avon Longitudinal Study of Parents and Children (ALSPAC) and Born in Bradford (BiB). In ALSPAC and BiB the outcomes were birthweight (BW; N = 9339) and BMI at age 1 (N = 8659) and 4 years (N = 7575), and in ALSPAC only we investigated BMI at 10 (N = 4476) and 15 years (N = 4112) and dual-energy X-ray absorptiometry (DXA) determined fat mass index (FMI) from age 10 to 18 years (N = 2659 to 3855). We compared MR results from several polygenic risk scores (PRS), calculated from maternal non-transmitted alleles at between 29 and 80,939 single nucleotide polymorphisms (SNPs). MV and MR showed a consistent positive association of maternal BMI with BW, but for adiposity at most older ages MR estimates were weaker than MV estimates. In MV regression a one standard deviation (SD) higher maternal BMI was associated with a 0.13 (95% confidence interval [CI]: 0.10, 0.16) SD increase in offspring BW. The corresponding MR estimate from the strongest PRS (including up to 80,939 SNPs) was 0.14 (95% CI: 0.05, 0.23), with no difference between the two estimates ($P_{difference}$ = 0.84). For 15 year BMI the MV and MR estimates (80,939 SNPs) were 0.32 (95% CI: 0.29, 0.36) and 0.13 (95% CI: 0.01, 0.24) respectively ($P_{difference}$ = 1.0e-3). Results for FMI were similar to those for adolescent BMI. As the number of SNPs included in the PRS increased, the MR confidence intervals narrowed and the effect estimates generally became closer to the MV estimates. Sensitivity analyses suggested the stronger effects with more SNPs were plausibly explained by horizontal pleiotropic bias away from zero. Consequently, the unbiased difference between the MV and MR estimates is probably greater than shown in our main analyses. Furthermore, MR estimates from IVs with fewer SNPs provided no strong evidence for a causal effect on adolescent adiposity.

Conclusions Our results suggest that higher maternal pre-/early-pregnancy BMI is not a key driver of higher adiposity in the next generation. Thus, they support interventions that target the whole

population for reducing overweight and obesity, rather than a specific focus on women of reproductive age.

Selected results

In confounder adjusted MV regression models maternal BMI was positively associated with all offspring outcomes (**Figure 10**). When we meta-analysed MV estimates from all three samples, a 1 SD higher age-adjusted maternal BMI was associated with a 0.13 (95% CI: 0.10, 0.16) SD higher sex adjusted BW; the corresponding estimates for 1 and 4 year age- and sex-adjusted BMI were 0.07 (0.04, 0.10) and 0.18 (0.15, 0.21), respectively. For ALSPAC, the MV estimate for 15 year BMI was 0.32 (0.29, 0.36), and estimates were similar for 10 year BMI and FMI from 10 to 18 years. Adjustment for potential confounders had a negligible impact on the estimates, aside from a small attenuation on adjustment for paternal BMI for outcomes after birth.

For the main MR analyses we did not select SNPs based on those reaching genome-wide significance but rather aimed to increase instrument strength and the statistical power of our analyses by selecting from across the whole genome. We used a method called lassosum which generates a polygenic risk score (PRS) from across the genome-wide summary statistics for a trait (in this example the BMI GWAS summary data). Specifically lassosum uses penalised regression to carry out shrinkage and selection on the base GWAS SNP effects and accounts for LD information from a reference panel. In our analysis the PRS included 80,939 SNPs for analyses in ALSAPC and 79,101 SNPs in BiB. For BW the MR estimate for the lassosum PRS for all three samples meta-analysed was 0.14 (0.05, 0.23), which was similar to the MV estimate; $P_{\text{difference (MV vs. MR)}}$ = 0.84)) (**Figure 10**). The corresponding lassosum MR estimates for 1 year BMI and 4 year BMI were -0.02 (-0.11, 0.07) and 0.01 (-0.08, 0.10) respectively, and there was moderate to strong evidence for an MR-MV difference ($P_{\text{difference}}$ = 0.10 and 1.3e-3, respectively). The MR estimates for 10 and 15 year BMI in ALSPAC (0.10 [-0.01, 0.21] and 0.13 [0.01, 0.24] respectively) were also smaller than the MV estimates ($P_{\text{difference}}$ = 1.4e-4 and 1.0e-3 respectively). Results for FMI were similar to those for BMI. We did not observe strong evidence for non-linearity or interaction by sex for either the MV or MR models, and results were similar when we (i) substituted BMI or ponderal index at birth for BW, (ii) natural log transformed skewed variables, (iii) removed cryptic relatedness from the, and (iv) used linear mixed models to adjust for population structure.

When we replaced the lassosum PRS with alternative IVs calculated from fewer SNPs, our MR estimates varied in a manner that was specific to the offspring outcome (**Figure 11**). For BW, including fewer SNPs in the IV did not result in large differences in the MR estimates, although the precision reduced markedly as we used fewer SNPs. For 1 and 4 year BMI, MR estimates increased as we used fewer SNPs, whereas for 10 year BMI they largely remained stable and for 15 year BMI they decreased. The patterns for FMI were similar to those for BMI. For all outcomes apart from BW, including more SNPs in the IV resulted in stronger evidence that MR estimates differed from MV estimates (i.e. smaller P_{dif}).

For the majority of outcomes (particularly in adolescence) there was moderate to strong statistical evidence that SNPs with smaller effect sizes gave larger (more positive) MR estimates, and this was not driven by weak instrument bias. When using only large-effect (genome-wide significance) SNPs, in general there was not strong statistical evidence for between-SNP MR estimate heterogeneity, nor was there strong evidence that the MR-Egger intercept differed from zero.

In ALSPAC there was strong evidence for correlation between maternal and paternal BMI (Pearson's *r*: 0.22, 95% CI: 0.16, 0.28, *P* = 7.9e-14), but no evidence for correlation between maternal non-transmitted allele and paternal lassosum BMI PRS (*r*: 0.02, 95% CI: -0.04, 0.07, *P* = 0.55). For comparison, a maternal lassosum BMI PRS that was calculated from both transmitted and non-transmitted alleles was slightly more strongly correlated with the paternal PRS (*r*: 0.04, 95% CI: -0.01, 0.10, *P* = 0.14).

Conclusions

In this study we explored the causality of associations between maternal pre-/early-pregnancy BMI and offspring BW and child/adolescent adiposity, using an MR approach with PRS calculated from maternal non-transmitted alleles. This approach yielded narrower confidence intervals compared with previous studies, and avoided sources of bias that may have affected previous work. We found no strong evidence for a causal effect of maternal BMI on offspring adiposity beyond birth, but strong evidence that confounder adjusted observational associations between maternal BMI and adolescent adiposity are affected by residual confounding. Although we cannot rule out a small or moderate causal effect on child/adolescent adiposity, the present study suggests that higher maternal pre-/early-pregnancy BMI is not a key driver of greater adiposity in the next generation. Thus, our results support interventions that target the whole population for reducing overweight and obesity, rather than a specific focus on women of reproductive age.

Figure 10: Mean difference in offspring outcomes (SD) per 1SD increase in maternal BMI, from MR (lassosum) and confounder adjusted multivariable regression (MV) models
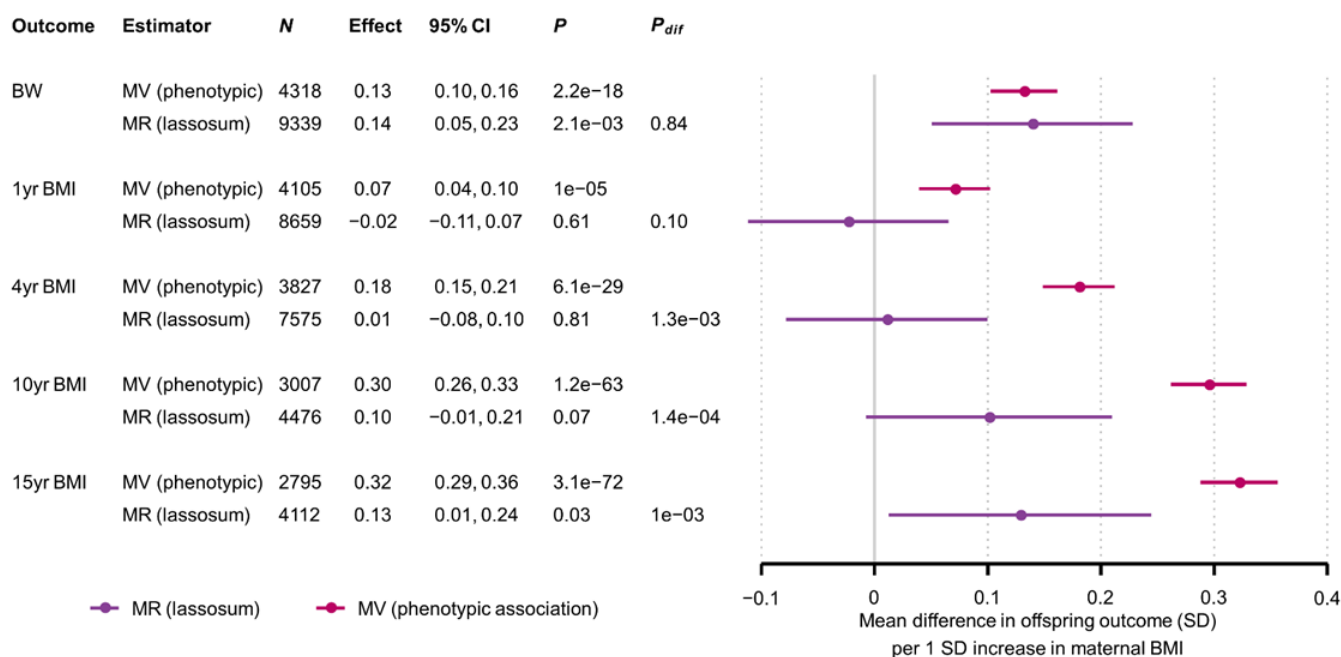
Outcome	Estimator	N	Effect	95% CI	P	P_{dif}
BW	MV (phenotypic)	4318	0.13	0.10, 0.16	2.2e−18	
	MR (lassosum)	9339	0.14	0.05, 0.23	2.1e−03	0.84
1yr BMI	MV (phenotypic)	4105	0.07	0.04, 0.10	1e−05	
	MR (lassosum)	8659	−0.02	−0.11, 0.07	0.61	0.10
4yr BMI	MV (phenotypic)	3827	0.18	0.15, 0.21	6.1e−29	
	MR (lassosum)	7575	0.01	−0.08, 0.10	0.81	1.3e−03
10yr BMI	MV (phenotypic)	3007	0.30	0.26, 0.33	1.2e−63	
	MR (lassosum)	4476	0.10	−0.01, 0.21	0.07	1.4e−04
15yr BMI	MV (phenotypic)	2795	0.32	0.29, 0.36	3.1e−72	
	MR (lassosum)	4112	0.13	0.01, 0.24	0.03	1e−03

Confounder adjusted multivariable regression (MV) estimates are from model three (Methods). *P*: *P*-value for the null hypothesis that the effect equals zero, P_{dif}: *P*-value for the null hypothesis that MR effect equals the MV effect. The lassosum MR analyses generates a PRS from across the genome (rather than select on the basis of genome-wide significant associations. Here 80,939 SNPs and 79,101 contributed to the genetic instrument (PRS) in ALSAPC and BiB, respectively.

Figure 11: Mean difference in offspring outcomes (SD) per 1SD increase in maternal BMI, from MR models using different SNP sets and confounder adjusted MV models

Outcome	Estimator	N	Effect	95% CI	P	P_{dif}
BW	MV	4318	0.13	0.10, 0.16	2.2e−18	
	Lassosum (MR)	9339	0.14	0.05, 0.23	2.1e−03	0.84
	Yengo (MR)	9339	0.17	0.04, 0.31	0.01	0.55
	Locke (MR)	9339	0.18	−0.01, 0.37	0.07	0.53
	Speliotes (MR)	9339	0.29	0.05, 0.53	0.02	0.16
	FTO (MR)	9339	0.15	−0.13, 0.43	0.29	0.89
1yr BMI	MV	4105	0.07	0.04, 0.10	1e−05	
	Lassosum (MR)	8659	−0.02	−0.11, 0.07	0.61	0.10
	Yengo (MR)	8659	0.12	−0.02, 0.26	0.10	0.53
	Locke (MR)	8659	0.25	0.02, 0.48	0.03	0.17
	Speliotes (MR)	8659	0.22	−0.01, 0.45	0.07	0.22
	FTO (MR)	8659	0.12	−0.21, 0.45	0.47	0.77
4yr BMI	MV	3827	0.18	0.15, 0.21	6.1e−29	
	Lassosum (MR)	7575	0.01	−0.08, 0.10	0.81	1.3e−03
	Yengo (MR)	7575	0.07	−0.09, 0.20	0.45	0.09
	Locke (MR)	7575	0.05	−0.16, 0.25	0.66	0.21
	Speliotes (MR)	7575	0.14	−0.09, 0.36	0.23	0.71
	FTO (MR)	7575	0.13	−0.15, 0.43	0.33	0.82
10yr BMI	MV	3007	0.30	0.26, 0.33	1.2e−63	
	Lassosum (MR)	4476	0.10	−0.01, 0.21	0.07	1.4e−04
	Yengo (MR)	4476	0.10	−0.09, 0.29	0.29	0.04
	Locke (MR)	4476	0.05	−0.21, 0.32	0.69	0.08
	Speliotes (MR)	4476	0.13	−0.14, 0.40	0.36	0.24
	FTO (MR)	4476	−0.01	−0.44, 0.43	0.97	0.25
15yr BMI	MV	2795	0.32	0.29, 0.36	3.1e−72	
	Lassosum (MR)	4112	0.13	0.01, 0.24	0.03	1e−03
	Yengo (MR)	4112	0.13	−0.06, 0.33	0.17	0.05
	Locke (MR)	4112	−0.13	−0.45, 0.19	0.43	0.01
	Speliotes (MR)	4112	−0.16	−0.51, 0.18	0.35	0.01
	FTO (MR)	4112	−0.49	−1.10, 0.12	0.11	0.01

Mean difference in offspring outcome (SD) per 1 SD increase in maternal BMI

Confounder adjusted multivariable regression (MV) estimates are from model three (Methods). *P*: *P*-value for the null hypothesis that the effect equals zero, P_{dif}: *P*-value for the null hypothesis that MR effect equals the MV effect, FTO: rs9939609 at the *FTO* locus, Speliotes, Locke, Yengo: GWS SNPs from the GWAS with the indicated first author, Lassosum: PRS calculated by the lassosum method. Colours separate outcomes at different ages.

2.1.2 Preliminary results from on-going projects where analyses have been largely completed

Below we present preliminary results from three projects where main analyses have been largely completed: (i) effects of assisted reproductive technology (a pre-/during- conception stressor) on growth adiposity and cardio-vascular/metabolically outcomes [these are presented below as two summaries as our plans are to submit as two separate papers, one focusing on growth and adiposity outcomes and the other on blood pressure, lipids and glycaemia related outcomes. (ii) Association of gestational age at birth with body size from infancy to adulthood, a collaboration between LifeCycle and the H2020 funded EUCAN-Connect project. (iii) Association of different types of stressors (socioeconomic, environmental and pathophysiological) with offspring adiposity from infancy to early adulthood. The latter two of these projects have been able to use DataShield with harmonized data and have been key demonstration projects of this approach.

Effects of assisted reproductive technologies (ARTs) on offspring cardio-metabolic/-vascular health.

Planned paper 1: Elhakeem A, Taylor A, Inskip H, co-authors, Lawlor DA. Effects of assisted reproductive technology on offspring growth and adiposity from infancy to early adulthood: a coordinated analysis of 26 multinational cohort studies. Currently being prepared for circulation to co-authors.

Abstract
Background: People conceived by in vitro fertilisation (IVF) make up a growing proportion of the world's population. Our aim was to compare long-term growth and adiposity patterns between IVF-conceived and spontaneously conceived (SC) offspring.
Methods: We did two-stage subgroup meta-analyses of individual participant data from 26 cohorts that had information on mode of conception and at least one postnatal growth and/or adiposity outcome in offspring. Multivariable regression models (adjusted for maternal age, parity, BMI, smoking, education, ethnicity, and offspring sex and age) were used to calculate differences in mean height, weight, BMI, waist circumference, body fat % and fat mass index in each cohort. Results were combined in random-effects sub-group (by age) meta-analyses. To tease out effects of IVF from any effects of parental subfertility, we repeated analyses comparing IVF offspring to SC offspring of sub-fertile parents (>12-months trying to conceive) and separately to offspring of fertile parents (≤12-months). We explored evidence of sex differences and of fertility treatment types by repeating analyses: (i) stratified by sex; (ii) comparing ICSI IVF and non-ICSI IVF each separately to SC; (iii) comparing fresh embryo transfer and frozen-thawed embryo transfer each separately to SC and (iv) comparing any medically assisted reproduction (including IVF) to SC.
Findings: A total of 26 cohorts with participants from Europe (n=20 cohorts), Australia (n=2 cohorts), and one each from New Zealand, China, Singapore, and Canada were included in this study. The number of participants included in each meta-analysis varied from 3,111 (151 IVF-conceived) to 152,833 (4,259 IVF). Compared to SC, IVF-conceived offspring were on average slightly shorter, lighter, and thinner in infancy, childhood, and early adolescence. For instance, mean differences (95%CI) in height, weight, and BMI at age '6 to ≤10 years' for IVF vs. SC were -0.07SDcm (-0.13 to -0.02), -0.08SDkg (-0.11 to -0.04), and -0.07SDkg/m2 (-0.13 to -0.01), respectively. Differences were largest at youngest ages and mostly attenuated with age. Patterns of association by age were broadly similar for waist circumference, body fat % and fat mass index, but estimates were less precise, e.g., mean difference in waist at '5 to ≤10 years' was -0.16SDcm (-0.27 to -0.05). Additional analyses showed these differences were mostly seen

for IVF involving fresh embryo transfers. There was some evidence that IVF may become associated with higher adiposity in adulthood.

Conclusions: Parents conceiving through IVF can be reassured that differences in growth and adiposity are small and particularly by older adolescence. Studies with larger samples and longer follow-up are needed to evaluate a possible risk of increased adiposity later in adulthood.

Selected results

Figure 12 shows the difference in mean BMI comparing IVF to SC for ages from infancy to adulthood. Mean BMI was lower in IVF than SC offspring up to age 10 to ≤14 years, with the magnitude being greatest at youngest ages. At some ages results were imprecise with wide CIs that included the null value. The difference in mean BMI was close to the null in older adolescence (14 to ≤18 year). Mean BMI in adulthood (>18 years) appeared higher in IVF than SC offspring, though these results were particularly imprecise and included the null value. Results for waist circumference, body fat %, and FMI were like those seen for BMI, with lower mean values in IVF than SC offspring in childhood and adolescence, though mean differences were imprecisely estimated for several timepoints (Figure 2).

Figure 12: Mean difference in body mass index between IVF and SC offspring.

body mass index

Age Group	I²	N St.	N SC	N IVF		Estimate [95% CI]
≤3 months	69.2%	9	85049	2229		-0.17 [-0.28, -0.05]
3 to ≤6 months	71.3%	10	145057	4152		-0.10 [-0.19, -0.02]
6 to ≤9 months	15%	12	87823	2658		-0.05 [-0.11, 0.01]
9 to ≤12 months	44.8%	8	102223	2717		-0.04 [-0.11, 0.03]
12 to ≤18 months	24.8%	16	116431	3505		-0.04 [-0.10, 0.01]
18 to ≤24 months	0%	6	31934	893		-0.08 [-0.15, -0.01]
2 to ≤3 years	16.3%	13	61113	1919		-0.07 [-0.12, -0.01]
3 to ≤4 years	5.6%	12	69575	1912		-0.09 [-0.14, -0.03]
4 to ≤6 years	0%	16	65323	1799		-0.10 [-0.15, -0.06]
6 to ≤10 years	42.6%	13	107750	3337		-0.07 [-0.13, -0.01]
10 to ≤14 years	38.1%	10	58353	1776		-0.07 [-0.16, 0.03]
14 to ≤18 years	0%	3	15203	178		0.01 [-0.13, 0.15]
>18 years	0%	3	10320	193		0.09 [-0.13, 0.32]

mean difference in SD units

Figure 13. Mean difference in waist circumference, body fat % and fat mass index between IVF and SC offspring

Age Group	I²	N St.	N SC	N IVF		Estimate [95% CI]
waist circumference						
≤5 years	0%	10	26247	620		-0.11 [-0.19, -0.02]
5 to ≤10 years	61.3%	11	70720	2228		-0.16 [-0.27, -0.06]
10 to ≤18 years	44.8%	6	23748	354		-0.08 [-0.22, 0.06]
>18 years	0%	3	10287	190		0.15 [-0.06, 0.37]
body fat %						
≤5 years	0%	3	5463	110		-0.06 [-0.24, 0.13]
5 to ≤10 years	0%	6	17318	213		-0.12 [-0.25, 0.01]
10 to ≤18 years	32.9%	6	15421	255		-0.15 [-0.31, 0.01]
>18 years	20.4%	3	5161	171		0.14 [-0.11, 0.39]
fat mass index						
≤5 years	0%	3	5899	106		-0.10 [-0.27, 0.08]
5 to ≤10 years	36.8%	7	14371	202		-0.24 [-0.42, -0.07]
10 to ≤18 years	36%	6	17551	266		-0.15 [-0.30, -0.00]
>18 years	0%	2	2960	151		0.24 [-0.03, 0.51]

mean difference in SD units

Conclusions

We did not find evidence that conception by IVF increased risk of obesity or higher adiposity between infancy and late adolescence. In general results suggested smaller size, particularly in infancy and early childhood, which is consistent with studies showing that IVF is related to small for gestational age. The smaller size in infancy and childhood attenuated was small at all ages and attenuated with increasing age, suggesting it is unlikely to have important adverse effects and that parents undergoing IVF conception can be reassured. There, was however some evidence of a possible increase in adiposity in early adulthood but this requires replication and assessment in larger sample sizes.

Planned paper 2: Elhakeem A, Taylor A, Inskip H, co-authors, Lawlor DA. Association of assisted reproductive technology with offspring cardio-metabolic health: an individual participant meta-analysis. Currently with lead writing group to prepare draft for subsequent circulation to co-authors.

Abstract
Background: Assisted reproductive technologies (ART), which mainly involve in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI), have seen a rapid uptake in developed countries over recent decades. There is growing concerns that ART may lead to adverse cardiovascular and metabolic health in the offspring. Our aim was to provide evidence from a large multicohort collaboration on the associations between ART conception and offspring cardio-metabolic health.

Methods: We did a two-stage individual participant data meta-analysis of up to 14 population-based cohort studies. The participants were mostly European community-dwelling apparently healthy young people aged from 3 months to 27 years with data on mode of conception, and ≥1 cardio-metabolic outcome measured after birth. The study outcomes were systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, glucose, insulin, and glycated haemoglobin (HbA1c). The main exposure was conception through ART (conventional in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI)) versus spontaneous conception (SC). Linear regression models were used to estimate mean difference and 95% CIs for each offspring cardio-metabolic outcome between ART and SC. Models were adjusted for confounders (maternal age, parity, BMI, smoking, education, and ethnicity) plus offspring sex and age at outcome assessment. To separate out effects of ART from any effects of parental subfertility, we repeated meta-analyses comparing ART to SC offspring of sub-fertile parents (>12-months trying to conceive) and separately to offspring of fertile parents (≤12-months). We explored evidence of sex differences and of ART treatment types by repeating analyses: (i) stratified by sex; (ii) comparing IVF and ICSI each separately to SC; and (iii) comparing fresh embryo transfer (ET) and frozen-thawed embryo transfer (FET) each separately to SC. The robustness of meta-analysis results to influential studies was investigated by a leave-one-out analysis where the meta-analysis models were performed leaving one of the studies out each time. We investigated if offspring and age at outcome assessment (up to 10 or older than 10 years) was a source of between-study heterogeneity using separate meta-regression models. A further assessment of whether effects sizes change with age was done by fitting linear spline multilevel models to repeated outcome measures taken from 3-26 years in offspring from the Avon Longitudinal Study of Parents and Children (ALSPAC). Evidence of change with age was assessed by testing ageXART interaction terms and inspecting the fitted trajectories for ART and SC offspring.

Findings: Between 35,780 (605 ART) and 4,502 (67 ART) participants were included in each meta-analysis. SBP (mean difference in SD units: -0.09SD; 95%CI: -0.21 to 0.03), DBP (-0.09SD; -0.24 to 0.05) and HR (0.00SD; -0.09 to 0.10) were similar in ART and SC offspring. TC (mean % differences: 2.5%; 0.5 to 4.6), HDL-C (4.2%; 1.8 to 6.6), and LDL-C (5.0%; 1.0 to 8.9) were modestly higher in ART compared to SC offspring, whereas triglycerides were more similar (-1.5%; -6.2 to 3.1). No clear differences were observed between ART and SC offspring for glucose (0.3%; -1.4 to 1.9), insulin (-5.0%; -13.2 to 3.1), or HbA1c (-0.07%; -0.14 to 0.0). Growth analysis in up to 7,380 (55 ART) ALSPAC offspring showed that blood pressure increased more rapidly in ART children and became higher by adulthood. For example, predicted SBP was 6.01 mm Hg (4.18 to 7.85) higher in ART compared to SC offspring at age 24.5y.

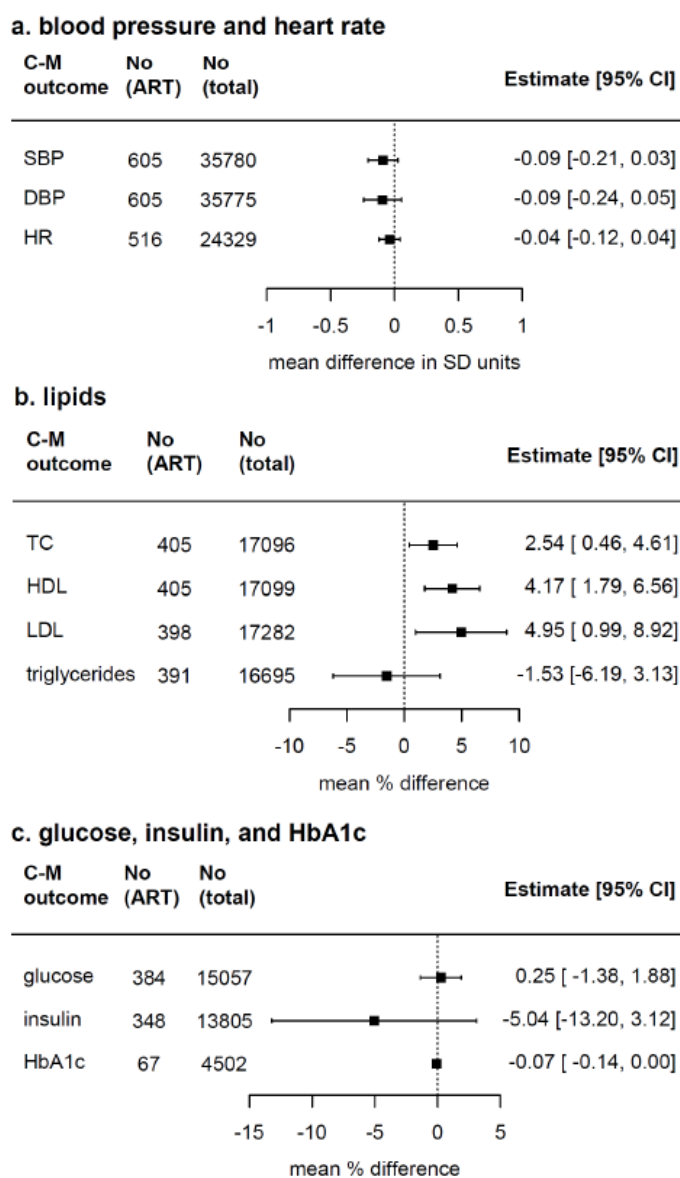**Conclusions:** Findings from this large multicohort study can provide reassurance to ART parents and offspring and prospective ART parents that there are unlikely to be clinically meaningful differences in blood pressure, heart rate, classical lipids, or glucose between their children and those conceived via SC. Studies with longer follow-up are needed to explore how results might change at older ages.

Selected results

Figure 14 shows differences in mean differences in SBP, DBP, HR, lipid and glucose-related measures between ART and SC offspring. Mean SBP, DBP, HR, and glucose related measures were similar in ART and SC offspring, whereas TC, HDL-C, LDL-C but not triglycerides were somewhat higher in ART compared to SC. Additional analyses showed that mean TC and LDL-C (and less so for HDL-C) were somewhat higher in frozen embryo transfer FET ART (vs. SC), as opposed to fresh embryo transfer (ET vs. SC) (**Figure 15**).

Overall numbers were too small to present results by age category. Meta-regression analyses suggested associations did not differ between those <10 and >= 10 years at outcome assessment. Growth modelling in ALSPAC (N =7,380 (ART=55) showed faster rises in SBP and DBP in ART (versus SC), and similar HR trajectories in both groups (**Figure 16**).

Figure 14: Mean differences in cardio-metabolic outcomes between ART and SC offspring

a. blood pressure and heart rate

C-M outcome	No (ART)	No (total)	Estimate [95% CI]
SBP	605	35780	-0.09 [-0.21, 0.03]
DBP	605	35775	-0.09 [-0.24, 0.05]
HR	516	24329	-0.04 [-0.12, 0.04]

mean difference in SD units

b. lipids

C-M outcome	No (ART)	No (total)	Estimate [95% CI]
TC	405	17096	2.54 [0.46, 4.61]
HDL	405	17099	4.17 [1.79, 6.56]
LDL	398	17282	4.95 [0.99, 8.92]
triglycerides	391	16695	-1.53 [-6.19, 3.13]

mean % difference

c. glucose, insulin, and HbA1c

C-M outcome	No (ART)	No (total)	Estimate [95% CI]
glucose	384	15057	0.25 [-1.38, 1.88]
insulin	348	13805	-5.04 [-13.20, 3.12]
HbA1c	67	4502	-0.07 [-0.14, 0.00]

mean % difference

Figure 15: Mean difference in TC, HDL, and LDL between ART and SC offspring, stratified by sex, sub-fertility, IVF/ICSI, and ET/FET

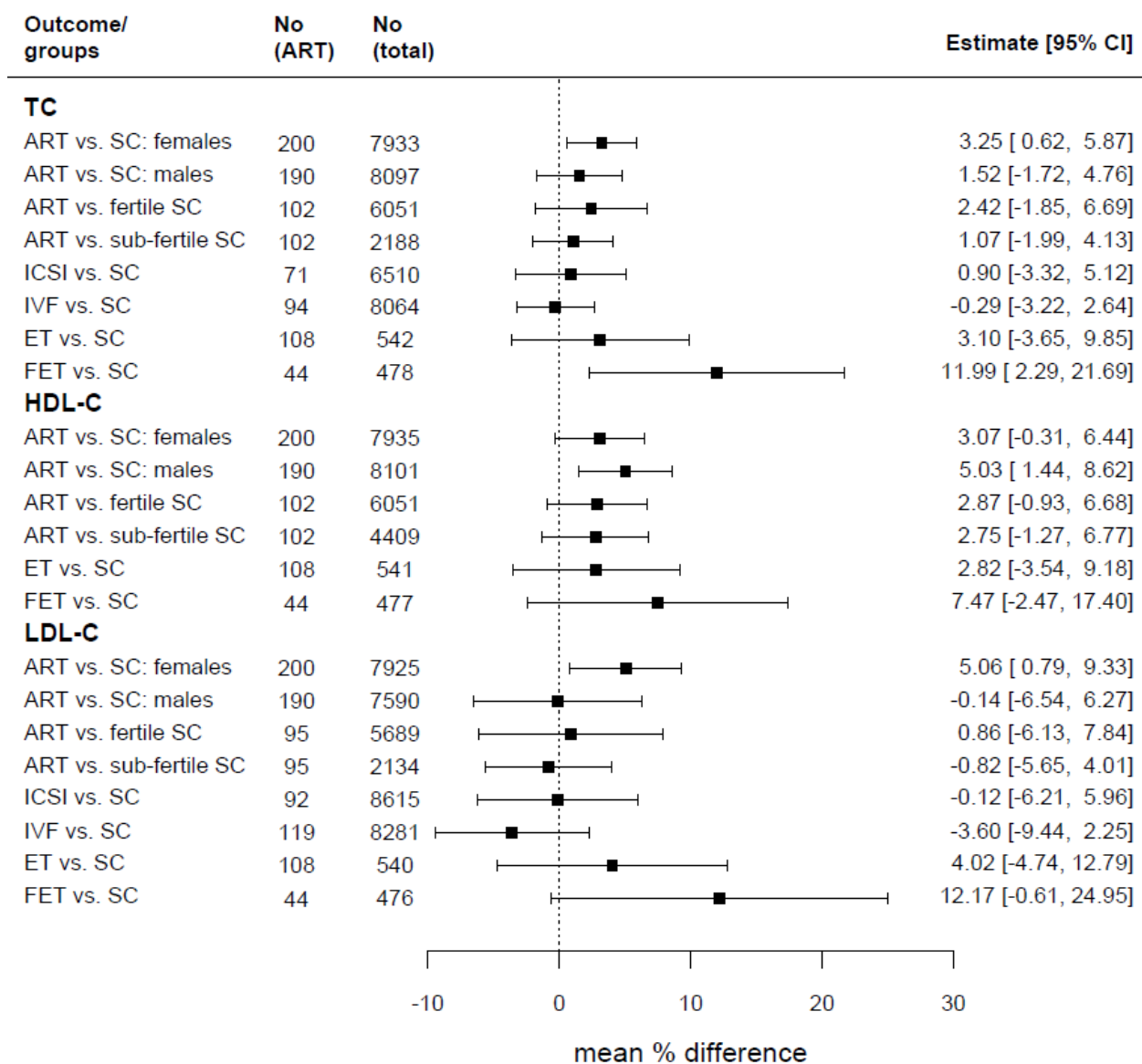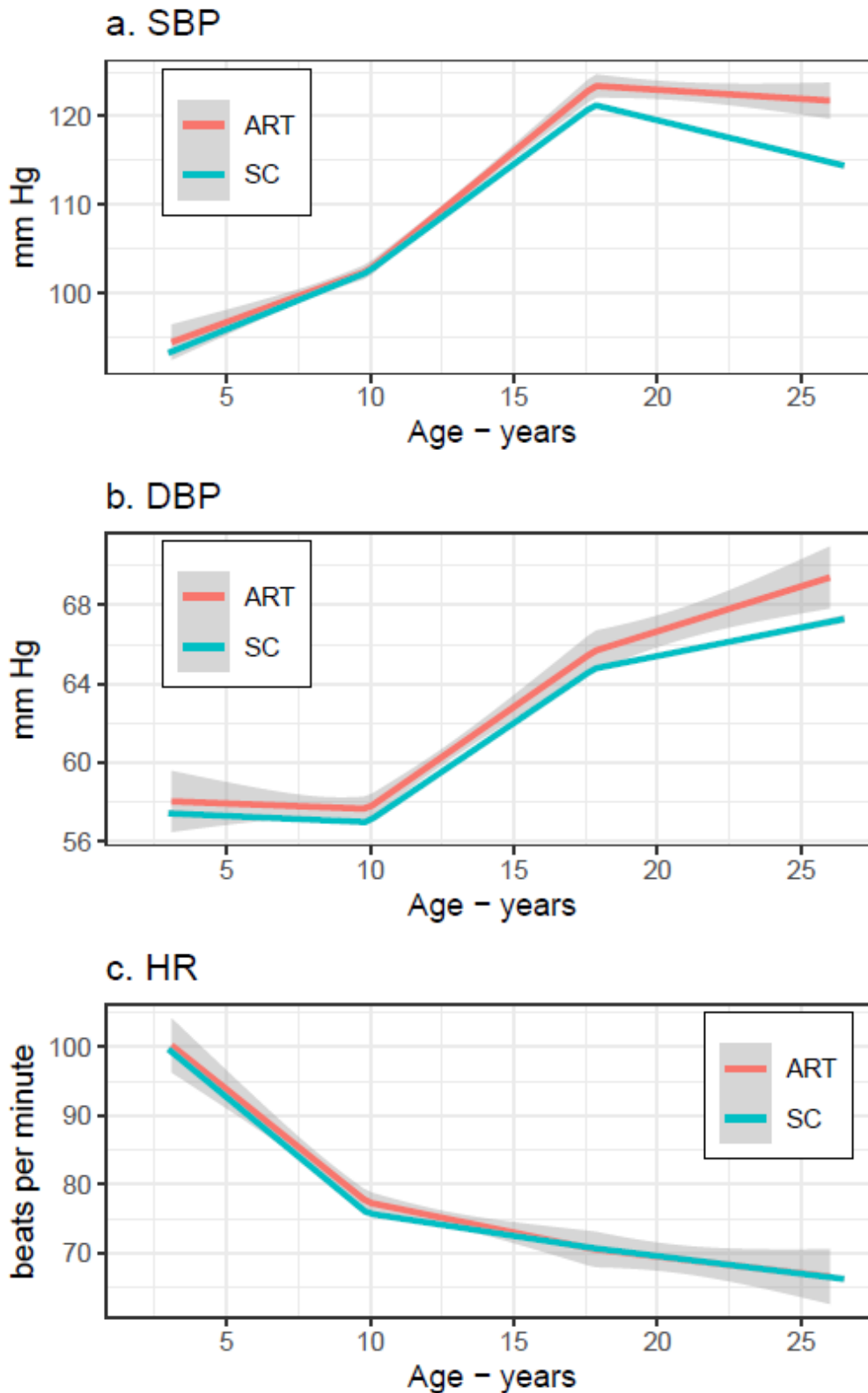| Outcome/ groups	No (ART)	No (total)	Estimate [95% CI]
TC			
ART vs. SC: females	200	7933	3.25 [0.62, 5.87]
ART vs. SC: males	190	8097	1.52 [-1.72, 4.76]
ART vs. fertile SC	102	6051	2.42 [-1.85, 6.69]
ART vs. sub-fertile SC	102	2188	1.07 [-1.99, 4.13]
ICSI vs. SC	71	6510	0.90 [-3.32, 5.12]
IVF vs. SC	94	8064	-0.29 [-3.22, 2.64]
ET vs. SC	108	542	3.10 [-3.65, 9.85]
FET vs. SC	44	478	11.99 [2.29, 21.69]
HDL-C			
ART vs. SC: females	200	7935	3.07 [-0.31, 6.44]
ART vs. SC: males	190	8101	5.03 [1.44, 8.62]
ART vs. fertile SC	102	6051	2.87 [-0.93, 6.68]
ART vs. sub-fertile SC	102	4409	2.75 [-1.27, 6.77]
ET vs. SC	108	541	2.82 [-3.54, 9.18]
FET vs. SC	44	477	7.47 [-2.47, 17.40]
LDL-C			
ART vs. SC: females	200	7925	5.06 [0.79, 9.33]
ART vs. SC: males	190	7590	-0.14 [-6.54, 6.27]
ART vs. fertile SC	95	5689	0.86 [-6.13, 7.84]
ART vs. sub-fertile SC	95	2134	-0.82 [-5.65, 4.01]
ICSI vs. SC	92	8615	-0.12 [-6.21, 5.96]
IVF vs. SC	119	8281	-3.60 [-9.44, 2.25]
ET vs. SC	108	540	4.02 [-4.74, 12.79]
FET vs. SC	44	476	12.17 [-0.61, 24.95]

mean % difference

29

Figure 16: Life-course blood pressure and heart rate trajectories for ART and SC offspring from the ALSPAC cohort

Report on the relationships of early-life exposures with trajectories of cardiovascular and metabolic
risk factors from birth to adulthood
Work package 4- Task 4.2 – Deliverable 4.2
Version Final (20/07/2021)

Conclusions and future plans for this work

Overall, neither study suggest concern in terms of ART resulting in greater adiposity or adverse cardio-vascular / - metabolic health across childhood and early adolescence. Higher levels of total cholesterol, HDL-C and LDL-C of 2.5 to 5 % warrant further investigation in larger cohorts.

This LifeCycle research was central to the successful award of an ERC-AG grant (ART-HEALTH), which will support larger collaborations, including national record linkage studies from several countries, to further explore the associations of ART on future offspring and maternal health, determine whether any associations are causal, for example using within sibship comparisons and any possible mechanisms using Mendelian randomization.

The relationship of gestational age at birth with body mass index

Vinther LV, Cadman T, co-authors, Nybo Anderson AM. Association of gestational age at birth with body size from early infancy to adulthood: a multi-cohort study from the EU Child Cohort Network in up to 157,880 individuals using federated analysis in DataSHIELD. Currently, initial feedback from co-authors being incorporated into the draft and possible additional analyses.

Summary

Aim

To determine the nature of the associations between gestational age and BMI from early infancy to late adolescence.

Methods and Findings

We used a federated analysis approach to perform Study-Level Meta-Analysis with linear and logistic regression models on up to 157,800 individuals from up to 12 cohorts (numbers varied by age category). Cohorts were from countries in Europe, North America and Australasia. Outcomes of interest were standardized mean BMI and overweight at five age-periods (early infancy >0-2 years, early childhood >2-4 years, adiposity rebound >4-9 years, puberty >9-14 years, and adolescence >14-18 years) representing key developmental periods of growth. Results suggested that gestational age was positively associated with BMI but with associations attenuating towards the null across the five age categories. Differences in mean BMI SDs per 1 completed week longer gestation (95%CI) across the five categories were: 0.07 (0.04, 0.09), 0.03 (0.02, 0.04), 0.02 (0.01, 0.03), 0.01 (-0.04, 0.02) and 0.00 (-0.02, 0.02), respectively.

Conclusions

Modest positive associations of longer gestation with higher mean BMI in infancy attenuate monotonically across age categories up to adolescence. These suggest that variation in gestational length may not be an important determinant of subsequent adiposity. However, it is important to note that difference cohorts contributed to different age categories, meaning that these may not directly comparable. Further analyses are required to explore between study heterogeneity in each age category and the potential effect of selection bias due to missing data.

Selected results

Figure 17 shows the difference in mean BMI per 1 completed week of gestation within each age category. This shows that a modest association of a 0.07 SD mean difference in BMI per 1 completed greater week of gestation (95% CI: 0.04, 0.09) in early infancy attenuates monotonically across the five increasing age categories to a point estimate of zero in adolescence. **Figure 18** shows the odds ratios of offspring overweight/obesity for a 1 week longer completed week of gestation, suggesting weak increases in odds of overweight/obesity with longer gestational duration in early infancy, but at most other ages the point estimates being close to the null. Both figures also illustrates that different cohorts

contribute to different age categories and so these may not be directly comparable. Furthermore, there appears to be between study differences in the associations within some of the age categories.

Conclusions, further analyses and plans for this work
These preliminary results suggest weak/modest associations of longer gestation with higher BMI in early infancy that attenuate with increasing age.

Additional analyses are being undertaken to explore between study heterogeneity, possible sex differences in associations and the possible influence of different studies and differential missing data on the differences in associations by age.
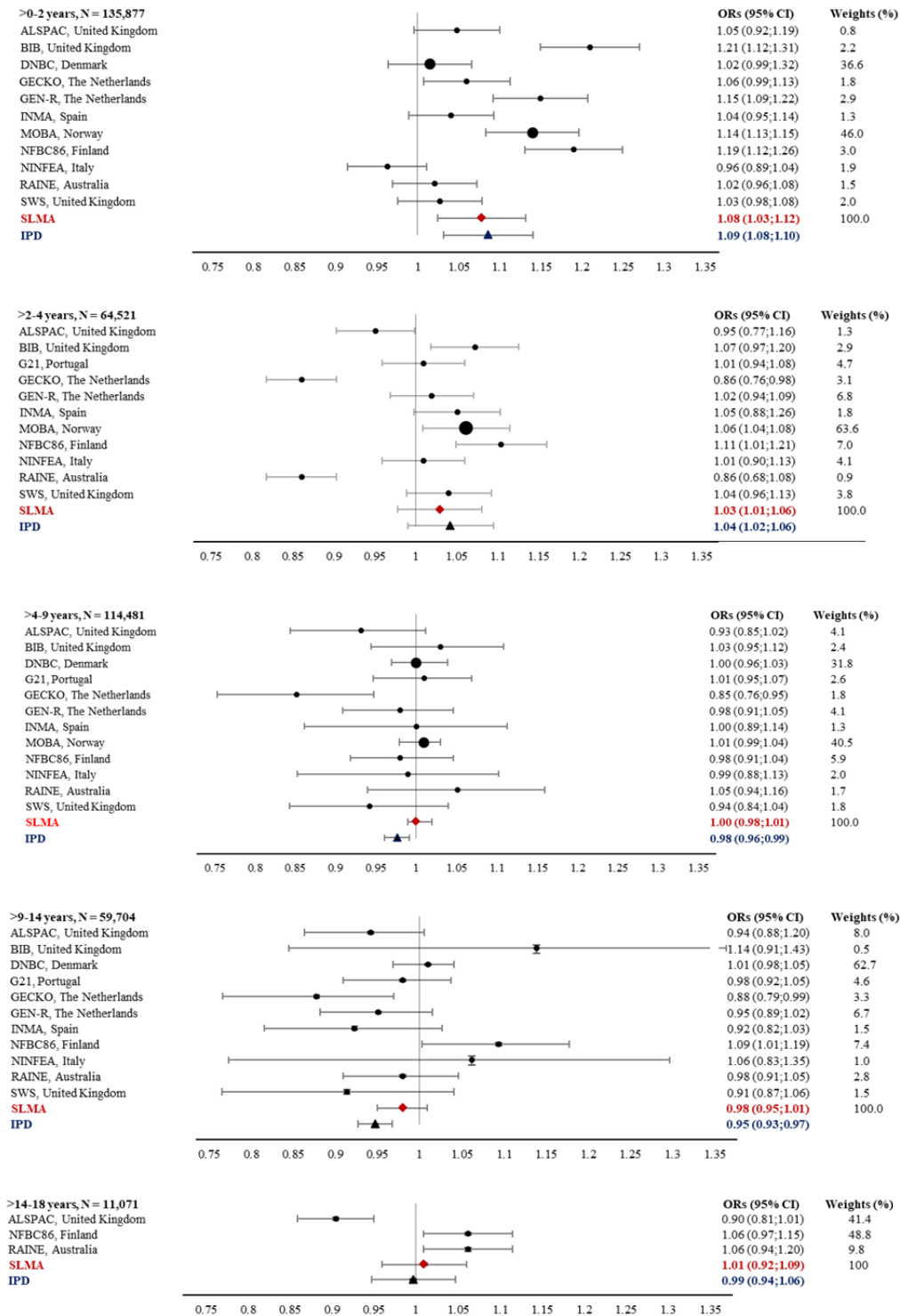
Figure 17: Associations of completed weeks of gestation with Body Mass Index at different ages
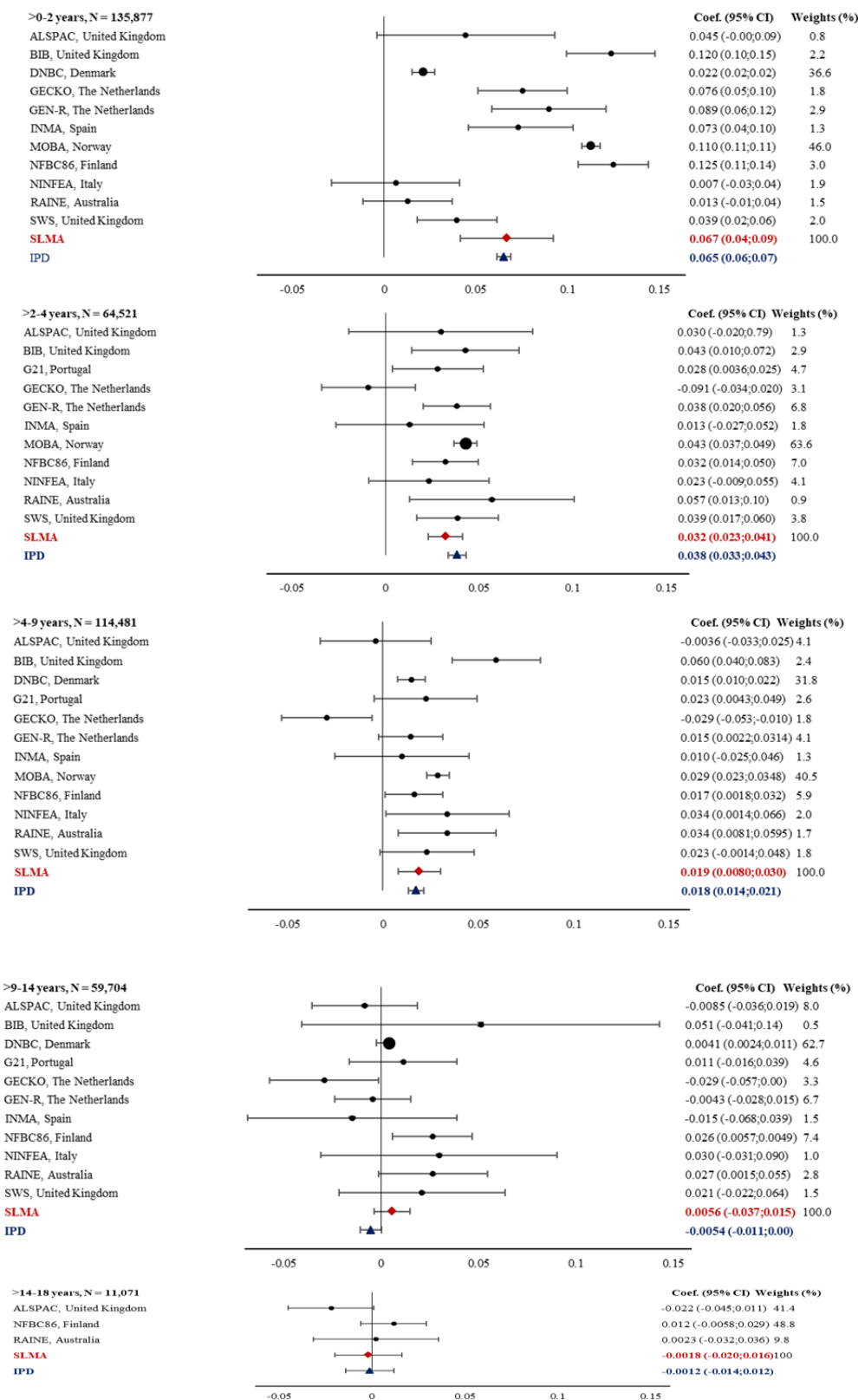
Associations are adjusted for maternal age, educational, pre-pregnancy BMI, smoking and parity

Figure 18: Associations of completed weeks of gestation with overweight/obesity at different ages

Associations of different early life exposures on offspring body mass index

Cadman T, Elhakeem A, co-authors, Lawlor DA. Associations of early life socioeconomic position, residential proximity to green space and gestational diabetes with offspring Body Mass index – preliminary results from federated (DataShield analyses) with up to 12 cohorts and 152,978 participants. Currently, a draft of the paper produced by Cadman with input from Elhakeem and Lawlor is being finalized, including with the addition of data from three additional cohorts, before circulating to all co-authors for comments.

Summary

Aim To explore the associations of exposures reflecting different domains of early life stressors (i.e. socioeconomic, environmental and pathophysiological) with offspring body mass index from childhood to adolescence using a federated data analysis approach.

Methods and results LifeCycle cohorts were eligible if they had at least one of the early life exposures and offspring weight and height (to calculate BMI) measured on at least one occasion after birth. The cohorts had to have these data harmonised according to the LifeCycle harmonisation procedures and to be accessible for analyse via DataShield. The exposures explored were selected to reflect different domains and exposures that have been hypothesised to influence future offspring adiposity: (i) maternal educational attainment at the time of pregnancy (individual family socioeconomic position indicator); (ii) residential neighbourhood deprivation (contextual socioeconomic indicator); (iii) residential proximity to green space (assessed by the Normalised Density Vegetation Index (NDVI) during the mothers pregnancy or offspring's first year of life (environmental indicator); and (iv) gestational diabetes (a pregnancy complication/pathology). A federated analysis approach was used to perform one-stage IPD meta-analysis was undertaken using DataShield. Analyses were undertaken in five age categories: >0 to 24 months, 25 to 48 months, 49 to 96 months, > 97 to 168 months and 169 to 215 months. Preliminary results were undertaken on up to 12 cohorts and 152,978 participants. A dose response (from high, medium to low) association of lower maternal education relating to higher mean BMI was observed from age 49 to 96 months. A similar pattern of association was observed for residential area deprivation. There was also evidence that exposure to maternal gestational diabetes was associated with higher mean BMI in the oldest age group for which results are available in the current analysis (>8-13 years) but not younger ages. There was no strong evidence of early life proximity to green space relating to offspring BMI at any age.
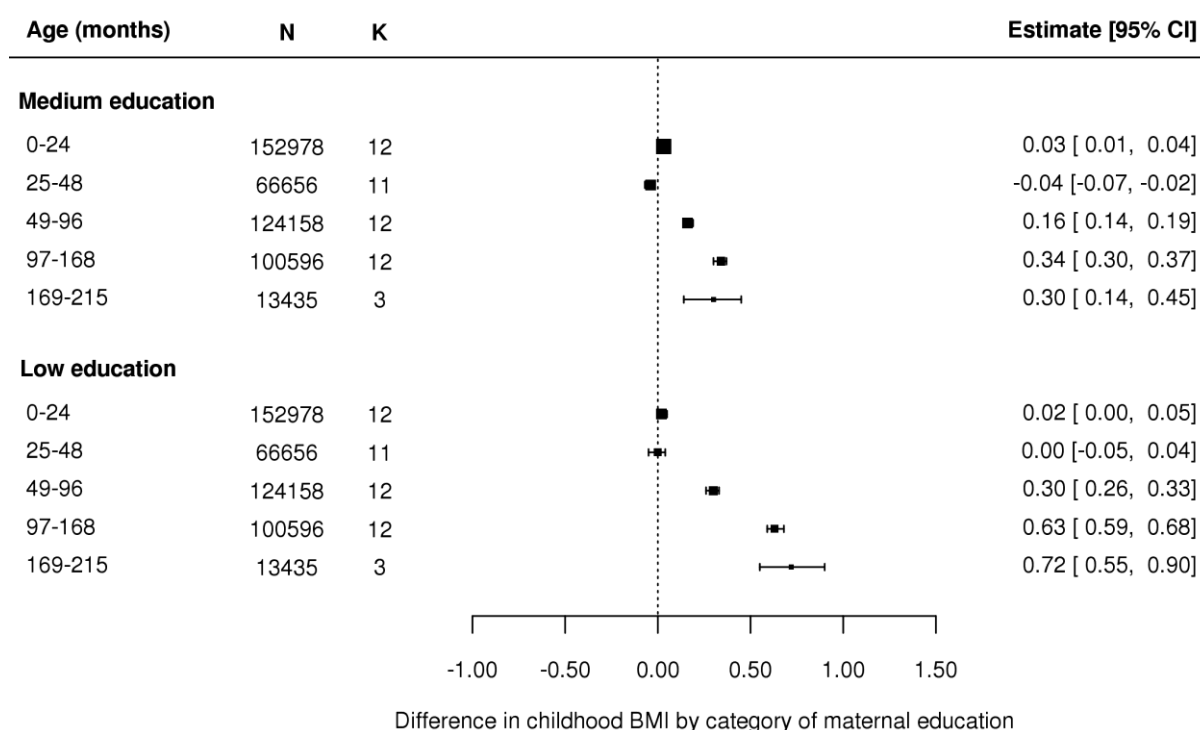
Selected results

Results presented here were undertaken on up to 12 cohorts and 152,978 participants depending on the exposure and age category. These results show a dose response (from high, medium to low) association of lower maternal education and higher family residential area deprivation with higher mean BMI from age 49 to 96 months, with differences before that age being very close to the null (**Figure 19 and figure 20**, respectively). There was some suggestion of a weak inverse association between proximity to more green space and BMI in early childhood (0-24 and 25-48 months), though with wide confidence intervals that included the null (**Figure 21**). Lastly, we find some evidence that exposure to maternal gestational diabetes is associated with higher BMI in the in late childhood/early adolescence (i.e. 97 to 168 months the oldest age group for which we had sufficient data to examine this association (**Figure 22**). This has some consistency with previous work showing an association with higher birth size that then appears null in infancy and earlier childhood to re-emerge later in childhood, though here we have not examined birth size.
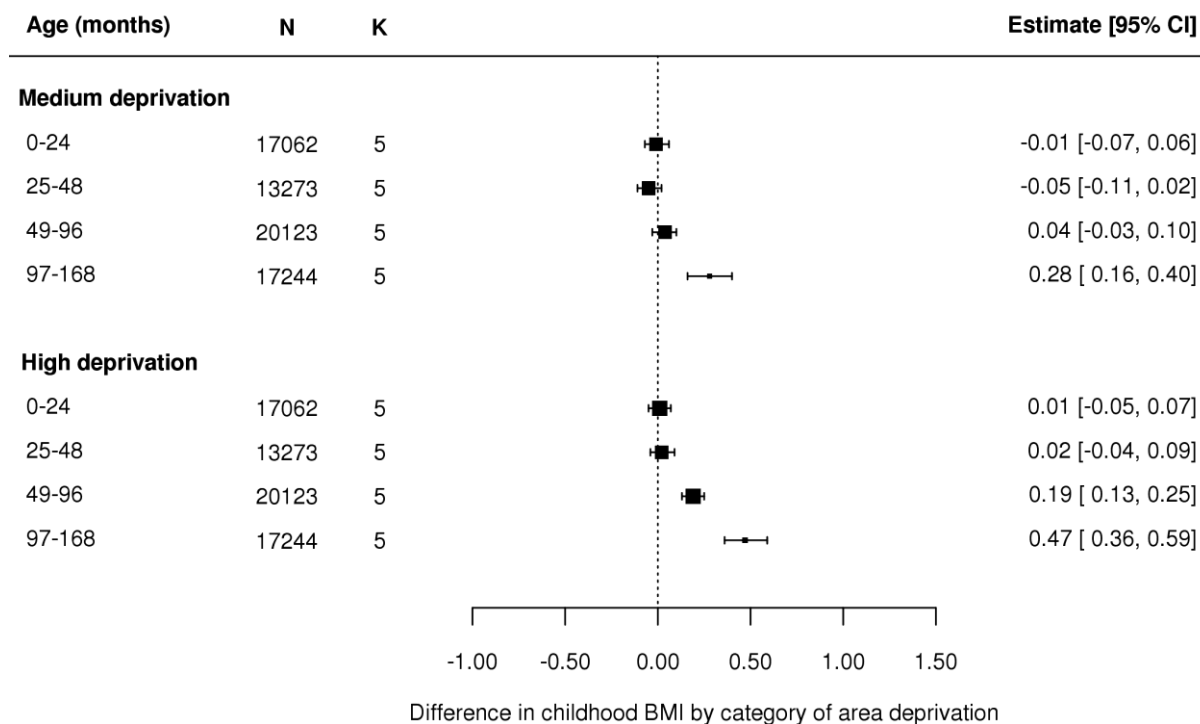
Conclusions

These preliminary results show the potential for federated analyses using DataShield to efficiently complete analyses across many cohorts. They suggest lower education, higher residential area deprivation and gestational diabetes are associated with higher BMI in later childhood/early. Proximity to greenspace in early life does not appear to influence later BMI. Results will be updated in the next 2-3 months with the addition of data from thee additional cohorts.

Figure 19: Associations between maternal education at birth and child BMI (reference category = high education

Age (months)	N	K	Estimate [95% CI]
Medium education			
0-24	152978	12	0.03 [0.01, 0.04]
25-48	66656	11	-0.04 [-0.07, -0.02]
49-96	124158	12	0.16 [0.14, 0.19]
97-168	100596	12	0.34 [0.30, 0.37]
169-215	13435	3	0.30 [0.14, 0.45]
Low education			
0-24	152978	12	0.02 [0.00, 0.05]
25-48	66656	11	0.00 [-0.05, 0.04]
49-96	124158	12	0.30 [0.26, 0.33]
97-168	100596	12	0.63 [0.59, 0.68]
169-215	13435	3	0.72 [0.55, 0.90]

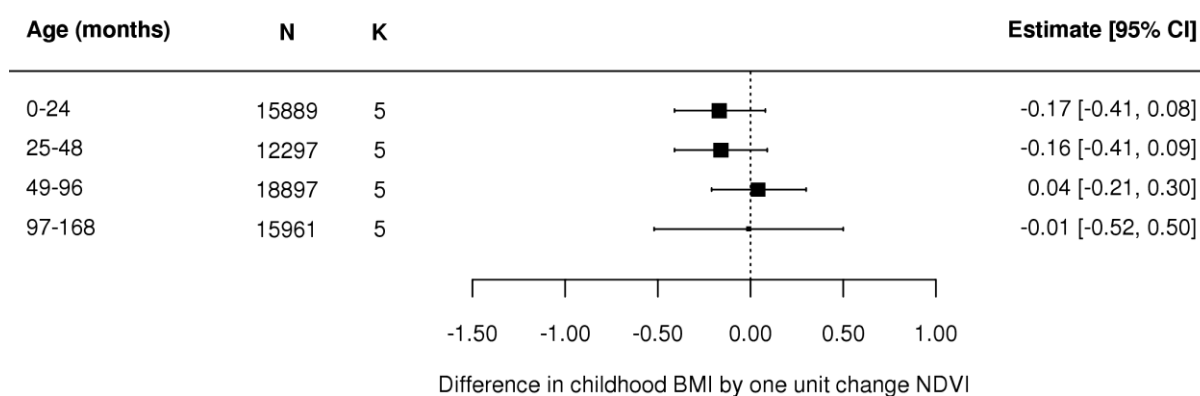Difference in childhood BMI by category of maternal education

N = number of participants; K = number of studies

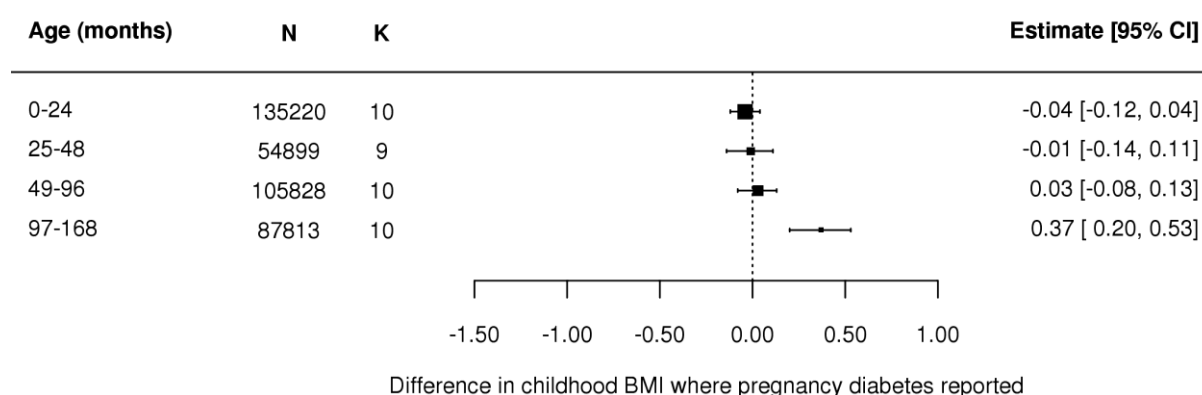## Figure 20: Associations between area deprivation in pregnancy and child BMI (reference category = low deprivation)

Age (months)	N	K		Estimate [95% CI]
Medium deprivation				
0-24	17062	5		-0.01 [-0.07, 0.06]
25-48	13273	5		-0.05 [-0.11, 0.02]
49-96	20123	5		0.04 [-0.03, 0.10]
97-168	17244	5		0.28 [0.16, 0.40]
High deprivation				
0-24	17062	5		0.01 [-0.05, 0.07]
25-48	13273	5		0.02 [-0.04, 0.09]
49-96	20123	5		0.19 [0.13, 0.25]
97-168	17244	5		0.47 [0.36, 0.59]

Difference in childhood BMI by category of area deprivation

N = number of participants; K = number of studies

Figure 21: Associations between exposure to green space (NDVI) in pregnancy and child BMI

Age (months)	N	K		Estimate [95% CI]
0-24	15889	5		-0.17 [-0.41, 0.08]
25-48	12297	5		-0.16 [-0.41, 0.09]
49-96	18897	5		0.04 [-0.21, 0.30]
97-168	15961	5		-0.01 [-0.52, 0.50]

Difference in childhood BMI by one unit change NDVI

N = number of participants; K = number of studies

37

Figure 22: Associations between gestational diabetes and child BMI

Age (months)	N	K	Estimate [95% CI]
0-24	135220	10	-0.04 [-0.12, 0.04]
25-48	54899	9	-0.01 [-0.14, 0.11]
49-96	105828	10	0.03 [-0.08, 0.13]
97-168	87813	10	0.37 [0.20, 0.53]

Difference in childhood BMI where pregnancy diabetes reported

N = number of participants; K = number of studies

2.1.3 Full list of published papers that have contributed to this deliverable

1. Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Ellis Voerman E, Felix JF, Kate Tilling KT, Lawlor DA. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. *PLoS Medicine* 2019, 16(11): e1002972. doi: 10.1371/journal.pmwd.1002972.
2. Brand JS, West J, Tuffnell D, Bird PK, Wright J, Tilling K, Lawlor DA. Gestational diabetes and ultrasound assessed fetal growth in South Asian and White European women: findings from a prospective pregnancy cohort. *BMC Medicine* 2018; **16**:203. doi: 10.1186/s12916-018-1191-7
3. Voerman E, Santos S, Inskip H, [59 additional co-authors] Godfrey K, Jaddoe VWV, Gaillard R. Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. *JAMA* 2019; 321:1702-1715
4. Voerman E, Santos S, Golab BP, [75 additional co-authors] Lawlor DA, Gaillard R, Jaddoe VWV. Maternal body mass index, gestational weight gain and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Medicine* 2019; **11**;16(2). doi: 10.1371/journal.pmed.1002744
5. Santos S, Voerman E, Amiano P, [77 additional co-authors] Lawlor DA, Gaillard R, Jaddoe Vwv. Impact of maternal body mass index and gestational weight gain on pregnancy complications: An individual participant data meta-analysis of European, North American and Australian cohorts. *British Journal of Obstetrics and Gynaecology* 2019;**126**:984-995. doi: 10.1111/1471-0528.15661
6. Warrington NM, Beaumont RN, Horikoshi M, [of 207 additional co-authors], Perry JRB, Evans DM, Freathy RM. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. *Nature Genetics* 2019; **51**:s804-814. doi: 10.1038/s41588-019-0403-1
7. Richmond RC, Timpson NJ, Felix JF, Palmer T, Gaillard R, McMahon G, Davey Smith G, Jaddoe VW, Lawlor DA. Using genetic variation to explore the causal effect of maternal pregnancy adiposity on future offspring adiposity. *PLoS Medicine* 2017 14(1): e1002221. doi:10.1371/ journal.pmed.1002221
8. Bond TA, Karhunen V, Wielscher M, Auvinen J, Männikkö M, Keinänen-Kiukaanniemi S, Gunter MJ, Felix JF, Prokopenko I, Yang J, Visscher PM, Evans DM, Sebert S, Lewin A, O'Reilly PF, Lawlor DA, Jarvelin M-R. Exploring the role of genetic confounding in the association between maternal and

offspring body mass index: evidence from three birth cohorts. *Int J Epidemiol*. 2019;doi: 10.1093/ije/dyz095

9. Bond TA, Richmond RC, Karhunen V, Cuellar-Partida G, Borges M-C, Zuber V, Couto Alves A, Mason D, Yang TC, Gunter MJ, Dehghan A, Tzoulaki I, Sebert S, Evans DM, Lewin AM, O'Reilly PF, Lawlor DA, Järvelin M-R. Exploring the causal effect of maternal pregnancy adiposity on offspring adiposity: Mendelian randomization using polygenic risk scores. *MedRxiv* 2021; doi: 10.1101/2021.04.01.21251414.

3. Conclusions

Published research for this deliverable has triangulated results from conventional multivariable regression, Mendelian randomization and paternal negative control analyses to show that smoking relates to slower growth of fetal head and abdominal circumference, femur length and estimated weight across gestation. Women who quit smoking in early pregnancy have fetal growth patterns similar to those who never started and there is a dose-response effect in those who continue to smoke. Fetuses of South Asian origin start smaller on all parameters and remain smaller throughout pregnancy. Those whose mothers are subsequently diagnosed with gestational diabetes have different growth patterns to those who are not, with similar patterns in South Asian and White European women. Taken together this results in fetuses of South Asian origin who have been exposed to gestational diabetes having almost identical fetal growth trajectories to those of White European origin whose mothers do not have gestational diabetes. We are extending this work by adding postnatal growth to the fetal growth trajectories to explore whether fetal growth 'sets' the pattern for postnatal growth and to explore the impact of pre-conceptual and intrauterine exposures on fetal through postnatal growth.

In a series of studies we have shown that maternal pre-/early-pregnancy BMI and gestational weight gain are positively associated with offspring birth size and subsequent increased risk of overweight and obesity across childhood and into adolescence, with stronger associations and predictive ability for maternal BMI than gestational weight. However, Mendelian randomization studies suggest that these associations are mostly explained by residual confounding. Maternal pre-conception and early pregnancy BMI may indicate which children are at greater risk of overweight/obesity but is not an important causal factor. Interventions to attain healthy weight need to target all family members / whole populations.

A novel approach to separating maternal and fetal genetic contributions to birth weight has enabled Mendelian randomization to further explore potential causal effects of the intrauterine environment on birth weight and subsequent cardiovascular/metabolic outcomes that have been related to lower birth weight. These analyses suggest the previously reported associations are unlikely to be causal. For example, the association of lower birth weight with higher blood pressure (one of the initial drivers of the developmental origins of health and disease) appears to be due to confounding, whereby maternal higher blood pressure results in lower birth weight and genetic (and environmental) predisposition to higher blood pressure in the mother is passed to the offspring, thus resulting in a non-causal link between lower birth weight and higher blood pressure.

In on-going research for which we present preliminary results, we find that conception by IVF/ICSI (a profound pre-conception stressor) does not seem to increase adiposity, blood pressure glycaemic traits or triglycerides across infancy and childhood. In early infancy and childhood there is evidence of lower

BMI, particularly with fresh embryo transfer. These are reassuring findings given narrative reviews and commentaries that have suggested conception by IVF/ICSI may increase cardiovascular risk. We did identify some evidence of higher total, LDLc, and HDLc levels and the possibility that BMI may be higher in adulthood. These require further replication in larger studies, which we aim to do in the recently funded ERC Advanced Grant ART-HEALTH, which builds on the success of the LifeCycle collaboration.

In further on-going work, preliminary analyses suggest that a longer gestation may increase offspring BMI in infancy, though the magnitude of the association is modest and with increasing age monotonically attenuates to the null. We also find that lower maternal education, higher residential area deprivation and exposure to maternal gestational diabetes in utero are associated with higher mean BMI in late childhood and early adolescence but not at earlier ages. By contrast early life residential proximity to green space does not appear to be associated with offspring BMI.

This work on gestational age, and maternal education, gestational diabetes and residential proximity to greenspace have been undertaken in DataShield using harmonised LifeCycle data. Preparing and harmonising the data needed for these analyses and developing code to run analyses in DataShield has been a major achievement of WP1 and 2, and for this task in particular, by the hard work of Tim Cadman and Johan Lerbech Vinther.

Preliminary results from the on-going research may change with additional data and analyses.