# Title: Integrating multiple lines of evidence to assess the effects of maternal BMI on pregnancy and perinatal outcomes

# Motivation and aim

Higher maternal pre-pregnancy body mass index (BMI) is associated with adverse pregnancy and perinatal outcomes. However, which of these associations are causal remains unclear. To explore the relation of maternal pre-pregnancy BMI with pregnancy and perinatal outcomes by integrating evidence from three different methods (i.e. multivariable regression, Mendelian randomization, and paternal negative control).

# Inclusion/exclusion criteria

The following exclusions will be made:

* Twins (or any multiple births)
* Known congenital anomalies
* Non-European ancestry [Note: for the main analysis, we will focus solely on European origin participants.]

# Exposure: maternal BMI

Maternal BMI in kg/m2 was calculated from measured or self-reported weight and height data. Weight data was collected before pregnancy in nine studies, before 20 weeks of gestation in two studies, and between 24 and 32 weeks of gestation in one study. Two studies did not have a measure of pre- or early-pregnancy BMI and could only contribute to the Mendelian randomization analyses.

# Outcomes

The following pregnancy and perinatal outcomes will be assessed:

## Continuous Outcomes (each converted to Z-score for analysis)

* Level of postpartum depression (see box 2 for definition of this variable)
* Offspring birthweight (g, converted to Z-score)
* Offspring birth length (cm, converted to Z-score)
* Offspring ponderal index (kg/m3 converted to z-score) (see box 2 for calculation of this variable)
* Gestational age at birth (weeks, converted to Z-score)

**Box 1: Transformation of variable to Z-score**

Z-score = [individual\_value – sample\_mean] / sample\_standard\_deviation

Please note for all variables that we request to convert to Z-scores this should be within your cohort (sample) and NOT standardised on any other variables (i.e. not standardised on age or gestational age)

## Binary Outcomes

* Preeclampsia (see box 2 for definition of this variable)
* Hypertensive disorder of pregnancy (see box 2 for definition of this variable)
* Gestational hypertension (see box 2 for definition of this variable)
* Caesarean delivery (binary: no/yes)
* Induction of labour (binary: no/yes)
* Stillbirth (binary: no/yes)
* Gestational diabetes (see box 2 for definition of this variable)
* Membrane rupture before onset of contractions (binary: no/yes)
* Postpartum depression (see box 2 for definition of this variable)
* Ever breastfed child (binary: yes/no)
* Postnatal anaemia (see box 2 for definition of this variable)
* Offspring low birthweight (<2500g)
* Macrosomia (≥4000g)
* Preterm birth (gestational age <37 completed weeks of gestation)
* Small-for-gestational age (based on within study gestational age and sex standardised z-scores and then defined as being <10th percentile in the study cohort)
* Large-for-gestational age (gestational age adjusted birth weight >90th percentile for sex and gestational age in the study cohort)
* Low Apgar score at 1 minute (low score defined as <7)
* Low Apgar score at 5 minutes (low score defined as <7)
* Admission to neonatal intensive care unit within the first week of life (binary: no/yes)

**Box 2: Definitions of required variables**

***Level of postnatal depression (continuous):***

Level of postpartum depression as a continuous score from any recognised depression/depression symptom scale (e.g. Edinburgh Postnatal Depression Score, Becks, and Hamilton). Use a measure as soon after the birth as possible up to 12-months (if you only have measurements after 12-months postnatal we will not include your data for this outcome) in within study z-score units. Information on the scale used to measure depression and the time from birth (in weeks or months) that the assessment was made will be reported.

***Postnatal depression (binary):***

Please use the scale used for the continuous measure of postnatal depression (above) and use the recommended cut off values for each measure. Cut off values for commonly used depression scales are:

Edinburgh Postnatal Depression Score: ≥13

Beck: ≥10

Hamilton: ≥12

If you are using a different measure, please get in contact stating the measure that you do have and we will provide you with a cut-off value to use.

Additionally, if your study does not have data from a recognised depression/depression symptom scale binary measures from GP records or self-report of a GPs diagnosis of depression will be sufficient for this variable. Use a measure as soon after the birth as possible up to 12-months

Information on the scale used to measure depression and the time from birth (in weeks or months) that the assessment was made will be reported.

***Calculation of ponderal index:***

Birthweight in kg / (birth length in m)3

(Values are normally between about 20 and 40 kg/m 3)

***Gestational diabetes:***

Defined as hyperglycaemia first diagnosed in pregnancy. As with hypertensive disorders of pregnancy (below) we recognise that different studies will have had different criteria applied for this diagnosis and different levels of detail. We will include any diagnosis based on fasting, oral glucose tolerance or oral glucose challenge test data, self-report of a diagnosis of gestational diabetes or abstraction of a diagnosis from medical records. Information on how the diagnosis was made, what thresholds of fasting and postload glucose were applied, the timing of any blood tests / challenge or tolerance tests in gestational weeks or for self-report how the question was asked and at what time (i.e. during pregnancy or post-natal) or for medical record abstraction who did the abstraction will be reported.

***Postnatal anaemia:***

Defined as a low haemoglobin up to 10 weeks after birth. If you have more than one measure/report please use the one nearest to birth. If you have actual measurements of haemoglobin then please apply these thresholds: <6.8mmol/l (<110g/L) for measures within the first 4-weeks after birth and < 7.5mmo/l (<120g/L) for measures between 4 to 10-weeks. In addition to cases defined by haemoglobin measurements that researchers have access to, self-report of being told they were anaemic within the first 10 weeks after birth or a diagnosis within the first 10 weeks after birth in the medical records are acceptable as a case will be reported.

**Box 2: Definitions of required variables continued…**

***DEFINITION OF HYPERTENSIVE DISORDERS OF PREGNANCY***

We recognise that there are different diagnostic criteria for hypertensive disorders of pregnancy (HDP) in different countries and that criteria have changed over time. We also recognise that different studies will have different levels and detail of data and definition. For example, in ALSPAC we have every repeat measure of blood pressure and proteinuria in pregnancy for participants and can apply any criteria to these (we have applied the International Society for the Study of Hypertension in Pregnancy criteria (ISSHP), which defines any HDP as SBP ≥140mmHg or DBP ≥90mmHg, measured on two occasions after 20 weeks gestation, with those who are then defined as having pre-eclampsia also having proteinuria (with the raised blood pressure) of at least 30g/Dl (equivalent of 1+ on most dip stix) and those defined as having gestational hypertension being those who do not meet criteria for pre-eclampsia. By contrast, in Born in Bradford we have a diagnosis extracted from medical records and cannot be certain how that was defined.

We want to be as inclusive as possible and so will apply the following criteria:

***Hypertensive disorder of pregnancy (HDP)***: Any woman with elevated blood pressure (defined by any criteria, but if researchers have measures of BP please apply the ISSHP criteria as above) on at least two occasions after 20 weeks of gestation; who self-reports being told that she had pre-eclampsia, pregnancy induced hypertension, gestational hypertension or hypertensive disorder of pregnancy; or any woman with a diagnosis of hypertensive disorder of pregnancy, pre-eclampsia, pregnancy induced hypertension or gestational hypertension extracted from medical records

***Pre-eclampsia (PE):*** Any woman with elevated blood pressure (defined by any criteria, but if researchers have measures of BP please apply the ISSHP criteria as above) on at least two occasions after 20 weeks of gestation together with proteinuria (at least 30g/Dl or 1+ on dip stix); who self-reports being told she had pre-eclampsia; or who has a diagnosis of pre-eclampsia extracted from medical records

***Gestational hypertension (GH)***: Any woman with elevated blood pressure (defined by any criteria, but if researchers have measures of BP please apply the ISSHP criteria as above) on at least two occasions after 20 weeks of gestation without proteinuria; or who self-reports being told they had pregnancy induced hypertension, gestational hypertension or hypertensive disorder of pregnancy; or any woman with a diagnosis of hypertensive disorder of pregnancy, pregnancy induced hypertension or gestational hypertension extracted from medical record.

***Controls for analyses with hypertensive disorders of pregnancy:***

For HDP – the control group are all other women who do not meet the criteria for HDP (i.e. women without PE and GH)

For PE – the control group are all other women without PE (i.e. women with GH are included as controls along with those who have neither PE or GH)

For GH – the control group are all women who do not meet the criteria for HDP (i.e. women with PE are excluded from these analyses)

Information on how HDP, PE and GH were defined and where data were obtained from, including for self-report how the question was asked and at what time (during pregnancy or post-natal it was asked) or for medical record abstraction who did the abstraction will be reported.

# Cohorts included

The following cohorts will be included:

ALSPAC, BiB, DNBC-GOYA, DNBC-PTB (controls), EFSOCH, FinnGen, GEN-3G, GenR, HAPO, INMA, MoBa, NFBC1966, NFBC1986, UK Biobank.

# Statistical analyses

All analyses will be conducted using Stata (StataCorp, College Station, TX) or R (R Foundation for Statistical Computing, Vienna, Austria). Results will be presented as odds ratio (OR) for each binary outcome per standard deviation (SD) of BMI to facilitate the comparison of results from different methods.

## Multivariable regression

We will adjust for the following potential confounders of the association between maternal BMI and the pregnancy and perinatal outcomes: maternal age, parity, education, smoking during pregnancy, and alcohol use during pregnancy. We will also adjust for offspring sex to improve statistical efficiency given its strong association with birthweight-related outcomes.

In the main analyses, we will use logistic regression with two sets of adjustments: (1) maternal age and offspring sex, and (2) additionally maternal education, parity, smoking during pregnancy and alcohol use during pregnancy where available. We consider the fully adjusted model to be the best causal estimate in these analyses due to including key confounders and present the minimally adjusted model in the supplementary material. Similar multivariable linear regression models will be used for the additional analyses with continuously measured outcomes. Study-specific results will be meta-analysed using inverse-variance weighted fixed-effects for the main analyses and random-effects (DerSimonian and Laird method) for sensitivity analyses.

## Paternal negative control

We will use paternal BMI as a negative control exposure to explore whether the associations of maternal BMI with pregnancy and perinatal outcomes could be explained by residual confounding due to shared familial environment influencing BMI in both partners. These analyses will include data from ALSPAC, MoBa and Gen-R cohorts and use the same multivariable regression approach as described above for model 2, adjusting for partner age, number of children, education, smoking and alcohol intake around the time of their partners pregnancy, as well as mutually adjusting for each parents BMI.

Results will then be contrasted between the adjusted maternal and paternal BMI (negative control) analyses. Similar estimates between maternal and paternal BMI analyses indicate maternal BMI is unlikely to be a cause of pregnancy and perinatal outcomes assuming comparable sources of biases. Conversely, associations that are specific or stronger in the maternal compared to the paternal BMI analyses are supportive of a causal effect of maternal BMI.

In sensitivity analyses, we additionally compare the maternal associations in the main analysis (say, analysis A) to maternal associations restricted to the sample that has paternal data (analysis B). As analysis B restricts to only those with paternal BMI data, we would expect analyses A and B to be similar if there was little to no selection bias.

## Mendelian randomisation

We will use two-sample Mendelian randomization, in which the effect of interest is estimated by combining summary data for the association of single nucleotide polymorphisms (SNPs) with BMI and with each outcome.(1) This approach allows us to maximise statistical power by including all 14 studies in the analyses even when data on pre- or early-pregnancy BMI was not available (i.e. FinnGen and UK Biobank).

We select SNPs previously reported to be strongly associated with BMI (P < ) from two genome-wide association studies (GWAS) conducted by the Genetic Investigation of ANthropometric Traits (GIANT) consortium.(2, 3) In 12 studies, data is available for the set of 97 SNPs reported in the larger GWAS(2), and in two (Generation R and INMA) for the set of 32 SNPs reported in the earlier GWAS(3).

We will estimate the strength of the genetic instruments using the mean F-statistic and total R2 for the SNP-BMI association in the GIANT GWAS results as previously described.(4, 5) We also examine the correlation between SNP-BMI estimates in non-pregnant (data from the GIANT consortium) and pregnant women (data from participating cohorts where information on pre- or early-pregnancy BMI was available).

Summary data for the SNP-outcomes associations will be obtained from each contributing study using logistic (or linear) regression assuming an additive model. For the 97 SNPs subset, we will metanalyse cohort-specific SNP-outcome associations using inverse-variance weighted fixed-effects for the main analyses and random-effects (*DerSimonian and Laird method*) for sensitivity analyses.

The main two-sample MR analyses will be carried out using the inverse variance weighted (IVW) method(6). In addition, we also conduct a leave-one-out analysis at the study-level where the pooled IVW estimate is re-computed removing one study at a time to check whether pooled results are driven by a single study.

We conduct a series of sensitivity analyses to explore the plausibility of the core Mendelian randomization assumption that any effect of SNPs on the outcomes is fully mediated by maternal BMI. We explore the potential presence of invalid instruments (e.g. due to SNPs affecting the outcomes through pathways not mediated by BMI) by: (i) assessing between-SNP heterogeneity and directional pleiotropy in effect estimates using Cochran’s Q-statistic and the MR-Egger intercept test(7), respectively; (ii) checking for the presence of outlying SNPs using leave-one-out analysis at the SNP level; (iii) using other Mendelian randomization methods that are more robust to invalid instruments than IVW (MR-Egger(7), weighted median(8) and weighted mode(9)). For offspring outcomes, we repeat the IVW analyses using summary data for the SNP-outcomes associations adjusted for offspring genotype since maternal BMI genetic variants might influence offspring outcomes (e.g. birthweight) due the fetus inheriting these variants from the mother rather than due to a causal effect of maternal BMI influencing the intra-uterine environment.(10-12)

# References

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