

Genetic evidence for causal relationships between maternal obesity-related traits and birth weight

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Structured abstract

Importance: Neonates born to overweight/obese women are larger and at higher risk of birth complications. Many maternal obesity-related traits are observationally associated with birth weight, but the causal nature of these associations is uncertain.

Objective: To test for genetic evidence of causal associations of maternal body mass index (BMI) and related traits with birth weight.

Design, Setting and Participants: We used Mendelian randomization to test whether maternal BMI and obesity-related traits are causally related to offspring birth weight. Mendelian randomization exploits the fact that genotypes are randomly determined at conception and are thus not confounded by non-genetic factors. Data were analysed on 30,487 women from 18 studies. Participants were of European ancestry from population- or community-based studies located in Europe, North America or Australia and participating in the Early Growth Genetics (EGG) Consortium. We included live, term, singleton offspring born between 1929 and 2013. We tested associations between a genetic score of 30 BMI-associated single nucleotide polymorphisms (SNPs) and (i) maternal BMI and (ii) birth weight, to estimate the causal effect of BMI on birth weight.

Analyses were repeated for other obesity-related traits.

Exposures: Genetic scores for BMI, fasting glucose level, type 2 diabetes, systolic blood pressure (SBP), triglyceride level, HDL-cholesterol level, vitamin D status and adiponectin level.

Main Outcome(s) and Measure(s): Offspring birth weight measured by trained study personnel (n=2 studies), from medical records (n= 10 studies) or from maternal report (n=6 studies).

Results: The genetic score for BMI was associated with a 2g (95%CI: 0, 3g) higher offspring birth weight per maternal BMI-raising allele (P=0.008). The maternal genetic scores for fasting glucose and SBP were also associated with birth weight with effect sizes of 8g (95%CI: 6, 10g) per glucose-raising allele (P=7x10⁻¹⁴) and -4g (95%CI: -6, -2g) per SBP-raising allele (P=1x10⁻⁵), respectively. A 1 standard

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deviation (1 SD \approx 4kg/m²) genetically higher maternal BMI was associated with a 55g (95% CI: 17, 93g) higher birth weight. A 1-SD genetically higher maternal fasting glucose (\approx 0.4mmol/L) or SBP (10mmHg) were associated with a 114g (95%CI: 80, 147g) higher or -208g (95% CI: -394, -21g) lower birth weight, respectively. For BMI and fasting glucose these genetic associations were consistent with the observational associations, but for SBP, the genetic and observational associations were in opposite directions.

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Conclusions and Relevance: This Mendelian randomization study supports a possible causal association between genetically elevated maternal BMI and blood glucose and higher offspring birth weight. Conversely, genetically elevated maternal systolic blood pressure was shown to be potentially causally related to lower birth weights. If replicated, these findings may have implications for counseling and managing pregnancies to avoid adverse weight-related birth outcomes.

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Introduction

Neonates born to overweight or obese women are more likely to be large for gestational age.¹ The precise mechanisms underlying this association and the extent to which confounding factors contribute are poorly understood. It is important to understand which maternal traits are causally associated with birth weight because this may (i) facilitate targeted development of interventions to be tested in randomized controlled trials, and (ii) enable clear, evidence-based recommendations in pregnancy.

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Maternal overweight and obesity are key risk factors for gestational diabetes.² Even in the absence of diabetes, obese women have higher glucose levels than normal weight women, despite a controlled diet.³ The association between gestational diabetes and higher birth weight is well documented⁴, and maternal glucose levels below those diagnostic of diabetes also show strong associations with birth weight.⁵

The fetus of an overweight or obese woman may be exposed to the consequences of higher maternal triglyceride levels and blood pressure, lower levels of HDL-cholesterol (HDLc) and adiponectin and lower vitamin D status^{1,6,7} (Box 1). These maternal obesity-related traits have been variably associated with birth weight in observational studies: higher triglycerides and lower HDLc with higher birth weight^{8,9}; higher blood pressure with lower birth weight¹⁰; lower vitamin D status with lower birth weight¹¹; and lower adiponectin with higher birth weight¹². However, associations are not always consistently observed and may be confounded, for example by maternal socioeconomic status and associated behaviours such as smoking and diet. Furthermore, the high inter-correlation of obesity-related traits complicates determination of causal relationships in an observational setting.

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Maternal genotypes may be used in a Mendelian randomization^{13,14} approach to provide evidence of a potential causal association between maternal traits and birth outcomes (Figure 1). Mendelian randomization is analogous to a randomized controlled trial: genotypes, which are randomly allocated at conception, are largely free from confounding and can be used to estimate the possible causal effects of maternal traits. Here, we have selected genetic variants to calculate genetic scores representing maternal BMI and each of 7 obesity-related maternal traits. The potential causal effect of maternal BMI and each related trait was estimated by testing associations between maternal genetic risk scores and offspring birth weights.

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Methods

Study participants

Single nucleotide polymorphism (SNP) genotype data were used from a total of 30,487 women from 18 population- or community-based studies located in Europe, North America or Australia. The birth weight of one child per mother was included (see eTable 1 for full details of participant characteristics and eTable 2 for genotyping information). Birth weight was measured by trained study personnel (n=2 studies), from medical records (n=10 studies) or from maternal report (n=6 studies). The offspring years of birth were from 1929 to 2013. Multiple births, stillbirths, congenital anomalies, births before 37 weeks gestation and individuals of non-European ancestry were excluded. Informed consent was obtained from all participants, and study protocols were approved by the local regional or institutional ethics committees.

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Selection of maternal obesity-related traits and SNPs

In addition to BMI, traits were selected that are associated with maternal obesity and may affect fetal growth through the intrauterine environment. Their effects were modelled in the directions hypothesised by their relationships to maternal BMI (Box 1)

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We selected SNPs robustly associated ($P < 5 \times 10^{-8}$) with BMI and each obesity-related trait. Full details of the selected SNPs are provided in eTable 3. SNPs associated with (i) fasting glucose and (ii) type 2 diabetes were used to represent maternal glycemia. We considered the type 2 diabetes SNPs to represent exposure to maternal diabetes in pregnancy, including gestational diabetes given overlap between type 2 and gestational diabetes genetic susceptibility variants.¹⁵ For blood pressure, SNPs were selected that are primarily associated with systolic blood pressure (SBP), though all also show strong evidence of association with diastolic blood pressure. For vitamin D status, two SNPs with hypothesised roles in vitamin D synthesis were used to represent 25(OH)D

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levels ([an indicator of overall vitamin D status](#)), as previously recommended.^{16,17} Further details of SNP selection are provided in the **eMethods**.

A weighted genetic score [was calculated](#) for each maternal trait (see **eMethods** for full details). Very few of the selected SNPs have been tested in pregnancy. Genetic scores [were validated by](#) confirming that each was associated with its respective maternal trait, measured during pregnancy (with the exception of BMI, for which the pre-pregnancy value was used). Maternal pre-pregnancy BMI was available from registry data (N=2 studies) or calculated from self-reported weight and height (N=3 studies). In the Avon Longitudinal Study of Parents and Children (ALSPAC) study, the self-report was validated with a clinic measure¹⁸. Details of traits measured in pregnancy and their sources are given in **eTable 4**. In each available study, we performed linear regression of the maternal trait (e.g. BMI) against the genetic score, adjusting for maternal age. To confirm that associations between each genetic score and its respective maternal trait were similar in the same individuals during and after pregnancy, [available data were used](#) from two longitudinal studies (the Avon Longitudinal Study of Parents and Children [ALSPAC] and the Exeter Family Study of Childhood Health [EFSOCH]). To check that [the](#) strategy for SNP selection had resulted in genetic scores that were specific to each maternal trait, we tested the association between each of the 8 genetic scores and the other maternal traits, and indicators of maternal socio-economic status and smoking.

Testing the hypothesis that maternal BMI and obesity-related traits influence birth weight through the intra-uterine environment.

For BMI and each related maternal trait, [two](#) Mendelian randomization approaches [were used to](#) test [the](#) hypothesis. First we tested associations between genetic scores, representing maternal traits, and offspring birth weight using the maximum number of participants (i.e. for each trait, those with genetic score and offspring birth weight data available, irrespective of whether they had the maternal trait measured). An association of the genetic score with birth weight would support a

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possible causal effect of the trait (e.g. pre-pregnancy BMI) on birth weight, but would not provide information on the size of that effect. Second, we performed analyses in those with the measured trait that enabled an estimate the size of a possible causal effect. The analyses took into account the association between each genetic score and the maternal trait it represented (e.g. BMI), in addition to the association between the same genetic score and birth weight. These two results were used to calculate an association between the maternal trait (e.g. BMI) and birth weight that was free from confounding. This second approach measures the relationship between variation in maternal BMI (or BMI-related trait) and birth weight that is attributable only to genetic factors (see **Figure 1** for an explanation of the method). For each approach, meta-analysis was used to combine data from individual studies (see **eMethods**).

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Using the first approach, we investigated the association between each genetic score and (i) birth weight and (ii) ponderal index (an index of neonatal leanness, measured in kg/m^3). Within each study, birth weight or ponderal index Z-scores were regressed against each maternal genetic score, adjusted for offspring sex and gestational age. Analyses using the type 2 diabetes genetic score were repeated after excluding participants with pre-existing and gestational diabetes. Analyses using the SBP genetic score were repeated after excluding participants with pre-eclampsia and existing or gestational hypertension.

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We compared our genetic estimate, from the second approach, of the association between each maternal trait and birth weight/ponderal index with the corresponding observational association. To obtain the observational estimates, linear regression was performed using birth weight or ponderal index as the dependent variable, and each of 7 maternal traits as independent variables, adjusting for sex and gestational age. There was insufficient information on maternal type 2 diabetes prevalence, so it was not possible to estimate the causal effect for that trait. Full details of the analysis are provided in the **eMethods**.

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Estimating how much of the association between maternal BMI and birth weight is mediated by fasting glucose

Available data were used to estimate the approximate causal effect of a 1 SD ($\approx 4\text{kg/m}^2$) higher maternal BMI on (i) fasting glucose and (ii) SBP. Using each of those estimates, the results of the Mendelian randomization analyses were rescaled to represent the effects of fasting glucose and SBP that could be directly compared with the causal effect of a 1 SD higher BMI on birth weight (see eMethods for a detailed description of the method).

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Correcting for direct fetal genotype effects

Genotypes of maternal-fetal pairs were available in up to 8 studies (N = up to 11,494). Analyses were repeated including the fetal genotype at each SNP in the model, to correct for potential confounding caused by direct effects of the fetal genotype.

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Results

The characteristics of included participants from the 18 contributing studies are shown in **Table 1**.

There was evidence of association between each genetic score and its corresponding maternal trait measured in pregnancy ($P \leq 0.002$; **Table 2**). For BMI, fasting glucose and SBP, data from multiple studies were meta-analysed, with similar effect estimates between studies for BMI and fasting glucose ($P_{het} > 0.05$) and weak evidence of heterogeneity for SBP ($P_{het} = 0.04$). The effect sizes of associations between maternal traits and their respective genetic scores were very similar when compared in the same individuals during and outside pregnancy, with the exception of the SBP genetic score which had a weaker effect during pregnancy (**eTable 5**). There was no evidence of association between any genetic score and potentially confounding variables. No individual genetic score was associated with any of the other maternal traits, except for the genetic score for BMI, which was positively associated with SBP ($P < 0.003$ Bonferroni-corrected for 15 tests; **eTable 6**).

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Genetic evidence for a possible causal association between higher maternal BMI and higher birth weight

The maternal BMI genetic score was associated with higher birth weight (**Table 3**) and ponderal index (**eTable 7**) with similar effect sizes before and after adjusting for possible effects of fetal genotype. Using the genetic score to quantify the possible causal association, a 1 SD genetically higher maternal BMI (equivalent to 4 kg/m^2) was associated with a 55g (95%CI: 17, 93) higher offspring birth weight. After adjusting for fetal genotype, the estimated effect was 104g (95%CI: 32, 176) (**Table 4**). These Mendelian randomization causal estimates were similar to the observational association of 62g (95%CI: 56, 70) per 1SD (4 kg/m^2) higher maternal BMI (**Figure 2**). Similar results were obtained for ponderal index (**eTable 8** and **eFigure 1**).

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Genetic evidence for a possible causal association between higher maternal fasting glucose and higher birth weight, but no association with maternal lipids or adiponectin

The maternal fasting glucose and type 2 diabetes genetic scores were associated with higher birth weight (**Table 3**) and ponderal index (**eTable 7**) with similar effect size estimates before and after adjusting for fetal genotype, and before and after excluding pre-existing and gestational diabetes. Using the genetic score to estimate the possible causal effect, a 1SD (0.4 mmol/L) genetically higher maternal glucose was associated with a 114g (95%CI: 80, 147) higher birth weight. After adjusting for fetal genotype, the association was 145g (95%CI: 91, 199) (**Table 4**). These genetic estimates were similar to the observational association of 92g (95%CI: 80, 104) per 1SD (0.4 mmol/L) higher maternal glucose (**Figure 2**). Similar results were obtained for ponderal index (**eTable 8** and **eFigure 1**).

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The maternal triglyceride genetic score was not associated with offspring birth weight (**Table 3**) or ponderal index (**eTable 7**). Using the genetic score to estimate the possible causal effect, a genetically higher maternal triglyceride level was not associated with offspring birth weight and the 95% confidence intervals around the genetic estimate excluded the observational association between maternal triglycerides and birth weight ($P=0.007$ testing difference between genetic and observational association; **Table 4**; **Figure 2**). Likewise, the genetic estimate of the possible effect of maternal adiponectin levels on offspring birth weight was different from the observational association ($P=0.002$). The genetic score for HDLc was not associated with birth weight or ponderal index and the analysis was consistent with no causal effect, however this could not be distinguished from the negative observational association between maternal HDLc and birth weight.

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Genetic evidence for a possible causal association between higher systolic blood pressure and lower birth weight

The maternal SBP genetic score was associated with lower birth weight (**Table 3**) and ponderal index (**eTable 7**) with similar effect size estimates before and after adjusting for fetal genotype, and before and after excluding maternal pre-eclampsia and hypertension. Using the genetic score to estimate

the possible causal effect, a 1SD (10 mmHg) genetically higher maternal SBP was associated with a -208g (95%CI: -394, -21) lower offspring birth weight. After adjusting for fetal genotype, the estimated effect was -151g (95%CI: -390, 89) (Table 4). The genetic estimate of the effect of maternal SBP on birth weight in the full sample of women was in the opposite direction to the observational association ($P=0.01$ for difference between genetic and observational associations; Table 4; Figure 2). Similar results were obtained for ponderal index (eTable 8 and eFigure 1).

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The maternal genetic score for lower vitamin D status was associated with lower birth weight ($P=0.03$; Table 3). However, the estimated causal effect was not significantly different from zero (the estimated change in birth weight for a 10% genetically lower maternal 25[OH]D level was -26g (95%CI: -54, 2); Table 4, Figure 2).

Associations between the genetic scores and birth weight were consistent across studies

Associations between maternal genetic scores and offspring birth weight were similar between studies in the meta-analysis (Table 3; $P_{het}>0.05$). Where we combined data from observational analyses, the associations between maternal fasting glucose or SBP and birth weight were similar ($P_{het}>0.05$), and there was weak evidence of heterogeneity for the BMI-birth weight observational association (Table 4; $P_{het}=0.03$).

Exposure of the fetus to higher maternal fasting glucose is unlikely to explain all of the association between higher maternal BMI and higher offspring birth weight

To estimate how much of the association between maternal BMI and birth weight might be mediated by fasting glucose, we used the BMI and fasting glucose genetic scores: a 1 SD ($\approx 4 \text{ kg/m}^2$) genetically higher maternal BMI was associated with a 0.34 SD ($\approx 0.14 \text{ mmol/L}$) higher maternal fasting glucose. From the Mendelian randomization analyses, 1 SD ($\approx 0.4 \text{ mmol/L}$) genetically higher maternal fasting glucose was associated with a 114g (95%CI: 80, 147) higher birth weight, so we

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would predict that a 0.34SD higher fasting glucose would be associated with a $114\text{g} \times 0.34 = 39\text{g}$ [95%CI: 27, 50] higher birth weight. This approximation is broadly similar to ~~the~~ total estimated effect of a 1 SD higher BMI on birth weight (55g [95%CI: 17-93]). However, using the same method with the BMI and SBP genetic scores we estimated that a 1SD higher maternal BMI would be associated with a -40g [95%CI:-75, -4] *lower* birth weight via its association with maternal SBP (**eFigure 2**), which would oppose the positive association with maternal fasting glucose.

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Discussion

This study provides evidence for a possible causal association between maternal BMI and offspring birth weight. A 4 kg/m² genetically higher maternal BMI (a 1 SD rise) was associated with a 55g (95% CI: 17, 93) higher offspring birth weight. In addition, a 0.4 mmol/l (1 SD) genetically higher circulating maternal fasting glucose was associated with a 114 g (95%CI: 80, 147) higher birth weight, while a 10 mmHg genetically higher maternal SBP was associated with a -208g (95%CI: -394, -21) lower birth weight. These results provide evidence of possible causal associations with birth weight of maternal fasting glucose and SBP in opposite directions. The estimated effects of these maternal traits on birth weight (either increased or reduced) are substantial and of clinical importance. They support efforts to maintain healthy gestational glucose and blood pressure levels to ensure healthy fetal growth. The positive association between maternal BMI and birth weight may be partially mediated by the effect of higher BMI on circulating maternal fasting glucose. There was no evidence of association with a genetic score for maternal triglycerides, which have also been hypothesised to be important contributors to higher birth weight in overweight or obese women. Other lipids, or specific subclasses of triglycerides, might be important but require further study.

Our results provide genetic evidence of a causal association between maternal glycemia and birth weight and ponderal index, even in women with no pre-existing or gestational diabetes, which is consistent with published observational data.⁵ A possible explanation for this finding is that women with a higher genetic score for type 2 diabetes have relatively higher glucose levels in pregnancy, as a result of inadequate beta cell compensation in response to gestational insulin resistance,^{19,20} leading to increased placental glucose transfer and fetal insulin secretion,²¹ and consequently higher birth weight.

Our data did not support a causal association between maternal triglyceride, HDLc or adiponectin levels and birth weight or ponderal index. The genetic associations between maternal triglycerides

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and adiponectin and birth weight were **null, in contrast to the** observational associations, suggesting that the observational associations seen here, and in other published studies^{8,9,12}, are confounded.

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The Mendelian randomization analysis showed that the positive observational association between SBP and birth weight is confounded, most likely by BMI, which is both an important risk factor for higher SBP in pregnancy and positively associated with birth weight.¹ Using genetic variants that are independent of confounding by BMI, we demonstrated that genetically higher maternal SBP is associated with lower birth weight, even after excluding pre-eclampsia and hypertension. **The precision of our estimate of the change in birth weight per 1 SD in maternal SBP could be affected by the heterogeneity between studies in the genetic score-SBP association ($P=0.04$, $I^2=76.0\%$; Table 2).** However, associations between the SBP genetic score and birth weight were consistent across all 13 meta-analyzed studies ($P=0.14$, $I^2=30.4\%$; Table 3) and supportive of a causal association between higher maternal SBP and lower birth weight. These findings support observational associations between maternal SBP and birth weight that were adjusted for a wide range of confounders,²² and are consistent with laboratory and population studies suggesting a link between hypertensive disorders of pregnancy and impaired fetal growth due to placental pathology.²³ There are increasing concerns about the effect the obesity epidemic might have on birth size, via greater maternal BMI. However, the focus of that concern has been largely on increased birth size as a result of greater maternal glucose and other fetal nutrients. Our findings **suggest** that there are opposing effects of maternal blood pressure and glucose.

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Published Mendelian randomization analyses provide evidence that higher BMI is causally associated with lower vitamin D status,⁶ and evidence from multiple observational studies suggests that lower maternal vitamin D is associated with lower birth weight.^{11,24} Our analysis of the vitamin D genetic score provided some evidence to support a possible causal association with birth weight, but this **requires** further exploration in larger numbers of pregnancies.

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Socio-economic factors and related behaviours such as smoking are key confounders of observational associations between maternal BMI (or BMI-related traits) and offspring birth weight, since they are associated with both variables (see **eTable 9** for a demonstration of these associations in the ALSPAC study). ~~The~~ genetic scores used in our analyses were not associated with socio-economic factors or smoking, and this illustrates a key strength of the Mendelian randomization approach: since genotypes are determined at conception, such confounding is avoided.

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There are some limitations to our study. Despite attempts to maximise specificity of the genetic scores, we cannot fully exclude the possibility that the selected genetic variants act on more than one maternal trait. Although ~~all~~ available information was used, ~~there was~~ limited power to detect associations between the genetic scores and other traits. ~~For example, the~~ known association between BMI-associated variants and triglyceride levels was not detected.²⁵ With the potential for high-throughput metabolomic studies and a growing public database of genetic associations,²⁶⁻²⁸ future studies will improve the specificity (for different lipid sub-fractions) of selected genetic variants.

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Despite our large sample, statistical power to detect causal effects was limited for some maternal traits (see **eMethods** and **eTable 10** for power calculations). ~~The~~ total sample provided >99% power to detect associations at $P < 0.05$ between birth weight and genetic scores such as fasting glucose and systolic blood pressure that explain at least 0.1% variance in birth weight. However, ~~larger~~ samples ($N > 80,000$) will be needed to confidently detect or rule out (i) the association with vitamin D status suggested by our data, or (ii) smaller positive or negative causal associations between maternal triglycerides, HDLc or adiponectin and birth weight.

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While adjusting for the fetal genetic scores was necessary to separate maternal effects from the direct effects of genetic variants in the fetus, this could potentially introduce bias via association with paternal genotypes. Assortative mating for BMI could additionally result in a correlation between maternal and paternal genotypes, leading to similar bias. However, a father's genetic score would only confound the Mendelian randomization estimates if the father's phenotype were related to birth weight, and we found only very weak associations of fathers BMI and related traits with offspring birth weight (eTable 11). Another potential bias could be induced by the use of the genetic score for SBP, which was derived from a genome-wide association study of blood pressure conditional on BMI. Since BMI is also associated with birth weight, this could bias the results.

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However, similar results were obtained using an alternative genetic score that was unadjusted for BMI (eMethods).

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In Mendelian randomization analysis, a weak statistical association between a genetic score and a maternal trait (due to low variance explained and/or small sample size) has the potential to cause weak instrument bias towards the observational results.²⁹ The proportions of maternal trait variance explained by the genetic scores are modest in our study (Table 2). However, the large overall sample size ensured that the possible causal associations identified are unlikely to be due to weak instrument bias (see eMethods).

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Our analyses assume that maternal BMI and related traits are linearly associated with offspring birth weight. We have not tested for non-linear associations which, in a Mendelian randomization design, would require very large numbers³⁰. However, for maternal BMI, fasting glucose and systolic blood pressure, there is observational evidence of such linear associations across the distribution, with no evidence of threshold or curvilinear associations^{5,10,31}.

Conclusions

This Mendelian randomization study supports a possible causal association between genetically elevated maternal BMI and blood glucose and higher offspring birth weight. Conversely, genetically elevated maternal systolic blood pressure was shown to be potentially causally related to lower birth weights. If replicated, these findings may have implications for counseling and managing pregnancies to avoid adverse weight-related birth outcomes.

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Deleted: genetic evidence in support of a possible causal association between higher maternal BMI and higher offspring birth weight. Some of this possible causal association may be mediated by fasting glucose. We further found genetic evidence in support of a possible causal association between higher maternal blood pressure and lower offspring birth weight. We found no evidence of association between genetic scores for maternal triglycerides, HDLc or adiponectin and birth weight. Our findings support promoting healthy levels of weight in women of reproductive age and also monitoring and controlling SBP and glucose levels in women with higher BMI. These results highlight the importance of findings from pilot and ongoing trials of metformin in obese pregnant women³¹ and of statins to prevent pre-eclampsia and small for gestational age in women at risk^{32,33}.

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ARTICLE INFORMATION

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Dr. Freathy and Profs Lawlor and Frayling had full access to all of the data in the ALSPAC, EFSOCH and HAPO (non-GWAS) studies and access to summary data from all contributing studies and take responsibility for the integrity of the data and accuracy of the data analysis.

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Role of the Sponsors

The funding agencies had no role in the design and conduct of the study; collection, management, analysis and interpretation of data; preparation, review, approval of manuscript; or decision to submit manuscript for publication.

Previous Presentations

This work was presented at the Diabetes UK Annual Professional Conference 2014, 5-7 March, Liverpool, UK.

Additional Contributions

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FIGURE/BOX LEGENDS

Box 1. The maternal obesity-related traits hypothesized to cause increased or decreased fetal growth, based on observational associations with birth weight: body mass index (BMI)⁴; fasting glucose⁵; type 2 diabetes³²; triglycerides⁹; HDL-cholesterol⁸; systolic blood pressure¹⁰; vitamin D status (as indicated by 25-hydroxyvitamin D, 25[OH]D level)¹¹; adiponectin¹².

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Figure 1

Principle of Mendelian randomization: If a maternal trait causally influences offspring birth weight, then a risk score of genetic variants associated with that trait will also be associated with birth weight. Since genotype is determined at conception, it should not be associated with factors that normally confound the association between maternal traits and birth weight (e.g. socio-economic status). Estimates of the genetic score-maternal phenotype association (w) and the genetic score-birth weight association (x) may be used to estimate the association between the maternal trait variation that is due to genetic score, and birth weight ($y = x/w$), which is expected to be free from confounding. If the estimated causal effect, γ , is different from the observational association between the measured maternal phenotype and birth weight, this would suggest that the observational association is confounded (assuming that the assumptions of the Mendelian randomization analyses are valid).¹⁴ The line connecting maternal trait with fetal growth has no arrow, to indicate that the causal nature of the association is uncertain. It is important to adjust for possible direct effects of fetal genotype (z).

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Figure 2. Comparison of the observational with the genetic change in birth weight (in grams) for a 1 standard deviation (SD) change in each maternal obesity-related trait. For 25[OH]D and adiponectin, we present the change in birth weight for a 10% change in maternal trait level because these variables were logged for analysis. The genetic change was estimated from Mendelian randomization analysis, in which a genetic score was used to estimate the possible causal effect of

the maternal trait on birth weight. The genetic estimate is presented twice: in the second case it was adjusted for fetal genotype using a subset of available studies. The error bars represent the 95% confidence intervals around the effect size estimates. For maternal pre-pregnancy BMI and fasting glucose, the 95% confidence intervals for both the observational and genetic approaches exclude the null, suggesting a positive possible causal effect of maternal BMI and fasting glucose on birth weight. For maternal SBP, the observational analysis suggested a weak positive association with birth weight, whereas the genetic analysis showed evidence of a negative possible causal effect. Observational analyses suggested that higher maternal triglyceride levels, lower maternal adiponectin and lower maternal HDL-cholesterol levels were associated with higher birth weight, while lower maternal vitamin D status was associated with lower birth weight, but none of these were supported by the genetic analyses.

Table 1. Key characteristics of participants by study (for full details, see eTable 1)

Abbreviated study name*	Country (sample source)	Offspring years of birth	N women with birth weight of one child / N offspring with genotype	Mean maternal age at delivery in years (SD)	Mean maternal pre-pregnancy BMI (SD) in kg/m ²	Mean offspring birth weight (SD) in grams
ALSPAC mothers	UK	1991-1992	7304 / 4913	28.5 (4.8)	22.93 (3.73)	3481 (475)
BBC mothers	Germany	2000-2004	1357 / 1357	30.1 (5.4)	22.78 (3.93)	3472 (511)
B58C-WTCCC	UK	1972-2000	855 / NA	26.2 (5.2)	NA	3325 (483)
B58C-T1DGC	UK	1972-2000	836 / NA	26.1 (5.4)	NA	3379 (469)
CHOP mothers	USA	1987-present	312 / NA	NA	NA	3440 (562)
COPSAC-2000 mothers	Denmark	1998-2001	282 / 282	30.4 (4.3)	NA	3560 (505)
DNBC-GOYA	Denmark	1996-2002	1805 / NA	29.2 (4.2)	23.57 (4.27)	3643 (495)
DNBC-PTB-CONTROL	Denmark	1987-2009	1649 / 975	29.9 (4.2)	23.57 (4.27)	3595 (497)
EFSOCH mothers	UK	2000-2004	746 / 332 ‡	30.5 (5.3)	24.07 (4.42)	3512 (480)
GEN-3G mothers	Canada	2010-2013	676 / NA	28.4 (4.4)	24.83 (5.63)	3448 (433)
Generation R mothers	The Netherlands	2002-2006	3810 / 2196	31.2 (4.5) †	23.12 (3.92)	3528 (494)
HAPO mothers (GWAS)	UK, Canada, Australia	2000-2006	1380 / 1300	31.5(5.3) †	24.5 (5.0)	3557 (517)
HAPO mothers (non-GWAS)	USA, UK, Canada, Australia	2000-2006	3590 / 2318	30.4 (5.4) †	24.63 (5.33)	3526 (463)
MoBa mothers	Norway	1999-2008	650 / 350	28.5 (3.3)	23.93 (3.94)	3679 (430)
NFBC1966	Finland	1987-2001	2035 / NA	26.5 (3.7)	NA	3525 (461)
NTR	The Netherlands	1946-2003	706 / NA	27.1 (3.7)	NA	3469 (529)
QIMR	Australia	1929-1990	892 / NA	24.5 (4.0)	22.79 (5.13)	3344 (532)
TwinsUK	UK	NA	1602 / NA	NA	NA	3365 (581)

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*Expanded study names: ALSPAC, Avon Longitudinal Study of Parents and Children; BBC, Berlin Birth Cohort; B58C-WTCCC, 1958 British Birth Cohort-Wellcome Trust Case Control Consortium; B58C-T1DGC, 1958 British Birth Cohort-Type 1 Diabetes Genetics Consortium; CHOP, Children's Hospital Of Philadelphia; DNBC-GOYA, Danish National Birth Cohort-Genetics of Obesity in Young Adults study; DNBC-PTB-CONTROLS, Danish National Birth Cohort Preterm Birth study Controls; EFSOCH, Exeter Family Study Of Childhood Health; GEN-3G, Genetics of Glycemic regulation in Gestation and Growth; HAPO, Hyperglycemia and Adverse Pregnancy Outcome study (GWAS, Genome-Wide Association Study); MoBa, the Norwegian Mother and Baby Cohort; NFBC1966, the Northern Finland 1966 Birth Cohort; NTR, Netherlands Twin Registry; QIMR, Queensland Institute of Medical Research.

†In Generation R, maternal age was recorded, on average, at 14.4 weeks of gestation; in HAPO, maternal age was recorded, on average, at 28 weeks of gestation.

‡Fetal genotype in EFSOCH available only for the fasting glucose genetic score.

NA, not available.

Table 2. Associations between maternal genetic scores and maternal obesity-related traits

Maternal obesity-related trait	Number of SNPs used to construct genetic score	Reference for primary GWAS paper for each set of SNPs	Estimate of % variance in maternal trait explained by genetic score in pregnant women	Total N women with trait measured during pregnancy (except BMI, for which the appropriate measurement is pre-pregnancy)	N studies	Estimated change in maternal trait per average weighted trait-raising/lowering* allele (95% CI)	P value	Heterogeneity P value (I ² %), where results from >1 study were meta-analysed
Higher pre-pregnancy Body Mass Index (BMI)	30	Speliotes et al., 2010, <i>Nat Genet</i> ³³	1.8% in ALSPAC	11,822	5	0.145 (0.126, 0.164) kg/m ²	< 2x10 ⁻¹⁶	0.18 (35.8)
Higher fasting glucose†	13	Dupuis et al. 2010, <i>Nat Genet</i> ³⁴	5% in EFSOCH	5,402	3	0.029 (0.025, 0.032) mmol/L	< 2x10 ⁻¹⁶	0.70 (0)
Higher odds of gestational diabetes and existing diabetes (SNPs associated with type 2 diabetes)	55	Morris et al. 2012, <i>Nat Genet</i> ³⁵	1.4% in ALSPAC	6,606 (54 Cases, 6,552 controls)	1	Odds ratio:1.08 (1.03, 1.14)	0.003	-
Higher triglycerides	17	Teslovich et al. 2010, <i>Nature</i> ³⁶	3% in EFSOCH	663	1	0.055 (0.032, 0.078) mmol/L	3x10 ⁻⁶	-
Lower HDL-cholesterol	4	Teslovich et al. 2010, <i>Nature</i> ³⁶	3% in EFSOCH	733	1	-0.050 (-0.072, -0.027) mmol/L	1x10 ⁻⁵	-
Higher systolic blood pressure	33	Ehret et al. 2010, <i>Nature</i>	1% in ALSPAC	8,450	2	0.186 (0.140, 0.231) mmHg	< 2x10 ⁻¹⁶	0.04 (76.0)
Lower vitamin D status, ln[25(OH)D]	2 ("Synthesis" score)	Vimalaswaran et al. 2013, <i>PLoS Med</i> ⁶	0.2% in ALSPAC	4,767	1	-0.024 (-0.039, -0.009) on log scale	0.002	-
Lower adiponectin, ln[adiponectin]	3	Yaghoobkar et al. 2013, <i>Diabetes</i> ³⁷	2% in HAPO	1,376	1	-0.17 (-0.23, -0.11) on log scale	1x10 ⁻⁸	-

*The decision to model the association in relation to the trait-raising or trait-lowering allele depended on the known direction of association of each trait with higher BMI (see **Box 1**). Column 1 specifies each of these directions of association.

†Removing the one study in which the rs10830963 SNP was poorly imputed ($r^2 < 0.8$), we obtained very similar results ($n=4026$; effect size = 0.028 (95%CI: 0.024, 0.032); $P < 2 \times 10^{-16}$; $P_{het} = 0.46$; $I^2 = 0\%$).

Levels of 25(OH)D and adiponectin levels were log-transformed to achieve normality before analyses. ALSPAC, Avon Longitudinal Study of Parents and Children. EFSOCH, Exeter Family Study Of Childhood Health. HAPO, Hyperglycemia and Adverse Pregnancy Outcome study.

Table 3. Associations between maternal genetic scores and birth weight of offspring

Maternal trait for which genetic score was constructed	N studies	Total N women	Estimated change in offspring birth weight (grams) per maternal trait-raising/lowering* allele (95% CI), to the nearest 1 gram†	P value	Heterogeneity P Value (I ² %) from meta-analysis	N studies with fetal genotypes	Total N offspring with genotypes available	Estimated change in birth weight (grams) per maternal trait-raising/lowering allele* (95% CI), to the nearest 1 gram†, adjusted for fetal genotypes	P value (adjusted for fetal genotypes)	Heterogeneity P Value (I ² %) from meta-analysis (adjusted for fetal genotypes)
Higher pre-pregnancy BMI	16	25,265	2 (0, 3)	0.008	0.84 (0)	7	10,964	4 (1, 6)	0.004	0.20 (30.5)
Higher fasting glucose	15	23,902	8 (6, 10)	7x10 ⁻¹⁴	0.11 (33.3)	8	11,493	11 (7, 14)	7x10 ⁻⁹	0.26 (21.6)
Higher odds of type 2 Diabetes	12	18,670	2 (0, 2)	0.06	0.22 (23.1)	5	7,769	4 (2, 6)	0.0004	0.81 (0)
Higher odds of type 2 Diabetes (excluding pre-existing and gestational diabetes)	6	13,029	2 (1, 3)	0.02	0.92 (0)	4	6,210	4 (1, 6)	0.006	0.93 (0)
Higher triglycerides	15	24,985	-2 (-4, 0)	0.12	0.83 (0)	6	11,031	-2 (-7, 1)	0.21	0.86 (0)
Lower HDL-cholesterol	15	22,167	0 (-3, 3)	1	0.52 (0)	6	9,176	0 (-5, 5)	0.98	0.85 (0)
Higher systolic blood pressure	13	20,062	-4 (-6, -2)	1x10 ⁻⁵	0.14 (30.4)	5	7,790	-3 (-6, 0)	0.09	0.50 (0)
Higher systolic blood pressure (excluding pre-eclampsia and hypertension)	7	13,271	-5 (-7, -3)	6x10 ⁻⁶	0.18 (32)	4	5,488	-4 (-8, 0)	0.04	0.16 (41.2)
Lower vitamin D status	18	30,340	-6 (-12, 0)	0.03	0.13 (37.1)	3	9,510	-14 (-25, 3)	0.01	0.77 (0)
Lower adiponectin	9	14,920	-2 (-16, 12)	0.76	0.90 (0)	5	7,820	7 (-16, 30)	0.55	0.71 (0)

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* The decision to model the association in relation to the trait-raising or trait-lowering allele depended on the known direction of association of each trait with higher BMI (see **Box 1**). Column 1 specifies each of these directions of association. Results are per average weighted allele, adjusted for sex and gestational age. †Standard deviation of birth weight averaged over a number of European studies (=484 g)³⁸ was used to generate these estimates from z-scores. We considered a 2-tailed P value of <0.05 to provide evidence against the null hypothesis.

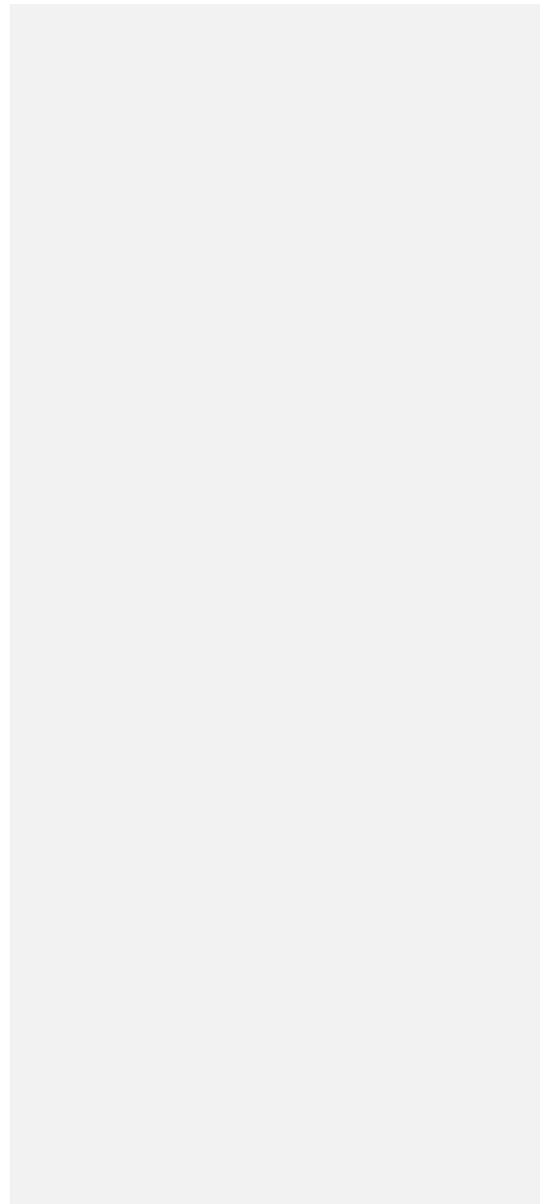


Table 4. A comparison of the observational with the genetic association between each maternal trait and offspring birth weight

Maternal trait (value of 1 SD with units)	Study/ies* used for observational estimates [Total N women]	N women for observational estimates	Observational estimate of the change in birth weight (g) per 1 SD (or 10% †) change in maternal trait, adjusted for sex and gestational age (95%CI)	Genetic estimate of the change in birth weight (g), adjusted for sex and gestational age, per 1 SD (or 10% †) change in maternal trait, unadjusted for fetal genotype (95%CI) [N women as in Tables 1 and 2]	P value‡ comparing observational with genetic birth weight associations (unadjusted for fetal genotype)	Genetic estimate of the change in birth weight (g), adjusted for sex, gestational age and fetal genotype , per 1 SD (or 10% †) change in maternal trait (95%CI) [N offspring as in Tables 1 and 2]	P value‡ comparing observational with genetic birth weight associations (adjusted for fetal genotype)
Higher pre-pregnancy BMI (4 kg/m ²)	ALSPAC Mothers, EFSOCH Mothers, HAPO Mothers	11,969	62 (56, 70)	55 (17, 93)	0.70	104 (32, 176)	0.28
Higher fasting glucose (0.4 mmol/L)	EFSOCH Mothers, HAPO Mothers	6,008	92 (80, 104)	114 (80, 147)	0.28	145 (91, 199)	0.09
Higher triglycerides (0.7 mmol/L)	EFSOCH Mothers	930	32 (7, 56)	-24 (-55, 8)	0.007	-33 (-86, 20)	0.03
Lower HDL-cholesterol (0.5 mmol/L)	EFSOCH Mothers	927	30 (3, 58)	0 (-33, 34)	0.17	-1 (-55, 54)	0.32
Higher systolic blood pressure (10 mmHg)	ALSPAC Mothers, HAPO Mothers	12,077	24 (15, 34)	-208 (-394, -21)	0.01	-151 (-390, 89)	0.14
Lower vitamin D status (10%)†	ALSPAC Mothers	4,710	-4 (-7, -2)	-26 (-54, 2)	0.13	-56 (-112, 1)	0.07
Lower adiponectin (10%)†	HAPO Mothers (GWAS only)	1,376	14 (9, 18)	-1 (-9, 7)	0.002	4 (-9, 17)	0.19

*Heterogeneity statistics from the meta-analyses of observational associations were: $P_{\text{het}} = 0.03$ and $I^2 = 67.7\%$ for BMI; $P_{\text{het}} = 0.09$ and $I^2 = 59.1\%$ for fasting glucose; $P_{\text{het}} = 0.54$ and $I^2 = 0\%$ for SBP. † For 25[OH]D and adiponectin, we present the estimated change in birth weight per 10% reduction in maternal trait level because these variables were logged for analysis.

‡P-values <0.05 are considered to indicate evidence that the genetic effect size estimate is different from the observational estimate, suggesting that the observational estimate is subject to confounding or bias.

ALSPAC: Avon Longitudinal Study of Parents And Children³⁹; EFSOCH: Exeter Family Study of Childhood Health⁴⁰; HAPO: Hyperglycaemia and Adverse Pregnancy Outcomes⁵; SD standard deviation

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