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Developmental overnutrition—an old hypothesis with new importance?*

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This paper is concerned with whether mothers who are more adipose or have higher glucose levels during pregnancy may overfeed their developing infants in utero and in doing so may set them on a pathway to greater adiposity throughout their lives. If this is the case, then the more adipose daughters of these mothers may also subsequently overfeed their infants in utero, thereby perpetuating the risk of greater adiposity across generations. I begin with the historical context of how gestational diabetes was first recognized and early evidence that diabetes in pregnancy results in increased birth size and adiposity. I then consider four questions, which are the main focus of the paper. Each of the four questions involves an exposure during pregnancy and evidence concerned with whether the exposure is causally related to offspring adiposity via intra-uterine mechanisms. The four related exposures are: (i) pregnancy diabetes; (ii) extreme maternal pregnancy obesity (≥40 kg/m² or meeting criteria for bariatric surgery); (iii) incrementally greater pre-/early pregnancy adiposity across the whole distribution seen in pregnant women; and (iv) gestational weight gain. Since randomized controlled trial evidence is not available I focus on methods that can provide the best causal evidence from observational data such as negative control studies, family comparisons and using genetic variants as instrumental variables (i.e. Mendelian randomization studies). Having addressed these four questions I go on to briefly discuss the possible role of epigenetic modification mediating any effects of maternal exposures on offspring outcomes. I conclude with a discussion about the future research and policy implications of evidence to date in this field.

Keywords Developmental overnutrition, causality, pregnancy, diabetes, adiposity
Introduction

One fortunate aspect about doing the John Pemberton lecture is that, like many of his generation, he worked in so many areas of relevance to population health that it would be easy for any contemporary epidemiologist (who is more likely to work in a focused area) to be able to find a link between their work and his. One of John Pemberton’s first publications was on malnutrition. It was written while he was still a medical student and was recently reprinted in IJE.1 His concern was with undernutrition in the poorest members of society and its adverse effects on early life growth and development.1 It is salutary that 77 years later my paper is also about malnutrition and developmental origins, but rather than undernutrition it reflects the major current concerns about the global epidemic of obesity and overnutrition. My focus is on whether mothers who are more adipose and/or have higher glucose levels during pregnancy overfeed their developing infants \textit{in utero} and in doing so set them on a pathway to greater adiposity throughout their lives—a phenomenon that has been called developmental overnutrition.2,3

Box 1 outlines the key questions that I aim to address and these are summarized in Figure 1, which also differentiates the ‘old’ central hypothesis and how this has been extended and interpreted recently. Before addressing these related questions I provide some historical context to this area of research.

Figure 1 The developmental overnutrition hypothesis. The pathways depicted by exposures, intermediates and outcomes in boxes with bold outlines and that are linked by dashed and heavily weighted arrows are those that were part of the ‘old’ (original) hypothesis, for which there is good causal evidence. Pathways linking boxes that do not have bold outlines with continuous light arrows represent the new extensions that now generate a link between maternal adiposity from early pregnancy through to offspring adiposity across their life course. The figure is illustrative of the focus of this paper and does not aim to show all possible relationships between the characteristics that are depicted.

Box 1 Four related causal questions on developmental overnutrition and offspring adiposity

Each of these questions involves a different exposure during pregnancy and is concerned with whether this exposure is causally related to offspring greater adiposity throughout their life course. The potential causal mechanisms are summarized in Figure 1. The four exposures are clearly related to each other but in this paper evidence for their effect on later offspring outcomes are considered separately.

(1) Does exposure to \textbf{maternal diabetes in pregnancy} cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

(2) Does exposure to \textbf{maternal extreme obesity in pregnancy} cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

(3) Does each ‘extra’ increment of \textbf{maternal pre-pregnancy or early pregnancy adiposity} cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

(4) Does each ‘extra’ increment of \textbf{maternal weight or fat gain in pregnancy} cause offspring to be more adipose throughout their lives through intrauterine mechanisms?
Evolution of the developmental overnutrition hypothesis

Figure 2 summarizes some key research milestones that are relevant to the evolution of the developmental overnutrition hypothesis.

Diabetes in pregnancy: its diagnosis and treatment

The developmental overnutrition hypothesis arose from the relationship between diabetes in pregnancy and infant size. To fully understand this hypothesis, and the results of studies examining it, it is important to understand the meaning of diabetes in pregnancy, its diagnosis and its treatment. A woman who becomes pregnant may have established type 1 or type 2 diabetes, or she may be diagnosed with gestational diabetes during pregnancy (Box 2), with gestational diabetes accounting for most diabetes in pregnancy. For example, of the 2–5% of pregnant women each year in the UK whose pregnancies are complicated by diabetes, ~88% have gestational diabetes, ~7% existing type 1 diabetes and ~5% existing type 2 diabetes.4 Women who enter their pregnancy with existing (type 1 or type 2) diabetes are likely to have had pre-conceptual counselling concerned with establishing good glycaemic control before becoming pregnant and they will be monitored and receive treatment aimed at maintaining tight glycaemic control from the very start of their pregnancy (Box 2).4,5 In contrast, those with gestational diabetes are likely to be diagnosed during the second trimester of pregnancy, or even later;4,5 consequently they will spend some of their pregnancy with high circulating glucose levels that they and their health care professionals are unaware of.

Gestational diabetes, its diagnosis and recognition as an important clinical phenomenon, have a long history (Figure 2). The notion that glucose tolerance during pregnancy differed to that outside pregnancy and that diabetes might first become apparent during pregnancy began to be discussed in the medical literature in the 1930s and 40s.13,14 In a seminal paper published in 1946, Hurwitz and Jensen reported the results of repeat oral glucose tolerance tests (OGTT) completed in 25 healthy women over the course of their pregnancy and early postnatal period.14 They found increased post-load glucose levels in pregnancy, particularly during the second and third trimester, which resolved postnatally (Figure 3).14 Importantly, it was observed that, if established criteria for gestational diabetes had been applied, a large proportion of these women would have been diagnosed with gestational diabetes.

Figure 2 Key points in the history of diabetes (lower parts of the figure below the central bar), diabetes in pregnancy (middle part of the figure just above the central bar) and the developmental overnutrition hypothesis (top part of the figure). IADPG, International Association for the Diabetes in Pregnancy Group
Box 2 Definitions of type 1, type 2 and gestational diabetes

**Type 1 diabetes**
Type 1 diabetes is characterized by the loss of pancreatic β-cells and hence lack of production of insulin. It usually has an onset in childhood, presents relatively acutely with polyuria, polydipsia, weight loss and ketoacidosis, and requires treatment with insulin.Women with existing type 1 diabetes who become pregnant will be treated with insulin throughout their pregnancy and will have their circulating glucose levels closely monitored from the start of their pregnancy. They are also likely to have been advised to avoid unplanned pregnancy and to have considered only trying to become pregnant when they had good pre-conceptual glucose control. For example, in England and Wales, NICE guidance on the management of diabetes in pregnancy recommends pre-conceptual counselling of women of reproductive age who have diabetes that emphasizes the need for good glucose control before trying to become pregnant. They state that ideally glycated haemoglobin should be maintained at below 6.1% before a woman tries to become pregnant and that women with a glycated haemoglobin above 10% should be strongly advised against becoming pregnant. During the antenatal period, these guidelines recommend careful and regular clinical monitoring, and, if it is achievable safely, that women with existing diabetes aim to maintain fasting blood glucose between 3.5 and 4.9 mmol/l and post-load glucose below 7.8 mmol/l antenatally.

**Type 2 diabetes**
Type 2 diabetes is characterized by resistance to the actions of insulin, which results in increased production of insulin by pancreatic β-cells and ultimately dysfunction in these. It presents insidiously and is often identified during general health screening, assessment of comorbidities in those with obesity, or as part of a differential diagnosis in individuals with non-specific symptoms. It is estimated that a substantial proportion of the general population have type 2 diabetes without knowing this. Until very recently, it was believed to be a disease of adults only (hence its previous name of ‘adult onset diabetes’), but with the emergence of the obesity epidemic it is now increasingly diagnosed in children and adolescents. Type 2 diabetes can be treated with lifestyle modification (weight loss, diet and physical activity), oral hypoglycaemic medication or insulin, usually with progression through these treatment options and monitoring of response. Women of reproductive age and those who become pregnant with existing diabetes will, generally, be advised about good glycaemic control pre-conceptually and will be closely monitored with the aim of maintaining good control antenatally. The guidelines for England and Wales, noted above, apply equally to women with existing type 2 and type 1 diabetes. The key difference is that pre-conceptually women with type 2 diabetes are less likely to be treated with insulin than those with type 1 diabetes (all of whom will be treated with insulin). Once pregnant (and in some pre-conceptually) women with type 2 diabetes are likely to be switched to treatment with metformin and/or insulin during pregnancy.

**Gestational diabetes**
Gestational diabetes is defined as any level of glucose intolerance with onset, or first diagnosis, in pregnancy. This means that someone with existing type 2 diabetes who has not previously been identified could be identified, through routine assessment and screening in pregnancy, and would be defined as having gestational diabetes. The fact that they had type 2 diabetes would only become apparent when the intolerance persisted after pregnancy. In terms of aetiology and pathology, gestational diabetes (including glucose intolerance during pregnancy that then resolves postnatally) resembles type 2 diabetes and the strong association with future risk of type 2 diabetes supports the notion that it is the same condition, which becomes unmasked by the ‘glucose metabolism challenge’ of normal pregnancy. Women with gestational diabetes are unlikely to be identified until the second trimester, or later in pregnancy, and are likely to be treated with lifestyle changes—primarily diet and physical activity. For example, in England and Wales, NICE guidance recommends initial risk factor screening at antenatal booking clinic, with a 75 g OGTT offered to women who have had a previous diagnosis of gestational diabetes at 16–18 weeks of gestation and to women with one of the other specified risk factors for gestational diabetes (BMI >30 kg/m²; previous delivery of an infant weighing >4.0 kg; first-degree relative with diabetes; or family (ethnic) origin with a high prevalence of diabetes) at 24–28 weeks of gestation. Thus, final diagnosis will be around 18 weeks in those with previous gestational diabetes and 28 weeks in those with other risk factors. In other countries where all pregnant women are routinely screened with a 75 g OGTT (for example in most states in the US), this is conducted between 24 and 28 weeks of gestation. In England it is estimated that approximately 80% of women with gestational diabetes are managed with lifestyle advice only. If control is not maintained with lifestyle changes then women with gestational diabetes may be treated with the oral hypoglycaemics metformin or glibenclamide or may be treated with insulin.
diagnosing type 2 diabetes (defined as 2-h post-load glucose levels of higher than 120 mg/100 ml in the paper) were applied to the second/third trimester glucose levels, 81% of pregnant women would be diagnosed with diabetes.14 This began an understanding that some level of glucose intolerance in pregnancy might be normal for healthy growth and development of the foetus, since it didn’t seem appropriate to accept that the vast majority of women had diabetes in late pregnancy.

Following on from this work, in the 1960s O’Sullivan and Mahan published the results of 100 g OGTTs in 752 pregnant women and used these to produce the first ‘normograms’ of glucose levels in pregnancy.15 With just minor subsequent modifications, this work informed the diagnostic thresholds used to identify women with gestational diabetes until the early 2000s.16 Throughout most of the second half of the 20th century, the criteria for diagnosing gestational diabetes were defined with the intention of identifying women at risk of developing type 2 diabetes after their pregnancy, rather than with the aim of identifying (and preventing) those at risk of adverse perinatal outcomes. Indeed, it is only very recently, with the results of the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) cohort study, that emphasis has shifted towards criteria for diagnosing gestational diabetes that aim to identify women at risk of adverse perinatal and longer-term offspring outcomes (discussed later in the section on policy at the end of this paper).16

Figure 3 Glucose tolerance curves of a typical woman during pregnancy and after delivery. The curves are obtained from glucose tolerance tests with the x-axis showing the hours since a glucose load. The + signs indicate levels of glycosuria closely matching blood glucose levels. Reproduced with permission from the New England Journal of Medicine.14
Pregnancy diabetes and ‘larger, fatter and more watery’ infants—an old hypothesis

From the 1930s onwards, reports were published showing that at birth the offspring of women with diabetes were larger than those of women without known diabetes.\(^{17,18}\) For the most part these studies were looking at the association of established diabetes with pregnancy outcome. Autopsy studies on infants who were stillborn or died shortly after delivery, and clinical examination of those who survived, indicated that infants of women with diabetes had greater fat mass and skeletal length and were more oedematous at birth,\(^{17,18}\) with Pedersen describing these infants as ‘larger, fatter and more watery’.\(^{19}\)

In the 1950s a number of competing hypotheses were proposed for explaining the association of maternal diabetes with offspring large size at birth. Several studies had demonstrated an association of previous pregnancy loss and larger than average birth size infants with subsequent diagnosis of diabetes in women.\(^{20–22}\) These pregnancy outcomes had often occurred many decades before the woman was diagnosed with diabetes and these findings led to the suggestion that the association between maternal diabetes and offspring birth size was inherited via genetic or familial environmental characteristics (i.e. that were present postnatally/through the mother and her offspring’s life and not just related to the mother’s pregnancy) that affected both diabetes risk and growth, rather than by intrauterine effects.\(^{20–22}\)

In order to test this hypothesis, Jackson, in 1954, compared the association of previous birth size of children with subsequent diagnosis of diabetes in both mothers and fathers. He argued that if inheritance of characteristics related to growth and diabetes explained the association, then one would expect to see a similar association of previous infant size in mothers and fathers. By contrast, an intrauterine mechanism would result in a stronger association in the mothers.\(^{23}\)

Figure 4 shows the distributions of previous infant birth weight amongst 398 fathers with diabetes, 396 control fathers, 428 mothers with diabetes and 819 control mothers. This shows very similar offspring birth weight distributions in control fathers and mothers and that both fathers and mothers with diabetes have larger birth weight infants on average. However, the difference for fathers is smaller than that between diabetic and control mothers. These findings led Jackson to conclude that: ‘The tendency to produce large babies [among women with diabetes in pregnancy] is partly an inherited characteristic combined with a tendency to diabetes, passed by the male as well as female, and partly an effect of maternal internal environment’.\(^{23}\)

Shortly after this publication, Pedersen published the results of two studies that also tried to ascertain

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Figure 4 Distribution of birth weights of previous (firstborn) children by whether the mother and father were subsequently diagnosed with diabetes. Produced using data from a paper by WP Jackson.\(^{23}\) NB: 1 lb = 0.454 kg, so e.g. 8 lb ~ 3.6 kg
the mechanism underlying the association of pregnancy diabetes with greater offspring size. In the first study he showed that the whole distribution of both infant height and weight were shifted upwards (to higher levels) in infants of mothers with diabetes during their pregnancy compared with control mothers. On the basis of those findings he concluded that, in addition to possible overseeding of the infant due to greater glucose supply (which would largely result in greater weight/adiposity), infants of diabetic women were exposed to the effects of a growth hormone, which resulted in skeletal growth and overall increased size.

In the second study he compared birth weight and length of offspring between two groups of women with diabetes and the same control group as that used in the first study. The two groups of diabetic women were described as ‘treated in the same way for a different length of time during the latter part of pregnancy’. Women in both groups had been admitted to hospital and treated with a strict diet of 1900 calories and high dose insulin for as long as possible during the last 53 days of pregnancy (based on their expected date of delivery). Of the long-term treatment group, 78% were treated for at least 30 days, whereas just 10% of the short-term treatment group were treated for this length of time. These differences occurred because of differences in when the women first presented for antenatal care, but the two groups appeared to be similar with respect to a number of other key characteristics. Both groups of diabetic mothers had babies who were heavier and longer than control non-diabetic mothers, but the difference was greater for the short-term compared with long-term treated diabetic mothers. These findings led to Pedersen’s suggestion that maternal glucose, together with foetal insulin (a growth hormone that was relatively suppressed in the long-term treated mothers with diabetes), may be the underlying mechanism responsible for the association of maternal diabetes in pregnancy with greater offspring size.

**Could developmental overnutrition drive the obesity epidemic?**

Since the work of Jackson and Pederson in the 1950s, a substantial amount of cellular, animal and human research has supported the hypothesis that pregnancy diabetes, particularly poorly controlled diabetes, results in larger and fatter infants at birth via intrauterine mechanisms that involve the delivery of greater amounts of glucose, and other nutrients such as fatty acids, to the infant and also through increased production of foetal insulin in response to exposure to greater levels of glucose. There is good evidence that glucose freely crosses the placenta, and together with fatty acids, is a key energy supply for the developing foetus, with greater energy provision resulting in the developing foetus accumulating more adipose tissue. Evidence also supports the hypothesis that a greater supply of glucose to the developing foetus results in increased foetal insulin secretion, which acts as a growth hormone. Maternal insulin does not cross the placenta. In recent studies in humans, foetal hyperinsulinaemia has been detected in the offspring of diabetic mothers both (assessed in samples of amniotic fluid) and immediately after birth (assessed in samples of cord blood) and this foetal hyperinsulinaemia is associated with greater foetal growth.

However, in recent years, this central hypothesis, concerned with maternal pregnancy diabetes and increased birth size of offspring, has been extended to suggest that maternal diabetes has long-term effects on offspring levels of adiposity in later life (not just at birth) and also to include earlier exposures in the mother, such as her pre- or early pregnancy adiposity across its whole distribution (Figure 1). This extension of the central ‘old’ hypothesis has led to the suggestion that developmental overnutrition could be driving the current obesity epidemic and could continue to do so across many generations even if effective means of obesity prevention were developed and implemented now.

With regards to the foetus/offspring it is suggested that exposure to maternal diabetes (i.e. greater circulating glucose levels) in pregnancy results, via intrauterine causal mechanisms, in greater risk of obesity in later life. One suggestion is that overnutrition of the developing foetus—i.e. the greater delivery of greater levels of glucose and other nutrients—affects the development of the foetal hypothalamus and related neuroendocrine systems, which are central to maintaining long-term energy balance postnatally. It has also been postulated that, since birth size tracks (i.e. size at one age correlates with size at a later age) over the lifetime, the simple fact that infants to diabetic mothers are fatter at birth predisposes them to be fatter throughout life.

With regards to the mother, it is suggested that it is not only maternal diabetes that predisposes to greater offspring adiposity at birth and throughout life, but that each increment of greater adiposity in early pregnancy and each increment of greater fat deposited during pregnancy, will result in greater adiposity in the offspring at birth and throughout life (i.e. women who are fatter at the start of pregnancy or who gain more fat during pregnancy will, on average, cause their offspring to be fatter at birth and throughout their lives). Higher BMI is one of the strongest risk factors for gestational diabetes and a range of other pregnancy complications, including large birth weight. This association is probably through maternal and foetal dysregulation of glucose, insulin, lipid and amino acids. There is a strong graded linear association of one’s own BMI (irrespective of whether this is during pregnancy or not) with circulating glucose levels. Therefore women who are fatter during pregnancy will on average have higher circulating glucose levels.
glucose levels. There is also a graded linear association of maternal circulating glucose levels (both fasting and post-load) during pregnancy with infant birth size and fat mass.\textsuperscript{29,50} Thus, the argument is that greater average maternal fatness during pregnancy will be associated with later offspring greater fatness through similar intrauterine mechanisms that are hypothesized to link pregnancy diabetes with greater offspring fatness.

Importantly, from a public health perspective, this extension of the central ‘old’ hypothesis raises the possibility that for each increment of greater fatness in pregnant women (either pre-existing or gained during pregnancy), the developing foetus will be on average more overfed with glucose and other nutrients, and as a consequence be fatter postnatally and in later life. For the female offspring, they will potentially go into their subsequent pregnancies fatter and thus there is the potential for a perpetual intergenerational cycle that is difficult to break.\textsuperscript{31,32} If this is true, then it places an important emphasis on pre-conception or antenatal interventions in women of reproductive age, that specifically target them for weight reduction and/or maintenance of a healthy weight.

The key question then is: ‘How good is the evidence that maternal exposures related to circulating levels of glucose in pregnancy result in lasting effects on offspring levels of adiposity in later life via intrauterine effects?’ This is the focus of the remainder of this paper. I will discuss in turn epidemiological evidence for a causal intrauterine effect of each of the four related exposures that form the research questions listed in Box 1 and illustrated in Figure 1. My starting point with respect to examining research in these areas is that currently randomized controlled trial evidence of the effect of these pregnancy exposures on long-term outcomes in offspring is not available and trials of interventions to maintain healthy weight (or even good glucose control in women with diabetes) throughout pregnancy that are sufficiently large to be able to detect long-term effects in offspring are unlikely to be completed for several decades, if ever. I, therefore, focus on methods that can provide the best causal evidence from observational data. These methods include negative control parental-offspring association comparisons, within-sibling associations and the use of genetic variants as instrumental variables (i.e. Mendelian randomization studies). Box 3 summarizes each of these methods, their underlying assumptions and limitations, provides a ‘proof of concept’ example, and summarizes some specific issues related to their use in addressing questions about the causal impact of developmental overnutrition. Applying two or more of these methods to address the same question is ideal. This is because they each have somewhat different underlying assumptions and limitations and therefore, if consistent results are obtained from different methods, our confidence that the result is the true causal effect is increased. Where different methods produce conflicting results it is important to consider the underlying assumptions of each method and the extent to which these are likely to have been violated in each method.

**Does exposure to maternal diabetes in pregnancy cause offspring to be more adipose throughout their lives through intrauterine mechanisms?**

Several observational studies have reported associations of maternal diabetes in pregnancy with greater offspring BMI, other indicators of greater adiposity, such as waist circumference and fat mass, and associated cardiometabolic risk factors, in infancy, childhood and early adulthood (most studies are in children, with mid-20s being the oldest ages at which these associations to date have been assessed).\textsuperscript{52–57} However, these associations are not necessarily due to intrauterine mechanisms. Genetic variants and shared familial lifestyles associated with greater adiposity would be related to greater diabetes risk in the mother and adiposity in the offspring, and could explain the link.

**Family comparison studies in the Pima Indians of Arizona**

Some of the strongest evidence for a causal intrauterine mechanism comes from studies of the Pima Indians of Arizona. The Pima Indians of Arizona are a population with very high levels of obesity, type 2 diabetes and gestational diabetes, resulting from recent lifestyle changes that related to White settlers moving into their native lands and changing the environment.\textsuperscript{58,59} In studies of this group of Pima Indians, mean BMI, fasting glucose and the risk of diabetes in children born to mothers who had diabetes during their pregnancy are greater than in children of mothers who developed diabetes later in their lives or those of mothers who never developed diabetes (outcomes assessed during childhood and up to age \textasciitilde 24 years).\textsuperscript{52,53,60} In a sibling study (182 individuals from 52 families), conducted in the Pima of Arizona, mean BMI and risk of type 2 diabetes (assessed when offspring ranged from 2 to 24 years; mean age 13 years) were greater among offspring born after the mother had been diagnosed with diabetes (i.e. so that she had diabetes during their pregnancy) than in their siblings born before their mother’s diagnosis.\textsuperscript{61} On average, mean BMI was greater in the sibling exposed to \textit{in utero} maternal diabetes by 2.6 kg/m\textsuperscript{2} (95% CI: 0.9–4.3) compared with the sibling born before their mother was diagnosed with diabetes, i.e. when she had normal glucose tolerance during pregnancy. The odds ratio for type 2 diabetes was 3.7 (95% CI: 1.3–11.3) comparing the
Box 3 Methods that have been used to try to assess causality using observational data in relation to the developmental overnutrition hypothesis

Negative control studies: parental-offspring association comparisons

Description

Parental-offspring association comparison studies are a form of ‘negative control’ study. In observational epidemiology, a ‘negative control’ study is one in which the results from the ‘real’ association of interest are compared with a study where there is either a different outcome (negative outcome control) or a different exposure (negative exposure control) that has been selected such that the proposed mechanism for the ‘real’ association of interest could not apply. In parental-offspring associations, the association of interest would be plausible in just one of the parents, and so the second parent is used as a negative control. This approach has intuitive value in the field of developmental origins of health and disease where intrauterine mechanisms (over and above any shared genetic or familial environmental mechanisms) are assumed. In this situation, the expectation is that if there are causal intrauterine mechanisms, then these are only plausible for maternal exposure-offspring outcome associations and paternal-offspring associations provide a negative exposure control. If the paternal-offspring association is of similar magnitude to the maternal-offspring association (after both are controlled for potential confounding factors) the assumption is that the maternal-offspring association is biased by residual confounding by shared genetic or familial lifestyle characteristics which also generate a paternal-offspring association.

Underlying assumptions

- There is no plausible mechanism by which the association could occur in the negative control parent.
- Specifically when testing causal intrauterine mechanisms, the assumption is that the exposure in the father could not generate an association that was due to intrauterine mechanisms.
- The assessment of the exposure in both parents is measured with the same level of accuracy.

Strengths (S) and limitations (L) of the method

- **S**: Tests the likelihood of alternative explanations for an association, including that of confounding. Therefore, has a different approach to exploring the likelihood that residual confounding explains the association from conventional multivariable regression approaches and is complementary to those approaches.
- **L**: Exposures may not be measured in fathers or may be measured with more error in the fathers than the mothers.
- **L**: A stronger magnitude of association for the maternal-offspring than paternal-offspring association provides some support for maternal-specific associations. These could be intrauterine, but it could also be that mothers have a stronger influence on their offspring behaviours postnatally than do fathers.
- **L**: It is plausible that non-paternity might contribute to a stronger maternal-offspring association if this is driven at least in part by genetic inheritance. Sensitivity tests to allow for differing degrees of non-paternity can be used to assess this.

Proof of concept example

The association of maternal smoking in pregnancy with lower offspring birth weight (a causal intrauterine association) is considerably stronger than the association of paternal smoking at the time of their partner’s pregnancy with offspring birth weight, with the weak paternal smoking-offspring birth weight association attenuating to the null when adjusting for maternal smoking.\(^{37,39}\)

Issues when used to examine the developmental overnutrition hypothesis

It has been suggested that a plausible mechanism exists that would generate a similar magnitude of association of maternal pre/early pregnancy BMI with offspring adiposity to that of paternal BMI with offspring adiposity (i.e. that mothers and fathers have similar intrauterine environmental influences on later offspring adiposity). The suggestion is that fathers influence future offspring adiposity via intrauterine mechanisms through genomic imprinting, i.e. that some foetal genes related to growth are differentially expressed depending on whether their copy of the gene is from their father or their mother through epigenetic effects. It is further suggested that these epigenetic effects from the paternal side influence offspring appetite, thus explaining the fact that maternal BMI-offspring birth weight associations are larger than paternal BMI-offspring birth weight associations, since intrauterine programming of appetite would only be expected to have an effect on size after birth.\(^{40}\) As yet, just how genomic imprinting might work to equalize parental influences is unclear,\(^{30,41}\) and we have argued that informal
or formal approaches to comparing explanatory models, which adopt the parsimony principles of Occam’s Razor,\textsuperscript{42} suggest that the likelihood of such perfectly mimicked effects, when produced by different mechanisms, is low.\textsuperscript{41} This issue does however, highlight the importance in such studies of clearly outlining the justification for believing the mechanism of the real association of interest is implausible in the negative control study and allowing that point to be debated and further tested.

**Within sibling associations**

**Description**

Within sibling comparisons can be considered as a natural experiment that allows for some control of unmeasured confounding (or for residual confounding due to inadequately measured cofounding).\textsuperscript{43}

**Underlying assumptions**

- Potential confounders of the association of interest will be the same or very similar for siblings.
- At the extreme, it is assumed that each sibling in a group has similar distributions of potential confounders and only differ by levels of exposure.

**Strengths (S) and limitations (L) of assumptions**

- **S**: Matches confounders within siblings. Therefore, has a different approach to testing the likelihood that confounding explains the association from conventional multivariable regression approaches and is complementary to those approaches.
- **S**: Able to control for unmeasured confounding for potential confounders that are similar or the same in siblings.
- **L**: Statistical power is lower than conventional multivariable regression approaches because only the discordant siblings contribute to the within sibling association.
- **L**: Siblings are not genetically identical (they share just 50% of their genome on average) and are likely to differ on many characteristics. If these are not likely to be confounders these differences may not introduce bias, but this is a strong assumption that could be more or less plausible for different research questions.

**Proof of concept example**

Birth weight of siblings exposed to maternal gestational diabetes is greater than that of their siblings who are not exposed, confirming the causal effect of gestational diabetes on birth size.\textsuperscript{44}

**Issues when used to examine the developmental overnutrition hypothesis**

One would ideally want siblings to be relatively close in age so that their postnatal exposures were likely to be similar, and therefore controlled for. For example, siblings who are just 2 years apart will spend most of their infancy and childhood in a similar family environment, whereas those who are 10 or more years apart are less likely to. For pregnancy diabetes, the exposed sibling is more likely to be the younger sibling (as women diagnosed with diabetes during pregnancy are at risk of having or developing type 2 diabetes which would be present in the next pregnancy) and this systematic age difference could generate age related/sibling order related confounding.

**Mendelian randomization**

**Description**

Mendelian randomization uses genetic variants, which are robustly associated with the exposure of interest, as an instrumental variable or proxy, for the causal effect of that exposure.\textsuperscript{45,46}

**Underlying assumptions**

- The genetic variant is associated with the exposure of interest with sufficient magnitude for ‘weak instrument’ bias not to occur.
- The association of the genetic variant with the exposure is not confounded.
- There is no pathway, other than through the association of the genetic variant with the exposure, by which the genetic variant can be related to the outcome.

**Strengths (S) and limitations (L) of assumptions**

- **S**: Reverse causality is unlikely since genetic variants are present from conception.
- **S**: Confounding by the many lifecourse environmental and lifestyle characteristics that might result in residual confounding in conventional multivariable regression approaches are unlikely because genetic variants are much less likely to have the multiple associations with other characteristics that are seen for non-genetic variables.\textsuperscript{47}
Sibling born following a pregnancy where the mother had diabetes with the sibling born during a normally glucose tolerant pregnancy.61

Sibling studies are a potentially powerful approach for determining causality as they inherently control for maternal genetic variation (including mitochondrial DNA and parent of origin effects) and any environmental or lifestyle exposures that have remained constant or are very similar across pregnancies and within siblings (Box 3).43 Of course children born when the mothers experienced diabetes in pregnancy (compared with their siblings born before there was evidence of pregnancy diabetes) will be younger, born when the mother is older, already has at least one child, and when she may, therefore, find maintaining weight, a healthy diet and a healthy level of physical activity postnatally more difficult. However, in this study of Pima Indians, siblings were selected so that the age difference was no greater than 3 years between siblings. Thus, for most of their infancy and childhood the siblings will have experienced similar postnatal maternal lifestyles. Having diabetes during pregnancy and having a greater risk of an assisted delivery may also influence the likelihood of

Proof of concept example

Genetic variants that influence levels of low density lipoprotein cholesterol (LDLc) demonstrate the causal effect of LDLc on coronary heart disease risk, which is established as causal on the basis of randomized controlled trials of LDLc-lowering (statin) therapy.48 Indeed this study suggested a stronger effect of lifelong lower LDLc (based on the notion that the genetic variants would affect levels across the whole of life) than the effect of statins given in mid life.

Issues when used to examine the developmental overnutrition hypothesis

Here the exposure of interest is maternal and the outcome is in the offspring. This has the advantage that Mendelian randomization results could not be biased by developmental canalization since the mother’s genetic instrument for her adiposity or diabetes status will only influence the developmental environment of the offspring through our exposure of interest. However, it has the disadvantage that there is clearly an alternative pathway from maternal genetic instrument to the offspring outcome, other than solely through the maternal intrauterine environment of interest; mother’s genotype will be related to offspring genotype which will influence offspring outcome. Specifically, if maternal adiposity variants are used to test whether incrementally greater maternal adiposity is causally related to offspring adiposity, a false-positive effect will be generated because of the association of maternal adiposity genotype with offspring adiposity genotype and of these with offspring adiposity. Being able to adjust for offspring genotype is therefore key in such studies.49 With respect to available variants for undertaking Mendelian randomization studies in this field, there are a number of genetic variants that are associated with fasting glucose, type 2 diabetes and gestational diabetes and also with incrementally greater BMI/fat mass, which could be used in combination in Mendelian randomization studies to increase statistical power and test some of the underlying assumptions of this method.50,51 In sufficiently large studies it might be possible to use the extremes of genetically determined adiposity (using the extremes of an allelic score) to mimic the exposure of extreme maternal obesity (>40 kg/m²). However, as yet I am not aware of genetic variants that are robustly associated with GWG. When using the Mendelian randomization approach to test the developmental overnutrition hypothesis, the assumption is that maternal genetic variants that are associated with exposures have similar magnitudes of association at the time of pregnancy as at other times in the life course. For pre-pregnancy BMI, this has been shown to be the case with the FTO variant.49
breastfeeding, and this might therefore differ between siblings discordant for maternal diabetes, and breastfeeding was not controlled for in the study of Pima Indians. However, randomized controlled trial and cross-cohort comparisons suggest that breastfeeding is not causally protective of obesity, but that confounding factors explain the association between the two.62,63 Thus, a difference in breastfeeding between siblings with different exposures to maternal diabetes in pregnancy is unlikely to be a causal explanation for any difference in their later outcomes.

Importantly, the Pima Indian study also found that within siblings there was no association of paternal diabetes around the time of their partner’s pregnancy with offspring BMI or diabetes (within sibling difference in mean BMI was 0.4 kg/m² (95% CI: −0.9 to 1.7) and within sibling odds ratio for type 2 diabetes was 1.1 (95% CI: 0.3–2.5).61 Comparing the association of paternal and maternal diabetes with offspring outcomes, as discussed above in relation to Jackson’s early study,23 is another approach to explore whether diabetes is causally associated with offspring outcomes via intrauterine mechanisms (Box 3).

Thus, two complementary approaches have been used in the Pima Indians, each with different assumptions and potential limitations (see Box 3) and yet both suggesting that exposure to maternal diabetes in pregnancy is causally related to later offspring greater BMI and type 2 diabetes risk. These findings suggest that, at least in a population at high risk for obesity and diabetes, intrauterine mechanisms make an important contribution to the link between pregnancy diabetes and offspring’s greater BMI and type 2 diabetes risk. Of importance, in the Pima Indian studies all family members are assessed every 2 years with an OGTT and, when analyses were restricted to only those whose mothers diabetes in pregnancy was determined by an OGTT results, were essentially the same as in the whole study.

There was some evidence in the sibling study in the Pima Indians of Arizona that the mean BMI difference within siblings increased with increasing age, and was only really apparent from age 12 years onwards.39 Since a key concern about developmental overnutrition is that it may perpetuate the obesity epidemic through generations, the possibility that any effect on later offspring BMI might be greater from adolescence and into early adulthood (i.e. when women are beginning their reproductive history) is important. However, given the overall sample size of this study, the numbers in each age stratum were small and no statistical evidence was provided of an age effect on the magnitude of the association. It is also important to note that the Pima Indians of Arizona have a considerably higher mean BMI and rates of obesity than do contemporary Western populations, with 75% of adult females having a BMI greater than 30 kg/m².30 It is unclear whether similar results would be found in leaner populations.

The influence of how gestational diabetes is diagnosed on its association with offspring adiposity

The associations of diabetes during pregnancy with offspring BMI and other measurements of adiposity, assessed in childhood and early adulthood, have been examined in European and North American populations, with the majority of these studies finding that offspring of mothers with diabetes during pregnancy have greater BMI, and higher fasting glucose and insulin levels, in later life.34–37 Some of these studies have reported that this association is confounded by maternal pre-pregnancy BMI since the association attenuates to the null with adjustment for maternal BMI.55 However, in the majority of these studies information on gestational diabetes was obtained from clinical records in populations where diagnostic tests for gestational diabetes were only completed in those considered by clinicians to be at high risk for this condition. Since there is a strong positive association between maternal pre-/early pregnancy BMI and gestational diabetes, women with higher BMI early in pregnancy will have been more likely to have been diagnosed with gestational diabetes than those with lower BMI but also with diabetes (i.e. there will be more normal weight diabetics who are misclassified as non-diabetic because they will have been unlikely to have been offered a diagnostic OGTT). Recent guidelines in the US, UK and other European countries where initial screening is by risk factors, specify a given level of first antenatal clinic BMI that should indicate referral for further testing; most commonly 30 kg/m². However, when many of these studies were conducted no such guidelines existed and clinicians had ‘freedom’ to decide when to refer for diagnostic testing, so we cannot assume a simple differential misclassification between those above 30 kg/m² compared with those below this threshold; a graded misclassification across most of the distribution is possible. This differential measurement error (by early pregnancy BMI) will mean that adjustment for early pregnancy BMI attenuates any association of diabetes with later outcomes more so than in the absence of this measurement error. This can be seen in Figure 5, in which the associations of gestational diabetes and glycosuria with offspring obesity assessed at age 9–10 years, in the ALSPAC cohort, a UK-based prospective birth cohort (http://www.bristol.ac.uk/alspac/)64,65 are shown. In that study the diagnosis of gestational diabetes was abstracted from medical records and, at the time these women were pregnant, diagnostic tests for gestational diabetes were undertaken only in those considered to be at high risk, with high risk defined by the woman’s health practitioners rather than any national guidelines. The association of gestational diabetes with offspring BMI is markedly attenuated with adjustment for maternal pre-pregnancy BMI, whereas the association of maternal glycosuria in pregnancy is very little attenuated.
with this adjustment. At the time of the study glycosuria was routinely assessed at all antenatal clinic visits and all of these results are available. There was a median of 12 measurements of glycosuria in the study sample (IQR 10–14) and the authors classified women as having glycosuria if they had at least ++ (equal to 13.9 mmol/l or 250 mg/100 ml) on at least two occasions at any time during the pregnancy. Because a urine test for sugar was undertaken at nearly every antenatal assessment, this exposure is not determined by health practitioner assessment of early pregnancy BMI, and so the lack of attenuation of its association with later offspring obesity when adjusted for early pregnancy BMI supports my suggestion that the attenuation of the association of clinically diagnosed gestational diabetes with offspring obesity/greater BMI in some studies may be the result of selection bias.

Family comparison studies in European origin populations

A recent large study of Swedish men (mean age 18 years) that included a within sibling analysis (280,866 men from 248,293 families) found that the BMI of men whose mothers had diabetes during their pregnancy was on average 0.94 kg/m² greater (95% CI: 0.35–1.52) than in their mothers born before their mother was diagnosed with diabetes. In that study maternal diabetes could include any of existing type 1, type 2 or gestational diabetes, with no means of distinguishing between them although, as noted above, the majority of diabetes in pregnancy is gestational diabetes. During the period included in this study there was no universal screening for gestational diabetes and women diagnosed with this are likely to have been selected for OGTT on the basis of existing risk factors. This exposure assessment will have been the same within sibling groups; nevertheless, it does mean that some siblings who are assumed to differ in terms of their exposure to maternal diabetes may not have differed that markedly in terms of circulating glucose levels. In part, this may explain the somewhat weaker within sibling association here than in the study of Pima Indians described above. There was no within sibling association of maternal early pregnancy BMI with offspring later BMI in this Swedish study, and adjustment for it did not alter the within sibling maternal diabetes-offspring BMI association. These results were in a relatively lean population (mean maternal early pregnancy BMI was 22 kg/m² and just 2.4% were obese and 13.5% overweight) and the results were largely unchanged when analyses were restricted to siblings with no more than 2 years age difference. These findings provide some evidence that, as in the Pima Indians of Arizona, intrauterine mechanisms contribute to the link between maternal diabetes in pregnancy and adi- posity in offspring in later life in a relatively lean European population, and that this association is not strongly confounded by early pregnancy BMI.

Does exposure to maternal extreme obesity in pregnancy cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

In contemporary clinical practice, obesity in pregnancy is assessed at the first antenatal visit. In research practice, either this first clinical measurement of weight or, if available, a recent pre-pregnancy assessment (including using maternal retrospective report of weight) is used. Pre-/early pregnancy BMI will be little affected by the pregnancy itself and, therefore, the definitions used to categorize pregnant women are the same as those used in the general population (overweight: between 25 kg/m² and 30 kg/m²; obese: between 30 kg/m² and 40 kg/m²; and morbidly obese: above 40 kg/m²). Much of the research regarding the impact of morbid obesity on perinatal and long-term offspring outcomes has been conducted in women eligible for and/or who have had bariatric surgery (including women with morbid obesity and those with a threshold BMI below that for morbid obesity, for example >35 kg/m², who also have obesity-related comorbidity). Therefore, I have used the term extreme obesity (indicating a BMI ≥40 kg/m² or women eligible for bariatric surgery) in this paper.

Whereas studies show continuous linear associations of BMI with blood glucose across the whole
distribution,\textsuperscript{36} and of blood glucose in pregnancy with offspring birth weight,\textsuperscript{29,36} extreme obesity may be associated with very marked metabolic disruption, but there may be too few participants at this extreme in general cohort studies to identify an obvious threshold effect at this level. Therefore, considering this as a distinct exposure from maternal BMI/adiposity across the whole distribution seems reasonable. Related to this, research in this area comes from studies of the impact of bariatric surgery in the extremely obese,\textsuperscript{66–69} and it may not be appropriate to extrapolate from the results of these studies to the impact of BMI/adiposity across the whole range of values seen in pregnant women.

In two elegant studies, offspring of mothers with extreme obesity born before and after their mothers had experienced marked weight loss following gastrointestinal bypass surgery were compared.\textsuperscript{68,69} In the first study 45 children (to 34 women) born before their mother’s surgery, when the mothers had a mean BMI of 48 (SD: 8) kg/m\textsuperscript{2}, were compared with 172 children who were born to 113 women (including the 34 with one or more previous births when they were pre-surgery) after their mothers’ surgery, when their mean BMI was 31 (SD: 9) kg/m\textsuperscript{2}.\textsuperscript{68} The children were aged 2 to 18 years at outcome assessment and the prevalence of overweight and obesity was higher in the children born before surgery than those born after surgery, with this difference being robust when analyses were restricted to within sibling comparisons.\textsuperscript{68}

In the second study, the same group of researchers completed a similar comparison of 54 children born before bypass surgery and 57 after surgery, and again included a nested within sibling study of 37 siblings born before surgery and 38 after surgery to 25 mothers. Both in the whole study population and within siblings, those born before surgery when the mother was severely obese, compared with those born after, had higher mean BMI and a higher prevalence of overweight or obesity; they also had higher body fat, waist:hip ratio, fasting insulin, glucose and triglycerides and lower HDLc (outcomes assessed when offspring were aged 2–26 years).\textsuperscript{69}

Sibling studies are useful for controlling for maternal genotype and many environmental/lifestyle characteristics that are similar during different pregnancies and with siblings (Box 3). Post-surgery, these women will have eaten less and there were notable differences in gestational weight gain (GWG) between pregnancies that occurred before the women had undergone surgery compared with pregnancies after surgery, with GWG much less after surgery, meaning that the associations could be driven by either maternal adiposity in general throughout pregnancy, GWG or a combination of both.\textsuperscript{69} Nonetheless, these studies provide some evidence that extreme maternal obesity during pregnancy is related to greater offspring adiposity and associated adverse cardiometabolic characteristics later in life, at least in part via intrauterine mechanisms.

### Does each ‘extra’ increment of maternal pre-pregnancy or early-pregnancy adiposity cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

By ‘each extra increment’ I am referring to a linear dose-response association of maternal pre-/early pregnancy fatness with later offspring outcomes—i.e. whether on average women with higher BMI (across the whole distribution) have offspring who are fatter through their lifespan. Most studies exploring this association have BMI as the only measure of maternal early/pre-pregnancy adiposity, as weight is commonly measured at the start of pregnancy. A number of studies have demonstrated that maternal pre- or early pregnancy BMI is positively associated with offspring BMI and other measures of offspring adiposity such as waist circumference and fat mass in later life (with studies assessing outcomes across childhood, adolescence and adulthood).\textsuperscript{35,70,71} However, these associations could be explained by shared maternal-offspring risk factors (genetic and lifestyle) for greater BMI as opposed to by intrauterine mechanisms. Evidence from studies comparing associations of maternal and paternal BMI across the whole distribution with offspring adiposity is conflicting (see Box 3 for a discussion of parental comparison studies). Some studies have found stronger associations of maternal BMI with offspring adiposity than of paternal BMI with offspring adiposity, though the differences in the magnitudes between the maternal and paternal associations in these studies are modest.\textsuperscript{49,72,73} In the larger studies, with outcomes assessed when offspring were aged 3–33 years, the magnitudes of associations of maternal BMI with offspring adiposity are similar to those of paternal BMI with offspring adiposity,\textsuperscript{74–77} suggesting that these associations are driven by shared familial genetic or lifestyle characteristics. The large Swedish sibling study, described above, found that maternal early pregnancy BMI was positively associated with offspring BMI (assessed at mean age 18 years) in the whole cohort and between non-siblings, but not within siblings.\textsuperscript{36} These results suggest that the association observed in the cohort as a whole, and in other studies, might be explained by confounding due to characteristics that are identical or very similar in siblings, such as maternal genotype, socioeconomic position, diet and patterns of physical activity.

In addition, a Mendelian randomization study, in which maternal genetic variation in the fat mass and obesity associated (\textit{FTO}) gene, conditional upon offspring \textit{FTO}, was used as an instrumental variable,\textsuperscript{46} to estimate the causal effect of exposure to greater maternal adiposity \textit{in utero} (see Box 3), did not provide support for incremental differences in maternal
BMIs in pregnancy causally affecting offspring adiposity. However, the instrumental variable analysis result was imprecisely estimated with very wide confidence intervals, and further replication of this study in a large cohort or with multiple genetic variants related to adiposity would be valuable.

Thus, for this exposure there is complementary evidence from three different types of study—several parental comparison/negative exposure control studies, a within sibling comparison and a Mendelian randomization study—which despite different underlying assumptions (Box 3) all suggest that incrementally greater early/pre-pregnancy maternal BMI across the whole distribution does not cause greater offspring adiposity through intrauterine mechanisms.

Does each ‘extra’ increment of maternal weight or fat gain in pregnancy cause offspring to be more adipose throughout their lives through intrauterine mechanisms?

The evidence above does not provide strong support for an incremental dose-response association of maternal pre-/early-pregnancy BMI with greater offspring adiposity, but there is evidence for an adverse effect of increased maternal adiposity on a wide range of adverse pregnancy and perinatal outcomes that has stimulated debate about pre-conceptual interventions to promote healthy weight in women of reproductive age. Pre-conceptual optimization of weight is difficult to achieve at a population level. For example, in the UK 50% of pregnancies are unplanned, and even in women planning a pregnancy, only a small proportion will follow nutritional and lifestyle recommendations. Consequently, focus has shifted to the possible beneficial effect (for pregnancy, perinatal and later offspring outcomes) of limiting GWG in pregnant women, since these are in regular contact with health professionals and may be motivated to change their lifestyles to benefit the health of the baby they are carrying.

Assessing gestational weight gain

Many studies examining the association of GWG with later offspring adiposity and related cardiometabolic outcomes categorize women on the basis of the US Institute of Medicine (IOM) categories of below, at, or above recommended GWG (Table 1). There are two concerns in categorizing GWG in this way, for research exploring its role in developmental overnutrition. First, the measure of GWG is based on weight towards the end of pregnancy (but before delivery) minus weight near the start of pregnancy, and hence includes not only weight/fat gain by the mother during pregnancy, but also her pregnancy-related volume expansion and the contribution of placenta, amniotic fluid and the growing foetus. As such it is impossible to distinguish between ‘maternal’ and ‘foetal’ contributions to any later outcome in mother or offspring. For example, although there is some evidence that greater GWG is associated with a greater risk of gestational diabetes, this could be due to reverse causality—i.e. gestational diabetes resulting in greater foetal growth (as expected) which is driving the greater GWG. Rather than GWG being an upstream driver of greater maternal glucose in pregnancy it may be a consequence of it and hence not a factor causing foetal overnutrition.

Second, the way in which the IOM categories are defined make it impossible to distinguish any effect of maternal pre- or early pregnancy BMI from GWG. This is because the recommendations are stratified by pre-/early pregnancy BMI, with considerably lower GWG recommended for those who are overweight or obese than for those who are normal or underweight. Whereas women who are more overweight at the start of pregnancy tend to gain less than those who are not, the extent of these differences is less than the differences between the IOM recommended levels. This is demonstrated in Table 1, using results from the ALSPAC cohort that I estimated for the purpose of this paper. It shows that women who are overweight or obese pre-pregnancy are considerably more likely to be over the recommended GWG level than are those who are normal weight. As such it is impossible to distinguish whether any association of these IOM categories with offspring or maternal outcomes is simply a reflection of maternal pre-pregnancy BMI with later outcomes, or an association of ‘true’ GWG.

In addition to these concerns, a recent study using simulated GWG data suggested that the dependency of GWG on gestational age is sufficient to mean that its association with any outcomes that are also influenced by gestational age (even if this association is weak or modest) may suffer from selection bias. Although this effect may be more problematic for inverse associations (rather than positive associations as in developmental overnutrition), this study is a further reminder of the difficulty of developing an evidence base in relation to whether GWG should be monitored and attempts made to control it in routine antenatal care.

Causal approaches to examining the association of GWG with offspring adiposity in later life

Several studies have examined associations of GWG with offspring adiposity and these have found positive associations with measures of offspring adiposity in childhood (up to age 12 years), adolescence (9–14 years) and adulthood (21 and 32 years). Most of these studies have used fairly crude assessments of GWG, usually based on just two measurements near the start and end of pregnancy, with at
least one of these being self-reported, and many have categorized GWG by IOM criteria. Some of the approaches described in Box 3, that try to understand whether there are causal intrauterine mechanisms linking maternal adiposity or diabetes to offspring outcomes, may not be possible with GWG. For example, few, if any, studies monitor paternal weight gain during a mother’s pregnancy and so comparing maternal with paternal exposure to see if there is a specific (greater) maternal association is not currently possible. To date genetic variants that are robustly associated with variation in GWG, and that could be used as instrumental variables to examine its causal association with later offspring adiposity, have not been identified. However, variants that have been shown to be associated with maternal and offspring adiposity appear not to be associated with GWG.

Two approaches have been attempted to untangle the question of whether greater GWG is causally, via intrauterine mechanisms involving developmental overnutrition, associated with later offspring adiposity. First, in the ALSPAC study, repeat measures of weight in pregnancy (median 10, interquartile range: 8–11) are available for ~12,500 women and these have been used to examine whether there is a specific association of GWG in early pregnancy (when maternal fat accumulation makes the greatest relative contribution to weight gain) with later offspring adiposity and related cardiometabolic outcomes. Here a ‘negative exposure control’ method is being employed (Box 3). The rationale is that, with respect to developmental overnutrition, it is the maternal fat deposition contribution to GWG that is of interest. Since this is the largest contributor to GWG in early pregnancy, with foetal growth and other pregnancy changes contributing more to GWG later in pregnancy, a stronger association of early GWG with offspring adiposity, in comparison with later GWG (negative exposure control), would support the hypothesis. The findings suggest that GWG in early pregnancy (up to 18 weeks of gestation) is linearly associated with offspring adiposity, whereas GWG in mid pregnancy (18–28 weeks) is associated with offspring adiposity only in women who gained >500 g per week, and after 28 weeks, there was no clear association with offspring adiposity. The findings suggest that maternal fat accretion early in pregnancy may be driving the associations of GWG with later offspring adiposity.

Second, the large Swedish sibling study, described earlier, examined the association between maternal weight retention—defined as the difference between maternal weight measured within 24 h of delivery of their baby (but after delivery) and maternal weight at the start of pregnancy—with offspring BMI at mean age 18 years within siblings. It found no evidence of an association of maternal weight retention with offspring BMI. The actual measurements are taken from the UK Avon Longitudinal Study of Parents and Children.

Table 1 US Institute of Medicine recommendations for gestational weight gain and actual weight gain in a UK pregnancy cohort

<table>
<thead>
<tr>
<th>Pre-pregnancy BMI</th>
<th>IoM recommendations</th>
<th>Actual results from a UK pregnancy cohort who were pregnant between 1991 and 1993*</th>
</tr>
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</table>
|                            | Recommended weight gain per BMI category (kg) | Predicted mean rate of weight gain in 2

The actual measurements are taken from the UK Avon Longitudinal Study of Parents and Children.
association within siblings (mean difference in offspring BMI = 0.00; 95% CI: −0.02 to 0.02 kg/m² per 1 kg difference in maternal weight retention) in mothers with a normal pre-pregnancy weight, but among overweight and obese mothers, greater weight retention was modestly positively associated with greater offspring BMI at 18 years (mean difference in offspring BMI = 0.06; 95% CI: 0.01–0.12). This study has two advantages over other studies. First, it uses a measure of weight gain by the mother during pregnancy (not including amniotic fluid, placental and foetal contributions). Second, it compares differences within siblings and so associations are unlikely to be confounded by characteristics, such as family socioeconomic position, maternal genetics or lifestyles that are the same or very similar for siblings (Box 3). The findings from this study raise the possibility that among normal weight women, the positive association of maternal weight gain in pregnancy with later offspring BMI in other studies is driven largely by shared familial (genetic and/or environment) risk factors, whereas in women who are overweight or obese in early pregnancy, greater weight gain appears to be associated with later greater offspring BMI via intrauterine mechanisms in addition to shared familial characteristics. This has some consistency with the finding in the within sibling study before and after bypass surgery, that reduced GWG following surgery in extreme obesity might mediate some of the impact of this maternal extreme obesity on later offspring outcomes. However, further exploration of findings in relation to GWG are important and whether the magnitude of the within sibling association in overweight/obese women found in the Swedish family study is of clinical importance is unclear.

In addition to these two studies, a recent editorial noted that ‘preliminary data on the 4 components [of GWG; maternal tissue, fluid accumulation, the placenta and the foetus] from the US Collaborative Perinatal Project indeed indicate foetal weight gain is the primary predictor of child BMI, whereas a mother’s tissue gain is the only predictor of her own post-partum weight retention’. The findings with respect to maternal postpartum weight retention have been published as a conference abstract, but those with respect to offspring BMI are, as yet unpublished. If this claim regarding offspring BMI is correct, then it suggests that any associations of GWG with offspring BMI are not largely driven by intrauterine mechanisms related to developmental overnutrition (i.e. due to maternal greater fat gain in pregnancy) but that they reflect foetal growth and its relationship to later size. This is based on the editorial suggesting that maternal tissue gain (which would reflect fat gain) was not associated with later offspring BMI, whereas foetal weight gain was. However, these results, together with the details of the study method, need to be verified.

Possible mechanisms
Pregnancy diabetes causes increased foetal fat accumulation through intrauterine mechanisms involving overnutrition of the foetus with glucose and other nutrients (see earlier section of this paper). Similar overnutrition of the foetus could result from extreme maternal obesity even in the absence of diabetes. The effect of this intrauterine overnutrition on infant size and adiposity at birth could result in greater fatness throughout life via tracking. Birth weight is positively associated with later offspring lean and fat mass, and it is possible that larger infants born to mothers with diabetes, or extreme obesity, simply become more adipose adults because of tracking of their size throughout life—i.e. they are born with more (and larger) fat cells on average and this remains the case throughout their lives.

Most association studies, including the sibling studies described above that assess outcomes in offspring ranging from age 2 years to mid 30s, find little evidence that birth weight is a major mediator of the association of pregnancy diabetes or extreme obesity with offspring greater adiposity. This is assessed in these studies by comparing associations in confounder-adjusted models with those in confounder plus birth weight (possible mediator) models. However, measurement error in birth weight, failure to fully account for all potential confounding factors between birthweight (i.e. the mediator) and later offspring adiposity, or to examine whether there are interactions between birthweight and offspring outcomes, could mean that these studies are unable to validly test the hypothesis that the effects are mediated by birth size.

An alternative hypothesis is that greater exposure of the developing foetus to glucose, fatty acids and other nutrients, together with the resultant increased secretion of foetal insulin, influence the development of the hypothalamic–endocrine system that controls appetite. However, direct evidence for this is currently lacking. In the developmental origins of health and disease (DoHAD) field, in general most mechanistic research has been concerned with the impact of developmental undernutrition (often indicated by lower birth weight) on later outcomes and mechanistic research conducted for in utero undernutrition may not be applicable to the issue here of overnutrition.

Does epigenetic modification mediate associations between indicators of developmental nutrition and offspring adiposity?
The general notion that in utero exposures might influence later offspring outcomes via epigenetic modification is gaining momentum in all aspects of DoHAD, to the extent that reports in the popular
press and some scientific journals assume that this is the case. It is, therefore, appropriate that I consider what current evidence is available regarding the role for epigenetic modification in mediating any associations of the developmental overnutrition exposures examined here with later offspring outcomes.

Environmentally responsive, mitotically stable epigenetic phenomena, such as DNA methylation, could potentially provide a plausible mediating mechanism for developmental overnutrition, since a large body of literature supports the influence of nutrition on the epigenome.97 Although an effect of epigenetic mechanisms has been reported in studies of animal models of developmental overnutrition,95,96 few human studies have provided evidence of a causal link between maternal exposures, neonatal or childhood epigenetic variation and subsequent offspring outcome differences. Exposure to the Dutch famine in utero was associated 60 years later with differences in peripheral blood DNA methylation at the IGF2 locus, when compared with that of same-sex siblings who were not exposed to the famine in utero.98 In a study in rural Gambia, where there are marked differences in preconceptual nutrition related to season of conception, season of conception was related to differences in DNA methylation patterns in peripheral blood at metastable epialleles (areas of the DNA in which methylation is likely to be stable for different tissues and cells) in childhood (age 3–11 years).99 Of direct relevance to developmental overnutrition in response to gestational diabetes, a study of 23 women with impaired gestational glucose tolerance (assessed at 24–28 weeks of gestation), compared with 25 age-matched normal glucose tolerance women, found no association of gestational glucose tolerance with DNA methylation of the leptin gene in placental tissue.100 However, in women with impaired glucose tolerance (but not in those with normal glucose tolerance) there was a positive correlation between 2-h post-load glucose levels and maternal-side placental tissue leptin gene DNA methylation, and an inverse correlation with foetal-side placental DNA methylation.100 The authors acknowledged that they were unclear why this association should differ between maternal-side and foetal-side placental tissue, and that their results needed further replication in other studies.100

Whereas these findings are interesting and support the hypothesis that different intrauterine nutritional exposures might affect placental and offspring DNA methylation, alone they do not provide evidence that these associations are causal or that DNA methylation status is an important mediator between maternal exposures, such as gestational diabetes and extreme obesity, and later offspring outcomes. Further research would need to show that these epigenetic modifications influence protein expression, that the changes to this expression influence offspring outcomes, and that each of the associations in the chain are causal; and to date I am unaware of any studies that have shown all of these steps. Other studies have examined the association of foetal DNA methylation patterns with later offspring outcomes. In one study, DNA methylation of the promoter regions of five candidate genes—RXRA, eNOS, SOD1, IL8 and PI3KCD—in umbilical cord tissue was related to DEXA-determined fat mass at age 9 years in an initial cohort of 78 individuals, with further replication in an independent cohort of 239.101 However, only the association of DNA methylation in RXRA with offspring fat mass at age 9 years was found to be replicated in an independent sample.101 In a second study, DNA methylation at 24 candidate gene sites in cord blood samples were related to a range of anthropometric outcomes. Differential methylation at five sites was associated with offspring BMI and/or fat mass at age 9–10 years, but none of these associations withstood adjustment for multiple statistical tests.102 As the authors of these studies101,102 and others103 have noted, such associations of DNA methylation with later outcomes do not prove causality and further replication and methods for determining causality are important.

One way of examining causation in this area is the use of genetic variants that are robustly associated with the environmental exposure of interest (e.g. maternal adiposity or glucose intolerance) and cis genetic variants that are robustly associated with DNA methylation in a two step Mendelian randomization design (Box 3 describes the principles of Mendelian randomization studies in general).103 A recent study, using this approach to examine whether DNA methylation and gene expression causally mediated the association between rapid early postnatal growth and later adiposity, found little supportive evidence,104 but to date this approach has not been used widely or at all in the field of developmental overnutrition.

Importantly, epigenetic signatures are tissue specific and the reliance of epidemiological studies on easily accessible sources of DNA (such as cord or peripheral blood) could limit the capacity to decipher important epigenetic changes in target tissues such as adipose tissue, liver, pancreas, muscle or brain.105 An important contribution can be made by animal studies in this regard, as these target tissues can be more readily interrogated for evidence of epigenetic perturbation than in human studies.

Conclusions and implications for policy and research

In summary, evidence to date from humans suggests that maternal diabetes and extreme obesity in pregnancy influence later offspring greater adiposity, at least in part via intrauterine mechanisms. These effects are likely to relate to increased delivery of nutrients such as glucose and free fatty acids to the developing
foetus, but just how this then results in greater adiposity in the offspring later in life (i.e. beyond birth) is unclear. Although there is currently considerable enthusiasm for the notion that epigenetic modification mediates developmental exposure influences on later offspring outcomes, it is too early to conclude that this is the case. There is a need for this enthusiasm to be balanced by a greater appreciation of some of the challenges to research in this area. Currently, there is little evidence that each ‘extra’ increment in a pregnant woman’s adiposity (across the whole distribution) results in more adiposity in her offspring through causai intrauterine mechanisms. Rather, the available evidence suggests that shared familial environmental or genetic characteristics explain associations of maternal adiposity across the whole distribution with offspring adiposity. Understanding the role of GWG as a key developmental overnutrition exposure that influences offspring adiposity is complicated by available measurements of GWG that are largely unable to distinguish maternal fat accumulation in pregnancy from other contributions to GWG.

The long-term follow-up of participants in ongoing or completed randomized controlled trials that aim to identify and treat women with gestational diabetes and limit weight gain and/or improve diet in obese or overweight pregnant women is an important research priority. These trials have been established to assess the impact of interventions on perinatal outcomes, but they provide an unprecedented opportunity for examining causal intrauterine mechanism related to developmental overnutrition and whether lifestyle modification and treatment with insulin (as appropriate) are effective in reducing long-term adverse outcomes in offspring. Similarly, long-term follow-up of existing birth cohorts that are able to apply some or all of the methods described in Box 3 is important.

In particular, follow-up that supports detailed intergenerational work is likely to be important in the future to understanding mechanisms related to developmental overnutrition and also related to intergenerational transmission of health and disease in general. For example, in the UK ALSPAC cohort, where the original offspring have now a mean age of 21 years and some have already become parents, the current identification, recruitment and assessment of that next generation will provide a uniquely detailed three-generational resource with genetic, phenotypic, antenatal, epigenetic and lifestyle information on three generations from the original parents, their children and now the children of the index children. To realize this potential, funders need to be persuaded of the value of this long-term follow-up and study investigators need to be prepared to work collaboratively across randomized controlled trials, as few will have adequate statistical power alone to precisely estimate long-term effects, and also to work across cohort studies where large sample sizes are required for some of the causal methods outlined in Box 3.

Research is also required to assess whether epigenetic modification is a causal mechanism that explains the effect of maternal diabetes and extreme obesity on future offspring outcomes; this needs to go beyond simple observational studies to studies that can validly assess causality. Such studies include examining the influence of randomized trial interventions targeted at developmental overnutrition exposures on DNA methylation, and examining the evidence that this mediates any long-term effects within those trials. They also include two-stage Mendelian randomization approaches in observational studies as recently described in this journal.

Additional studies that aim to understand whether there is a causal association between GWG and a range of health outcomes are necessary because guidelines for clinical practice in some countries already recommend restricting GWG, despite the current evidence of a health benefit for the mother or her offspring of this policy being limited. Ideally, these studies need to include more repeat measurements of weight, assessments using imaging to compartmentalize different contributions to weight at different stages of pregnancy, and designs such as those described in Box 3 that might better establish causality than conventional association methods.

Despite limited evidence of a causal influence on perinatal or offspring outcomes, or of its ability to identify those at risk, assessment of GWG and attempts to maintain this within IOM recommended levels are applied across the US. But in other areas research in the developmental overnutrition field has appropriately influenced recommendations for antenatal care. The recent recommendations of the International Association of Diabetes and Pregnancy Study Group (IADPG) for diagnosing gestational diabetes have been influenced by research highlighting the potential importance of developmental overnutrition through gestational diabetes. In a notable shift from previous decades, where the thresholds used to define gestational diabetes were directed towards reducing the future risk of maternal type 2 diabetes, the newly proposed IADPG thresholds are directed towards reducing birth size and future offspring adiposity. If these thresholds are widely accepted in clinical practice they would result in an increase in the number of women identified with gestational diabetes in pregnancy. Eighteen percent of the multi-ethnic HAPO cohort would be diagnosed with gestational diabetes based on the newly recommended IADPG diagnostic thresholds of 5.1 mmol/l (92 mg/dl), 10 mmol/l (180 mg/dl) and 8.5 mmol/l (153 mg/dl) for fasting, 1-h and 2-h post-load glucose, respectively.

More generally, the evidence discussed in this paper highlights the importance of identifying and appropriately treating women with diabetes and extreme obesity in pregnancy, not only to improve short-term perinatal...
outcomes, but also because these exposures may (via intrauterine mechanism) affect future risk of greater adiposity in offspring. If the new IADPG criteria for diagnosing gestational diabetes are widely applied, ongoing research and monitoring will be necessary to determine whether these do indeed improve perinatal and future offspring outcomes.

The importance of developmental overnutrition to the current obesity epidemic is unclear. Given the lack of a dose-response intrauterine effect of each increment of maternal adiposity with offspring adiposity and the fact that too few pregnant women had extreme pregnancy obesity or gestational diabetes in the 1940s and 50s to have driven the start of the obesity epidemic in the 1970s in Western countries, it is unlikely that developmental overnutrition played a key role initiating the epidemic. However, as the epidemic has progressed and there are more women entering pregnancy with extreme obesity and being diagnosed with gestational diabetes, it is possible that developmental overnutrition will contribute to the continuation of this epidemic.

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KEY MESSAGES

- Pregnancy diabetes causes increased foetal skeletal growth and fat accumulation through intrauterine mechanisms involving overnutrition of the foetus with glucose and other nutrients, and through increased foetal insulin secretion.
- The long-term consequences of this developmental overnutrition are unclear, but it has been suggested that greater maternal adiposity at the start of pregnancy, greater fat gain during pregnancy and pregnancy diabetes influence future offspring adiposity levels.
- Evidence to date from humans suggests that maternal diabetes and extreme obesity in pregnancy influence later offspring greater adiposity, at least in part, via intrauterine mechanisms.
- There is little evidence that each ‘extra’ increment of a pregnant woman’s adiposity (across the whole distribution) results in more adiposity in her offspring through causal intrauterine mechanisms.
- Understanding the role of gestational weight gain as a key developmental overnutrition exposure is complicated by the fact that most studies are unable to distinguish maternal fat accumulation in pregnancy (the biologically proposed exposure for developmental overnutrition) from foetal and other maternal contributions to gestational weight gain.

References


