DIABETIC Medicine

INVITED REVIEW

Developmental programming by maternal obesity: Lessons from animal models

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Abstract

The obesity epidemic has led to more women entering pregnancy overweight or obese. In addition to adverse short-term outcomes, maternal obesity and/or gestational diabetes predispose offspring to developing obesity, type 2 diabetes and cardiovascular disease in adulthood through developmental programming. Human epidemiological studies, although vital in identifying associations, are often unable to address causality and mechanistic studies can be limited by the lack of accessibility of key metabolic tissues. Furthermore, multi-generational studies take many years to complete. Integration of findings from human studies with those from animal models has therefore been critical in moving forward this field that has been termed the 'Developmental Origins of Health and Disease'. This review summarises the evidence from animal models and highlights how animal models provide valuable insight into the maternal factors responsible for developmental programming, potential critical developmental windows, sexual dimorphism, molecular mechanisms and age-related offspring outcomes throughout life. Moreover, we describe how animal models are vital to explore clinically relevant interventions to prevent adverse offspring outcomes in obese or glucose intolerant pregnancy, such as antioxidant supplementation, exercise and maternal metformin treatment.

KEYWORDS
animal models, exercise, interventions, metabolic syndrome, metformin, obesity, preclinical

1 | MATERNAL OBESITY

The prevalence of maternal obesity is rising globally. In the developed world, up to 30% of women of reproductive age were obese in 2009 and this number is likely to increase in the future.[1] Although genetics, a sedentary lifestyle and unhealthy diets contribute to this trend, the global rise in obesity and its comorbidities cannot be explained by genetics and current lifestyle alone. In recent years, it has become widely accepted that the environment that we are exposed to in the womb and during the first months of life may influence our risk of becoming obese as well as developing its comorbidities, such as type 2 diabetes. Indeed, epidemiological evidence has shown that obesity during pregnancy, in addition to detrimental pregnancy and perinatal outcomes (including the development of gestational

diabetes mellitus [GDM]), is associated with long-term effects on offspring health such as increased risk of obesity, cardiovascular disease and the metabolic syndrome (reviewed in Alfaradhi et al.[2] and Blackmore et al.[3]). This is not entirely explained by genetics or the shared postnatal environment, as siblings born after bariatric surgery have lower rates of obesity, insulin resistance and hypertension than their siblings born before maternal weight loss. The prenatal obesogenic environment can thus program offspring predisposition to metabolic and cardiovascular abnormalities. This has been referred to as 'developmental programming' or 'the Developmental Origins of Health and Disease' hypothesis (DOHaD).[2,3]

2 | ANIMAL MODELS OF MATERNAL OBESITY

2.1 | Why animals?

Human epidemiology studies are vital in identifying associations between intrauterine insults and long-term offspring outcome. However, it is challenging to demonstrate causal relationships in such studies, and they are not suitable for elucidation of potential mechanisms as it is unethical to manipulate human pregnancy and clinically accessible tissues are limited. In addition, many offspring outcomes of *in utero* exposure to obesity, such as cardiometabolic disease, develop with age and the required life-course studies would take decades to complete. Animal models thus provide a valuable alternative. Species used to study developmental programming include rodents, sheep and to a lesser extent non-human primates. The latter are most closely related to humans, but their gestation period is long and they are expensive to maintain.[2] Sheep are used for their similarities to humans with respect to maturity at birth as well as the ability to carry out complex foetal physiology studies.[4,5] Nevertheless, rodent models are often preferred due to their relatively short gestation and lifespan thus allowing experiments across the life-course. Moreover, their identical genetic background allows to specifically demonstrate non-genetic effects in a controlled environment. Animal models of maternal overfeeding or obesity have replicated epidemiological associations with cardiometabolic disease, converging on offspring adiposity, insulin resistance and hypertension (Figure 1).[2]
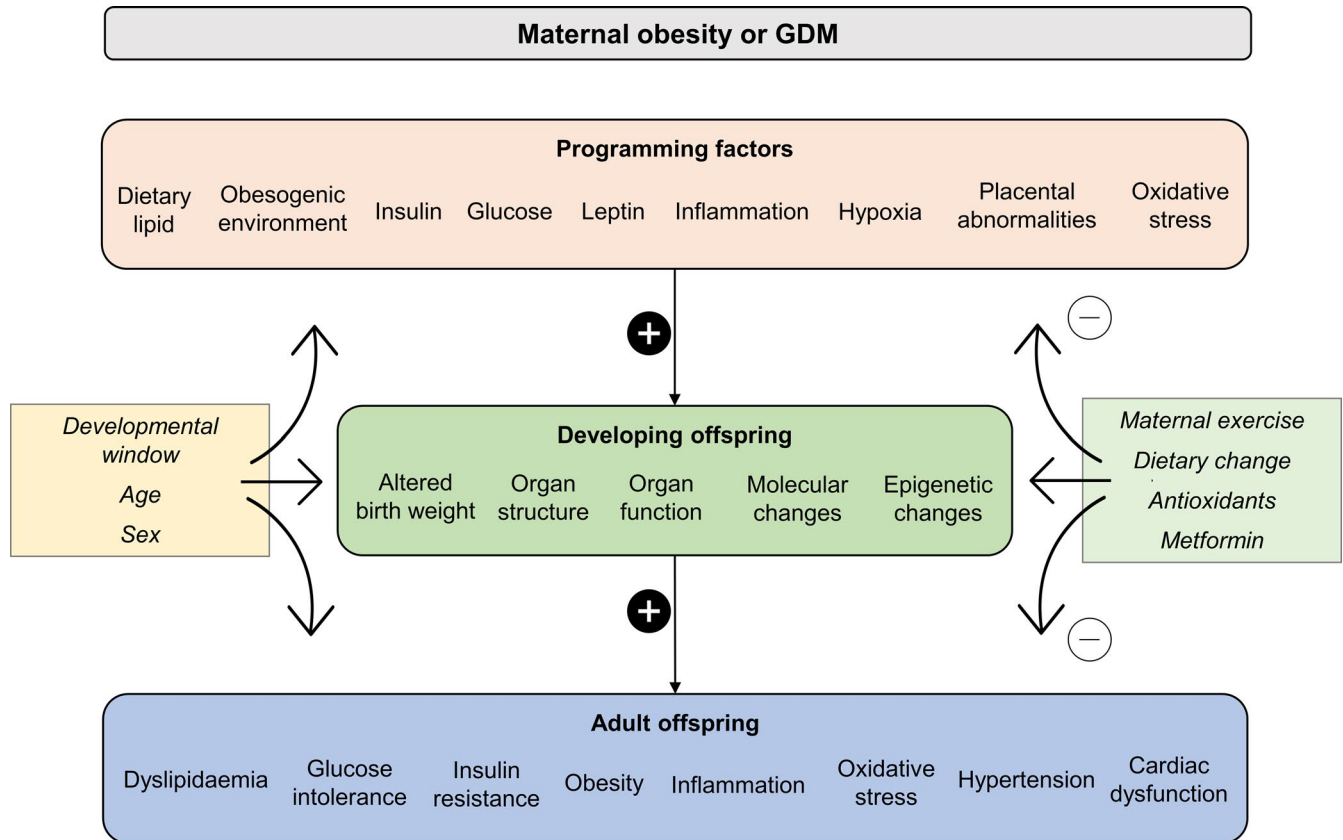
2.2 | Types of animal models

There are several ways to study programming by maternal obesity or GDM in rodent models. Genetic models of obesity can be used, such as the Lepr$^{db/+}$ model, where

Novelty statement
- Epidemiological studies show that maternal obesity during pregnancy is associated with risk of offspring developing obesity, type 2 diabetes and cardiovascular disease later in life.
- Animal models help to define causal mechanisms underlying the long-term effects of development in an *in utero* obesogenic environment.
- This review highlights the importance of animal models in studying the long-term age- and sex-dependent effects of maternal obesity and potential clinically relevant interventions on offspring outcome.

dams heterozygous for the leptin receptor are bred with wild-type males to generate offspring exposed to a GDM-like environment *in utero* (reviewed in Vambergue et al.[6]). However, genetic models often have limited translational impact as they do not reflect common causes of human obesity. Alternatively, maternal obesity can be induced by overfeeding the same diet as the control group, such as in a sheep model of gestational overfeeding where sheep were provided with 150% of food as required by veterinary guidelines, compared to 100% in the control group.[4] Other models use experimental diets to reflect a human Western diet, either initiated before gestation to induce pre-conception obesity[7] or closer to conception.[8] The timing of exposure can help distinguish between the impact of long-term consequences of factors such as pre-pregnancy maternal obesity (including endocrine changes at the time of conception and impact on oocyte development) and the direct effects of the diet on the foetus and/or exposure to an altered *in utero* metabolic milieu independent of maternal obesity. Experimental diets can also be introduced or replaced with control diets during lactation or offspring can be cross-fostered to enable lactation by dams on a different diet, thereby targeting specific developmental windows.[2] Experimental diets include high-fat diets (HFD), usually ranging from 40% to 60% kcals from fat.[9,10] Despite their ability to introduce metabolic alterations, HFD pellets are less palatable than chow and HFD-fed rodents often match chow-fed animals in caloric intake by suppressing the amount of pellet they eat.[7] Alternative diets include those high in both fat and sugar (HFHS) or diets consisting of highly palatable human foods termed 'junk food' or 'cafeteria diets' to cause maternal obesity.[7,11]

Genetic or diet-induced maternal obesity models often lead to maternal insulin resistance and/or glucose intolerance and can thus act as models for obese GDM as well. However, models specific for maternal hyperglycaemia/

Maternal obesity or GDM

Programming factors

| Dietary lipid | Obesogenic environment | Insulin | Glucose | Leptin | Inflammation | Hypoxia | Placental abnormalities | Oxidative stress |

(+)

Developmental window
Age
Sex

Developing offspring

| Altered birth weight | Organ structure | Organ function | Molecular changes | Epigenetic changes |

Maternal exercise
Dietary change
Antioxidants
Metformin

(−)

(+)

(−)

Adult offspring

| Dyslipidaemia | Glucose intolerance | Insulin resistance | Obesity | Inflammation | Oxidative stress | Hypertension | Cardiac dysfunction |

FIGURE 1 Developmental programming by maternal obesity or GDM. An obese or diabetic intrauterine environment is associated with altered maternal nutritional and endocrine status, placental abnormalities and increased inflammation, hypoxia and oxidative stress. These can all act as programming factors leading to long-term changes in organ structure and function in developing offspring, including at the molecular and epigenetic levels. These changes, which are heavily dependent on the timing of the exposure as well as sex and age of the offspring, predispose offspring to developing the metabolic syndrome in adulthood. Interventions such as maternal exercise, dietary improvements, antioxidant supplementation and metformin treatment may influence programming factors and consequently outcomes in exposed offspring. (+) refers to the effects of maternal obesity, (−) refer to interventions negating these effects

diabetes, but not necessarily obesity, are also informative and help address effects of exposure to increased glucose *in utero*. These include models of hyperglycaemia, either via genetic models such as the type 1 diabetes-like 'non-obese diabetic' mouse or by injection of wild-type mice with streptozotocin, a toxin that preferentially attacks pancreatic β-cells (reviewed in Vambergue et al.[6]). Some models show normal or mildly altered glucose homeostasis before mating but develop into full-blown diabetes when pregnant. These include models of streptozotocin injections at a later timepoint in gestation and the Lepr$^{db/+}$ mouse.[6]

3 | OFFSPRING OUTCOMES IN ANIMAL MODELS

3.1 | Obesity

Animal research clearly shows that offspring body composition is adversely affected by exposure to an obese environment *in utero*. A meta-analysis looking at various animal models of maternal pre-conception obesity found significantly increased body weight (123 studies, $n = 5772$), body fat percentage (32 studies, $n = 1284$) and absolute fat mass (10 studies, $n = 205$) in offspring of obese pregnancy. Importantly, all offspring were fed healthy chow postnatally, and the association held true when stratified by sex, species and offspring age.[12] Notably, body weight in chow-fed male offspring exposed to maternal obesity was similar to body weight of control offspring given HFD from adulthood, indicating the effect of maternal obesity is of equal magnitude to that of postnatal diet.[13] Moreover, the effects of maternal and postnatal diet were additive, with highest adiposity in the 'double-exposed' group. Adipocyte hypertrophy and hyperplasia have been implicated in the developmental programming of later obesity by maternal overnutrition or GDM in rodent models and occurs prior to changes in body weight.[2,14] Exposure to maternal obesity can introduce permanent changes in adipose tissue function

as well as mass. Rat offspring of dams fed a HFD before and during pregnancy (but not lactation) developed alterations in both the plasma and adipose tissue lipidome characterised by elevated circulating triglycerides despite *de novo* lipogenesis, suggesting inability of the adipose tissue to adequately store lipid.[15] Interestingly, offspring adiposity and adipose tissue dysfunction may not be programmed in the same critical developmental window: male offspring of mice fed a HFD pre-pregnancy showed exaggerated adiposity when challenged with a HFD in adulthood, whereas age-matched offspring from dams where the HFD was continued in pregnancy showed adipose tissue inflammation and glucose intolerance without differences in fat mass.[16]

Altered energy homeostasis likely contributes to adiposity in offspring of obese pregnancy. A rat study of maternal HFHS-induced obesity in pregnancy and lactation showed adiposity and basal hyperphagia in both male and female chow-fed offspring,[17] although other studies required a 'stressor' such as a postnatal unhealthy diet or fasting and refeeding to observe offspring hyperphagia.[11,18] These effects may be related to programmed changes in the hypothalamic circuits regulating appetite and energy expenditure. Upregulation of orexigenic peptides (NPY and AgRP) and downregulation of anorexigenic peptides (POMC) in the hypothalamus of offspring exposed to maternal overnutrition have been reported (reviewed in Dearden et al.[19]). Additionally, offspring of obese rat dams exhibit resistance to the appetite suppressing and hypothalamic signalling effects of leptin that may contribute to the development of hyperphagia and obesity.[17]

3.2 | Type 2 Diabetes

Hyperglycaemia and hyperinsulinaemia are common outcomes in animal studies looking at adult offspring of obese or HFD-fed dams. A meta-analysis of animal models of maternal pre-conception obesity found that exposed offspring have significantly higher glucose (68 studies, $n = 1980$) and insulin levels (70 studies, $n = 1975$) as well as increased insulin resistance as assessed by HOMA-IR (13 studies, $n = 554$ animals).[12] Changes in insulin sensitivity precede alterations in glycaemia. Rat offspring from obese Western diet-fed dams had higher serum insulin concentrations compared to controls at birth, which was maintained at 2 weeks and 2 months of age, at which point they developed glucose intolerance.[14] Moreover, in addition to systemic insulin resistance, tissue-specific insulin resistance in liver, brain, muscle and adipose tissue has been described in offspring exposed to maternal overfeeding.[2]

The effect of maternal obesity on offspring insulin secretion remains less well studied, despite impaired insulin secretion being required for progression from glucose intolerance to type 2 diabetes. Male offspring of HFHS-fed obese mice were insulin resistant at 3 months of age, evidenced by elevated serum insulin and pancreatic insulin content without differences in glucose. By 6 months of age, they had developed a type 2 diabetes-like phenotype with decreased serum and pancreatic insulin resulting in fasting hyperglycaemia, indicative of β-cell exhaustion and insulin secretory impairment.[7] Unlike males, 6-month-old female offspring had increased pancreatic insulin content and a comparable glucose tolerance to controls, suggesting sexual dimorphism in the timing of programmed outcomes.[7] Defects in insulin secretory capacity may be programmed directly by maternal obesity. A study of maternal HFHS-induced obesity found signs of islet malfunction in 8-week-old male offspring. These mice maintained normal glucose-stimulated insulin secretion at this age, but underlying defects detected may predispose to future β-cell failure.[20] Consistent with the sex differences in 6-month-old offspring described above, an improvement in islet function was observed in female offspring, suggesting that exposure to an intrauterine obesogenic environment may have prepared females for a nutrient-rich postnatal life, at least in the short term.[20] Whether this protection is long-lived remains unclear, since glucose-stimulated insulin secretion was decreased in 9-month-old female offspring of HFD-fed rats.[21] Maternal obesity therefore increases type 2 diabetes risk by affecting both insulin resistance and insulin secretion.

3.3 | Cardiovascular disease

The aforementioned meta-analysis of pre-conception maternal obesity models also found a significant increase in offspring systolic blood pressure by tail cuff plethysmography (9 studies, $n = 251$) as well as dyslipidaemia (elevated triglycerides [46 studies, $n = 1337$], total cholesterol [27 studies, $n = 795$] and LDL-cholesterol [4 studies, $n = 158$ animals]) independent of species, sex or age.[12] Since hypertension and dyslipidaemia are both independent risk factors for cardiovascular disease, this suggests maternal obesity can program predisposition not only to type 2 diabetes, but cardiovascular pathologies as well. Alongside adiposity, 3- and 6-month-old mouse offspring exposed to maternal HFHS-induced obesity showed elevated systolic and diastolic blood pressure in the dark (active) phase.[7] Although adiposity is a risk factor for hypertension, changes in offspring BP often precede offspring obesity and thus seem independently programmed.

Indeed, studies in young adult offspring have shown elevated systolic blood pressure in male offspring exposed to maternal obesity at 8 weeks of age, when body weight remained similar to controls.[22] Moreover, male and female offspring of obese glucose intolerant dams showed hyperreactivity of mesenteric arteries to noradrenaline stimulation and impaired endothelium-dependent relaxation to acetylcholine.[7] Programmed changes in offspring vascular endothelial function by maternal obesity could thus contribute to their risk of hypertension.

Maternal obesity also affects offspring hearts. Maternal HFHS-induced obesity increased left ventricular weight in 8-week-old male and female offspring.[23] Studies using a similar mouse model found increased heart weight as early as 3 weeks of age in male offspring, accompanied by increased cardiomyocyte size and re-expression of foetal cardiac genes, indicative of pathological hypertrophy.[24] This occurred before the development of obesity, suggesting direct cardiovascular programming by maternal obesity independently of postnatal adiposity, and these changes may persist throughout life as this phenotype was also observed at 8 weeks of age.[24] Accordingly, cardiac morphological changes were also an early phenotype in sheep foetuses exposed to gestational overfeeding.[4] With regard to function, *ex vivo* assessment of heart function using the isolated Langendorff heart perfusion preparation showed systolic and diastolic dysfunction with sympathetic dominance in 12-week-old offspring of obese mouse dams.[24] Similarly, impaired systolic and diastolic functions have been shown *in vivo* using echocardiography in rodent offspring.[10,22]

3.4 | The effect of age

Programmed differences in adiposity and glucose tolerance have been shown to become greater with age. For instance, female offspring of non-obese HFD-fed rats showed no difference in body weight or serology at 80 and 180 days but were obese, hyperglycaemic and dyslipidaemic by 360 days of age.[25] A mouse study of maternal diet-induced obesity demonstrated deterioration of insulin secretion in male offspring of obese dams between 3 and 6 months of age, indicating ageing was required for progression from glucose intolerance to type 2 diabetes in this model.[7] Similarly, despite normal glucose tolerance for the first 3 months of life, both male and female mouse offspring of dams fed a HFD from 2 weeks pre-conception developed glucose intolerance by 36 weeks, which persisted until 52 weeks of age and was accompanied by hyperinsulinaemia and whole-body insulin resistance.[26,27] Both basal and stimulated hepatic glucose output were increased throughout life but drastically increased between 12 and 24 weeks of age in female offspring of HFD-fed dams, clearly showing an ageing effect.[27] Moreover, hepatocytes isolated from female offspring at 6, 12 and 24 weeks of age were resistant to the inhibitory effect of insulin on glucose production, indicating that peripheral insulin resistance may precede and contribute to changes in whole-body insulin resistance observed later in life.[27] A different murine study of maternal HFD feeding without overt obesity found glucose intolerance and insulin resistance in female offspring at 9 but not 6 or 12 months of age,[28] stressing the importance of investigating several timepoints across the life-course.

Although body weight, fat mass and glucose tolerance were not altered in in 8-week-old male offspring of HFHS-induced obese mouse pregnancy,[22,24] they developed increased adiposity and metabolic abnormalities by 6 months of age.[29] These 6-month-old male offspring showed epididymal fat expansion with adipocyte hypertrophy, altered expression of lipogenic enzymes, endoplasmic reticulum stress, systemic and epididymal fat insulin resistance as well as glucose intolerance during a glucose tolerance test.[29] Few studies have investigated the effects of maternal obesity (rather than gestational HFD feeding) in offspring aged for 12 months or longer. A study in rats showed that both male and female offspring of obese dams showed excessive age-related adiposity, insulin resistance, hyperinsulinaemia, hyperleptinaemia and fatty liver disease at 12 months of age.[30] Moreover, age-related patterns of worsening phenotypes were initiated earlier in these animals indicative of accelerated ageing accompanied by shortened lifespan.[30]

Accelerated ageing was also evident in female mouse offspring of dams fed a HFD from one week pre-mating: offspring were heavier as adults (12 weeks) but reached their maximum weight sooner and lost relatively more fat mass with further ageing when compared to offspring of control-fed dams.[31] They also showed increased prevalence of senescence-associated T cells as well as hepatic inflammation and fibrosis at 70 weeks of age,[31] indicative of immunological ageing. In another study of maternal overfeeding, increased fur thinning and markers of osteoporosis were indicative of accelerated ageing in female offspring of HFD-fed mice.[9]

4 | FACTORS MEDIATING EFFECTS OF MATERNAL OBESITY

Several factors may mediate developmental programming by maternal obesity, including diet and lipids, glucose homeostasis and hyperinsulinaemia, adipokines, inflammation, placental dysfunction and oxidative stress (Figure 1).

4.1 | Maternal diet

Feeding pregnant rats a 'junk food' diet from conception (thus avoiding pre-conception obesity) to weaning induced obesity and altered food preferences in 10-week-old offspring, illustrating the influence of maternal diet during gestation and lactation that is independent of any pre-pregnancy effect on the oocyte.[11] Maternal HFD initiated at conception is sufficient to program obesity, insulin resistance and glucose intolerance in mouse offspring indicating a role for dietary lipid specifically including potential direct effects of the *in utero* milieu on the foetus that are independent of maternal obesity.[8] Indeed, a study comparing offspring of dams fed HFD without obesity (HFD in pregnancy and lactation only) to offspring of pre-conception obese HFD-fed dams found comparable degrees of obesity and hyperinsulinaemia in adult offspring from both groups, suggesting the maternal lipid intake as a crucial factor.[32] In contrast, another study found increased body weight and fat mass in offspring when dams were fed a HFD *ad libitum*, but not when isocaloric to control dams, indicating that the obese intrauterine environment itself is important for the programming of offspring obesity.[13]

4.2 | Glucose homeostasis

Maternal fasting insulin (but not lipids or adiposity) directly correlates with insulin in 8-week-old male offspring from HFHS-fed obese mouse dams, pointing towards a key role for maternal hyperinsulinaemia in the programming of offspring type 2 diabetes risk.[33] Accordingly, intrauterine exposure to maternal genetic insulin resistance (without maternal obesity or glucose intolerance) induces hyperinsulinaemia, insulin resistance and glucose intolerance and fatty liver disease in wild-type offspring.[34] These offspring did not show changes in body weight or adiposity up to 6 months of age, suggesting factors other than maternal hyperinsulinaemia or insulin resistance were responsible for the programming of obesity.[34] Direct effects of maternal insulin on the developing foetus are unlikely as insulin does not cross the placenta. Programming effects may instead occur secondary to increased placental insulin signalling, which could promote placental lipid deposition as well as directly alter placental function.[33] In addition, enhanced placental transfer of glucose (which freely crosses the placenta) in hyperglycaemic pregnancies stimulates foetal insulin secretion, leading to fat accretion and consequent adiposity as seen in foetal rhesus monkeys chronically infused with insulin in late gestation at a concentration similar to that expected in

human diabetic pregnancy.[35] Maternal hyperglycaemia may also contribute to cardiovascular disease risk in offspring. Neonatal rat offspring of both HFD-fed and streptozotocin-injected dams displayed cardiac dysfunction, but offspring exposed to the combination of maternal HFD feeding and diabetes had the most severe impairment, indicating a role for both diet and glycaemia.[10] Lastly, insulin is a neurotrophic factor and elevated foetal insulin levels could impact on the development of hypothalamic circuits regulating energy balance and food intake (reviewed in Dearden et al[19]).

4.3 | Leptin

Maternal obesity is associated with excess circulating levels of the adipokine leptin, as is also seen in animal models of obese pregnancy.[7,13] Late gestation leptin infusion in foetal sheep led to alterations in adipose tissue morphology and transcriptome, indicating effects of leptin on developing adipose tissue.[5] Leptin administration in mid-gestation was recently shown to affect placental size and transcriptome, suggesting leptin may also influence foetal development through actions on the placenta.[36] In adults, leptin suppresses food intake and promotes energy expenditure through actions on the hypothalamus. Leptin levels in rodents rise sharply in the second week of postnatal life, giving rise to a 'neonatal leptin surge' that occurs independently of neonatal adiposity and does not impact on body weight or glucose homeostasis. During this time, leptin regulates the development and organisation of hypothalamic circuits involved in food intake.[19] Abnormal leptin levels in offspring exposed to maternal obesity, such as the prolonged and exaggerated leptin surge observed in offspring of HFHS-fed obese dams, may thus lead to permanent changes in hypothalamic control of energy balance.[17] Like maternal obesity, neonatal leptin injections also increased offspring BP in adulthood, suggesting alterations in the leptin surge may program both hyperphagia and hypertension by driving sympathetic hyperactivation in the brain independent of its effects on food intake and obesity.[37]

4.4 | Inflammation, hypoxia and oxidative stress

Obese pregnancy is associated with elevated levels of inflammatory markers in serum and adipose tissue, indicating dams enter pregnancy in a pro-inflammatory state that may be transmitted to the fetus.[38] Accordingly, inflammatory markers are also found in the placenta and

in several tissues of both foetal and offspring exposed to maternal obesity.[2] Notably, a study of HFD-induced obesity in a mouse model proposed that increased placental IL-6 signalling was directly responsible for the decreased fetoplacental vasculature and endothelial cell damage observed in this model.[38]

Placental abnormalities described in animal models of maternal obesity may lead to decreased nutrient and oxygen delivery to the foetus, leading to intrauterine growth restriction.[38] Furthermore, levels of hypoxia-inducible factor 1 alpha were elevated in late gestation placentas from obese mouse pregnancies and correlated with maternal insulin, suggesting a direct effect of the maternal metabolic state on placental function.[33] Intrauterine hypoxia likely contributes to adverse outcomes in offspring, as shown by experimental models of maternal and foetal hypoxia using hypoxic chambers which have demonstrated cardiovascular dysfunction in adult rat offspring.[39] Paradoxically, some of the programming effects of hypoxia and/or maternal obesity may be mediated by oxidative stress, since supplementation of antioxidants during maternal hypoxia ameliorates offspring outcomes.[39] Placentas of obese mouse dams show excess lipid deposition, thus increasing the risk of placental oxidative stress with maternal obesity.[33] Accordingly, markers of lipid peroxidation were found in isolated cardiomyocytes from newborn rat offspring of HFD-fed (but not of diabetic) dams.[10] Oxidative stress in exposed offspring may be an early effect as evidence of oxidative stress was already present in pre-implantation embryos, foetuses and newborns from Western diet-fed dams.[14]

5 | INTERVENTION STRATEGIES

The concept of DOHaD offers a new and early intervention window for the prevention of non-communicable diseases. In order to prevent the negative effects of the obesity epidemic being transmitted to the next generation, we need interventions that can be easily implemented in pregnancy with beneficial effects on mother and baby. It is especially important to break the vicious cycle of maternal obesity, referring to the effect that daughters of obese mothers are at increased risk of being obese in pregnancy themselves due to developmental programming and therefore potentially transmit poor cardiometabolic health to their children. Clinically relevant interventions aim to target the abovementioned programming factors, with most studies focusing on improving maternal glycaemia, insulin sensitivity and/or weight. These can be broadly classified into lifestyle and pharmaceutical interventions (Figure 1).

5.1 | Lifestyle interventions

5.1.1 | Dietary interventions

Lifestyle interventions in humans often involve dietary advice. Animal models of maternal diet manipulation show beneficial effects of dietary change in obese pregnancy and/or lactation. In rats, switching dams from a junk food to a control diet after birth prevented hyperphagia and obesity when offspring were weaned onto a junk food diet.[11] Similarly, after 2–4 years of HFD in non-human primates, placing mothers on a healthy diet for a few months before pregnancy attenuated the maternal insulin response to a glucose tolerance test (in the absence of changes in body weight), prevented foetal growth restriction and significantly attenuated the increase in foetal hepatic triglycerides and gluconeogenic gene expression.[40] These data demonstrate that the pre-conception and early postnatal periods, in addition to pregnancy itself, are promising time windows for early life intervention. However, although pair-feeding hyperphagic Lepr$^{db/+}$ mice to wild-type dams improved maternal and foetal outcomes, restricting intake to 70% of controls led to intrauterine growth restriction.[41] Therefore, improving diet quality rather than caloric content may be a more successful approach.

Research has also turned to dietary supplements as an intervention. Gestational dietary supplementation with the antioxidant Quercetin in a murine diet-induced maternal obesity model attenuated the hyperinsulinaemia, hyperglycaemia, obesity and hypertension observed in female offspring,[9] and antioxidant supplementation to Western (but not control) diet-fed rat dams decreased offspring adiposity, leptin and insulin levels, and improved glucose tolerance.[14] Interestingly, the latter intervention prevented maternal hyperinsulinaemia but did not affect maternal body composition, indicating that programming effects can be prevented without changes in maternal adiposity.[14]

5.1.2 | Exercise interventions

Moderate exercise during obese pregnancy can correct placental hypoxia, maternal insulin levels and glucose tolerance in pregnancy without affecting maternal weight, adiposity or leptin.[22,33] These maternal improvements also corrected the hyperinsulinaemia and adipose tissue insulin resistance in 8-week-old male offspring and prevented the cardiac hypertrophy and LV dysfunction observed in offspring of untreated obese dams through improved calcium homeostasis and cardiac contractility.[22,33] Interestingly, the exercise intervention had no

effect on offspring blood pressure.[22] Cardiac function and blood pressure may thus be programmed through different mechanisms and will therefore likely need different intervention strategies to correct them. Maternal exercise was also shown to prevent adiposity and hepatic steatosis in 4-month-old offspring of Western-diet fed obese mouse dams.[42] Gestational exercise also prevented the hyperinsulinaemia and insulin resistance observed in 12-month-old female mouse offspring of HFD-fed dams.[27] Moreover, although training in gestation alone was sufficient to improve glucose tolerance in young adult males, both male and female offspring were protected against glucose intolerance and adiposity until 12 months of age when the exercise intervention was initiated 2-week pre-gestation, illustrating the importance of the timing of interventions.[26,27]

Lifestyle interventions are extremely useful since they are affordable and easy to implement. However, poor compliance in humans means these often lead to only modest improvement in maternal body weight or metabolic health, and differences in offspring obesity risk can disappear with time. Therefore, research has addressed potential pharmacological interventions, such as metformin.

5.2 | Metformin intervention

Metformin is an oral glucose-lowering agent that is increasingly used in type 2 diabetes- and GDM-complicated pregnancies where glucose levels cannot be controlled by lifestyle alone.[43] Metformin is an attractive candidate to prevent programming by maternal obesity as it is cheaper and easier to administrate than insulin, limits weight gain in humans, has anti-inflammatory and vasculoprotective effects and may reduce oxidative stress.[43] However, metformin readily crosses the placenta, and although the intervention does not lead to birth defects, concerns regarding long-term effects of direct exposure *in utero* have been raised.[43] Longitudinal follow-up of human trials investigating metformin use in pregnancy is limited by the age of the exposed offspring and long-term outcomes will not be reported on for many years. Animal models are therefore crucial to explore such long-term consequences.

Treatment with a clinically relevant dose of metformin during pregnancy improved maternal metabolic health and placental function in a mouse model of diet-induced obesity but did not rescue foetal growth restriction.[44] To date, few studies have published metabolic outcomes in adult offspring exposed prenatally to metformin, and with conflicting results. Salomäki et al. provided metformin during chow-fed pregnancy and found that metformin introduced IUGR at E18.5.[45]

Although no difference was seen in chow-fed offspring until 10 weeks of age, metformin-exposed offspring developed adiposity and sex-specific metabolic dysfunction after a 10-week HFD challenge, with hypercholesterolaemia in females and hyperglycaemia, glucose intolerance and adipose tissue insulin resistance in male offspring.[45] A follow-up study by the same group found that when metformin was given to pregnant obese dams fed a HFD before and during pregnancy (but not lactation), female offspring gained less weight and fat mass on HFD and male offspring had improved lipid profiles.[46] Maternal metformin also prevented the worsening glucose tolerance normally seen after 7 weeks of HFD feeding, suggesting offspring were protected against HFD-induced obesity and glucose intolerance in adulthood.[46] These contrasting results highlight the importance of the maternal metabolic state with regard to offspring outcome. A latter study by the same group using a genetic model of obesity (selective NPY overexpression in the brain and sympathetic nervous system) showed protection against HFD-induced obesity and hyperinsulinaemia in 7-month-old males, compared to exacerbated obesity and metabolic dysfunction in female offspring.[47] In addition to confirming the importance of the maternal environment in determining offspring response to metformin, this study highlighted the importance of studying both sexes in a programming context. Interestingly, although in these studies the adverse or beneficial effects of prenatal metformin intervention on offspring adiposity and metabolic health emerged only after 10–20 weeks of age, it is unclear what changes were due to ageing or postnatal HFD, respectively. Further studies are thus required to determine the long-term consequences of metformin intervention in these models.

In a different model of HFD-induced obese pregnancy, metformin treatment attenuated adiposity and glucose intolerance and prevented muscle metabolic dysfunction in 8-week-old male offspring weaned onto HFD, suggesting protective effects on offspring metabolic health.[48] In contrast, increased adiposity and insulin was found in female offspring using a similar model in the rat.[49] These studies provided metformin treatment during both pregnancy and lactation, which differs from human clinical settings. The only study to date investigating gestational (but not lactational) metformin treatment in obese glucose intolerant pregnancy found adiposity and adipose tissue inflammation in male, but not female, young adult offspring.[50] The heterogeneity of evidence highlights that post-weaning diet, timing of metformin exposure and the interaction between metformin and the maternal environment are critical. In addition, long-term follow-up in both male and female offspring will be vital to explore potential sexual dimorphism and age-related effects.

6 | CONCLUSION

In conclusion, animal models provide an extremely useful tool to study offspring outcomes in models of developmental programming by maternal obesity and/or GDM. Specifically, they are able to address causality, allow the longitudinal assessment of cardiometabolic outcomes across the life-course and the investigation of sex differences, and they can help determine the critical windows during which offspring are particularly vulnerable to programming effects. Moreover, the evidence presented highlights the importance of addressing both short- and long-term effects of interventions on both mother and offspring. Animal studies therefore provide an ethical first step to investigate clinically relevant interventions that can be implemented during pregnancy, which is specifically relevant when assessing age-related outcomes such as type 2 diabetes and cardiovascular disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Poston L, Caleyachetty R, Cnattingius S, et al. Preconceptional and maternal obesity: epidemiology and health consequences. *Lancet Diabetes Endocrinol*. 2016;4(12):1025-1036. doi:10.1016/S2213-8587(16)30217-0
2. Alfaradhi MZ, Ozanne SE. Developmental programming in response to maternal overnutrition. *Front Genet*. 2011;2:27. doi:10.3389/fgene.2011.00027
3. Blackmore HL, Ozanne SE. Programming of cardiovascular disease across the life-course. *J Mol Cell Cardiol*. 2015;83:122-130. doi:10.1016/j.yjmcc.2014.12.006
4. Fan X, Turdi S, Ford SP, et al. Influence of gestational overfeeding on cardiac morphometry and hypertrophic protein markers in fetal sheep. *J Nutr Biochem*. 2011;22(1):30-37. doi:10.1016/j.jnutbio.2009.11.006
5. Yuen BSJ, Owens PC, Muhlhausler BS, et al. Leptin alters the structural and functional characteristics of adipose tissue before birth. *FASEB J*. 2003;17(9):1102-1104. doi:10.1096/fj.02-0756fje
6. Vambergue A, Fajardy I. Consequences of gestational and pregestational diabetes on placental function and birth weight. *World J Diabetes*. 2011;2(11):196. doi:10.4239/wjd.v2.i11.196
7. Samuelsson A-M, Matthews PA, Argenton M, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension*. 2008;51(2):383-392. doi:10.1161/HYPERTENSIONAHA.107.101477
8. Yokomizo H, Inoguchi T, Sonoda N, et al. Maternal high-fat diet induces insulin resistance and deterioration of pancreatic β-cell function in adult offspring with sex differences in mice. *Am J Physiol – Endocrinol Metab*. 2014;306(10):E1163-E1175. doi:10.1152/ajpendo.00688.2013
9. Liang C, Oest ME, Prater MR. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. *Birth Defects Res Part B – Dev Reprod Toxicol*. 2009;86(5):377-384. doi:10.1002/bdrb.20206
10. Mdaki KS, Larsen TD, Wachal AL, et al. Maternal high-fat diet impairs cardiac function in offspring of diabetic pregnancy through metabolic stress and mitochondrial dysfunction. *Am J Physiol – Hear Circ Physiol*. 2016;310(6):H681-H692. doi:10.1152/ajpheart.00795.2015
11. Bayol SA, Farrington SJ, Stickland NC. A maternal "junk food" diet in pregnancy and lactation promotes an exacerbated taste for "junk food" and a greater propensity for obesity in rat offspring. *Br J Nutr*. 2007;98(4):843-851. doi:10.1017/S0007114507812037
12. Menting MD, Mintjens S, Beek C, et al. Maternal obesity in pregnancy impacts offspring cardiometabolic health: systematic review and meta-analysis of animal studies. *Obes Rev*. 2019;20(5):675-685. doi:10.1111/obr.12817
13. White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. *Am J Physiol Regul Integr Comp Physiol*. 2009;296(5):R1464-R1472. doi:10.1152/ajpregu.91015.2008
14. Sen S, Simmons RA. Maternal antioxidant supplementation prevents adiposity in the offspring of western diet-fed rats. *Diabetes*. 2010;59(12):3058-3065. doi:10.2337/db10-0301
15. Martin MJ, Manzano M, Bueno-Vargas P, et al. Feeding a slowly digestible carbohydrate diet during pregnancy of insulin-resistant rats prevents the excess of adipogenesis in their offspring. *J Nutr Biochem*. 2018;61:183-196. doi:10.1016/j.jnutbio.2018.05.018
16. Chang E, Hafner H, Varghese M, et al. Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. *Sci Rep*. 2019;9(1): doi:10.1038/s41598-019-52583-x
17. Kirk SL, Samuelsson A-M, Argenton M, et al. Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. *PLoS One*. 2009;4(6):e5870. doi:10.1371/journal.pone.0005870
18. Dearden L, Buller S, Furigo IC, Fernandez-Twinn DS, Ozanne SE. Maternal obesity causes fetal hypothalamic insulin resistance and disrupts development of hypothalamic feeding pathways. *Mol Metab*. 2020;42: doi:10.1016/j.molmet.2020.101079
19. Dearden L, Ozanne SE. Early life origins of metabolic disease: developmental programming of hypothalamic pathways controlling energy homeostasis. *Front Neuroendocrinol*. 2015;39:3-16. doi:10.1016/j.yfrne.2015.08.001
20. Nicholas LM, Nagao M, Kusinski LC, Fernandez-Twinn DS, Eliasson L, Ozanne SE. Exposure to maternal obesity programs sex differences in pancreatic islets of the offspring in mice.

Diabetologia. 2020;63(2):324-337. doi:10.1007/s00125-019-05037-y

21. Taylor PD, McConnell J, Khan IY, et al. Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(1):R134-R139. doi:10.1152/ajpregu.00355.2004

22. Beeson JH, Blackmore HL, Carr SK, et al. Maternal exercise intervention in obese pregnancy improves the cardiovascular health of the adult male offspring. *Mol Metabol*. 2018;16:35-44. Published online June 18, 2018. doi:10.1016/j.molmet.2018.06.009

23. Ferey JLA, Boudoures AL, Reid M, et al. A maternal high-fat, high-sucrose diet induces transgenerational cardiac mitochondrial dysfunction independently of maternal mitochondrial inheritance. *Am J Physiol – Heart Circ Physiol*. 2019;316(5):H1202-H1210. doi:10.1152/ajpheart.00013.2019

24. Blackmore HL, Niu Y, Fernandez-Twinn DS, Tarry-Adkins JL, Giussani DA, Ozanne SE. Maternal diet-induced obesity programs cardiovascular dysfunction in adult male mouse offspring independent of current body weight. *Endocrinology*. 2014;155(10):3970-3980. doi:10.1210/en.2014-1383

25. Khan IY, Taylor PD, Dekou V, et al. Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension*. 2003;41(1):168-175. doi:10.1161/01.HYP.0000047511.97879.FC

26. Stanford KI, Lee M-YY, Getchell KM, So K, Hirshman MF, Goodyear LJ. Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring. *Diabetes*. 2015;64(2):427-433. doi:10.2337/db13-1848

27. Stanford KI, Takahashi H, So K, et al. Maternal exercise improves glucose tolerance in female offspring. *Diabetes*. 2017;66(8):2124-2136. doi:10.2337/db17-0098

28. Laker RC, Lillard TS, Okutsu M, et al. Exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring. *Diabetes*. 2014;63(5):1605-1611. doi:10.2337/db13-1614

29. de Almeida-Faria J, Duque-Guimarães DE, Ong TP, et al. Maternal obesity during pregnancy leads to adipose tissue ER stress in mice via miR-126-mediated reduction in Lunapark. *Diabetologia*. 2021;64(4):890-902. doi:10.1007/s00125-020-05357-4

30. Rodríguez-González GL, Reyes-Castro LA, Bautista CJ, et al. Maternal obesity accelerates rat offspring metabolic ageing in a sex-dependent manner. *J Physiol*. 2019;597(23):5549-5563. doi:10.1113/JP278232

31. Imai A, Fujimoto E, Tamura K, Utsuyama M, Sato K. A maternal high-fat diet may accelerate adipo-immunologic aging in offspring. *Life Sci*. 2019;219:100-108. doi:10.1016/j.lfs.2019.01.008

32. Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *J Physiol*. 2009;587(4):905-915. doi:10.1113/jphysiol.2008.163477

33. Fernandez-Twinn DS, Gascoin G, Musial B, et al. Exercise rescues obese mothers' insulin sensitivity, placental hypoxia and male offspring insulin sensitivity. *Sci Rep*. 2017;7:44650. doi:10.1038/srep44650

34. Isganaitis E, Woo M, Ma H, et al. Developmental programming by maternal insulin resistance: hyperinsulinemia, glucose intolerance, and dysregulated lipid metabolism in male

offspring of insulin-resistant mice. *Diabetes*. 2014;63(2):688-700. doi:10.2337/db13-0558

35. Susa JB, Schwartz R. Effects of hyperinsulinemia in the primate fetus. *Diabetes*. 1985;34(suppl. 2):36-41. doi:10.2337/diab.34.2.s36

36. Denisova EI, Kozhevnikova VV, Bazhan NM, Makarova EN. Sex-specific effects of leptin administration to pregnant mice on the placentae and the metabolic phenotypes of offspring. *FEBS Open Bio*. Published online November 25, 2019:0-1. doi:10.1002/2211-5463.12757

37. Samuelsson A-M, Mullier A, Maicas N, et al. Central role for melanocortin-4 receptors in offspring hypertension arising from maternal obesity. *Proc Natl Acad Sci*. 2016;113(43):12298-12303. doi:10.1073/pnas.1607464113

38. Kretschmer T, Schulze-Edinghausen M, Turnwald E-M, et al. Effect of maternal obesity in mice on IL-6 levels and placental endothelial cell homeostasis. *Nutrients*. 2020;12(2):296. doi:10.3390/nu12020296

39. Giussani DA, Camm EJ, Niu Y, et al. Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. *PLoS One*. 2012;7(2):e31017. doi:10.1371/journal.pone.0031017

40. McCurdy CE, Bishop JM, Williams SM, et al. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. *J Clin Invest*. 2009;119(2):323-335. doi:10.1172/JCI32661

41. Yamashita H, Shao J, Qiao L, Pagliassotti M, Friedman JE. Effect of spontaneous gestational diabetes on fetal and postnatal hepatic insulin resistance in Leprdb/+ mice. *Pediatr Res*. 2003;53(3):411-418. doi:10.1203/01.PDR.0000049667.58071.7D

42. Kasper P, Breuer S, Hoffmann T, et al. Maternal exercise mediates hepatic metabolic programming via activation of AMPK-PGC1α axis in the offspring of obese mothers. *Cells*. 2021;10(5):1247. doi:10.3390/cells10051247

43. Nguyen L, Chan SY, Teo AKK. Metformin from mother to unborn child – Are there unwarranted effects? *EBioMedicine*. 2018;35:394-404. Published online August 27, 2018. doi:10.1016/j.ebiom.2018.08.047

44. Hufnagel A, Fernandez-Twinn DS, Blackmore HL, et al. Maternal but not fetoplacental health can be improved by metformin in a murine diet-induced model of maternal obesity and glucose intolerance. *J Physiol*. 2021. doi:10.1113/JP281902

45. Salomäki H, Vähätalo LH, Laurila K, et al. Prenatal metformin exposure in mice programs the metabolic phenotype of the offspring during a high fat diet at adulthood. *PLoS One*. 2013;8(2):e56594. doi:10.1371/journal.pone.0056594

46. Salomäki H, Heinäniemi M, Vähätalo LH, et al. Prenatal metformin exposure in a maternal high fat diet mouse model alters the transcriptome and modifies the metabolic responses of the offspring. *PLoS One*. 2014;9(12):e115778. doi:10.1371/journal.pone.0115778

47. Salomäki-Myftari H, Vähätalo LH, Ailanen L, et al. Neuropeptide Y overexpressing female and male mice show divergent metabolic but not gut microbial responses to prenatal metformin exposure. *PLoS One*. 2016;11(9):e0163805. doi:10.1371/journal.pone.0163805

48. Tong JF, Yan X, Zhao JX, Zhu MJ, Nathanielsz PW, Du M. Metformin mitigates the impaired development of skeletal muscle in the offspring of obese mice. *Nutr Diabetes*. 2011;1(5):e7. doi:10.1038/nutd.2011.3

49. Álvarez D, Ceballo K, Olguín S, et al. Prenatal metformin treatment improves ovarian function in offspring of obese rats. *J Endocrinol*. 2018;239(3):325-338. doi:10.1530/JOE-18-0352

50. Schoonejans JM, Blackmore HL, Ashmore TJ, Aiken CE, Fernandez-Twinn DS, Ozanne SE. Maternal metformin intervention during obese glucose-intolerant pregnancy affects adiposity in young adult mouse offspring in a sex-specific manner. *Int J Mol Sci*. 2021;22(15):8104. doi:10.3390/IJMS22158104