Interventions to prevent adverse fetal programming due to maternal obesity during pregnancy

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Maternal obesity is a global epidemic affecting both developed and developing countries. Human and animal studies indicate that maternal obesity adversely programs the development of offspring, predisposing them to chronic diseases later in life. Several mechanisms act together to produce these adverse health effects. There is a consequent need for effective interventions that can be used in the management of human pregnancy to prevent these outcomes. The present review analyzes the dietary and exercise intervention studies performed to date in both altricial and precocial animals, rats and sheep, with the aim of preventing adverse offspring outcomes. The results of these interventions present exciting opportunities to prevent, at least in part, adverse metabolic and other outcomes in obese mothers and their offspring.

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INTRODUCTION

Worldwide, nearly 1.5 billion people are overweight (body mass index [BMI] >25 kg/m²) or obese (BMI >30 kg/m²), and the distribution among countries is broad. For example, in Mexico, 32% of women of reproductive years are obese⁵; in the United States, 35% of women of reproductive years are obese¹; in Brazil, 50% of the population is overweight or obese⁵; in the United Kingdom, 33% of pregnant women are overweight or obese⁵; in India, 26% of women of reproductive years are overweight and 8% are obese⁵; in China, 16% of women are overweight or obese⁵; and in Ghana, 64.7% of women are either overweight or obese¹⁰. The World Health Organization has declared obesity one of the top ten adverse health risk conditions in the world and one of the top five in developed nations.

As noted in another review in the present supplement, animal studies indicate maternal obesity predisposes offspring to a wide variety of chronic, later-life diseases through fetal programming.¹¹ In order to gain a better understanding of such developmental programming, investigation of the various factors involved in its challenges, mechanisms, and outcomes is required. Reports of experimental interventions in animal models in the setting of obesity are scarce.¹²,¹³ In some experimental animal models, the use of nutritional or targeted therapeutic interventions during windows of developmental programming has shown the potential to reverse unwanted metabolic outcomes in offspring.¹³ For example, in neonatal female rats born to undernourished mothers, treatment with leptin prevented development of metabolic compromise in adulthood¹²; maternal antioxidant supplementation in rats fed a Western diet partially prevented adiposity and normalized glucose tolerance in offspring.¹³ In other studies, genistein supplementation in mice during gestation was shown to protect offspring from susceptibility to obesity.¹⁴ This review focuses on animal studies in which interventions were designed to...
illuminating mechanisms by which a change in maternal diet or level of exercise may act to improve maternal and offspring outcomes.

There are many reasons why animal intervention studies are needed. Importantly, animal studies are much more controllable than human clinical interventions, which is the parallel human approach to hypothesis-driven animal research. In addition, a greater depth of mechanistic interrogation is possible, resulting from tissue retrieval and multiple rounds and forms of testing, and results are obtained much more quickly than in human studies. Reproducibility and independent confirmation, the indispensable requirements of scientific certainty, are also generally easier to achieve in animal studies.

Carefully designed clinical trials to determine the effects of interventions and to improve maternal health in pregnancy and offspring outcomes are now in progress. However, in addition to the length of time needed to obtain the required data, clinical trials have to contend with multiple confounds related to the mother’s socioeconomic status and prepregnancy health; this not only makes analysis and interpretation difficult, it may also limit the usefulness of findings for determining mechanisms. There is a pressing need, as a recent report from the Institute of Medicine indicates, for the development of evidence-based interventions that both inform and motivate pregnant women to adopt a healthy lifestyle before and during pregnancy. Currently, there is much interest in both maternal diet and exercise as potentially modifiable factors to target through interventions. The optimal timing and extent to which the adverse effects of the maternal metabolic phenotype, resulting from maternal obesity and associated high-calorie diets, can be prevented and/or possibly reversed by these interventions remain to be determined, and are of considerable physiological interest and importance in clinical obstetric management. Most authorities believe that interventions introduced before conception will have the best results. However, it should always be born in mind that poor maternal nutrition also programs adverse offspring outcomes and sudden and excessive restriction of maternal and fetal nutrient availability may well introduce new dangers. Thus, firm scientific data are needed to guide interventions.

When considering the goals of specific interventions to beneficially impact developmental programming outcomes, a distinction must always be made between interventions designed to prevent negative offspring outcomes and interventions conducted at later stages of an offspring’s life to reverse adverse health outcomes. Clearly, prevention is a better strategy than reversal. The present review focuses on animal studies that have evaluated maternal interventions to prevent negative offspring outcomes due to maternal obesity.

PREPREGNANCY DIETARY INTERVENTION STUDIES IN THE OBESE RAT

Methods

Investigations of adverse offspring programming due to maternal obesity require that investigators ensure initial phenotypic homogeneity within the different groups of mothers studied, i.e., controls, obese mothers, and mothers in which interventions are introduced. Care is necessary to achieve this important goal when females who will be the study mothers are purchased from commercial vendors. Specific information should be obtained as to the lineage of all females purchased to avoid the inclusion of sibling females in the same subgroup. The following procedures used in previous studies can help to avoid this and related problems. The first requirement is to maintain a colony of nonpregnant females, which are then bred to deliver the female pups that will be recruited as mothers into future studies. All rats should be maintained on the same laboratory chow unless they are being exposed to an experimental diet. At delivery (postnatal day [PND] 0), litters should be culled to 10 pups, with each litter containing at least four female pups. This standardization is important since programming effects have been shown in offspring according to the different sizes of litters being rear by mothers during lactation.

At weaning (PND 21), female offspring were randomly assigned either a control (C) group fed normal laboratory chow or to a maternal obesity group (MO) fed a high-energy, obeseogenic diet containing 23.5% protein, 20% animal lard, 5% fat, 20.2% polysaccharide, 20.2% simple sugars, 5% fiber, 5% mineral mix, 1% vitamin mix (w/w), and energy 4.9 Kcal/g. Only one female from any one litter was assigned to any study group. At PND 90, 1 month before breeding, half of the obese females were selected at random for the dietary intervention (DINT) group and placed back on the C diet for the rest of the study, including during pregnancy and lactation. The remaining obese females continued on the high-fat diet during pregnancy and lactation. Females were bred at 120 days as it has been shown that at younger breeding ages the mothers are still growing, albeit not as fast as earlier in life, and key components of offspring outcomes, such as growth, triglyceride levels, and leptin levels, are affected by maternal age as well as the nutritional challenge (unpublished data). At PND 120, all three groups, C, MO, and DINT, were bred and fed their prepregnancy diet throughout pregnancy and lactation. All mothers delivered by spontaneous vaginal delivery. Day of delivery was considered as PND 0.
Changes in maternal and offspring phenotypes resulting from maternal obesity and dietary intervention

In one study that followed the procedures outlined above, MO females were 16% heavier than controls, which is equivalent to a pregnant woman increasing her BMI from 25 (the top of the normal BMI range) to 30.5 (the lower end of the obese range). DINT females were 9% heavier than controls (equivalent to BMI in the mid-overweight range) at breeding. Maternal serum leptin at the end of lactation was higher in the MO group than the C group. Leptin levels in the DINT group were similar to controls.28

The effects of MO and DINT have only been reported in male offspring.28 No differences in body weight were seen between pups at birth and at weaning. At weaning, MO offspring had more subcutaneous fat tissue and higher serum triglyceride and leptin levels than C offspring, showing dysregulation of lipid metabolism; offspring from the group receiving prepregnancy maternal DINT did not show the increases observed in MO offspring. This important finding shows the limitations of weight alone at any age as an assessment of outcomes. Body composition is much more important for predicting future offspring health. Serum glucose did not differ among the three offspring groups; however, serum insulin was elevated in MO offspring but not in C or DINT offspring, indicating the presence of insulin resistance in MO offspring.28

At PND 120, male MO offspring had elevated resting serum glucose and insulin and increased insulin resistance compared with C offspring. Insulin remained elevated in DINT compared with C offspring, while blood glucose in the DINT group did not differ significantly from either the C or MO groups.28 It is important to note that all offspring were on the same post-weaning diet. Thus, there was no opportunity for any post-weaning increase in offspring dietary intake to act as a second hit in addition to the developmental programming that had already occurred.

PREPREGNANCY EXERCISE INTERVENTION STUDIES IN THE OBSESE RAT

Methods

Recently, we have published the effects of an exercise intervention in mothers and offspring of obese and control rats. General management of the pregnancies and lactation were as described above for the dietary intervention. At PND 90, i.e., 1 month before breeding, one half of the C group of non-pregnant females and half of the MO female groups were selected at random to continue on their diet and begin wheel-running exercise (C exercise intervention, CEx; MO exercise intervention, MOEx) (Figure 2). Mothers continued to be placed in the wheel throughout pregnancy. All females continued on their respective diets. A training session lasted 15 min, which was established as the optimum running schedule that was always completed, and it was followed by a 15 min rest period and a second 15 min run. Rats were allowed to rest for 2 days per week according to the following regimen: 2 days running, 1 day resting, 3 days running, 1 day resting. Before pregnancy, all rats completed the 30-min total running regimen, while during pregnancy rats were placed in the wheel for only one 15-min session per day, and the amount of voluntary exercise completed varied among the animals, especially in late gestation. During lactation, mothers nursed their pups and did not exercise. Therefore, lactating mothers were maintained on their pregnancy diet and not placed in the wheel.29
Changes in maternal phenotype and fertility resulting from maternal exercise prior to and during pregnancy

Calorie and food intake per day were similar in all four groups and exercise did not affect calorie or food intake in any group. Exercise had no effect on maternal weight at any stage except for in the CEx group in which exercise decreased body weight in comparison with the C group at parturition. There were no differences among the four groups in average distance run per session before pregnancy or in the first 15 days of pregnancy but, interestingly, MOEx ran further than CEx rats in the last few days of pregnancy. This difference is hypothesized to result from lower circulating estrogen levels in the MOEx group, since a negative correlation has been shown to exist between estrogen levels and physical activity.30

As in the DINT study mentioned above, at the end of lactation, maternal insulin, glucose, HOMA, leptin, triglycerides, and cholesterol were all elevated in MO mothers. Exercise did not alter these variables in C mothers but it prevented changes from occurring in all of the variables except leptin in MOEx mothers.

Effect of maternal obesity and exercise on male offspring metabolism at postnatal day 36

Offspring outcomes were evaluated since the goal of the interventions in MO pregnancy was to improve both maternal and offspring outcomes. At offspring PND 36, one male offspring from each litter \((n = 8)\) was chosen at random, fasted for 4 h, and euthanized by decapitation; trunk blood samples were obtained and fat depots were excised and weighed. Litter size, litter weight, individual pup body weight, and litter sex ratio at birth were not affected by either MO or exercise. In male offspring, body weight, cholesterol, and insulin were not different at PND 36 among the four groups. Maternal obesity increased offspring leptin, triglycerides, and fat mass in males. In the MOEx group, maternal exercise prevented the leptin increase seen in male offspring of MO mothers who did not exercise and it partially prevented the increase in triglycerides. In the CEx group, maternal exercise reduced glucose and HOMA in the male offspring. The importance of paying attention to the phenotype of study mothers is shown by the interesting observation that maternal exercise decreased weight and cholesterol in control offspring, indicating that even animals recruited as controls may be affected by experimental protocols.29

Maternal voluntary exercise intervention has been previously reported in lean pregnant rats in two different models; male, but not female, offspring of exercised mothers showed increases in percent lean mass and decreases in fat mass percent compared to male offspring from controls, showing that maternal exercise can affect offspring.31,32 These effects on the metabolic phenotype of offspring show similarities to the effects observed in control mothers in the study described above. In another study, normal, lean, pregnant rats performed voluntary exercise,33,34 training from 42 days before pregnancy and continuing on to day 19 of gestation, with the result that
maternal plasma antioxidant status was improved. Both of these studies provide important data for designing future studies in obese mothers.

**Comparison of diet intervention and exercise models**

One of the important differences between the DINT and the exercise intervention model is the maternal weight. In the DINT model, the increased maternal body weight of the DINT group at breeding was partially prevented, and there were no differences in maternal body weight during pregnancy and lactation between the DINT and C groups. In contrast, maternal exercise intervention did not modify the maternal body weight at any stage. The weight of MOEx mothers was the same as MO mothers before and during pregnancy as well as during lactation. However, in both models, the mothers undertaking the intervention presented a better metabolic and hormonal maternal environment with regard to offspring outcomes than the MO mothers not receiving an intervention.

In a completely independent study, maternal dietary and exercise interventions were applied to subsets of MO and C groups and all groups were compared. Corticosterone was found to be increased in serum of MO mothers prior to breeding. Maternal DINT and exercise interventions before pregnancy decreased maternal corticosterone concentration, but values in the MOEx group did not return to those of the C group (Figure 3A). A similar picture was seen at the end of lactation (Figure 3B) and in the neonate and young adult male offspring (Figure 3C,D). These changes in corticosterone levels may be protective mechanisms to help prevent future metabolic problems in the MO offspring.

Both types of maternal intervention resulted in reductions in the adverse metabolic outcomes caused by MO in offspring, but the degree and type were different in the two models. Maternal DINT partially prevented increases in glucose, insulin, HOMA, fat, and fat cell size, and it completely prevented leptin increases in MO offspring. The maternal exercise intervention partially prevented increases in fat and insulin and completely prevented increases in glucose, HOMA, and fat cell size in MO offspring.

For both models, the data indicate there were different changes in metabolism in various tissues, since no differences in food intake and body weight were found in the young adult offspring. The two intervention models appear to benefit offspring metabolism in diverse ways, suggesting dissimilar maternal mechanisms. Excessive gestational weight gain has been associated with adverse maternal pregnancy outcomes, and Catalano has demonstrated that maternal prepregnancy BMI is a major factor in determining maternal and offspring outcomes. One important finding of the exercise intervention study was that the adverse offspring outcomes produced by MO were the result of maternal metabolic changes and/or increased maternal corticosterone and not maternal body weight, since MOEx prevented many of the adverse MO offspring outcomes without producing any change in maternal calorie intake or body weight. This finding again shows that outcomes are related more to body composition than to body weight. The overwhelming evidence in favor of the importance of body composition clearly indicates that the most successful interventions will contain at least a component of prepregnancy intervention. Additional interventions during pregnancy will further build on the interventions performed prior to pregnancy.

In previous reports on the effects of maternal dietary intervention on offspring outcomes in the setting of obesity the following was written, which statement bears: “There is a need to determine optimal timing, nature and extent of interventions. We have taken the view – as others have done – that the optimal time for recuperation would be prior to pregnancy and have sought to develop
a model to show the ability and extent of the simplest of interventions, reducing global intake, to produce beneficial results. ... The available evidence indicates that women do not spontaneously alter their dietary patterns when they discover they are pregnant. Interventions in pregnancy, as in any other major health area, therefore need to be based on firm, reproducible scientific evidence.

Obese women contemplating pregnancy need to be provided with firm information as to the benefits that accrue from decreasing their BMI both before and during pregnancy for at least two reasons. First, they need to be aware of the biological reasons that maternal obesity is harmful to themselves and their baby in many ways. Second, they need to be confident that appropriately lowering their BMI and food intake will provide significant benefit to themselves and their children.

**Influence of interventions on offspring aging**

Maternal low-protein diets accelerate aging in rat offspring. For example, increased adiposity index and leptin, and triglyceride levels have been found in male offspring of non-intervention MO mothers during young adult life (PND 110), but these were not accompanied by statistically significant differences in body weight. However, by PND 650, more rapid aging was evident in some metabolic indices, such as body weight (Figures 4–6), fat, and adiposity index, while such increases were not apparent in the offspring of MO mothers that received a maternal dietary and exercise intervention (Figure 4).

One good example of positive features of programming is seen in the offspring of CEx mothers, which had a better metabolic phenotype at an early stage of aging (PND 650) than the rest of the groups, including the control group.

**Figure 4** Comparison of three measures in rat male offspring from a diet and exercise intervention study at postnatal day 650: (A) body weight, (B) fat, and (C) adiposity index. Values are mean ± SEM; n = 6; P < 0.05 for groups not sharing at least one letter. Abbreviations: C, control diet; MO, maternal obesity; DINT, maternal dietary intervention; MOEx, maternal obesity exercise intervention.

**Figure 5** Representative pictures of male offspring from dietary intervention study at postnatal day 650. Abbreviations: C, control diet; MO, maternal obesity; and DINT, maternal dietary intervention.

**Figure 6** Representative pictures of male offspring from exercise intervention study at postnatal day 650. Abbreviations: C, control diet; CEx, control diet + maternal exercise intervention; MO, maternal obesity; and MOEx, maternal obesity + maternal exercise intervention.

**STUDIES ON DIETARY INTERVENTION IN OBESE PREGNANT SHEEP**

As discussed in another review of developmental programming in the present supplement, there are differences...
between pregnancy in altricial, polytocous mammals, such as the rat, and precocial, monotonocous species, such as humans, sheep, and nonhuman primates.\textsuperscript{11} The pregnant sheep has been extensively investigated to determine the impacts of decreased maternal nutrition, but fewer studies have been conducted on the effects of maternal overnutrition/obesity on fetuses and offspring in this important, precocial, experimental species.\textsuperscript{38–46} Although there are differences in some capabilities, such as locomotion, both sheep and pregnant women produce well-developed precocial offspring, exhibit similar newborn-to-maternal weight ratios, and have a temporal pattern of fetal tissue and organ development. Further, investigators worldwide have utilized the fetal sheep as a biomedical model to design studies of human pregnancy, such as fetal behavior, heart rate, and sleep states.\textsuperscript{47–50}

Methods

Some studies on the impact of maternal overnutrition/obesity in the ewe on fetal growth and development and offspring health have been conducted with animals from a well-characterized, closed flock at the Center for the Study of Fetal Programming, University of Wyoming.\textsuperscript{11} For those studies, ewes of similar size and breeding were maintained in the source flock developed from lambs born within the flock whose mothers were fed according to National Research Council (NRC) feed requirements throughout pregnancy and lactation. The ewe lambs were then maintained on the same diet and used as the mothers in all studies; they were housed together and fed only NRC-compliant feed from weaning to maturity. This management policy provides assurance that animals have not been exposed to highly variable environments prior to any investigation and thus limits the chance of markedly different environmental (epigenetic) influences on study results, as well as other problematic influences, such as sibships within groups.

A model of maternal overnutrition/obesity (MO) has been developed and characterized, whereby ewes are fed a highly palatable pelleted diet at 150\% of NRC requirements from 60 days before conception throughout pregnancy. On this diet, ewes become obese by the time they are bred and continue to gain additional weight throughout pregnancy; their fetuses show a definitive endophenotype.\textsuperscript{38–41,45,46,51,52} In humans, overweight and obesity at conception in pregnant women has been shown to have the greatest impact on increasing adiposity of infants at birth, which leads to insulin resistance and exhibition of obesity in later life.\textsuperscript{35} In our model of diet-induced MO, lambs are born with increased adiposity, and by 19 months of age they exhibit hyperphagia, glucose and insulin dysregulation, and increased adiposity compared to offspring of ewes fed only to requirements.\textsuperscript{46} Previous studies have demonstrated that maternal undernutrition (50\% global undernutrition) starting at day 28 of gestation resulted in delivery of offspring that exhibited metabolic disturbances as adults (i.e., they were hyperphagic, insulin resistant, and obese).\textsuperscript{35} It was, therefore, hypothesized that a dietary intervention in which the obesogenic diet is reduced from 150\% to 100\% of NRC requirements (MO intervention [MO-I]) beginning on day 28 of gestation would be early enough to, at least in part, prevent the negative impacts of maternal overnutrition/obesity on the fetus and resulting offspring. Further, day 28 of gestation in the sheep is equivalent to approximately day 50 in human pregnancy, which is about the time when women confirm they are pregnant and early enough for their obstetrician to provide overweight/obese women with a corrective dietary regimen if deemed necessary.

MOI eliminated MO-induced fetal macrosomia at midgestation, and either reduced (right ventricular weight, liver weight) or prevented MO-induced increases in organ weight (left ventricular weight, total kidney weight, pancreatic weight, and total perirenal fat weight). At day 135, while fetal weight was similar among the C, MO, and MOI fetuses, the MOI fetuses exhibited greater left ventricular weights and thicknesses, right ventricular thicknesses, total kidney weight, and total perirenal fat, as well as reduced pancreatic weight, in comparison with C fetuses. The weights and thicknesses of these organs and tissues in the MOI fetuses returned to C levels.\textsuperscript{54} These data provide the first indication that alterations in fetal organ and tissue growth as well as endocrine changes (see below) can, at least in part, be prevented by early pregnancy MOI in the face of maternal obesity.

To date, changes in cortisol levels have only been evaluated in the MOI sheep model in order to observe any similarities with findings from the obese rat model described above (Figure 3). MO increased both maternal and fetal cortisol at 50\% and 90\% gestation, and this increase was prevented by MOI at both timepoints. Interestingly, while the maternal increases in cortisol were accompanied by increased ACTH, this was not so in the fetus where cortisol, but not ACTH, was higher in the MO group than the C group at both ages (Figure 7). Two possible mechanisms for this finding are hypothesized: 1) MO may change adrenal sensitivity to ACTH; and 2) much of the fetal cortisol in the setting of maternal obesity is produced in peripheral fetal tissues by increased activity of 11BHSD1, converting inactive cortisone to active cortisol. It has been shown that the 11BHSD1 system is upregulated in fetal female perirenal fat and fetal male liver in the setting of maternal undernutrition, which supports a potential role for increased 11BHSD1 activity in response to maternal dietary challenges.\textsuperscript{35} Importantly, the extent to which inhibition of the increase in fetal cortisol will prevent adverse side effects of MO on offspring remains to
be seen. These findings illustrate clearly the value of comparing results of studies performed in precocial and altricial species. Current studies in sheep are evaluating C, MO, and MOI offspring to determine whether reducing maternal nutrition to recommended levels in early pregnancy of overnourished/obese ewes prevents endocrine and metabolic disturbances in offspring in adult life.

**Communicating the need for interventions to the general public: Lessons from antismoking campaigns**

Improved women’s health and, especially, the institution of effective corrective measures is vitally important for obtaining optimal obstetric outcomes in the face of the current epidemic of obesity in women of reproductive years. There may be lessons that can be learned from the success in the developed world in decreasing the incidence of smoking through antismoking campaigns. One of the most persuasive pieces of scientific evidence in the antismoking campaign was the demonstration that while smoking from early adult life tripled mortality rates, giving up smoking at age 50 halved the mortality risk, and stopping at age 30 removed virtually all mortality risk. Changing this self-destructive behavior has taken decades, but the eventual decrease in smoking is estimated to have saved thousands of lives. The parallel between smoking and MO would be the potential to avoid a likely negative outcome through behavior and lifestyle modification. For MO, the persuasive message would be the evidence given

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**Figure 7** Comparison of cortisol and ACTH levels in obese maternal sheep and their offspring at 75 and 135 days gestation: A) fetal ACTH, B) fetal cortisol, C) maternal ACTH, and D) maternal cortisol. Values are mean ± SEM; n = 6; P < 0.05 ** vs C and MO, * vs C and MOI. Abbreviations: C, control (open); MO, maternal obesity (solid); MOI, maternal obesity dietary intervention (striped).
here that lifestyle adjustments aimed at reducing obesity would have the potential to prevent adverse maternal and offspring outcomes.

CONCLUSION

Epidemiological studies have shown that once a woman knows she is pregnant, she does not typically modify her lifestyle. Thus, the earlier a positive lifestyle intervention is undertaken, the better the outcome. As the important IOM report and reviews by several clinical and basic science leaders indicate, studies such as those described here are essential for determining the mechanistic targets in the mother that can guide the development of predictive and clinical tools for use in human pregnancy. Other potential interventions remain to be investigated to permit evidence-based changes in clinical management. These include diets supplemented with polyunsaturated fatty acids or antioxidants. Different interventions to improve outcomes may act through different mechanisms. If so, a combination of approaches may lead to even better results for obese mothers and their offspring.

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