Mechanisms by which maternal obesity programs offspring for obesity: evidence from animal studies

Elena Zambrano and Peter W Nathanielsz

Maternal obesity can profoundly affect offspring phenotype and predisposition to obesity and metabolic disease. Carefully controlled studies in precocial and altricial mammalian species provide insights into the involved mechanisms. These include programming of hypothalamic appetite-regulating centers to increase orexigenic relative to anorexigenic drive; increasing maternal, fetal, and offspring adrenal and peripheral tissue glucocorticoid production; and increasing maternal oxidative stress. Outcomes often show offspring sex differences that may play a role in the differential susceptibility of males and females to later-life obesity and other related metabolic diseases.

INTRODUCTION

In developed and developing countries around the world an obesity epidemic is occurring in the general population as well as among women of reproductive age. Worldwide, over 30% of women of reproductive age are obese. The global extent of the problem is reflected in reports from numerous countries, including Mexico, the United States, Bangladesh, Nepal and India, Brazil, the United Kingdom, India, and Ghana.

Several studies in human mother–child cohorts have found an association between high maternal BMI or gestational weight gain and disrupted childhood metabolism and cardiovascular function. Many aspects of this relationship are addressed in other articles in this supplement. This particular article addresses the relationship between maternal obesity and developmental programming in offspring, as revealed in animal studies. While there are many definitions of developmental programming, it defined here as the response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development with resulting effects on health that persist throughout life.

MATERNAL OBESITY AND DEVELOPMENTAL PROGRAMMING

Studies of developmental programming of offspring phenotype by suboptimal maternal nutrition initially focused on the adverse outcomes that occur when maternal nutrition is reduced. This emphasis was driven by interest worldwide on the adverse outcomes resulting from cohorts of intrauterine growth-restricted (IUGR) babies, e.g., the Dutch Hunger Winter, the Leningrad famine, and the Chinese famine. These studies also provide valuable information on programming challenges that result in offspring obesity since the offspring of nutrient-restricted mothers are predisposed to obesity if their postnatal diets are rich in calories and fat, as is typically the case in the modern, westernized diet. Thus, developmental programming of offspring obesity is one of the adverse outcomes of reduced maternal nutrient availability, which is inevitably accompanied by reduced fetal and neonatal nutrient availability. The mechanisms involved in the predisposition of offspring of nutrient-restricted mothers to later-life obesity have been reviewed extensively, and much can be learned from these studies in

Affiliations: EZambrano is with the Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico. PW Nathanielsz is with the Center for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, The University of Texas Health Science Center San Antonio, San Antonio, TX, USA.

Correspondence: EZambrano, Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán 14000, Mexico. E-mail: zamgon@unam.mx. Phone: +52-55-5487-0900 ext 2417. Fax: +52-55-5655-9859.

Key words: altricial mammalian species, maternal obesity, offspring phenotype, offspring programming mechanisms, precocial mammalian species

doi:10.1111/nure.12068
relation to the mechanisms and outcomes. However, this article focuses mainly on evidence from carefully controlled animal studies that throw light on the extent to which, and the mechanisms by which, maternal obesity alters offspring phenotype, predisposing the next generation to obesity.

Any analysis of the effects of maternal obesity on the developing fetus and neonate must assess the complex, interacting mechanisms involved. First, it is crucial to consider the independent and interactive effects of the obese maternal phenotype per se and the diet inevitably associated with that phenotype. Second, it is now clear that many different mechanisms play a role in a developmental-stage-related fashion. Some mechanisms that have a significant effect on offspring phenotype appear to occur even before pregnancy, acting through altered gamete function; others occur during pregnancy, acting through the impaired placental development and function that occurs in response to poor maternal nutrition; yet others occur in the neonatal period, acting by affecting lactation and maternal care.

Thus, variations in outcomes show temporal specificity, since the developing organism passes through multiple critical periods of development at different stages of fetal and neonatal life, and the vulnerability of various organ systems to a specific challenge differs according to its stage of development at the time of the insult. These critical stages of vulnerability usually represent periods at which the maturing organ is undergoing key stages of proliferation or differentiation.

When considering developmental programming, it is useful to separate and consider the interrelationships of the challenges and outcomes followed by the mechanisms involved. In addition, controlled animal studies allow investigators to introduce an intervention designed to address a causal hypothesis, as described more fully elsewhere in this supplement. In the human setting, epidemiological studies based on carefully collected cohorts of offspring for whom much developmental data are available have given much impetus to the study of the relationship between maternal obesity and programming of offspring for obesity, but unless an intervention is introduced, epidemiological studies can only indicate associations. To demonstrate causality, interventions with changes in maternal diet, exercise, or pharmacological agents are required. Of importance, animal studies allow the investigator a much greater degree of control over potential confounding variables, such as lifestyle differences, and can throw light on potential interventions that may have benefit before or during pregnancy and lactation in women. Understanding the challenges and outcomes and their related mechanisms is essential for the translation of this exciting new area of developmental biology to obstetric, neonatal, adult, and even geriatric medicine.

Comparative physiology of precocial and altricial species in relation to developmental programming

The millennia of evolution have produced a very broad spectrum of pregnancy strategies, trajectories of fetal development, and neonatal offspring developmental patterns that optimize each mammalian species’ ability to survive and reproduce in its particular environmental niche. Rodents are, in general, altricial species born after relatively short pregnancies and requiring considerable maternal care in the immediate postnatal period to regulate basic neonatal functions, such as maintenance of offspring body temperature, while they develop the ability to thermoregulate. However, this generalization does not apply universally to rodents. There are exceptions, such as the guinea pig, which is born at a very mature stage and able to eat solid food from birth. Precocial species such as humans, nonhuman primates, and sheep are more mature at birth. However, even in precocial species, not all physiological systems are equally mature at birth. Humans are considered to be precocial in many respects, but with regard to organized locomotion or ability to control their body temperature, human newborns are far less advanced than newborn lambs or monkeys. All these species provide valuable data, since comparative physiology provides the opportunity to observe the different ways in which a particular developing system, adrenal steroid production for example, responds to a challenge during development, what growth factors are recruited or inhibited, what neural connections are impaired, and, most importantly, what gene changes occur and which of the epigenetic marks placed on the genes will persist into postnatal life and lead to a persistent change in phenotype.

Although the separation into altricial and precocial species is not all-embracing, it is of value because many maturational changes that occur postnatally in altricial rodents occur during gestation in precocial species, i.e., when the levels of key regulatory and metabolic factors present in the offspring’s blood are very different from the levels in the postnatal period. The prenatal environment differs greatly from the postnatal environment. Before birth, developing tissues are exposed to an arterial P02 of about 40 mmHg. After birth, arterial P02 is 100 mmHg. This difference potentially has consequences for programming mechanisms, such as oxidative metabolism and production of oxidative stress, which is discussed below as a potential mechanism for both normative and programmed development. Similarly, before birth, fetal glucose concentration is half that in postnatal life, and the fetus is exposed to a very different endocrine milieu before versus after delivery. In addition, the fetus is exposed to hormones and metabolites produced by the placenta while the neonate is not. As a result of all these differences, challenges during the key windows of vulnerability
referred to above, occurring at different times in relation to birth and in the presence of very different environments, may result in perturbation of different cellular mechanisms with different outcomes in precocial and altricial species.

There are also major differences among species in the nutritional burden the mother bears during pregnancy and lactation. Humans are monotonous species—meaning that women generally bear only one fetus, though 1 in 80 natural pregnancies results in twins and experience with assisted reproductive techniques clearly indicates the ability of women to conceive and carry more fetuses to term. In contrast, rodents are polytocous species bearing large litters. Thus, even under optimal feeding conditions, the nutritional demands of pregnancy and lactation on the litter-bearing rodent mother are much greater than on mothers in monotonous species. The feto-placental biomass nurtured by a pregnant rat is equivalent to a pregnant woman bearing a 25–30 kg baby. In addition, there are qualitative differences in the metabolism of key nutrients in rodents compared with primates. Two key examples can be given of differences in micronutrient metabolism between rodents and primates that have potential significant effects on programming mechanisms: 1) the methionine cycle, which is important in gene methylation, and 2) vitamin C, which is a powerful antioxidant. The rodent methionine cycle, which is central to epigenetic transformation resulting from altered methylation, differs from that of primates. Folic acid reduction to active tetrahydrofolate by dihydrofolate-reductase in human liver is <2% of rat liver. In addition, rodents can synthesize their own vitamin C. The biochemical differences between these species present several interesting issues that will clearly produce differences in mechanisms and outcomes between rodents and primates in response to nutritional challenges, such as maternal overnutrition or low-protein diets lacking methionine.

Differences also occur in key metabolic systems. There are two forms of the key gluconeogenic enzyme phosphoenolpyruvatecarboxykinase, one mitochondrial (PEPCKM) and the other cytosolic (PEPCKC). Hepatic PEPCKC comprises 90% of PEPCK in rats and mouse livers and, as a result, PEPCKM only represents 5–10% of the total PEPCK activity. In human liver, 50% of the PEPCK activity is PEPCKM, and this balance is seen in most mammalian species. The mRNA, or protein, level for PEPCKC is often used as a good index of the rate of gluconeogenesis, which is an important fetal response to nutrient deficiency. However, the impact of specific nutritional challenges on both of these isoforms requires studies in species that are similar to humans and have both forms of the enzyme. An enormous amount of very valuable metabolic information has been generated over the past 25 years using the techniques of mouse genetics combined with metabolic analysis, but there is also a need for studies in precocial species, such as sheep and nonhuman primates, for comparative evaluation and translation to the human situation.

With respect to lipid metabolism, differences have also been demonstrated in key hepatic enzymes between rodents and humans. Acetyl-CoA carboxylase (ACC) exists as two isoforms, ACC1 and ACC2. In rats, ACC1 is mainly expressed in lipogenic and ACC2 in oxidative tissues. In humans, ACC2 is the major enzyme in both oxidative and lipogenic tissues.

**ANIMAL STUDIES**

Several experimental animal studies have been performed to examine the effects of maternal obesity during pregnancy and lactation on offspring development; these have been conducted in different animal species, but mostly rodents, sheep, and nonhuman primates. Several adverse effects of maternal obesity on offspring metabolism have been demonstrated, including the following: increased adult body weight and fat mass, reduced insulin sensitivity, increased blood glucose and triglycerides levels, increased lipid deposition and defects in fatty acids metabolism in adult liver, as well as increased leptin levels and hypothalamic alterations of appetite-regulating neuropeptides. The high-fat-diet-induced maternal obesity model has been the most utilized in rodents to study offspring metabolic abnormalities.

In the rat and nonhuman primates, maternal obesity alters liver metabolism in offspring. Maternal consumption of a Western style, junk-food diet that is rich in energy, fat, and sugar during pregnancy and lactation induces nonalcoholic fatty liver disease in adult life. Male offspring showed hepatic steatosis-altered hepatic expression of genes associated with insulin sensitivity with increased lipogenesis and lipid oxidation, whereas females demonstrated a gene expression profile that was indicative of hepatic insulin resistance. In one study, a cafeteria-style diet during pregnancy and lactation caused liver steatosis in adult offspring. Male offspring of obese mother rats have a significant increase in serum glucose, insulin, leptin, VLDL, apolipoprotein B100, and lipid levels, as well as a permanent reduction in hepatic β-oxidation and an increase in hepatic lipogenesis.

McCurdy et al. used a nonhuman primate model to study the consequences of high-fat diets and maternal obesity in offspring. Surprisingly, fetuses in this study were slightly growth restricted. Fetuses of obese mothers had signs of fatty liver disease, increased triglycerides, and PEPCK in the liver compared to offspring from control mothers. These metabolic changes observed in the liver of obese offspring in fetal life, persisted in later life (at 180 days of age) and were accompa-
nied by a twofold increase in percent body fat. When the maternal high-fat diet was changed to a low-fat diet in a later pregnancy, fetal hepatic triglycerides and PEPCK were partially normalized while maternal body weight was unchanged. Fetuses of obese mothers showed increased oxidative stress, irrespective of the type of diet consumed.

**MECHANISMS**

As discussed above, maternal high fat intake and obesity increase the offspring’s risk of juvenile obesity and metabolic diseases. However, the mechanism(s) whereby excess maternal nutrition affects fetal and postnatal development remain poorly understood. Obesity results from an imbalance between energy intake and output. Both sides of the equation represent multiple components, all or some of which may be changed in the setting of obesity. For example, exercise decreases reactive oxygen stress in animal models.39 Obese individuals almost invariably exercise less and are thus likely to generate more reactive oxygen species.

One fundamental question regarding mechanism is whether the acute in utero and long-term developmental programming effects of maternal obesity are due to either, or both, the increased dietary intake, usually fatty food, eaten by the mother or the obesity per se? In an interesting model of maternal obesity in the Japanese macaque,33 the monkeys were fed a high-fat diet. Triglycerides increased in the livers of the fetuses regardless of whether the mother was lean or fat. Fetal livers showed signs of oxidative stress similar to those seen in nonalcoholic fatty liver disease. These and other changes that were observed independent of maternal phenotype would suggest that fat in the maternal diet can play an independent role in programming metabolic dysfunction. If this is so, then determining the optimum dietary intake and balance between saturated and unsaturated fats will be very important. In one study, a maternal high-fat diet in the Japanese macaque reduced maternal and fetal plasma n-3 fatty acids in late gestation.40 Western diets have evolved greatly over the last decades and now have a much greater n-6:n-3 ratio – a change that is generally considered disadvantageous to good health.41

The need to consider potential independent effects of the diet and maternal obesity is indicated by important studies in non-human primates, which showed that when obese monkeys who had been on a high-fat diet were provided a normal diet during pregnancy, several of the fetal hepatic changes that occurred in the obese mothers remaining on the fat high-diet were, at least in part, prevented.33,42

**Epigenetic mechanisms**

Epigenetics is a term coined by Waddington33 to describe heritable gene function changes without a changed DNA base sequence. Epigenetics has become a central mechanism of developmental programming. Epigenetic mechanisms include activation and inactivation of genes by DNA methylation, histone acetylation, and micro-RNAs. For example, DNA methylation suppresses gene expression. Epigenetic mechanisms have a profound effect on phenotype and health risk predisposition.

It has been shown that in an IUGR fetal baboon model resulting from reduced maternal nutrient availability, the promoter for PEPCK1 is hypomethylated with a resultant increased translation of PEPCK protein production.39 When these baboons were studied just before they reached puberty, juvenile offspring showed a prediabetic metabolic phenotype consisting of increased fasting glucose and fasting insulin as well as β cell responsiveness accompanied by decreased peripheral glucose disposal.44

**Maternal obesity and the methionine cycle**

One area of epigenetics that has received very little attention is the potential alteration of the methionine cycle by high-calorie diets and obesity. The methionine cycle is essential to methyl group transfer to a variety of substrates including methylation of DNA resulting in epigenetic changes. In a baboon model of maternal obesity, it has been shown that the methionine cycle is disrupted, resulting in increased levels of maternal and fetal homocysteine, which is a pathological marker for cardiovascular and other chronic diseases. In addition, methyl donor supplementation prevents transgenerational passage of obesity.45 Interestingly, in both the obese mothers and their fetuses, circulating folate was increased while vitamin B12 was decreased (Figure 1). It is hypothesized that this unexpected situation represents a lack of use of folate to generate methyl groups in the setting of obesity.

**MicroRNAs**

MicroRNAs (miRNAs) represent another method of regulating gene activity, in part by altering mRNA stability. In a model of maternal obesity in sheep, non-pregnant ewes received either a control diet (100% of National Research Council [NRC] recommendations) or an obesogenic diet (150% of NRC recommendations) from 60 days before to 75 days after conception, i.e., mid-gestation; when the fetal longissimus dorsi muscle was sampled and miRNA expression analyzed at mid-gestation, muscle from the fetuses of obese sheep showed reduced expression of the miRNAlet-7g. Let-7g function
was tested in C3H10T1/2 cells for its role in adipogenesis and cell proliferation. Overexpression of let-7g in C3H10T1/2 cells reduced their proliferation rate and decreased the expression of adipogenic markers, formation of adipocytes, and expression of inflammatory cytokines. In the muscle of the fetuses of fat mothers, reduced expression of miRNA let-7g was correlated with higher expression of its target genes. It was concluded that fetal muscle miRNA expression is altered due to maternal obesity and, specifically, that downregulation of let-7g may enhance intramuscular adipogenesis during fetal muscle development in the setting of maternal obesity.46

**Effects of maternal obesity on development of hypothalamic centers that control appetite**

The hypothalamic arcuate nuclei (ARH), located in the third ventricle, contains neurons that form the basis of a negative feedback system that, under normal conditions, balances energy intake and output to maintain normal body weight and composition. Regulation is accomplished by the production of two orexigenic proteins, neuropeptide Y (NPY) and Agouti-related protein, and two anorexigenic peptides, proopiomelanocortin and cocaine and amphetamine-related transcript. These neurons have widespread afferent and efferent connections throughout the brain, but especially important are those to the nearby hypothalamic paraventricular nucleus.47,48 Several studies show that this complex energy and appetite control system develops postnatally in altricial species.49–51 Much less is known about their development in precocial species such as sheep52 and non-human primates51 in which there are some indications that maturation begins in fetal life.

In rats, the wiring of connections within the ARH occurs in the first 3 weeks of life coincident with a peak in leptin in the newborn pups’ peripheral blood.29 In pups born to normal-weight mothers, the peak lasts 4 or 5 days. Pups born to obese mothers have a distorted plasma leptin peak, which has a higher amplitude and is longer lasting (Figure 2A). The source of this leptin has yet to be determined, as increased leptin in the blood of offspring of obese mothers is not associated with an increase in total fat in the newborn body; however, there is evidence that adipose tissue in offspring of fat mothers produces more leptin mRNA per unit weight.29 It may be that specific adipose depots are overactive, but this possibility remains to be explored. When pups delivered by obese mothers reach adulthood, they have an increased appetite and become obese; this may be related to the development of resistance to the feedback of leptin. They are also hypertensive as a result of overactivity of the sympathetic nervous system.35

Recent studies53 have shown that newborn lambs also have a leptin peak in their blood during the first week of life. In lambs born to obese mothers, the leptin peak is slightly earlier and lower in amplitude (Figure 2C). When grown, if food is available in unlimited amounts, the offspring of obese mothers eat more and become fat.

Leptin and insulin regulate feeding behavior. Leptin acts on the ARH to increase energy expenditure and reduce hunger. Leptin administration in postnatal mice downregulates NPY and stimulates anorectic proopiomelanocortin neurons.54 Maternal obesity induced by a high-fat diet prior to and during pregnancy alters leptin and insulin signaling and the expression of orexigenic and anorexigenic peptides in term fetal hypothalami in the rat.28 Hyperinsulinemia and hyperleptinemia in late gestation of obese rat mothers compared to a control group was associated with higher serum leptin of their fetuses. Fetal hypothalami of obese progeny showed elevated levels of mRNA of leptin and NPY, indicating that orexigenic neuropeptides are already upregulated by fetal stage. These findings, observed in the late fetal period,34,55 may be related to hyperphagic behavior that carries over to the development of obesity in postnatal life.30

---

![Figure 1](image1.png)

**Figure 1** Maternal (MAT) and fetal (FET) serum methionine cycle metabolites in the pregnant baboon at 90% gestation. Open histogram: mothers on normal baboon chow diet; closed histogram: mothers on a high-fat, high-energy diet. Mean ± SEM; P < 0.05 * vs chow Mat; † vs chow FET; n = CTR Mat 11–22; HF-HED Mat 5; CTR FET 11–21; HF-HED FET 5. Abbreviations: MET, methionine; HCY, homocysteine.
It has been observed that the offspring of obese rats display an amplified and prolonged neonatal leptin surge, which is accompanied by elevated leptin mRNA expression in their abdominal white adipose tissue in adult life.\textsuperscript{29} Using a model of maternal chronic nutritional excess in mice, it was observed that offspring became hyperphagic with reduced locomotor activity, and this was associated with increased abdominal adiposity compared with a control group. Also, fasting insulin was raised at 3 months and by 6 months fasting plasma glucose was elevated.\textsuperscript{30} To study postnatal overnutrition in rodents, it is very common to reduce the litter size post birth to increase the food intake exposure; this results in hyperphagia, hyperinsulinemia, and cardiovascular risk in the pups.\textsuperscript{56} Rat offspring from small litters show increased NPY expression and a reduced responsiveness to leptin.\textsuperscript{57,58}

These findings suggest that the programming effects of an altered intrauterine environment induced by consumption of a high-fat diet may be related to hypothalamic alterations of energy balance signals in early life.

\textbf{Role of glucocorticoids: Evidence that maternal obesity during pregnancy alters maternal, fetal, and offspring pituitary adrenal function}

In precocial species, glucocorticoids play a critical role during late gestation in the processes that aid maturation of a variety of fetal organs for their new functions in postnatal life. Major mechanisms of glucocorticoid action include stimulating differentiation and inhibiting growth and proliferation. Figure 3 shows the rise in glucocorticoids in late gestation in five precocial species.\textsuperscript{59}

Increased fetal cortisol has been clearly shown to play a role in the initiation of parturition in ruminant species; in sheep, for example, destruction of the fetal paraventricular nuclei prevents the elevation of fetal ACTH and cortisol and prolongs pregnancy.\textsuperscript{60} While the effect on parturition does not appear to operate in humans and non-human primates, the effect on maturation of fetal organs is separate and appears universal, as shown by the ability of glucocorticoids to stimulate lung development in preparation for air-breathing after birth\textsuperscript{61} – a vital preparation all mammals must make in order to

\textbf{Figure 2:} A: Rat serum leptin in offspring of control mothers (open bars) and obese mothers (closed bars); and B: adipose leptin mRNA expression in abdominal fat. P < 0.05, ** P < 0.01, and *** P < 0.01 versus offspring of control dams for the same period (n = 3–6). For longitudinal comparisons, a significant difference (P < 0.05) from the preceding period. C: Circulating plasma leptin levels (mean ± SEM) from birth until postnatal day 11 in lambs from obese mothers (closed circles; n = 6) and control mothers (open circles; n = 6). Plasma leptin *P < 0.01 control vs obese lambs at the same time point. Figures 2A and 2B reproduced from Kirk et al.\textsuperscript{29} with permission; Figure 2C reproduced from Long et al.\textsuperscript{53} with permission.
survive in their new, extrauterine environment. Carefully controlled studies in the chronically instrumented sheep model have shown that the physiological term rise in fetal cortisol matures the fetal lung,61 thyroid axis,62 and heart function.63

There is still much controversy regarding the role of the maternal and fetal pituitary adrenal axes in developmental programming in general and in programming resulting from maternal obesity in particular. As noted in one publication: “It is tempting to conclude that disturbances of pituitary adrenal axis function may help explain fetal programming, particularly as such disturbances have long been associated with obesity.”64 However, data in the various models of maternal undernutrition are mixed on the question of involvement of the maternal and fetal pituitary adrenal axes and developmental programming. Research in the rat has shown that a low-protein diet leads to elevated maternal and offspring corticosterone.65 In the setting of maternal obesity, maternal corticosterone is elevated.66 Corticosterone is also raised in offspring of obese mothers at 2 and 110 days after birth (Figure 4).

The sheep model is very powerful for the study of fetal physiology, since it is possible to place catheters in the fetus and mother under general anesthesia as early as 100 days of pregnancy (term is approximately 150 days) and, following their recovery from surgery, to study both normative and perturbed changes in both the fetus and the mother. This method was used to show that a rise in fetal cortisol begins about 20 days before delivery allowing a gradual effect.67 In studies performed in collaboration with Dr. Stephen Ford, elevated maternal and fetal cortisol levels were observed at 90% of gestation in obese ewes (Figure 5).68 It has been hypothesized that increased fetal and neonatal cortisol is responsible for the altered leptin peak observed in the offspring of obese mothers. There is some support for this hypothesis, since leptin levels in fetal sheep plasma and in perirenal adipose tissue mRNA increase in the last 15 days of gestation.69 Fetal adrenalectomy in sheep prevents these changes, while exposure of the intact fetus to high cortisol levels late in gestation increases fetal leptin levels.

Peripheral tissue production as a mechanism of increased cortisol production in the setting of obesity

Cortisol is produced locally in adipose tissue by reduction of inactive cortisone to active cortisol by the enzyme 11 beta hydroxysteroid dehydrogenase-1 (11β-HSD1). NADPH provision as a result of hexose-6-phosphate-dehydrogenase (H6PD) activity is essential for 11β-HSD1

Figure 3 Mean fetal concentrations of plasma cortisol with respect to time (d) from delivery in sheep (closed circles), pig (open circles), human (solid triangles), guinea-pig (open squares), and horse (open triangles). Hatched vertical line represents birth. Reproduced from Fowden et al.59 with permission.

Figure 4 Rat plasma corticosterone: A) maternal at the time of breeding; B) offspring values (male and female combined) on neonatal day 2; C) offspring values at postnatal day 110. Mean ± SEM; n = 6. C control diet, MO obese maternal diet; * P < 0.05 vs C.
reductase activity. All of the components required for local generation of cortisol are present in fetal baboon adipose tissue. Circulating maternal and fetal plasma cortisol levels were increased in a fetal IUGR baboon model, but increased local cortisol production was also observed in adipose tissue in female IUGR fetuses and liver in male IUGR fetuses. The importance of the increased local and systemic fetal cortisol levels observed in these IUGR fetuses is that increased active cortisol generation by 11\(\beta\)-HSD1 in peripheral tissues provides a mechanism for increasing cortisol production in the body without the need for ACTH stimulation of the fetal adrenal. Such a mechanism can explain the data shown in Figure 5, in which fetal cortisol is elevated at term in fetuses of obese ewes in the presence of normal fetal plasma ACTH. Indeed, if cortisol is produced locally and is released from the fat into the bloodstream and into the plasma, it will depress ACTH by negative feedback, thereby leading to the interesting possibility of elevated fetal cortisol in the presence of normal, even decreased, ACTH.

There are no published studies of increases in either maternal or fetal cortisol in human pregnancy. One small pilot study showed that human cord blood cortisol was significantly elevated in babies born by elective cesarean section to overweight and obese mothers (Figure 6).

Influence of fetal sex on outcomes. A general principle of programming is that outcomes from fetal challenges usually depend on fetal sex, as shown above with regard to 11\(\beta\)-HSD1. Effects specific to the sex of offspring have been shown in many physiological systems. Relevant to the role of glucocorticoids and the setting of the level of activity of the hypothalamo-pituitary adrenal axis in offspring, researchers from Toronto University have elegantly shown differences in male and female offspring with regard to programming at the level of the brain.

Several laboratories have shown developmental programming of offspring metabolism following maternal administration of synthetic glucocorticoids that cross the placental barrier and expose the fetus to levels of steroid that are inappropriately high for the current stage of gestation. Studies have been conducted in several species of animals including rats, guinea pigs, sheep, and primates. The studies performed by our group have focused on sheep. One study examined the multigenerational metabolic effects of fetal synthetic glucocorticoid on F1 and F2 female offspring of founder-generation (F0) ewes.
At 70% gestation, the ewes were given four injections of dexamethasone (2 mg, circa 60 μg/kg/day 12 h apart) or saline (control). The first filial generation (F1) of female offspring was bred to produce a second filial generation (F2) of female offspring. F1 and F2 ewe lambs showed similar levels of increased fasting glucose and decreased IVGTT β-cell responses, indicating incipient failure of insulin secretion. In a recent study conducted using the same dexamethasone treatment protocol, post-pubertal pituitary adrenal function was tested in F1 and F2 offspring. Dexamethasone increased baseline responses but reduced stimulated pituitary adrenal responses in F1 and F2 female offspring. Thus, the effects of fetal glucocorticoid exposure on postnatal pituitary-adrenal phenotype can be multigenerational. Other investigators have shown the effects of maternally administered synthetic glucocorticoids on the glucocorticoid function of F1 offspring. These studies provide compelling evidence supporting the view that maternal glucocorticoids may play a fundamental role in fetal developmental programming in the face of multiple challenges; these include, stress, nutrient restriction, IUGR, maternal obesity, and fetal exposure to inappropriate levels of glucocorticoid relative to its stage of maturation. In each of these situations, the fetus receives inappropriate levels of glucocorticoid.

Changes in placenta function

One mechanism whereby maternal obesity affects offspring phenotype is by altering placental nutrient transport and, hence, nutrient availability to the fetus for general growth, but also micronutrients for very specialized cell functions (see above section on the methionine cycle). Just prior to delivery, the placentas of the obese mice that were fed a high-fat diet for 8 weeks before mating, resulting in maternal obesity and a nearly 50% increase in fetal weight, showed a fivefold increase in transplacental transport of glucose and a tenfold increase for neutral amino acids in vivo. Placentas of the obese mice also showed a fivefold increase in microvillous plasma membrane protein expression of glucose transporter 1 (GLUT 1) and a ninefold increase in protein expression of sodium-coupled neutral amino acid transporter 2 (SNAT2). Other transporters (GLUT 3 and SNAT4) were unaltered. The researchers propose that placental nutrient transporter increases are one mechanism by which maternal obesity and high-fat diet lead to macrosomia. Increased transport may occur in a species- and gestational stage-related manner.

In spontaneously obese pregnant baboons, decreased system A placental amino acid transport has been observed. The mononuclear cells (PBMC) transcriptome was altered in fetuses from obese compared with non-obese mothers. Interestingly, the effects of maternal obesity were similar in humans and baboons with regard to increased placental and adipose tissue macrophage infiltration, increased CD14 expression in maternal PBMC, and maternal hyperleptinemia. Fetal weight was not increased in these pregnancies, but the changes in placental and fetal phenotype are consistent with those described for large-for-gestational-age human fetuses.

In a parallel study of pregnant women at 39 weeks gestation, placental function was compared between mothers who were lean (BMI < 25) and obese (BMI ≥ 30) at entry. Placental SNAT activity, maternal hyperleptinemia, and syncytiotrophoblast expression of leptin receptor and SNAT-4 were all decreased in the placentas of obese mothers, despite similar measures of maternal weight gain and offspring birth weight in the two groups.

Maternal obesity is associated with placental inflammation in human pregnancy. It is, therefore, likely that while placental function is initially unimpaired, and even enhanced, by maternal obesity, at some stage of pregnancy the level of inflammation present in the setting of obesity will eventually impair placental function.

Mechanisms in specific tissues

Fat cells and skeletal muscle. Male offspring from fat mother rats provided with normal laboratory chow after weaning show an increase in fat cell size and mass, serum triglycerides, and leptin and insulin concentrations, leading to developmental programming of offspring who are predisposed to obesity, diabetes, hypertension, appetite disorders, and other chronic disease. In sheep, maternal obesity downregulates fetal myogenesis and impairs skeletal muscle development. When nonpregnant ewes were fed an obesogenic diet 60 days before pregnancy until 90% gestation, fetal weight was found to be elevated at 50% gestation but not at term. This observation supports the view presented above that maternal obesity initially leads to fetal overnutrition, with placental damage then decreasing nutrient flow to the fetus. In this model, fat cell numbers and size were increased in fetal skeletal muscle, with infiltration of adipocytes into muscle fibers and an inflammatory response, together with higher fetal insulin serum levels.

Changes in fetal thyroid axis. Normal maturation of the fetal thyroid axis plays a role in fetal growth and in preparing the fetus for extrauterine life, particularly for temperature regulation in the immediate neonatal period. Fetal exposure to a maternal high-fat diet in a non-human primate model reduced fetal levels of free T4 in comparison with non-obese subjects. It has also been shown that fetal cortisol plays a central role in maturation of the fetal
thyroid axis, and the changes in fetal cortisol shown above in the setting of maternal obesity will likely result in dysfunction of the perinatal thyroid axis.

Heart and cardiovascular system. In the sheep model of maternal obesity described above, fetal glucose and insulin were increased in fetuses of obese ewes. Fetal cardiac function was impaired at 90% gestation, both at the cellular molecular level and when whole heart function was evaluated in the Langendorff in vitro system. In hearts from fetuses of obese mothers, phosphorylation of AMP-activated protein kinase, a cardioprotective signaling pathway, was reduced, while the stress-signaling pathway p38 MAPK was upregulated compared with controls. Phosphorylation of c-Jun N-terminal kinase and insulin receptor substrate-1 were increased in the hearts of fetuses of obese mothers, and this was associated with lower downstream PI3K-Akt activity, indicating impaired cardiac insulin signaling. Although the whole hearts of fetuses of obese mothers exhibited normal contractile function when examined using in vitro Langendorff preparation during basal perfusion, they showed an impaired heart-rate-left-ventricular-developed pressure product in response to high workload stress (Figure 7). Taken together, these maternal obesity-induced changes would predispose the offspring to later-life insulin resistance and cardiac dysfunction.91

In keeping with these markers of impaired heart development, maternal obesity is associated with fibrosis and deposition of extra adipose tissue, which would result in local increases in lipids, cytokines, and other factors that impair organ function.92,93

Maternal reactive oxygen and reactive nitrogen species

Maternal reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive free radicals containing an unpaired electron capable of reacting with another free radical to produce molecules containing an unpaired electron. One consequence of this reaction is oxidative/nitrate damage to proteins, lipids, and nucleic acids.84 As a result, the physiological or pathophysiological effects of ROS/RNS are rapidly enhanced. ROS/RNS do not readily cross cell membranes and, thus, may act locally. Local effects depend on the vulnerability of cell components and the balance of many physiological and pathological systems including obesity and glucocorticoids. Under normal circumstances, ROS/RNS are continually produced by prooxidant mechanisms, particularly in the mitochondrial electron transfer chain.95,96 ROS/RNS levels reflect a balance between production and antioxidant defenses, especially superoxide dismutase, catalase and glutathione peroxidase, and molecules such as vitamins E and C and melatonin.95 Physical activity reduces NADPH oxidase activity and superoxide anion production, which, in turn, decreases ROS generation.97

While ROS/RNS have documented pathological effects, they appear to also have physiological functions. For example, they participate in responses to small physiological levels of hypoxia, are involved in apoptosis and mitosis,96 and maintain lung ventilation-perfusion matching. These physiological functions are in keeping with observations that adult nonpregnant female rats at 120 days of life have significant levels of malondialdehyde in their blood and livers as well as ROS (Figure 8). Nitrotyrosine, a well-characterized marker of nitrative stress, is also present. All of these markers of ROS are raised in obese females who have been on a high-fat high-energy diet for 99 days since weaning (Figure 8). The importance of the balance in the physiological and pathophysiological roles of ROS is shown by a study in which overproduction of the antioxidant enzyme, glutathione peroxidase initially produces beneficial changes in key pancreatic islet genes PDX1 and UCP2, followed by chronic hyperinsulinemia.98

Intrauterine oxidative stress can be generated by maternal overnutrition, which increases risk of adult disease.99 Mitochondria are very sensitive to early developmental programming,74 and mitochondrial dysfunction has been reported in mouse embryos of obese mothers, which show increased ROS production and oxidative phosphorylation.100 Dysfunction of the electron transport chain in skeletal muscle and liver have also been reported in offspring of obese mice.100 Alteration in mitochondrial function negatively impacts cells that have a high energy requirement, such as the β-cell. Beta cells are
especially vulnerable to ROS because expression of antioxidant enzymes in the pancreatic islets is very low and β-cells have a high oxidative energy requirement.\textsuperscript{101} ROS production gradually increases in rat IUGR pancreatic islets, which damages mitochondrial DNA with subsequent deterioration in mitochondrial function and decline in β-cell function predisposing to diabetes.\textsuperscript{102} Antioxidant supplementation (vitamins A, E, C, and selenium) provided to rats fed a Western diet was shown to reduce oxidative stress and inflammation in the embryos. Further, restoration of the antioxidant balance during pregnancy in the mothers receiving a Western diet decreased adiposity in offspring and improved glucose tolerance test results at 2 months of age.\textsuperscript{103}

Inflammation is now thought to be an important factor in the pathophysiology of obesity. There is evidence that feeding female mice with a high-fat diet before and during pregnancy increases maternal and fetal weight accompanied by chronic hypoxic stress and an inflammatory response in placentas.\textsuperscript{104}

CONCLUSION

The developmental programming effects of maternal obesity are now well accepted based on results from compelling human epidemiological and animal studies. In the ongoing search for mechanisms, studies are required in both precocial and altricial species addressing the many mechanisms reported here. Only by understanding the mechanisms will it be possible to conduct appropriate studies to determine effective interventions in human pregnancy.

Acknowledgments

Funding. This work was partially supported by CONACyT (Consejo Nacional de Ciencia y Tecnología) 155166, México, Sociedad Mexicana de Nutrición y Endocrinología and HD 21350 from the National Institute of Child Health and Human Development.

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES


30. Samuelsson AM, Matthews PA, Argenton M, et al. Diet-induced obesity in


17. Nathanielsz PW. Maternal Obesity

16. Barker D. Developmental programming of the


12. Benkeser RM, Biritwum R, Hill AG. Prevalence of overweight and obesity and


6. Balarajan Y, Villamor E. Nationally representative surveys show recent increases


48. Bouret SG. Developmental origins of obesity: energy balance

47. Hillebrand JJ, de Wied D, Adan RA. Neuropeptides, food intake and body


45. Waterland RA, Travisano M, Tahiliani KG, et al. Methyl donor supplementation

44. Choi J, Li C, McDonald TJ, et al. Emergence of insulin resistance in juvenile

43. Bouanane S, Merzouk H, Benkalfat NB, et al. Hepatic and very low-density lipo-

42. Suter M, Bocock P, Showalter L, et al. Epigenetics: maternal high-fat diet expo-


40. Ten X, Triche EW, Hogan JW, et al. Prenatal factors for childhood blood pres-

39. Roque FR, Briones AM, Garcia-Redondo AB, et al. Aerobic exercise reduces ox-

38. Bouanane S, Merzouk H, Benkalfat NB, et al. Hepatic and very low-density lipo-


35. Long NM, Ford SP, Nathanielsz PW. Maternal obesity eliminates the neonatal

34. Morris MJ, Chen H. Established maternal obesity in the rat reprograms hypo-

33. Farrow AM, Morris MJ, Saha V, et al. Maternal obesity and anorexigenic (alpha-MSH, CART) neuropeptides of paraventricular hypo-


30. Samuelsson AM, Matthews PA, Argenton M, et al. Diet-induced obesity in


25. Long NM, Ford SP, Nathanielsz PW. Maternal obesity eliminates the neonatal

24. Burns JJ. Missing step in man, monkey and guinea pig required for the biosyn-


15. Armitage JA, Khan IY, Taylor PD, et al. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evi-


12. Benkeser RM, Biritwum R, Hill AG. Prevalence of overweight and obesity and


6. Balarajan Y, Villamor E. Nationally representative surveys show recent increases


4. Colcherio MA, Sosa-Rubi SG. Heterogeneity of income and lifestyle determin-


S53


94. Carter MF, Dudley DJ, Nathanielsz PW. Fetal cortisol is evaluated in maternal obesity (MO). Reprod Sci. 2011;18:139A.


