45 Fetal programming

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INTRODUCTION

The prenatal period requires vigilance to insure a safe environment for the fetus to grow and develop appropriately. Great lengths are taken during prenatal care to avoid exposures known to have teratogenic potential. Fetal programming is the theory that environmental events in utero during sensitive windows of development can have permanent and long-lasting effects. The fetus has the capacity for developmental plasticity. That is, one genotype can lead to different phenotypes depending on environmental factors (1). In other words, the environment interacts with genes in the developing organism to permanently program gene expression in adulthood. Changes in the fetal environment can program childhood and adult response pathways. Therefore, the developmental period represents a critical opportunity for irreversible effects from the fetal environment, whether these are from pharmaceuticals, nutrition, microorganisms, the maternal hormonal milieu, and/or environmental chemicals.

There is a strong precedence for the importance of the perinatal environment. Clinicians are often among the first to recognize the deleterious effects of teratogenic pharmaceuticals. To that effect, a five-tier classification system designed to rate the safety of medications during pregnancy and lactation has greatly improved pharmaceutical counseling during the prenatal and perinatal periods. However, while many effects resulting in obvious birth defects have been documented, such as limb defects during the era of thalidomide use, the potential for pharmaceuticals to alter the fetal hormonal environment and alter adult disease susceptibility is a relatively new area of study. The long-term consequences of these types of effects are difficult to identify and are largely unknown. In addition to pharmaceuticals, maternal nutrition is known to affect fetal development. A physician, DJ Barker, was among the first to put forth the *Developmental Origins of Adult Health and Disease* hypothesis, highlighting the potential for maternal nutrition to impact the offspring's risk for coronary heart disease (CHD) and type II diabetes (1). Although not the focus of this chapter, an extensive body of literature has used animal models of development to confirm cause and effect relationships initially found in epidemiologic studies supporting the *Developmental Origins* hypothesis.

Altered fetal programming may manifest as physical, developmental, or neuropsychiatric abnormalities that often have long latencies and may not become evident until childhood or even adulthood. It is difficult to pinpoint a specific exposure during pregnancy responsible for a certain behavioral pattern or health condition that presents years after the exposure, especially when considering the vast number of potential variables, both physical and psychologic (i.e., emotional nurturing). However, a number of epidemiologic studies have found associations with altered fetal environ-

ments. An area of great interest within fetal programming is the molecular changes within the fetus in response to maternal stress, medications, and exogenous hormone exposure.

We know that pharmaceuticals can program fetal development and cause effects that can be seen at birth. In addition, there is a large and growing body of literature demonstrating that the fetal environment can program the fetus and result in long-latency effects associated with increased risk of adult disease. The developmental origins of adult disease hypothesis evolved to explain this type of programming. In this chapter, we will focus on the long-term consequences of changes within the uterine environment during pregnancy, including nutritional, hormonal, pharmaceutical, and other environmental factors.

BIRTH WEIGHT AND ADULT DISEASE RISK

It is known that maternal nutrition is important for healthy fetal development; however, only recently has a link between maternal and fetal nutrition been made with adult disease (Box 1). The developing fetus is dependent on its mother to receive the proper nutrients required for normal growth and development. Low birth weight has historically been associated with an increased risk of death from CHD. Barker et al. first identified this association in a large population of men born in Hertfordshire, England, in 1911 to 1930 (3). As a baby's weight at birth or year 1 decreased, the risk of death from ischemic heart disease rose. People who were the lightest at birth (≤ 5.5 pounds) and also lightest at 1 year of age (≤ 18 pounds) had the highest standardized mortality ratio (SMR = 200; 95% CI 114–384) (3). Furthermore, Barker et al. also reported that systolic blood pressure, a risk factor for cardiovascular disease, was inversely related to birth weight in 10- and 36-year-olds born and residing in Great Britain (3).

The initial finding that birth weight was associated with CHD in adulthood led Barker to propose the *Developmental Origins of Adult Health and Disease* hypothesis, suggesting that changes that occurred during development that caused a baby to be born small may also impact adult disease risk. For example, low-birth weight babies are known to have increased blood pressure in adulthood (4,5). It has been suggested that decreased blood flow to the kidney during key developmental periods may lead to decreased cell numbers in the glomeruli. Each glomerulus is therefore subjected to increased blood flow, and although blood pressure can initially be maintained, hyperfiltration eventually leads to glomerulosclerosis. This, in addition to the normal loss of glomeruli that occurs with aging, results in a feed-forward loop of increasing blood pressure and glomerular loss (6). Low birth weight is also associated with impaired glucose tolerance (5). In a similar mechanism, an undernourished fetus directs glucose to the developing brain at the expense of the muscles thereby resulting in less muscle mass at birth. As muscle is the primary

Box 1 Consequences in Adult Life Associated with Low Birth Weight

- Hypertension
- Type 2 diabetes
- Obesity and the metabolic syndrome
- Coronary heart disease
- Stroke
- Osteoporosis
- Depression and psychosis
- Age-related cognitive impairments
- Chronic renal failure
- Altered gonadal responses
- Altered immune responses
- Overall reduced life expectancy

Source: From Ref. 2.

location of insulin action, decreased muscle composition in adulthood results in lifelong insulin resistance (7). Another possibility is that there are fewer β -cells in the pancreas of low-birth weight babies.

Low birth weight has also been associated with diseases of the metabolic syndrome in adulthood. Curhan et al. found that low birth weight was associated with an increased risk of hypertension and diabetes in a longitudinal study of 51,529 U.S. males born from 1911 to 1946. Compared with men born with average size (7.0–8.4lb), men born weighing less than 5.5 lb were 26% more likely to have hypertension and 75% more likely to have diabetes (8). Curhan et al. also examined data from the Nurses’ Health Studies I and II and found that low birth weight was associated with hypertension in women (8). Additionally, in a study of the Pima Indian population in Arizona, McCance et al. found that low birth weight was strongly associated with increased risk of diabetes (9). Hales et al. also found increased risk of diabetes or insulin resistance with decreasing birth weight in the Hertfordshire studies discussed above (5).

Barker further refined his *Developmental Origins* hypothesis with the introduction of the concept of the *thrifty fetus*. He hypothesized that the fetal environment could provide a “weather forecast” of the nutritional status outside of the womb. His *thrifty phenotype* hypothesis suggests that declines in nutritional status while in the womb can program a fetus to be “thrifty” and to store and ration energy accordingly (10). Problems arise, however, when individuals programmed in utero to be “thrifty” are introduced to an environment of plenty during early adolescence and adulthood. Being “thrifty” in development allows a fetus to conserve energy for important tissues like the brain at the expense of other tissues such as muscle and liver. Barker further suggests that the tissues and organs that were sacrificed during development have a diminished ability to properly respond to a “world of plenty” in adulthood. This hypothesis suggests that catch-up growth creates problems for those who were “thrifty” during development.

As an example of the *thrifty phenotype*, Barker and colleagues have extensively studied the case of people who were in utero during the Dutch Famine, which occurred from October 1944 to May 7, 1945, as a result of the German occupation in the West Netherlands during World War II. This discrete famine has allowed researchers to directly study

the effects of maternal malnutrition on adult disease risk. During the famine, daily caloric intake fell to 300 to 800 calories/day. Fortunately, recovery was swift and the caloric intake rebounded to pre-famine levels by June 1945. Because of the relatively short length of the famine, researchers have been able to determine whether adult-onset diseases are linked to nutritional insufficiency during very specific times of development. Ravelli et al. examined obesity among 19-year-old military draftees who were exposed to the famine compared with those born just before or just after the famine or born during the same time but in unaffected locations. They found that men exposed to the famine during the first two trimesters of pregnancy had a significantly higher prevalence of obesity compared with men born in cities unaffected by the famine. Conversely, men exposed to famine during the third trimester of gestation through the first 3 to 5 months of postnatal life had a decreased prevalence of obesity compared with men born outside of the region of famine (11).

Another series of studies examined 297 people exposed to the Dutch famine during gestation and 444 born just before or just after the famine. Children who were exposed to the famine during mid or late gestation were lighter, shorter, and thinner with smaller heads and placentas than those unexposed and had reduced glucose tolerance following a 2-hour oral glucose test (12,13). Conversely, those exposed to famine in early gestation were heavier and longer at birth than those unexposed (12). They also had a more atherogenic lipid profile, higher low-density lipoprotein to high-density lipoprotein ratio, higher body mass index (BMI), and a higher prevalence of CHD (12,14,15). Since high blood pressure is related to low birth weight, it was expected that it would also be related to famine exposure, particularly late exposure that tended to produce low-birth weight babies. Researchers found, however, that while increased blood pressure was related to low birth weight, it was overall independent of famine exposure (12). They were further able to elucidate that increased systolic blood pressure was inversely related to the protein/carbohydrate ratio in the third trimester regardless of famine exposure (16).

The Dutch Famine occurred for a short, discrete period of time and was followed by a quick return to nutritional sufficiency. By contrast, the famine that was incurred during the Leningrad Siege in what is presently known as St. Petersburg, Russia, from September 1941 to January 1944 lasted much longer and those exposed remained nutritionally deprived throughout much of their early adolescence. In examining those who were exposed in utero (born January 1, 1941–June 30, 1941; $n = 169$) or during infancy (born November 1, 1941–June 30, 1942; $n = 192$) or those born during the same period but outside of the area of the siege, Stanner et al. found very little differences in adult disease risk factors; there was a correlation with BMI and systolic and diastolic blood pressure in females in both exposure groups and von Willebrand factor was decreased in the infant group compared with the in utero group (17). Overall, the authors concluded that there was no effect on glucose concentration or insulin-like molecules or risk for hypertensive or cardiovascular disease with exposure to the Leningrad siege famine (17). The fact that there were very little differences found in the study of those exposed to the Leningrad Siege yet there were

extensive differences found in the study of the Dutch Famine may serve to further highlight Barker's thrifty phenotype hypothesis that catch-up or compensatory growth further attenuates the low-birth weight baby's adult disease risk.

As discussed above and demonstrated in the comparison of the two famine studies, problems arise when the postnatal environment differs from the prenatal in utero environment and catch-up growth occurs. For example, in a study of 3641 men born in Helsinki from 1924 to 1933 for which there were serial measurements of height and weight from 6 to 16 years of age, Eriksson et al. found that men who were born with the lowest ponderal index but that subsequently had the highest BMI at age 11 were the most likely to have died from CHD (18). Forsen et al. reported on the corresponding cohort of 3447 women born in Helsinki during the same time period (19). In contrast to the data on the men, Forsen et al. reported that the risk of CHD was not associated with childhood BMI in women. Instead, in women, an increased risk was related to length at birth and increased placental weight. Women who were shortest at birth but tallest at age 7 (i.e., had experienced "catch-up growth") had the highest risk of CHD. Low birth weight followed by catch-up growth has also been shown to be a risk factor for adult-onset type II diabetes and hypertension (20). Along with others, these studies indicate not only the importance of catch-up growth, but also the important differences between how the sexes develop, namely, that females experience slower prenatal growth than males (21,22). Furthermore, recent work has shown sex-specific alterations in DNA methylation (epigenetic) patterns of genes involved in metabolism in people exposed to the Dutch Famine (23).

While the increase risk of adult disease in association to low birth weight continues to be a major concern in both developing and developed countries, an equally important association with the effects of being born large for gestational age must also be studied, especially in light of the growing obesity epidemic. Large for gestational age is typically defined as birth weight greater than the 90th percentile for gestational age and is often associated with maternal obesity and/or diabetes (24). When excess maternal glucose crosses the placenta, the fetus becomes hyperglycemic and then experiences hyperinsulinemia. As insulin is an important growth factor for the developing fetus, aberrant circulating levels negatively affect adipose, muscle, and cardiac tissues resulting in a large or macrosomic baby (24). Large babies and those born to diabetic mothers are more likely to experience complications during delivery and, as adolescents, are more likely to be obese and to develop type II diabetes (8,9,24–27).

Although birth weight has historically been used as a predictor in the developmental origins of adult health and disease, several studies indicate that it might not fully explain the complex interactions that have occurred to produce a baby of any given size. The utilization of additional markers, such as maternal birth weight, third-trimester maternal weight and weight gain, maternal arm fat area, maternal height, maternal energy intake, socioeconomic status, ponderal index, birth length, placental weight, and postnatal growth rate, may help to further understand how the perinatal environment programs adult disease risks. To this end, Kuzawa and Adair have developed a novel model to address

the importance of fetal growth potential (genetics) versus fetal nutrient supply by relating maternal height to the potential fetal growth demand and third-trimester maternal arm fat area to the potential maternal supply. They found that male offspring born to tall mothers with low arm fat (lowest energy reserves) had the highest risk of cardiovascular disease as measured by lipid levels at age 14 (28). In female offspring, there was no difference in lipid levels, further supporting the idea that fetal programming of CHD risk from nutrition differs between the sexes. As we move forward in our examination of how the maternal and early postnatal nutritional environment affects adult disease risk, we must continually allow for new variables that help clarify the environment in which the fetus is developing. Furthermore, as clinicians, it is important to understand all potential ramifications of counseling new mothers regarding proper nutrition, including effects that may not be seen until their children reach adulthood.

PHARMACEUTICAL TERATOGENS AND BIRTH DEFECTS

Teratogens cause fetal programming that can result in severe birth defects. Throughout history, severe malformations caused by teratogens have been documented. One of the most well-known cases is thalidomide, which was initially used as a sedative and antiemetic and resulted in 10,000 to 20,000 victims worldwide who were born with missing or severely malformed limbs. While thalidomide has both antiangiogenic and anti-inflammatory properties, it has recently been demonstrated that it is the antiangiogenic nature of the drug that prevents the development of newly forming blood vessels necessary to support limb development (29). That is, a chemical exposure permanently altered the course of development and resulted in unformed limbs in those exposed.

Another suspected teratogen, isotretinoin (trade name Accutane), is a pharmaceutical prescribed for the systemic treatment of acne and is a derivative of retinoic acid. Retinoic acid, more generally known as vitamin A, is essential during development. A concentration gradient along the anterior-posterior axis regulates the expression of homeobox genes that subsequently direct the correct timing of developmental events within a growing organ system. The timing and concentration of retinoic acid is exquisitely regulated and involved in the proper development of bone, vision, brain, face, heart, and spinal cord. Because of the molecular similarities with retinoic acid, isotretinoin exposure alters the delicate balance of retinoic acid in the fetus and can interfere with the normal patterns of development causing severe malformations. Today doctors must extensively counsel women prescribed isotretinoin on the risks of severe fetal malformations and the need to use two methods of contraception. Unfortunately, despite their best efforts, more than 2000 women have become pregnant while on isotretinoin treatment (30).

LONG-LATENCY EFFECTS FROM NON-TERATOGENIC PHARMACEUTICALS

Selective Serotonin Reuptake Inhibitor Use During Pregnancy

Treating depression in pregnancy is a difficult decision for patients and physicians; both treatment and lack of treatment are associated with increased fetal risks. Untreated maternal

depression negatively affects secondary to neonates increased maternal tobacco use, substance abuse, inadequate prenatal care, and poor maternal–fetal bonding postnatally. Previous data also suggest an increased risk of preterm labor and fetal growth restriction in fetuses born to mothers with untreated depression. Furthermore, maternal stress and mood disorders can permanently program the fetal adaptive stress response mediated by the hypothalamic–pituitary–adrenal (HPA) axis. Exposure to stress results in an increase in corticosterone levels in humans. In animal models, exposure of fetuses to stress in the prenatal and early postnatal periods resulted in exaggerated cortisol production in response to mild stressors in adulthood (31). Maternal stress and poor mothering behaviors postnatally have also been shown to alter fetal and infant glucocorticoid metabolism through epigenetic mechanisms involving alterations of DNA methylation (32–34). Maternal stress increases cortisol within the fetal HPA axis, resulting in shorter gestations, prematurity, lower birth weights, increased congenital malformations, smaller head circumferences, and depressed neonatal neurologic functioning (35). Importantly, infant and adolescent maltreatment are associated with altered cortisol levels and impaired response to psychologic stressors (36). Specifically, maternal stress and malnutrition have been linked to increased incidence of schizophrenia in offspring (37). The decision not to treat or stop treatment during pregnancy may also increase the risk of postpartum depression (38).

On the other hand, fetal exposure to selective serotonin reuptake inhibitors (SSRIs) also carries certain risks. Certain antidepressants are linked with increased risk of congenital anomalies, such as cardiac defects, omphaloceles, and other midline defects as well as persistent pulmonary hypertension. However, the absolute risk of these conditions secondary to SSRIs (the most commonly prescribed class of antidepressants during pregnancy) is very low (38,39). Although overall risk of anomalies are low, transient side effects as a result of serotonin withdrawal at the time of delivery, such as agitation, altered muscle tone, breathing, and suction problems, are commonly reported (40). These effects typically resolve spontaneously within 2 weeks and are conventionally thought to pose less severe long-term consequences to the fetus than those associated with uncontrolled maternal depression. However, subclinical effects of perinatal exposure to SSRIs on fetal neurodevelopment may be subtle and remain undiagnosed until adolescence or early adulthood. More recently, an association with fetal SSRI and autism has been suggested, as increased serotonin causes dysfunction of the amygdala, which plays a role in emotion perception, and suppressed oxytocin production, a hormone proposed to promote “pro-social” behavior (41). Furthermore, SSRIs enter placental circulation and therefore increase fetal serotonin levels, an effect that may program the fetal HPA axis to function properly only in the presence of elevated serotonin, a state removed at the time of delivery.

Progestin Use During Pregnancy

Progesterone is a necessary component to maintain a healthy pregnancy. From the earliest stages of pregnancy, progesterone assists in implantation and supports pregnancy by formation of the corpus luteum. While endogenous progesterone is

essential, it is still unclear what long-term effects may occur from the use of exogenous progesterone.

Progestin is a component of oral contraceptive pills (OCP) and limited exposure during the periconceptual period occurs with failure of oral contraception. Continued use of OCPs until detection of pregnancy occurs in 2% to 5% of pregnancies and can expose fetuses to exogenous hormones during critical windows of fetal development (42). In addition, emergency contraception exposes fetuses to even higher doses of exogenous hormones and is not 100% effective at preventing implantation. Prior to the advent of modern pregnancy tests that detect human chorionic gonadotropin in urine, the major indication for progestins in pregnancy occurred from progestin administration for 3 to 5 days to induce withdrawal menstruation. Today, the major indications for progestin therapy are to prevent preterm birth and treat recurrent pregnancy loss. Progestin supplementation is also utilized in assisted reproduction protocols to promote endometrial development favorable for implantation of a transferred embryo.

Several studies have assessed the risk of hypospadias following progestin exposure, the results of which seem to be inconclusive (43). One complication is that many studies examine boys born 20 or more years ago, when different progestins were indicated during pregnancy than those in current use. However, a more recent case-control study by Carmichael et al. of children born from October 1997 to December 2000 found a 3.7-fold increased risk of second- and third-degree hypospadias when women took any progestin to help become pregnant or to prevent pregnancy complications or loss for any length of time, but not from perinatal OCP exposure (44). Furthermore, in a meta-analysis of 14 articles, Raman-Wilms et al. found no increased risk of urogenital malformations with hormonal exposure during the first trimester. In this case, exposure was from OCP, hormonal pregnancy tests, as well as treatment for threatened abortion (45).

To date, 17-hydroxyprogesterone is the formulation of greatest efficacy in preventing preterm deliveries in women with a previous spontaneous preterm birth. In a retrospective cohort study by Waters et al., women receiving weekly injections of 17-hydroxyprogesterone caproate were 3.3 times more likely to develop gestational diabetes, controlling for maternal age, BMI, and maternal race (46). Progesterone may increase insulin resistance by affecting glucose transport at the glucose transporter type 4 (GLUT-4) receptor (46). Gestational diabetes can lead to insulin resistance in utero and permanently modify expression of genes responsible for glucose transport and beta cell development, predisposing fetuses to type II diabetes mellitus in adulthood (47). As use of progesterone to prevent preterm birth continues to gain momentum, the postnatal effects will continue to be elucidated as exposed fetuses reach adolescence and adulthood.

Glucocorticoid Use During Pregnancy

At proper concentrations, glucocorticoids are essential for fetal growth and development. Typical fetal cortisol levels are much lower than maternal levels due to an enzyme within the placenta, 11-beta-hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which catalyzes rapid metabolism of corticosteroids into inactive metabolites (48). Exogenous steroid administration to preterm infants is frequently utilized to

promote lung maturity and decrease complications of preterm delivery including necrotizing enterocolitis, intraventricular hemorrhage, and pulmonary hypoplasia. The two steroids most commonly used, dexamethasone and betamethasone, are utilized because they are poorly metabolized by 11β -HSD2 and easily diffuse through the placenta, thus enhancing the protective benefits by maximizing exposure (2). However, prolonged fetal exposure to exogenous steroids has been associated with an increased incidence of growth restriction, elevated cortisol levels, and reduced placental 11β -HSD2 activity (49). These effects are exacerbated by placental insufficiency, which can be seen with maternal hypertension and diabetes, or with modification of the 11β -HSD2 receptor (2). Conditions that typically occur during preterm labor or preterm premature rupture of membranes, such as increased progesterone, estrogen, nitric oxide, prostaglandins, and inflammatory cytokines, can temporarily reduce activity of placental 11β -HSD2 activity, thereby increasing the susceptibility to negative side effects to the population at greatest risk (2).

While the benefits to the developing lung are obvious, epidemiologic and animal model data suggest that there are negative side effects, such as altered brain development, particularly in the HPA, following multiple steroid treatments and may be worse in dexamethasone- than betamethasone-treated individuals (50,51). Neuropsychiatric disorders are also prevalent in exposed individuals, including memory impairment, increased emotionality, unsociability, avoidance, and behavioral problems (52,53). Furthermore, Drake et al. recently reported that the effects of in utero exposure of rats to dexamethasone on low birth weight and glucose dyshomeostasis are also transmitted to second-generation offspring that are not directly exposed (54). The safety of exogenous steroids given postnatally for use in respiratory distress syndrome and bronchopulmonary dysplasia has also been questioned. Meta-analyses suggest that postnatal steroid use may increase the risk of cerebral palsy by 63% and increases neurologic impairment up to 40% (51). An important question is whether fetal growth restriction is an effect of glucocorticoid exposure in utero or whether it is the primary cause in warranting steroid administration. This question will be difficult to answer unequivocally.

In laboratory rats, developmental exposure to betamethasone led to altered partner preference, sexual behavior, and reduced testosterone in adulthood (55). The authors suggest that “prenatal treatment with betamethasone, by increasing maternal corticosteroid level, may have diminished testosterone peak in male pups, a peak crucial to brain sexual differentiation.”

Diethylstilbestrol

Because teratogen exposure often results in specific malformations that can be identified at birth, a cause and effect relationship between exposure and birth defect can often be established. However, linking exposure with fetal programming that results in long-latency effects is more challenging. One of the best-known examples is the case of diethylstilbestrol (DES) daughters. From 1940 to 1971, millions of women were prescribed the synthetic estrogen DES in the mistaken belief that it would prevent spontaneous abortion and promote a healthy pregnancy. The negative effects of developmental DES exposure were not known until 1971 when the physician AL Herbst published a case report of eight young girls with vaginal clear cell adenocarcinoma, a rare type of cancer that previously had only afflicted much older women. Herbst found that seven of the eight presenting cases had been exposed to DES during the first trimester (56) and later confirmed this on a larger scale (Fig. 1). Additionally, women exposed in utero to DES had increased incidence of various reproductive abnormalities including T-shaped uteri, transverse vaginal ridges, and cervical collars (58–62). Today, it is known that both men and women exposed to DES in utero have increased risk of adult-onset diseases, several of which are discussed below. Furthermore, it appears that the risks of exposure to DES did not stop with those that were exposed in utero, but extend to a third generation providing evidence for transgenerational effects (63–67).

The phenotype seen in women from fetal programming by DES has been reproduced in laboratory mice developmentally exposed to DES (68,69). In mice, the T-shaped uterus appeared to be caused by posterior shift in the expression of homeobox A genes whose sequential expression are necessary for proper tissue development (70). When the discovery that

Figure 1 Market sales of 25mg DES versus cases of vaginal clear cell adenocarcinoma by year of diagnosis. Source: From Ref. 57 and Market share Litigation exhibit.

developmental exposure to an estrogen pharmaceutical created long-latency developmental effects existed, it opened the door to the exploration of fetal programming. Additional sources range from natural variation in endogenous fetal estrogens to exogenous exposure to chemicals in the environment with hormonal activity, termed endocrine disruptors, among a variety of other pharmaceuticals that are just beginning to be scrutinized for potential fetal programming.

ENDOGENOUS AND EXOGENOUS FETAL ESTROGEN AND ADULT DISEASE

Altered fetal serum estrogen has been associated with fetal programming and altered risk of adult disease (Table 1). It is known that adult estrogen exposure is associated with endometriosis and breast cancer. Conditions associated with *increased* fetal exposure to estrogens are also associated with an increased risk of endometriosis and breast cancer in adulthood. Daughters whose mothers took the synthetic estrogen DES during pregnancy have an 80% increased risk of endometriosis and up to 300% increased risk of breast cancer (58,71). Dizygotic twins, who have two placentas, circulate 60% to 80% higher levels of estradiol than singletons (75). Dizygotic twins subsequently have approximately 70% increased risk of endometriosis and 50% increased risk of breast cancer in adulthood (58,72). Monozygotic twins do not have an increased risk of breast cancer, suggesting that it is not being a twin that raises the risk but having two placentas (72). In addition, maternal estrogen concentrations are positively associated with maternal age and offspring birth weight, and breast cancer risk is also positively associated with maternal age and birth weight (reviewed in 72). High ponderal index at birth is associated with increased circulating estradiol in adulthood (76).

Conditions associated with *decreased* fetal exposure to estrogens are associated with reduced risk of endometriosis and breast cancer in adulthood. Smoking inhibits the estrogen-synthesizing enzyme aromatase and is associated with reduced estrogen levels (77). For example, in adult women smokers, there is a 20-fold reduction in the incidence of the estrogen-dependent disease endometriosis (73,74). Importantly, placental aromatase activity is reported to be reduced in smokers (78). Daughters born to women who smoked during pregnancy have over a fourfold reduced risk of endometriosis and have a threefold reduced risk of breast cancer (73,74). Additionally, maternal estrogens are lower in pre-eclamptic patients versus controls (79,80). Daughters born

to pre-eclamptic mothers have a twofold decreased risk of breast cancer in adulthood (72). No data were found on the risk of endometriosis. Taken together, the fetus appears to be sensitive to permanent programming due to an altered fetal hormonal environment.

ENDOCRINE-DISRUPTING CHEMICALS

While women no longer intentionally take synthetic estrogens during pregnancy, pregnant women and their fetuses are exposed to a wide variety of environmental chemicals that may alter normal development. Many of these chemicals can surreptitiously modulate the endocrine system and have been termed endocrine disruptors or endocrine-disrupting chemicals (EDCs). Hundreds of chemicals, both natural and manmade, have been described with the ability to pirate the body’s own endocrine system causing altered hormone signaling. Like teratogens, endocrine disruptors can program the fetus and result in permanently altered phenotypes (81). However, unlike teratogens, EDCs can affect both adults and developing organisms. Whereas the effects of endocrine disruptors in adulthood tend to be reversible, the effects on the developing organism tend to be permanent. For this reason, a considerable amount of research has focused on this sensitive period, where exposure can result in permanent fetal programming.

Teratogens cause severe fetal programming that results in malformations present at birth, but there are probably other persistent changes that are subtler and therefore not attributed to the exposure. Teratogens and EDCs probably represent a continuum of effects from more severe effects often with short latency to more subtle effects often with long latency. Severity can be related not only to higher exposure to teratogens but also to different mechanisms of action. Unfortunately, effects seen at high doses cannot necessarily predict those seen at low doses. The effects of endocrine disruptors most often exhibit a non-monotonic dose–response where qualitatively and/or quantitatively different effects have been described at low doses versus high doses (82,83). For example, in mice developmental exposure to low doses of DES increases the adult estrogen responsiveness in the uterus while exposure to high doses decreases the responsiveness (84). This adds to the difficulty in establishing cause and effect relationships between chemical exposure and adult disease.

To date, there has been a large amount of research in the laboratory demonstrating developmental programming by EDCs. As illustrated above, conditions associated with altered hormonal environments during human pregnancies are

Table 1 Altered Fetal Estrogen and Adult Disease			
Fetal or maternal condition	Fetal estrogen	Breast cancer	Endometriosis
DES	Increased	3.00 (1.01–8.98) ^a	1.8 (1.2–2.8) ^c
Twins	Increased	1.48 (1.01–2.16) ^b	1.7 (1.2–2.5) ^f
Smoking	Decreased	0.35 (0.12–0.99) ^c	0.22 (0.06–0.82) ^g
Pre-eclampsia	Decreased	0.48 (0.30–0.78) ^d	N/A ^h
Ratio with (95% confidence interval). ^a Incidence rate ratio for cancers occurring at ages ≥ 50 years (71). ^b Hazard ratio in meta-analysis of case-control studies for women who have a dizygotic twin (72). ^c Adjusted (for age, nulliparity, and active smoking) relative rate for daughters born to women who smoked on average 15 cigarettes/day (73). ^d Relative risk calculated from meta-analysis of case-control studies for daughters born to pre-eclamptic mothers (72). ^e Rate ratio for daughters who were part of a multiple gestation controlling for birth weight (58). ^f Rate ratio for daughters with any prenatal DES exposure (58). ^g Odds ratio for daughters born to women who smoked on average 15 cigarettes/day (74). ^h No analysis found. Abbreviation: DES, diethylstilbestrol.			

associated with altered disease risk in adulthood. While long-latency effects from hormonal chemicals are difficult to detect, humans have been shown to be sensitive to this type of fetal programming (85). Because of the prevalence of EDCs in our environment and the potential for EDCs to cause developmental programming in humans, the Endocrine Society recently released a position statement on endocrine disruptors (86): “The evidence for adverse reproductive outcomes (infertility, cancers, malformations) from exposure to endocrine disrupting chemicals is strong, and there is mounting evidence for effects on other endocrine systems, including thyroid, neuroendocrine, obesity and metabolism, and insulin and glucose homeostasis.” They further suggest partnering of scientific societies to further research the effects in humans. Similar statements have been adopted by the American Medical Association, American Chemical Society, and others.

The U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening and Testing Program was established in 1998 in response to the Food Quality Protection Act and was charged with the goal of developing screening and testing methods for endocrine-active compounds. Over 10 years later, the final selection of chemicals to be tested to validate screening and testing methods was chosen (87). Recently, the Endocrine Disruptor Prevention Act of 2009 was introduced to strengthen regulation of EDCs.

MECHANISMS OF EDCS

The U.S. EPA defines an EDC as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.” A detailed discussion of the mechanisms of EDC action is beyond the scope of this chapter but has been reviewed elsewhere (81,86). Summarized below are potential consequences resulting from developmental disruption of normal estrogen, androgen or thyroid hormone signaling by EDCs in adulthood.

Estrogens

To date, the largest group of environmental chemicals with hormonal activity interferes with normal estrogen signaling. Those chemicals that bind directly to the estrogen receptor are termed xenoestrogens. They can have estrogenic, antiestrogenic, or mixed receptor activity. While hundreds of xenoestrogens have been described, a few selected ones are described below.

Bisphenol A is an estrogen receptor ligand and selective estrogen receptor modulator (88,89). It has been shown to disrupt the endocrine system in >100 reports in different animal systems (82). Bisphenol A is the monomer that is polymerized to form polycarbonate plastic (hard, clear plastic) and is one of the top 50 chemicals in worldwide production, with annual production of >6 billion pounds (90). It is released from polycarbonate products, such as water and baby bottles (91,92), the resin lining of metal food cans (93–95), some dental sealants (96), eyeglasses, CD cases, and polycarbonate laboratory animal cages and water bottles (97,98). Bisphenol A was shown to comprise 84% of the estrogenic activity in landfill leachate (99).

Bisphenol A is an almost ubiquitous chemical in our environment and in people (100). Calafat recently reported that >92% of Americans have detectable levels of bisphenol A

in urine (101). Importantly, children had twice the level as adults (4.5 vs. 2.5 $\mu\text{g/L}$) and women had higher levels than men. It was recently estimated that infants drinking out of polycarbonate bottles would receive a daily exposure of up to 2 $\mu\text{g/kg}$ (102). Of particular concern, bisphenol A alters normal development at this exact dose (and lower) in animal models (82,98,103–115).

Ethinyl estradiol is an orally active form of estradiol, and it is a component of OCPs. While ethinyl estradiol is not considered a teratogen, evidence from laboratory studies suggest that it is an endocrine disruptor. Millions of women use OCP, and approximately 2% to 5% will conceive while taking them due to noncompliance (116). Some of these women deliver infants exposed to 0.2 to 0.5 $\mu\text{g/kg}$ of this potent estrogen during the critical window of sexual differentiation in the first trimester of pregnancy. This dose has been shown to alter mouse development (105,117). Additionally, ethinyl estradiol has been measured in waste water influent and effluent and may be a source of human exposure (118).

Genistein is a naturally occurring phytoestrogen and the predominant soy isoflavone. Adults consuming soy-based foods, particularly vegetarians, circulate 50 to 200 ng/mL total isoflavones. Infants fed soy-based infant formula circulate approximately 700 ng/mL genistein (119). While soy-based foods have been promoted as having beneficial effects in adults, there is increasing evidence that developmental exposure during differentiation of the uterus may be detrimental (120–123).

Importantly, xenoestrogens are found in the human fetus. Pregnant women and their fetuses tend to have higher levels of bisphenol A (124–126), and bisphenol A has been found in human maternal and fetal serum at 0.2 to 10 ng/mL (125–127). Olea and colleagues recently measured total xenoestrogen bioactivity in human placentas. By separating endogenous from xenoestrogens, it was estimated that 16% of the total activity was due to xenoestrogens (128). There is a considerable amount of literature relating to EDCs: on mechanisms of action, controlled laboratory studies, and human health trends. Taken together, these strongly support fetal programming by these chemicals. Outlined below are many of these trends with supporting associations with EDCs.

Puberty

A trend toward earlier onset of puberty has recently been described, and EDCs, particularly early exposure to xenoestrogens, are thought to be a major contributing factor (129–133). Earlier puberty (defined as development of secondary sexual characteristics before 8 years of age) is a risk factor for breast and testicular cancer, polycystic ovarian syndrome, and metabolic disorders (133). Thigpen et al. found that rodent chow containing various low doses of genistein and another soy isoflavone, daidzein, advanced vaginal opening in Wistar rats and CD-1 mice (134). Other xenoestrogens including DES and bisphenol A and various other EDCs have also been shown to advance puberty in rodents at very low doses (25 ng–200 $\mu\text{g/kg/day}$) (104,105,135–138). In humans, early pubertal onset has been associated with neonatal exposure to the polybrominated biphenyl FireMaster, which was accidentally introduced into the Michigan food supply in 1973 when

it was confused with the nutritional supplement Nutri-Master (139,140). Furthermore, precocious thelarche before age 2 has been associated with elevated serum levels of phthalate esters in Puerto Rico. One phthalate in particular, di-(2-ethylhexyl) phthalate, was detected in patients at levels 6.4-fold higher than controls (141). Importantly, a trend toward altered timing of pubertal onset may affect later disease risk (i.e., increased cumulative estrogen exposure increases breast cancer risk), and also how clinicians medically and pharmacologically treat children with precocious or delayed puberty.

Fertility

The rates of infertility and recurrent pregnancy loss have increased over the last 50 years. While delayed childbearing in women is a clear and strong factor in decreased fertility, it has been postulated that developmental xenoestrogen exposure may also play a role (142). McLachlan and colleagues reported reduced fertility in mice following developmental DES exposure (68). Jefferson and Newbold recently showed that developmental 500 µg/kg genistein exposure resulted in impaired fertility in female mice in adulthood (121). This dose of genistein is an order of magnitude lower than that consumed by infants fed exclusively soy infant formula (143).

Breast Cancer

Breast cancer is the second most fatal cancer for women and one out of eight women will be diagnosed in their lifetimes (144). While interpreting the trends in breast cancer incidence is complicated by many factors (145), it appears that incidence has increased in the second half of the twentieth century (146,147). For example, in Hong Kong age-adjusted risk increased two- to threefold for women born in 1960 compared with 1900 (Fig. 2 (147)). Ravdin et al. recently reported a decline in breast cancer incidence in the United

States starting around 2002, but it is still unclear what factors have primarily accounted for the change (146,148).

In humans, exposure to steroidal estrogens is associated with increased breast cancer risk, and xenoestrogen exposure has been associated with increased breast cancer risk in adulthood. As discussed above, increased fetal estrogen exposure, endogenous or exogenous, is associated with increased adult breast cancer risk, while decreased exposure is associated with reduced risk. Developmental DES exposure has clearly been associated with increased breast cancer risk (71). While it is difficult to study unknown exposures with long-latency diseases 40 to 70 years later, developmental xenoestrogen exposure has been associated with altered mammary gland development and cancer risk in laboratory animals (107,149–161).

Male Reproductive Tract

There is some evidence that reproductive tract abnormalities in boys are influenced by developmental estrogen exposure. Cryptorchidism, hypospadias, and testicular cancer incidence are higher in first-born boys. Since maternal estrogens are higher in first pregnancies, these data are compatible with the hypotheses linking excessive pregnancy-estrogen exposure to conditions more common among first-born individuals (162). Additionally, cryptorchidism and testicular cancer increase in boys born to older mothers (163). However, unlike breast cancer and endometriosis, cryptorchidism does not appear to be related to maternal smoking (164).

Vegetarians tend to consume greater quantities of soy-based products than their omnivorous counterparts. Thus, vegetarians are exposed to increased levels of phytoestrogens, chemicals in plants that mimic estrogens. A prospective cohort study in the United Kingdom that sampled 7928 males born in 1991 and 1992 found an odds ratio for hypospadias of 4.99 for boys who were born to vegetarian mothers (165). An increased incidence of hypospadias has also been reported in DES grandsons (63–66).

Figure 2 Relative risk estimates of incidence of breast cancer by birth cohort. Based on full age-period-cohort model with period constraints. Bars around the point estimate indicate the 95% confidence intervals. Source: From Ref. 147.

Metabolic Programming

Newbold and colleagues have also shown that developmental exposure to DES at a dose of 1 µg/kg/day but not at higher doses (10–100 µg/kg/day) can cause obesity in adulthood in mice (reviewed in (166)). At 2 months of age, prior to a difference in size between DES-treated and control mice, the DES mice had elevated levels of leptin, adiponectin, IL-6, and triglycerides. By 6 months of age, densitometry studies indicated that the DES-exposed mice had increased abdominal fat, which was not likely caused by a slightly increased food intake and/or decreased activity. Furthermore, Newbold et al. have also shown that DES-exposed animals have a high level of islet cell hyperplasia, indicating that they may have problems with glucose homeostasis (reviewed in (166)).

Androgens

There are several environmental chemicals that interfere with androgen action. Chemicals that bind to the androgen receptor and prevent androgen action are known as androgen receptor antagonists, including DDE (a metabolite of the pesticide DDT), vinclozolin, a fungicide, and several phthalates. Phthalates are esters of phthalic acid and are used in hundreds of products, including plasticizers, PVC resins, and cosmetic and personal care products such as shampoos and soaps. As of 2004, manufacturers produced about 800 million pounds of phthalates each year. They contribute 10% to 60% of plastic products by weight (167).

The incidence of male reproductive tract disorders, including cryptorchidism, hypospadias, testicular cancer, and low sperm count, has increased over the last 30 to 50 years (168–174). It has been postulated that these disorders comprise a testicular dysgenesis syndrome (TDS) and may have a common etiology of deficient androgen production or action during development of the testes in fetal life (175).

Normal sexual differentiation of the male reproductive tract is androgen dependent. Exposure to antiandrogens during sexual differentiation produces malformations of the reproductive tract in humans and rodents. For example, the fungicide vinclozolin is an antiandrogen. Developmental exposure to vinclozolin during sexual differentiation in rats resulted in reduced anogenital distance, retained nipples, cleft phallus with hypospadias, cryptorchidism, a vaginal pouch, epididymal granulomas, and small-to-absent sex accessory glands (176).

Anogenital Distance (AGD)

AGD is a marker for sexual differentiation. In humans, AGD is measured from the anus to the base of the scrotum in males

and from the anus to the genitals in females and is about twice as long in males as in females (177). AGD is androgen dependent and developmental exposure to antiandrogens reduces AGD. Many laboratory studies have shown that developmental exposure to EDCs with antiandrogenic activity, such as di-*n*-butyl phthalate, bis(2-ethylhexyl)phthalate, linuron, procymidone, and DDE (a metabolite of the pesticide DDT) can reduce AGD (176).

Phthalates have been shown to have estrogenic activity and antiandrogenic activity, and to alter androgen production. Importantly, Swan et al. prospectively examined the relationship between developmental phthalate exposure and age-adjusted AGD (anogenital index) in infant boys (178). AGI was inversely related to four phthalate metabolites in maternal circulation during pregnancy (Table 2). Boys with the highest developmental exposure were 10 times more likely to have a shorter AGD than boys with the lowest prenatal exposure (178).

Testicular Cancer

Testicular cancer incidence is associated with higher serum levels of EDCs in adulthood (179) and maternal levels of EDCs (180,181). Over the past 50+ years, the incidence of testicular cancer has risen at an alarming rate. In a number of studies from around the world, it is estimated that between approximately 1982 and 2005, incidence of testicular cancer rose 2.4% per year in Spain (182), 2.6% per year in Australia (183), 2.3% to 3.4% per year in the Nordic countries, and by 5% in Poland and Germany (184). For men in six European countries born after 1945, the risk of testicular cancer increased in all six countries. Relative risks for those born around 1965 were 3.9 in Sweden and 11.4 in East Germany (185).

Cryptorchidism

The incidence of cryptorchidism doubled in the United States between 1968 and 1993 and has been associated with developmental exposure to several EDCs (168). During a period of peak use of the pesticide DDT in the United States between 1959 and 1967, babies born to mothers with the highest serum DDT levels had nearly a twofold increased risk of cryptorchidism when compared with those whose mothers had lowest serum DDT levels (186).

Similarly, the concentration of 21 persistent and non-persistent EDCs in the breast milk of Finnish and Danish mothers between 1997 and 2001, as measured in a series of case reports from a prospective birth cohort, was associated with an increased risk of cryptorchidism (187–189). A combined statistical analysis for the eight most prevalent persistent EDCs (*p,p'*-DDE, *p,p'*-DDT, β-HCH, HCB,

Table 2 Mean (Median) Phthalate Monoester Metabolite Levels by Anogenital Index Category

Monoester metabolite	AGI category [mean (median; ng/mL)]		
	Long ^a (n = 17)	Intermediate ^b (n = 43)	Short ^c (n = 25)
MBP	13.1 (11.5)	22.2 (13.1)	38.7 (24.5)
MBzP	10.6 (6.6)	15.1 (7.7)	25.8 (16.1)
MEP	124 (47.1)	592 (112)	1,076 (225)
MiBP	2.3 (1.5)	3.3 (2.1)	7.7 (4.8)

^aLong, AGI ≥75th percentile of expected AGI. ^bIntermediate, 25th percentile ≤ AGI <75th percentile of expected AGI. ^cShort, AGI <25th percentile of expected AGI. Abbreviations: MBP, mono-butyl phthalate; MBzP, mono-benzyl phthalate; MEP, mono-ethyl phthalate; MiBP, mono-isobutyl phthalate. Source: From Ref. 178.

α -endosulfan, cis-HE, oxychlordane, and dieldrin) revealed higher concentrations in the breast milk of case mothers. This indicates that a mixture of low concentrations of EDCs may be more important when assessing the risk of cryptorchidism than the concentration of any single chemical (187). The concentration of the sum of seven polybrominated diphenyl ethers, which are commonly found in flame-retardants, was also significantly higher in the breast milk of mothers of cryptorchid boys compared with controls (188). Additionally, phthalate levels in the mothers' breast milk were associated with altered levels of hormones related to Leydig cell function, which might have led to decreased virilization in this cohort (189).

Hypospadias

In 1997, the CDC reported a doubling of hypospadias in the United States from 1968 to 1993 (169). European countries also reported increases in hypospadias but increases were not seen in less affluent countries (168). Exposure to industrial and agricultural EDCs during development is associated with increased risk of hypospadias as demonstrated by Dolk et al. (190) and Carbone et al. (191).

Phthalate exposure during sexual differentiation has been shown to lead to TDS in laboratory animals (192). One hypothesis for the increased incidence of male reproductive tract disorders in humans is developmental exposure to EDCs that can alter androgen signaling. Wilson et al. reviewed different groups of EDCs that converge on androgen signaling. While the exact mechanisms of these EDCs differed, the end result was a reduction in testosterone signaling through androgen receptor (193).

Sperm Count

Sperm counts have declined over the past 60 years. In a meta-analysis, Swan et al. found a twofold reduction in sperm counts from 1938 to 1990 (172). Since Swan et al. have confirmed their initial findings and expanded the scope of years examined, the number of studies examined, and further analyzed confounders and statistical methods used for approximating the decline (171,173), adult and developmental exposure to environment chemicals has been linked to lower sperm counts. Swan et al. compared semen characteristics, including sperm concentration, from samples collected at four centers in the United States (Columbia, Missouri; New York, New York; Minneapolis, Minnesota; and Los Angeles, California). All samples were collected and analyzed following very strict protocols, and it was found that the men from Missouri had the lowest sperm concentrations, which in turn was associated with higher urinary concentrations of five pesticides, including alachlor and atrazine, which are commonly used herbicides (170,194). The data suggest that residence in a rural community with high pesticide exposure may affect sperm concentrations.

Developmental exposure to anabolic steroids or xenobiotics in beef has been linked to low sperm count in adulthood. Fertile men whose mothers consumed seven or more beef meals per week during pregnancy had 24% lower sperm count, were more likely to have a history of infertility, and there was a threefold increase in the proportion of these men with less than 20 million sperm per mL (195).

THYROID HORMONE

Unlike xenoestrogens and environmental antiandrogens that bind directly to the estrogen and androgen receptor, EDCs that disrupt thyroid function do so without binding to the thyroid receptor. Howdeshell extensively reviewed EDCs for their potential to interfere with thyroid hormone signaling and found over 150 chemicals with the ability to disrupt thyroid hormone action (196). EDCs have been shown to disrupt nearly every step in the synthesis and metabolism of thyroid hormone (196). For example, EDCs have been shown to alter thyroid hormone synthesis, uptake of iodine by the thyroid gland, and binding to specific thyroid-binding proteins in blood.

Polychlorinated biphenyls (PCBs) are a group of chemicals used in a variety of manufacturing applications, for example, in coolants, transformers, plasticizers, oils, adhesives, and so on. PCBs are persistent organic pollutants that are highly toxic, similar in action to dioxin, and used widely until their ban in 1976 in the United States. In addition to acute toxicity, PCBs are endocrine disruptors and interfere with normal hormone action, particularly estrogen and thyroid hormone signaling.

Adequate thyroid hormone during development is essential and inadequate levels are associated with cognitive deficits in children born to pregnant women with hypothyroidism (197). PCBs have been shown in the laboratory to interfere with multiple steps in thyroid hormone physiology (reviewed in (196)). Many studies of prenatal exposure to PCBs have been undertaken in various parts of the world and have shown negative effects on neurodevelopment (198–208). For example, Jacobson et al. examined infants in the Lake Michigan area selected based on their mothers' fish consumption. Because PCBs are persistent and lipophilic, they bioaccumulate in the food chain and have been found in high concentrations in fish and fish eaters. Infants with high levels of PCBs showed impaired visual memory (204). In 4-year-olds, prenatal PCB exposure was associated with lower scores in verbal and numerical memory (203), visual discrimination processing, and impaired short-term memory scanning (209). In 11-year-olds, prenatal PCB exposure was correlated with lower full-scale and verbal IQ scores. On average, IQ scores were 6.2 points lower in the highest exposure group than their lesser-exposed peers (202). It was also estimated that PCB-exposed children were up to 7.2 months behind in word comprehension (202). Since PCBs were banned in 1979, human exposure to these persistent chemicals has slowly declined. However, PCBs are very persistent chemicals and cognitive dysfunction is still reported, suggesting that environmentally relevant doses of endocrine-disrupting chemicals may alter human neurodevelopment (210).

SUMMARY

There is a great need for research to define the mechanisms of fetal programming. These mechanisms involve epigenetic changes, that is, the effects do not result from DNA mutations. Intense research centers on elucidating the mechanisms responsible for fetal programming and the epigenetic alterations regulating gene expression and adult disease. Epigenetic mechanisms include DNA and histone methylation, and histone acetylation (211,212). These marks can retain fetal programming information into adult life.

While high-dose/pharmacologic exposures have been associated with birth defects and severe long-latency effects (DES), it is likely that current human exposure to other pharmaceuticals and industrial chemicals may program the fetus and result in more subtle alterations in adult phenotype. Establishing cause and effect relationships between exposures and these subtle effects is much more difficult. Despite the difficulty in demonstrating such cause and effect relationships with subtle long-latency effects, these associations have been found in controlled experiments in human and laboratory animal studies and it is clear that subtle fetal programming occurs in people and alters adult disease risk. It is likely that only the tip of the iceberg has been described to date, and it is largely unknown the extent that the fetal environment programs lifelong phenotypes. Given this backdrop, every effort should be made to use the precautionary principle in counseling pregnant women about pharmaceutical use and minimizing exposure to environmental chemicals, such as indoor pesticides and painting while pregnant. Maternal risk of withholding medication must be considered, but when potential fetal risks outweigh maternal benefit, the precautionary principle should be employed.

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