

# Developmental exposure to endocrine-disrupting chemicals programs for reproductive tract alterations and obesity later in life<sup>1–4</sup>

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## ABSTRACT

Many chemicals in the environment, especially those with estrogenic activity, are able to disrupt the programming of endocrine signaling pathways established during development; these chemicals are referred to as endocrine-disrupting chemicals. Altered programming can result in numerous adverse consequences in estrogen-target tissues, some of which may not be apparent until later in life. For example, a wide variety of structural, functional, and cellular effects have been identified in reproductive tract tissues. In addition to well-documented reproductive changes, obesity and diabetes have joined the list of adverse effects that have been associated with developmental exposure to environmental estrogens and other endocrine-disrupting chemicals. Obesity is a significant public health problem reaching epidemic proportions worldwide. Experimental animal studies document an association of developmental exposure to environmental estrogens and obesity. For example, a murine model of perinatal exposure to diethylstilbestrol has proven useful in studying mechanisms involved in abnormal programming of differentiating estrogen-target tissues, including reproductive tract tissues and adipocytes. Other environmental estrogens, including the environmental contaminant bisphenol A, have also been linked to reproductive problems and obesity later in life. Epidemiology studies support similar findings in humans, as do studies of cells in culture. Together, these findings suggest new targets for abnormal programming by estrogenic chemicals and provide evidence supporting the scientific concept termed *the developmental origins of adult disease*. Furthermore, the association of environmental estrogens with obesity and diabetes expands the focus on these diseases from intervention or treatment to include prevention or avoidance of chemical modifiers, especially during critical windows of development. *Am J Clin Nutr* 2011;94(suppl):1939S–42S.

## INTRODUCTION

A complex series of events is involved in the development of the mammalian fetus and neonate. Processes including cell division, proliferation, differentiation, and migration are all involved and are closely regulated by hormonally active substances that communicate information between specializing cells, tissues, and organs. Although embryonic and fetal development was once thought to occur by the “unfolding of a rigid genetic program,” for which environmental factors played no significant role (*see* reference 1 for a review), this strict interpretation of developmental events has changed because numerous experimental and epidemiologic studies point to the developmental plasticity of the fetus and neonate. Mounting evidence suggests

that environmental factors, such as chemical toxicants, can drastically alter developmental programming cues and result in permanent long-term consequences (2). Research now focuses on the role of environmental factors during critical windows of perinatal growth and development and the mechanisms involved (3). It has become obvious that the placenta is not impenetrable, ie, it cannot completely protect the fetus from the outside world and, in many cases, the fetus and neonate are more sensitive than the adult to the same environmental insults. Reports identifying a cocktail of environmental chemicals in amniotic fluid, cord blood, and breast milk only serve to heighten concern for exposures during development (*see* reference 4 for a review).

The term *the fragile fetus* was coined by Howard Bern in 1992 to denote the extreme vulnerability of the developing organism to perturbation by environmental chemicals, particularly those with hormone-like activity (5). Bern pointed out that rapid cell proliferation and cell differentiation coupled with complex patterns of cell signaling contribute to its unique sensitivity and therefore makes the fetus prone to chemical insult. Exposure to environmental chemicals during development can result in death in the most severe cases or in structural malformations and/or functional alterations in the embryo or fetus. Unlike adult exposures that can result in reversible alterations, exposure to environmental chemicals or other factors during critical windows of development can cause irreversible consequences. Some of these consequences, such as birth defects, are seen fairly immediately after exposure. For example, prenatal exposure to thalidomide, which was used to treat maternal anxiety and depression, resulted in limb deformities in the exposed offspring; this chemical is probably the best known example of a prenatal teratogen. Other consequences of developmental exposure may not be seen until later in life: prenatal exposure to

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the potent synthetic estrogen diethylstilbestrol, which was prescribed in the 1940s–1970s to prevent miscarriage, is a well-known example whereby a multitude of adverse consequences were not seen until puberty or later in life (5–7). In fact, the full extent of the consequences of this chemical exposure is still unfolding as the diethylstilbestrol population ages, and it may also include multigenerational effects (8–10). The concept that developmental exposure to drugs and chemicals such as diethylstilbestrol can cause permanent functional changes that are not overtly toxic, such as ionizing radiation, or teratogenic effects, such as thalidomide, but still result in increased susceptibility to disease or dysfunction later in life, even at low environmental exposure levels, has greatly expanded the field of “developmental toxicology.”

Interestingly, the concept that there is a developmental origin of adult health and disease also has roots in epidemiology studies that have examined maternal nutrition; an altered nutritional status leading to low-birth-weight infants was shown to be associated with the latent appearance of disease in adult life, including increased susceptibility to noncommunicable diseases, coronary heart disease, obesity or overweight, type 2 diabetes, osteoporosis, and metabolic dysfunction (11). Chronic stress was also shown to be associated with similar latent responses; for example, experimental studies using macaque monkeys showed that early life stress resulted in obesity and increased incidences of metabolic diseases later in life (12). Maternal smoking, another example of a fetal stressor, was also shown to be linked to the subsequent development of obesity and disease later in life (13). These studies represent just a few examples that have led to a substantial research effort focusing on perinatal influences and subsequent chronic disease (14). These topics are covered in detail in other articles in this supplement issue. Taken together, epidemiologic studies describing an association of restricted fetal nutrition with the subsequent development of obesity and metabolic diseases and experimental studies showing a correlation of perinatal exposure to endocrine-disrupting chemicals (EDCs) with multiple effects on reproductive tract tissues and obesity provide an attractive framework for understanding delayed functional effects of toxicant exposures. The mechanisms involved in how environmental factors—eg, nutrition, stress, or EDCs—can affect developmental events are not completely understood but most likely involve numerous pathways including the following: 1) changes in the neuroendocrine system, whereby the developing nervous system communicates information from the environment to the developing endocrine system; 2) epigenetic mechanisms, whereby environmental signals alter the methylation or modify the histone patterns of genes, causing their transcriptional activities to be altered; 3) and/or direct effects on gene expression, particularly with regard to hormonally active environmental agents (15).

#### **EXPOSURE TO DIETHYLSTILBESTROL VERIFIES THE DEVELOPMENTAL ORIGIN OF THE DISEASE OR DYSFUNCTION CONCEPT**

Diethylstilbestrol, a synthetic nonsteroidal chemical with estrogenic activity, is an EDC that was used in the 1950s–1960s as a feed additive to enhance weight gain in cattle and poultry. However, its notoriety was due to its widespread clinical use to prevent miscarriage and other complications of pregnancy in the

1940s–1970s. In 1971, a hallmark report associated prenatal diethylstilbestrol treatment with a rare form of reproductive tract cancer, vaginal clear cell adenocarcinoma, which was detected in a small number (<0.1%) of adolescent daughters of women who had taken the drug while pregnant (16). Later, diethylstilbestrol was also linked to more frequent benign reproductive tract problems in >95% of the diethylstilbestrol-exposed daughters; reproductive tract malformation and dysfunction, poor pregnancy outcome, and immune system disorders were just some of the reported effects. Likewise, prenatally diethylstilbestrol-exposed men experienced a range of reproductive tract abnormalities, including hypospadias, microphallus, retained testes, and increased genital-urinary inflammation (*see* references 6, 17, and 18 for review and update). Although an increased incidence in prostatic and testicular cancers was proposed, thus far the diethylstilbestrol-exposed population has not reported an increase in these diseases relative to unexposed men, but rigorous studies await a definitive conclusion.

Thus, diethylstilbestrol became a well-documented example of the developmental origin of disease/dysfunction. It had the dubious distinction of being the first example of a human transplacental carcinogen; it was shown to cross the placenta and to induce a direct carcinogenic effect on the developing fetus. Diethylstilbestrol caused a major medical catastrophe that still continues today. Although it is no longer prescribed to prevent miscarriage, a major concern remains that as diethylstilbestrol-exposed individuals age and reach the time when the incidence of reproductive organ tumors normally increases, they will have a much higher incidence of lesions than will unexposed individuals. For example, diethylstilbestrol-exposed women have been reported to have a higher incidence of breast cancer as they age than do unexposed individuals (19). Another concern is that additional organ systems (eg, urinary, immune, cardiovascular, brain/nervous, gastrointestinal, bone, and adipocytes) may be affected. Furthermore, the possibility of multigenerational effects, as suggested by experimental animal (8) and human studies (9, 10, 20), suggests that another generation may be at risk of developing health problems associated with the diethylstilbestrol treatment of their grandmothers. The diethylstilbestrol episode is a salient reminder of the potential toxicity that may be caused by EDCs if exposure occurs during critical windows of susceptibility.

#### **DEVELOPMENTAL EFFECTS OF DIETHYLSTILBESTROL ON THE REPRODUCTIVE TRACT**

Questions about the mechanisms involved in diethylstilbestrol-induced abnormalities in humans prompted the development of numerous experimental animal models to study the adverse effects of estrogens and other EDCs on genital tract differentiation. The perinatal (prenatal or neonatal) mouse model has been particularly successful in duplicating and predicting many adverse effects observed in humans with similar diethylstilbestrol exposures (*see* reference 7 for a review). These murine models have also been successfully used to study the molecular mechanisms involved in diethylstilbestrol-adverse effects (21–24).

In general, prenatal diethylstilbestrol treatment caused a high incidence of malformation and a low, but significant, increase in reproductive tract tumors; whereas, neonatal treatment causes a low incidence of malformation, but a high incidence of reproductive tract neoplasia. Predictably, it is apparent that the

timing of exposure and the stage of tissue differentiation determine the subsequent resulting abnormalities. Furthermore, because many developmental events for the reproductive tract that occur in the mouse during prenatal and neonatal life, happen entirely prenatally in humans, the prenatal plus neonatal mouse model can be useful in predicting what happens prenatally in humans. In humans, the timing of exposure was also shown to be an important factor for cancer risk in diethylstilbestrol daughters; research showed that exposure early in pregnancy was associated with a greater risk of vaginal cancer than was exposure later in pregnancy (6, 18).

#### DEVELOPMENTAL EFFECTS OF DIETHYLSTILBESTROL AND OTHER EDCs ON OBESITY

Obesity and overweight have dramatically increased in prevalence in wealthy industrialized countries over the past 2 to 3 decades and also in poorer underdeveloped nations, where it often coexists with undernutrition (25, 26). Obesity has now reached epidemic proportions in the United States, although a recent study found that its increase has stopped its upward spiral in the past few years; however, there is no indication of any decreases in prevalence (27). Common causes of obesity have usually been attributed to high-calorie, high-fat diets and a lack of exercise combined with a genetic predisposition for the disease. However, the current alarming rise in obesity cannot be solely explained by only these factors; an environmental component must be involved. It has been suggested that exposure to EDCs during critical stages of adipogenesis is contributing to the obesity epidemic (28–32). The term *obesogens* has been coined for environmental chemicals that stimulate fat accumulation, referring to the idea that they inappropriately regulate lipid metabolism and adipogenesis to promote obesity (33).

Experimental animal studies support the idea of involvement of EDCs in obesity; developmental exposure to numerous chemicals—including diethylstilbestrol, other estrogens (32), and other chemicals, such as tributyl tin (33)—has been associated with obesity or overweight and adipogenesis. Recently, there has been much interest in the chemical bisphenol A (BPA) because of its high production volume and its potential for widespread environmental contamination (34). Numerous studies have now shown an association of BPA exposure with increased body weight and adiposity (35–43). The later study suggests that an increase in body weight is sex specific, but that timing and dose may contribute to the complexity of these findings because other investigators report effects in both males and females. Interestingly, a recent article describes similar increases, as previously reported, in the body weights of pups obtained from moms fed BPA in their diets during pregnancy; the doses were low and were considered “ecologically relevant” at 1  $\mu\text{g}$  BPA/kg diet (1 ppb) (44). However, unlike previous reports, the differences in body weight at weaning disappear as the mice age (44). This is probably due to the palatability of the diet, which was substituted at weaning because both control and BPA mice did not continue to gain weight on the new diets.

In vitro studies with BPA provide additional evidence of a role for this chemical in the development of obesity and further suggest specific targets; BPA causes 3T3-L1 cells (mouse fibroblast cells that can differentiate into adipocytes) to increase differentiation (45) and, in combination with insulin, accelerates

adipocyte formation (46, 47). Other in vitro studies have shown that low doses of BPA, similar to diethylstilbestrol, impair calcium signaling in pancreatic  $\alpha$  cells, disrupt  $\beta$  cell function, and cause insulin resistance (48, 49). Low environmentally relevant doses of BPA have also been reported to inhibit adiponectin and stimulate the release of inflammatory adipokines, such as interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), from human adipose tissue, which suggests that BPA is involved in obesity and the related metabolic syndrome (50, 51). Furthermore, other studies have linked BPA exposure to disruption of pancreatic  $\beta$  cell function and blood glucose homeostasis in mice (52), which suggests changes indicative of the metabolic syndrome.

Epidemiologic studies also support an association of BPA with obesity. BPA was detected at higher concentrations in both nonobese and obese women with polycystic ovarian syndrome than in nonobese healthy women, which suggests the possible involvement of BPA in polycystic ovarian syndrome and/or obesity (53).

#### CONCLUSIONS

The data included in this article support the idea that developmental exposure to EDCs are contributing to disease and dysfunction later in life; the adverse consequences from EDCs that have been identified in various developing organ systems include, but are not limited to, reproductive tract tissues and adipocytes. Together, these data show the extreme sensitivity of the developing organism and emphasize the need for identification and avoidance of EDCs, especially during critical windows of prenatal and neonatal development. Additional mechanistic studies are essential to determine critical windows of susceptibility for various target tissues, effects of dose, potential additivity, or synergy of effects from mixtures of EDCs and altered programming of developmental pathways so that future generations can look forward to a healthy future.

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