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TOXICOLOGICAL HIGHLIGHT

Endocrine Disruptors and the Obesity Epidemic

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The highlighted article brings together research on the site and mechanism of endocrine-disrupting chemicals that have the potential to have a significant impact on human health and research on obesity, which is known to seriously affect human health. The effect of exposure to endocrine-disrupting chemicals on the initiation or exacerbation of obesity, which may significantly alter the perception of the etiology of obesity and impact intervention and prevention efforts, is an important emerging area needing even further research emphasis.

Obesity is a growing concern worldwide. The prevalence of obesity has risen dramatically in developed countries over the past 2 to 3 decades (Oken and Gillman, 2003). Obesity has reached epidemic proportions in the United States with more than 20% of adults defined as clinically obese and an additional 30% defined as overweight. In 1999, 13% of children aged 6–11 years and 14% of adolescents aged 12–19 years in the United States were overweight. This prevalence has nearly tripled for adolescents in the past two decades (U.S. Department of Health and Human Services, 2001). Since overweight adolescents have a 70% chance of becoming overweight or obese adults (80% if even one parent is obese or overweight) the prognosis for the future health of Americans is declining. In American society, the immediate consequence of being overweight is social discrimination, often causing poor self-esteem and depression. Overweight or obese adults, especially those with central or trunk adiposity, are also at risk for a number of chronic diseases, including heart disease, type 2 diabetes, high blood pressure, and some forms of cancer. Indeed, type 2 diabetes, previously considered an adult disease, has increased dramatically in children and adolescents along with the increase in obesity.

Obesity is mainly considered to be caused by overeating and lack of physical activity on a background of genetic predisposition. However, there is still much uncertainty related to the etiology of obesity. What is clear is that obesity is notoriously difficult to treat; thus, prevention is critical. In this regard, a new paradigm for prevention has emerged in recent years that evolved from the idea that environmental factors in early life and *in utero* can have profound influences on lifelong health (e.g., the fetal basis of adult disease [Oken and Gillman, 2003]). While the focus of this research area has been on the relationship between fetal experiences and later risk for adult chronic diseases, there is recent information regarding the fetal origins of obesity. Initial work in this area focused on the role of *in utero* nutrition and its effects on birth weight. A large number of epidemiological studies have demonstrated a direct relationship between birth weight and body mass index (BMI) attained later in life. Lower birth weight seems to be associated with later risk for central obesity, especially when it is associated with catch-up growth in the first few years of life. In addition, higher birth weight is associated with higher attained BMI. "We are faced with the seeming paradox of increased adiposity at both ends of the birth weight spectrum—higher BMI with higher birth weight and increased central obesity with lower birth weight" (Oken and Gillman, 2003). Thus prevention of childhood and adult obesity must start *in utero*.

The major environmental influence on birth weight has been considered to be *in utero* nutrition. Therefore, maternal nutrition has been the focus of research into the fetal basis of diseases including obesity. However, nutrition is not the only environmental influence that may have an effect on adult diseases. There is increasing evidence that *in utero* exposure to environmental chemicals at environmentally relevant concentrations may alter developmental programming via alterations in gene expression or gene imprinting that do not result in either low birth weight or malformations but in functional deficits that do not become apparent until later in life where they surface as increased susceptibility to disease. With regard to the role of environmental exposures *in utero* and obesity, there are a number of cross-sectional and cohort studies that demonstrate that childhood obesity is associated with maternal smoking in pregnancy (Toschke *et al.*, 2002). Animal studies have also shown that prenatal nicotine exposure not only

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disrupts cholinergic and catecholaminergic systems but also results in postnatal weight gain (Levin, 2003).

These data suggest a role for toxicology in the etiology of obesity. This role has received additional support from a recent review (Baillei-Hamilton, 2002) that presents a provocative hypothesis to explain the global obesity epidemic: chemical toxins. This article presents data showing that the current epidemic in obesity cannot be explained solely by alterations in food intake and/or decrease in exercise. There is a genetic predisposition component of obesity; however, genetics could not have changed over the past few decades, suggesting that environmental changes might be responsible for at least part of the current obesity epidemic. Indeed, the level of chemicals in the environment is purported to coincide with the incidence of obesity, and examples of chemicals that appear to cause weight gain by interfering with elements of the human weight control system—such as alterations in weight-controlling hormones, altered sensitivity to neurotransmitters, or altered activity of the sympathetic nervous system—are noted. Indeed, many synthetic chemicals are actually used to increase weight in animals. This article provides fascinating examples of chemicals that have been tested for toxicity by standard tests that resulted in weight gain in the animals at lower doses than those that caused any obvious toxicity. These chemicals included heavy metals, solvents, polychlorinated biphenols, organophosphates, phthalates, and bisphenol A. This is an aspect of the data that has generally been overlooked.

Thus, the data on the fetal basis of adult disease, along with the above-referenced chemical hypothesis of obesity, add significance to examination of exposure to environmental chemicals as likely candidates to be tested for an effect on obesity. Chemicals having endocrine-disrupting activity rise to the top of the list as most act via receptors linked to activation of transcriptional activity. The state of the science in the area of endocrine disruptors, including data on their mechanism of action has recently been reviewed (Damstra *et al.*, 2002). This World Health Organization- (WHO-)sanctioned review clearly shows that endocrine disruptors, especially those with estrogenic activity, act via alterations in gene expression and that many of these changes are imprinted and remain even into the next generation. The focus of endocrine-disruptor research around the world has been on the reproductive, immune, and nervous systems as evidenced by the WHO review. There has been little information of the possible direct effect of endocrine-disrupting chemicals on fat cell differentiation or physiology. Neither has there been an attempt to link effects of endocrine-disrupting chemicals on the immune or nervous system with effects on fat cell metabolism via alterations in hormonal or nervous system control of adipose tissue.

While many hormones and growth factors control hormonal regulation of adipose tissue differentiation and metabolism, it is evident that that fat cell precursors and adipocytes themselves contain estrogen receptors. Both estrogen receptors (ER) alpha and beta are expressed in human adipose tissue. In the adult, loss of circulating estrogen due to ovariectomy leads to increased body and adipose tissue weights. Estrogen receptor alpha knockout mice have a significantly increased body fat content, and estrogen decreases the activity of lipoprotein lipase (referenced in Naaz *et al.*, 2003). In the adult rat, estrogen administration induces a depletion of triglyceride stores (referenced in Masumo *et al.*, 2002). Thus, in the adult, estrogen is antilipogenic. Indeed, there are data showing that dietary genestein, a phytoestrogen that binds to ER alpha and beta, produces antilopogeneic effects in mice, resulting in reduced size of adipocytes (Naaz *et al.*, 2003).

The effects of estrogens on the development of fat cells and adipose tissue are not as clear. The studies by Masuno *et al.* address the issue of the role of estrogens and endocrine-disrupting chemicals with estrogenic activity on the *in vitro* development of adipocytes from precursor cells. In these publications, the authors use a clonally isolated cell line of mouse fibroblasts (3T3-L1) that can differentiate into adipocytes when confluent cultures are treated with insulin, dexamethasone, and 1-methyl-3-isobutylxanthine for two days. They used expression of lipoprotein lipase and glycerol 3-phosphate dehydrogenase activity as well as triglyceride accumulation in the cells as markers of differentiation into adipocytes. In an earlier publication (Masumo *et al*., 2002), the authors showed that the estrogenic endocrine-disrupting chemical bisphenol A at concentrations as low as 2 μg/ml, in the presence of insulin, stimulated differentiation of the 3T3L1 cells into adipocytes. In the present publication, the focus is another estrogenic environmental chemical, 4-nonylphenol (NP), a byproduct of the wastewater treatment-mediated cleavage of alkylphenol ethoxylates, components of plastics, surfactants, paints, and insecticides.

The authors have shown that following hormonal induction of differentiation of 3T3L1 cells into adipocytes, the addition of either 5 or 10μ/ml of NP increased the DNA content and the number of bromodeoxyuridine (BrdU) staining cells in the cultures. These data indicate that NP stimulated the proliferation of fully differentiated 3T3L1 cells (e.g., adipocytes). These data were collaborated by the decrease in triglyceride content and lipoprotein lipase activity as well as mRNA levels of lipoprotein lipase and adipocyte-specific fatty acid binding protein. The authors also showed that the effect of NP could be mimicked, albeit with less potency, by 4-tert-octylphenol, another estrogenic environmental chemical. The fact that an environmental chemical has the potential to stimulate growth of "preadipocytes" has enormous implications for the area of obesity and its control. This suggests the intriguing possibility that developmental exposure to environmental estrogens could alter the pathway of adipocyte development. Differentiation could be inhibited and more potential fat cells could be formed, as seems to be the case with NP, or differentiation could be stimulated, as appears to be the case with BPA. In both cases, the result is more adipocytes with the differences, perhaps due to the timing of exposures and the hormonal milieu. If these

effects were to be shown to occur *in vivo*, the result would be an animal that would have the tendency to become obese. The authors of this article have opened the door to a potentially very exciting new area of research on the action of estrogenic endocrine-disrupting chemicals: one that has enormous implications for public health.

As with any article, this one also asks more questions than it answers. For example, why are the effects of NP only partially inhibited by the estrogen receptor antagonist ICI182,780? It would have been helpful if the effects of estrogen were measured in this system and the effects of ICI182,780 tested on the estrogen response. Measurement of the estrogen receptor in these cells across the differentiation process would also be helpful. Since these analyses were not done, it is not clear if the effect of NP and OP in this article or that of BPA in a previous article (Masuno *et al.*, 2002), in which ICI182,780 was not tested, mediated their effects via an estrogen receptor. In light of the potential impact of this research, answers to these questions must be forthcoming. Other questions relevant to this article include consideration of the use of serum that contains steroids in the cultures and the use of plastic culture dishes and their impact on the data.

There are also other, more global questions that need answers: Will these results extrapolate to the *in vivo* situation in rodents and other animal models? Will the results shown with high concentrations *in vitro* be replicated *in vivo* with low environmentally relevant concentrations? Will humans be sensitive to the *in utero* exposure to environmental estrogens with regard to the development of adipocytes? Will toxicology and environmental health sciences play a major role in addressing the obesity epidemic via reduction in exposures to environmental chemicals *in utero* and throughout life? Will this area of research be a fruitful area for intervention and prevention studies of obesity? Only time and more research will tell, but the door has been opened by the novel work being highlighted.

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