Organizational Effects of Perinatal Exposure to Bisphenol-A and Diethylstilbestrol on Arcuate Nucleus Circuitry Controlling Food Intake and Energy Expenditure in Male and Female CD-1 Mice

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The endocrine disrupting compound bisphenol-A (BPA) has been reported to act as an obesogen in rodents exposed perinatally. In this study, we investigated the effects of early-life BPA exposure on adult metabolic phenotype and hypothalamic energy balance circuitry. Pregnant and lactating CD-1 dams were exposed, via specially prepared diets, to 2 environmentally relevant doses of BPA. Dams consumed an average of 0.19 and 3.49 μg/kg per day of BPA in the low and high BPA treatments prenatally and an average of 0.36 and 7.2 μg/kg per day of BPA postnatally. Offspring were weaned initially onto a normal (AIN93G) diet, then as adults exposed to either a normal or high-fat diet (HFD). Males exposed to the high dose of BPA showed impaired glucose tolerance on both diets. They also showed reduced proopiomelanocortin fiber innervation into the paraventricular nucleus of the hypothalamus, and when exposed to HFD, they demonstrated increased neuropeptide Y and Agouti-related peptide expression in the arcuate nucleus (ARC). Females exposed to the high BPA dose were heavier, ate more, and had increased adiposity and leptin concentrations with reduced proopiomelanocortin mRNA expression in the ARC when consuming a HFD. BPA-exposed females showed ARC estrogen receptor α expression patterns similar to those seen in males, suggesting a masculinizing effect of BPA. These results demonstrate that early-life exposure to the obesogen BPA leads to sexually dimorphic alterations in the structure of hypothalamic energy balance circuitry, leading to increased vulnerability for developing diet-induced obesity and metabolic impairments, such as glucose intolerance. (***Endocrinology*** **154: 1465–1475, 2013)**

Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure

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Bisphenol A (BPA) is an estrogenic endocrine disruptor widely used in the production of plastics. Increasing evidence indicates that in utero BPA exposure affects sexual differentiation and behavior; however, the mechanisms underlying these effects are unknown. We hypothesized that BPA may disrupt epigenetic programming of gene expression in the brain. Here, we provide evidence that maternal exposure during pregnancy to environmentally relevant doses of BPA (2, 20, and 200 μg/kg/d) in mice induces sex-specific, dose-dependent (linear and curvilinear), and brain region-specific changes in expression of genes encoding estrogen receptors (ERs; ERα and ERβ) and estrogen-related receptor-γ in juvenile offspring. Concomitantly, BPA altered mRNA levels of epigenetic regulators DNA methyltransferase (DNMT) 1 and DNMT3A in the juvenile cortex and hypothalamus, paralleling changes in estrogen-related receptors. Importantly, changes in ERα and DNMT expression in the cortex (males) and hypothalamus (females) were associated with DNA methylation changes in the ERα gene. BPA exposure induced persistent, largely sex-specific effects on social and anxiety-like behavior, leading to disruption of sexually dimorphic behaviors. Although postnatal maternal care was altered in mothers treated with BPA during pregnancy, the effects of in utero BPA were not found to be mediated by maternal care. However, our data suggest that increased maternal care may partially attenuate the effects of in utero BPA on DNA methylation. Overall, we demonstrate that low-dose prenatal BPA exposure induces lasting epigenetic disruption in the brain that possibly underlie enduring effects of BPA on brain function and behavior, especially regarding sexually dimorphic phenotypes.

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The effect of perinatal exposure to ethinyl oestradiol or a mixture of endocrine disrupting pesticides on kisspeptin neurons in the rat hypothalamus

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Early life exposure to endocrine disruptors is considered to disturb normal development of hormone sensitive parameters and contribute to advanced puberty and reduced fecundity in humans. Kisspeptin is a positive regulator of the hypothalamic–pituitary–gonadal axis, and plays a key role in the initiation of puberty. In the adult, *Kiss1* gene expression occurs in two hypothalamic nuclei, namely the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC), which are differentially regulated by peripheral sex steroid hormones. In this study we determined the effects on puberty onset and Kiss1 mRNA levels in each of the two nuclei after long-term perinatal exposure of rats to ethinyl oestradiol (EE_2) or to five different pesticides, individually and in a mixture. Rat dams were per orally administered with three doses of EE_2 (5, 15 or 50 μg/kg/day) or with the pesticides epoxiconazole, mancozeb, prochloraz, tebuconazole, and procymidone, alone or in a mixture of the five pesticides at three different doses. Kiss1 mRNA expression was determined in the AVPV and in the ARC of the adult male and female pups in the EE_2 experiment, and in the adult female pups in the pesticide experiment.

We find that perinatal EE_2 exposure did not affect Kiss1 mRNA expression in this study designed to model human exposure to estrogenic compounds, and we find only minor effects on puberty onset. Further, the Kiss1 system does not exhibit persistent changes and puberty onset is not affected after perinatal exposure to a pesticide mixture in this experimental setting. However, we find that the pesticide mancozeb tends to increase Kiss1 expression in the ARC, presumably through neurotoxic mechanisms rather than *via* classical endocrine disruption, calling for increased awareness that Kiss1 expression can be affected by environmental pollutants through multiple mechanisms.

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ORIGINAL ARTICLE *Puberty, aging and HRT*

Phthalates may promote female puberty by increasing kisspeptin activity

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STUDY QUESTION: Is there an association between exposure to phthalates and the timing of female puberty?

SUMMARY ANSWER: Our study suggests that the early onset of puberty is related to increased kisspeptin secretion.

WHAT IS KNOWN ALREADY: Girls are maturing earlier than in past decades and the quantity of phthalates used in consumer products has concurrently risen. The hypothesis that exposure to phthalates may increase kisspeptin secretion and thereby cause early-onset puberty is unexplored.

STUDY DESIGN, SIZE, DURATION: This case–control study ran from 2006 to 2009. We enrolled 104 girls. Girls in the central precocious puberty (CPP) (case) group were recruited from a pediatric endocrinology policlinic in Taiwan; prepubescent controls were recruited from local elementary schools and all were categorized based on a pediatrician's diagnosis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The physical characteristics of puberty were assessed and levels of LH, FSH estradiol and kisspeptin-54 in blood samples were evaluated using radioimmunoassay. Reversed-phase high-performance liquid chromatography-tandem mass spectrometry was used to analyze seven urinary phthalate metabolites. Non-parametric analyses, trend tests and linear regressions were performed on the data.

MAIN RESULTS AND THE ROLE OF CHANCE: All seven urinary phthalate metabolites in the CPP group were significantly ($P < 0.05$) higher than in prepubescent controls. Serum kisspeptin-54 levels were higher ($P = 0.022$) in the CPP group than controls and were still significantly higher after adjusting for age ($P = 0.03$). There was a significant increasing trend ($P_{trend} = 0.005$) between levels of kisspeptin and the stages of puberty. The concentration of kisspeptin-54 did not change in girls treated with leuprorelin acetate. There was a significant positive correlation between kisspeptin-54 and urinary mono-n-butyl phthalate (ng/ml: $R^2 = 0.251$, $P < 0.001$; μg/g-creatinine: $R^2 = 0.109$, $P = 0.024$).

LIMITATIONS, REASONS FOR CAUTION: The study duration was short and the sample size relatively small; therefore, we were unable to collect sufficient evidence to support the temporality between exposure to phthalates and the subsequent occurrence of PP.

WIDER IMPLICATIONS OF THE FINDINGS: Kisspeptin may promote the onset of puberty in girls who are exposed to a high level of phthalates, especially di-n-butyl phthalate. These data suggest that developing a kisspeptin antagonist might be an alternative strategy for treating PP.

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