Fetal exposure to very low doses of ethinyl estradiol increases prostate size and androgen receptors in CF-1 and CD-1 mice

RL Ruhlen, KA Thayer, DE Preziosi, KL Howdeshell, WV Welshons, FS vom Saal,
University of Missouri (Columbia), Columbia MO 65211

It is estimated that ten-million women worldwide become pregnant each year while taking oral contraceptives (due to missed pills), and then continue taking oral contraceptives for variable periods during early pregnancy. The most common dose of ethinyl estradiol (EE), the estrogen used in oral contraceptives in the USA, is 500 ng/kg. We fed (in corn oil) female CF-1 mice EE at doses of 0 (vehicle control), 2, 20, 200, and 2000 ng/kg body weight/day, beginning on the day of mating (Day 0) and ending on Day 17 of pregnancy (equivalent to approximately the end of the first trimester of pregnancy in women). Higher doses of EE were also examined, but were found to block implantation. In adulthood, the entire prostate was removed from male offspring, weighed, and snap frozen. Prostates were homogenized in 20 µl modified gel loading buffer per mg prostate weight. To measure protein and DNA, aliquots of the homogenates were precipitated with TCA, and total protein and total DNA were measured. Androgen receptors were measured by western blot by loading a constant amount of protein (20 µg) in each lane. Ten animals from separate litters from each dose group were examined. A prostate from each dose group was examined on each of ten separate gels. Also examined on each gel (in duplicate) were homogenates from a pool of prostates collected from untreated males, which provided the standard (100%) to which other values on that gel were compared. Standard curves revealed linearity within the range of protein loading.

A significant ($P<0.05$) increase in prostate weight (approximately 15%) occurred at 20, 200, and 2000 ng/kg EE doses relative to controls. However, at only one dose (20 ng/kg) were androgen receptors per prostate increased (by 2-fold; $P<0.01$), androgen receptors per protein increased (by 47%; $P<0.05$), androgen receptors per DNA increased (by 58%; $P<0.05$), and DNA per prostate increased (by 22%; $P<0.05$). Protein per prostate was significantly increased at both 20 ng/kg (by 23%; $P<0.01$) and 200 ng/kg (by 20%; $P<0.05$). These findings reveal that while prostate weight increased across a hundred-fold range of doses of EE, an increase in androgen receptors and the number of cells (as measured by DNA) only occurred in a much narrower dose range. The finding of inverted-U dose-response curves for prostatic androgen receptors, protein and DNA per prostate provides additional evidence that effects at high doses of hormones and hormone mimicking chemicals do not predict effects at lower doses. Also, organs that show the same weight may show functional differences due to developmental exposure to estrogenic chemicals.

A second experiment was conducted in which EE was administered to pregnant CD-1 mice, with doses of 0, 2, 20, 200, 2000, and 20,000 ng/kg body weight/day fed to mice beginning on Day 7 and ending Day 17 of pregnancy. The altered dosing regime allowed us to examine higher doses than in the previous study, since dosing started after implantation. In adulthood, a significant ($P<0.05$) increase in prostate and seminal vesicle weight occurred at the 200 and 20,000 ng/kg/day doses relative to controls, while spleen weight was significantly increased at 200 – 20,000 ng/kg/day. A significant increase in body weight only occurred at the 20 ng/kg dose. Developmental exposure to another estrogenic drug, diethylstilbestrol (DES), in mice has proven to be highly predictive of effects in humans, and damage to internal reproductive organs often occurred in the absence of external malformations in DES sons and daughters. These findings suggest a risk to offspring of exposure during the initial period of

reproductive organ development due to the continued use of oral contraceptives by women who are unaware that they are pregnant.