Introduction
Neonatal exposure to Diethylstilbesestrol (DES) has been shown to adversely affect a number of estrogen sensitive tissues, including bone. Effects of in utero DES exposure have also been shown to affect the third generation. Environmental exposure to estrogens was shown to be higher than anticipated. The aim of this study is to determine the effect of in utero exposure to DES on the intervertebral disc (IVD) of adult mice.

Methods
Animal Manipulation - Pregnant C57/bl mice were injected with either vehicle (peanut oil) or one of three doses of DES (Sigma Aldrich, Oakville, Ontario) (0.1, 1.0, and 10.0 µg/kg/day) at 11-14 days of gestation. A minimum of 3 dams was set for each group. Pups were then allowed to grow to adulthood without further intervention until three months of age. At this point mice were randomized into two groups; one with a once daily swimming regimen which started as five min. and was escalated to a maximum of 1 h. The second group were left to their normal activity level and considered sedentary. All animals were sacrificed at exactly 4 months of age, weighed and frozen at -20°C.

Histology - The lumbar segment of the spines were dissected from 3 animals per group for histologic evaluation, fixed in 10% formalin for 18 h, decalcified in formic acid for a maximum of 48 h, dehydrated in graded solutions of ethanol in water, infiltrated with xylene, and finally paraffin embedded. Sections of 4 µm were cut with a microtome and placed on glass slides. After deparaftinization they were stained with 0.1% Safranin O and counter stained with 0.02% Fast Green. All slides were scanned and measurements of intervertebral disc height were taken using the NDP view software (Hamamatsu Corp., Japan). Three measurements of disc height were taken per disc and the means compared for control vs. DES exposed mice.

Proteoglycan content - Whole intervertebral discs were isolated and digested overnight at 56°C with proteinase K (BioShop) at 56°C. They were then vortexed and centrifuged for 30 min at 2800 × g. Mucopolysaccharides were then extracted using the chondroitinase (Sigma Aldrich, Cambridge, MA) was used for the standard curve.

Statistical Analysis - All statistical analyses were done using ANOVA and Fischer’s Least Significant Difference Post Hoc test, except for histological quantitative analysis where the Mann Whitney test was used. A p value of 0.05 was set to determine statistical significance.

Results
Parameters associated with IVD degeneration were found to have higher scores in DES exposed mice vs. controls (Figure 1). Clefts in the NP were only significantly increased in the female swim group at 0.1 µg/kg/day (p=0.007) whereas the female sedentary group showed significant increases at all three doses of DES (p <0.001 for all three). NP clefting was less evident in males, where the male swim group showed a significant increase at 0.1 and 1.0 µg/kg/day (p = 0.002 and p = 0.02) and the male sedentary group showed a significant increase at 10.0 µg/kg/day (p = 0.002). The male sedentary group showed a significant increase at 0.1 and 1.0 µg/kg/day (p=0.002 and p=0.03). The male sedentary group showed significant increases at 1.0 and 10.0 µg/kg/day (p<0.0001 and p<0.0007). No significant changes were seen in the male swim group compared to control. DES had little effect on male IVDs than those of females. Swimming caused an overall worsening of scores in females. Males were less affected by swimming than females.

Proteoglycan content in the intervertebral disc was significantly decreased at 0.1µg/kg/day and 1.0 µg/kg/day. A significant increase was noted at 10.0 µg/kg/day as compared to control (Figure 2).

Discussion
The intervertebral disc was recently shown to be an estrogen-sensitive tissue. This had a clinical effect in obese postmenopausal women undergoing hormone replacement therapy. Our study shows that DES in utero exposure can cause nuclear extrusion and affect the intervertebral disc. The mechanisms of the effects of DES are however not well understood and further studies are necessary.

Significance
This study might shed light on the possible increased risk of disc degeneration in the sons and daughters of mothers exposed during gestation, as well as their children. Furthermore, this new found environmental exposure to estrogen agonists makes it important to understand the full effects that such estrogen agonists may have.

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