Effects of Estradiol (17-alpha) on Osteoarthritis and Bone Development in Genetically Heterogeneous Het3 Male Mice

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INTRODUCTION:
Estradiol 17-alpha is an isoform of E2 estrogen that has recently gained attention for its ability to extend life-span and health-span in male animals without the feminization associated with estradiol 17-beta administration. Estrogens, especially E2, have long been implicated in the maintenance of both the bone and cartilage aspects of the skeletal system. However, there are often sexually dimorphic effects most likely due to the relation and relative importance of testosterone to estrogen. Recent studies have indicated 17-alpha administration can prevent cancellous deterioration following ovariectomy in 8-week-old mice [1]. However, to our knowledge similar studies have not been done in males [2]. On the other hand, the effects of 17-alpha administration on males for osteoarthritis has been investigated. These studies showed little effects, but the mice were on average 2.5 years old, which is the equivalent of an 85 year old human. The average age of OA onset in humans is approximately 55. Thus, it could be beneficial to investigate intermediate timepoints to detect treatment efficacy. We sought to fill these knowledge gaps by assessing bone and knee outcomes over the natural aging process with and without 17-alpha administration. We hypothesized that 17-alpha administration to males would slow age-related declines in bone and joint health.

METHODS: All animal procedures were done with the approval of our institutional IACUC. Treated Male Het3 mice were given 14.4ppm estradiol (17-alpha E2) in standard chow for 19 weeks and prior to euthanasia at 60 or 105 weeks old (middle and old, respectively). Untreated young (20 weeks old), middle, and old males were given only standard chow. Following euthanasia, the left knees and the right femora were harvested. The knees were fixed with 10% neutral buffered formalin and stored in 70% ethanol before microCT scanning (10um resolution), decalcification, and histological processing. The three-dimensional microCT renderings were blindly assessed by an attending physician who specializes in joints and joint replacements on a 0 to 3 scale (0= healthy, 3 = severe deterioration). Then the knee micro-CT scans were segmented into medial/lateral femoral condyle, medial/lateral tibial plateau, and proximal tibial cancellous regions for analysis with Dragonfly's bone wizard module. The paraffin sections will be stained with Safranin-O and fast-green to qualitatively and quantitatively assess cartilage degradation and synovitis. The femurs were immediately frozen in PBS-soaked guaze. Micro-CT scans of the midpoint and distal diaphysis were analyzed to determine cortical and cancellous bone parameters, respectively. The femurs will undergo 3-point mechanical testing to assess bone strength. Because a young treated group is not currently available, data for middle and old groups was first analyzed with a two-way ANOVA (factors: age, treatment). Then the young vehicle group was comparted to the vehicle and treated groups with separate one-way ANOVAs (factor: age).

RESULTS SECTION: Data collection is still on-going. The microCT measurements collected to date indicate no significant differences between treatment groups for OA score, condyle BV/TV, condyle BMD, tibial cancellous BV/TV, tibial cancellous BMD, or femoral mid-point BA/TA (Table 1, n=3 to 12/group). For simplicity, only measurements collected for the femoral and tibial medial condyles are presented. The results' trends and conclusions were the same for all other condyle regions. The expected increases in total size with increasing age were present for condyle, cancellous, and cortical regions as was advancing knee joint degradation.

DISCUSSION: While 17-alpha E2 estrogen shows great promise to extend life in male animals while also improving function of many organ systems, the effects on skeletal deterioration appear to be minimal at best. This is in line with a recent study demonstrating little effect of 17-alpha treatment on osteoarthritis in significantly older animals (~130weeks) of the same strain [2]. However, the mouse strain used here is genetically diverse being a cross of 4 standard strains. Thus if repeated in more homogenous strains, the results could be different. Further, this study has some promising trends when compared to the young vehicle group and data collection is still on-going. It could be that mechanics or micro-scale analysis is sensitive enough to reveal improvements not captured at the tissue-scale. Further, both studies are relatively simple. Clinically the most efficacious treatments are sometimes combinations, which would certainly be warranted here given the close association of testosterone and estrogen.

SIGNIFICANCE/CLINICAL RELEVANCE: 17-alpha E2 estrogen is gaining interest for its ability to improve life-span and health-span ,in males without the feminizing features associated with 17-beta E2 estrogen treatment. It would be advantageous if 17-alpha has similar effects on the skeletal system during the aging process.

IMAGES AND TABLES:

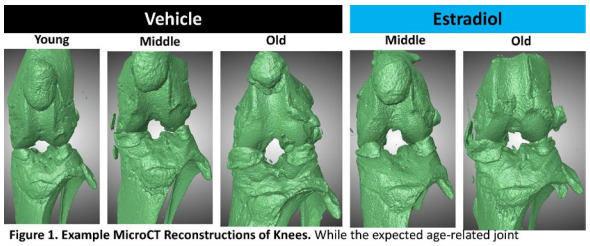

Figure 1. Example MicroCT Reconstructions of Knees. While the expected age-related joint deterioration is present, it was highly variable and was not strongly inhibited by estradiol 17-alpha administration.

Table 1. Selected MicroCT Outcomes.

Group	n	Blinded OA Score	n	Medial Femoral Condyle		Medial Tibial Condyle		n	Femur Midpoint
				BV/TV	BMD (mgHA/ccm)	BV/TV	BMD (mgHA/ccm)		BA/TA
Young Vehicle	12	0.67 ± 0.78	6	0.65 ± 0.06	824 ± 110	0.77 ± 0.06	945 ± 128	12	0.80 ± 0.07
Middle Vehicle	9	1.00 ± 0.71	6	0.65 ± 0.06	860 ± 81	0.77 ± 0.07	977 ± 144	7	0.74 ± 0.04
Old Vehicle	9	1.78 ± 1.10	6	0.61 ± 0.09	864 ± 60	0.74 ± 0.05	982 ± 63	9	0.79 ± 0.08
Middle Estradiol	8	1.25 ± 0.71	3	0.64 ± 0.10	884 ± 100	0.80 ± 0.04	1027 ± 88	10	0.79 ± 0.04
Old Estradiol	5	1.40 ± 1.14	4	0.62 ± 0.01	772 ± 33	0.76 ± 0.04	925 ± 33	6	0.83 ± 0.07

REFERENCES: [1] Shivani N. Mann, Kevin S. Pitel, Molly H. Nelson-Holte, Urszula T. Iwaniec, Russell T. Turner, Roshini Sathiaseelan, James L. Kirkland, Augusto Schneider, Katherine T. Morris, Subramaniam Malayannan, John R. Hawse, Michael B. Stout, 17α-Estradiol prevents ovariectomy-mediated obesity and bone loss, Experimental Gerontology,Volume 142, 2020.
[2] Dave Ewart, Lindsey Harper, Amy Gravely, Richard A. Miller, Cathy S. Carlson & Richard F. Loeser (2020) Naturally occurring osteoarthritis in male mice with an extended lifespan, Connective Tissue Research, 61:1, 95-103,