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Effect of Anastrozole Therapy on Bone Formation and Growth of Orchidectomized Pubertal Male Rats

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Abstract

Our study was designed to evaluate the effect of aromatase inhibition in orchidectomized rats and determine the effect of lack of estrogen and gonadal androgen in bone. Thirty male Sprague Dawley rats age six weeks were used for the study, 20 of them were bilaterally orchidectomized, and 10 of them served as wild-type controls. The orchidectomized rats were randomly subdivided into two different groups. The first group consisted of ten Orchidectomized Control Rats (ORX) and the second composed of ten Orchidectomized Rats Treated with Anastrozole (ANA). The duration of the study was six weeks (age 7 weeks to 13 weeks). The ANA group showed an increased longitudinal growth rate ($p < 0.009$) and femoral length ($p < 0.04$) compared to ORX. The T.Ar was increased by 29% ($p < 0.005$) and Tb.Sp decreased by 49.7% ($p < 0.004$) compared to ORX, with no difference in TB.Th. The periosteal BFR ($p < 0.02$) and CT.Ar ($p < 0.03$) were also increased in ANA compared to ORX. Interestingly, the maximal (breaking) load of the femur was 17.5% higher in ANA than ORX ($p < 0.001$) and was similar to controls ($p < 0.6$). Surprisingly anastrozole improved bone fragility, mainly by reducing bone modeling in the cancellous bone and possibly by increasing periosteal BFR in the cortical bone. We hypothesized that these results could be secondary to an increase in non-gonadal androgen production, increased IGF-1 receptor expression at the bone level or possibly a combination of both.

Keywords: Anastrozole, Orchidectomized rats, Bone formation, Testosterone, Aromatase inhibitor, Cortical bone

Abbreviations: CON: Wild type Controls Rats; ORX: Orchidectomized Rats; ANA: Orchidectomized Rats treated with Anastrozole; T.Ar: Trabecular Area; T.Sp: Trabecular Space; Tb.Th: Trabecular Thickness; CT.Ar: Cortical Area; P.Pm: Periosteal Perimeter; E.BFR: Endosteal Bone Formation Rate; P.BFR: Periosteal Bone Formation Rate

Introduction

Sex hormones are important for the development and maintenance of the skeletal system [1-6]. Mutations of the aromatase gene or estrogen receptor can lead to delayed epiphyseal closure and tall stature, indicating that estrogen and not testosterone is responsible for epiphyseal fusion [7,8].

This discovery prompted an increase in off label use of aromatase inhibitors as a potential therapeutic tool in the treatment of children with growth disorders and precocious puberty [9-13]. However, the possibility that these medications could potentially reduce bone mineral acquisition [1,7,9] lead to studies that showed no adverse effects on bone mineralization after 2 years of treatment [14,15]. The findings could presumably be explained by a compensatory increase of gonadal and adrenal androgen.

To address this question, the current study was designed to evaluate the effect of aromatase inhibition

on orchidectomized rats, where gonadal androgen and estrogen production is negligible. We hypothesized that aromatase inhibition in orchidectomized rats may increase non-gonadal androgens at the bone level and could potentially prevent bone loss.

Material and Methods

Thirty male Sprague Dawley rats age 6-weeks-old were ordered from Hilltop Animals Inc; 20 animals were bilaterally orchidectomized under anesthesia by the vendor, and 10 rats served as Controls (CON). The orchidectomized rats were randomly subdivided into 2 groups of 10 animals each as follows: Orchidectomized (ORX) and ORX plus Anastrozole (ANA).

Anastrozole was kindly supplied by Astra Zeneca. It was suspended in 5% dextrose solution and administered orally every day at a dose of 1 mg/kg/day. The animals were allowed to acclimate in the laboratory for 1 week. The duration of the study was 6 weeks (rat ages 7 weeks to 13 weeks).

All rats were given water ad libitum and were allowed free access to standard pellet chow diet (Rodent laboratory Chow 5001; Ralston Purina's Louis, MO). They were housed in single cages under vivarium conditions (temperature of 23.8 °C and 12 hours on/off light cycle). All animals were maintained in accordance with the National Institutes of Health Guide for the Care and Use of laboratory animals, and animal protocols were approved by the Laboratory Animal Care Committee of Winthrop University Hospital.

Preparation of specimens

All rats were labeled with calcein (Sigma Chemical Co) at a dose of 10 mg/kg SC, 10 days and 3 days prior to sacrifice. They were anesthetized with an intraperitoneal injection of Xylazine 12 mg/Kg and Ketamine 80 mg/kg and euthanized by exsanguination. The right femur of each animal was dissected free of soft tissue and used for measurement of the femoral length and to perform a three-point bending test.

The right tibia was dissected and cut into three equal parts. The right proximal tibia and shaft were fixed in 70% ethanol solution for 2 days and immersed in Villanueva Osteochrome Bone stain (Polysciences, Warrington, PA) for 5 days.

The specimens were dehydrated by sequential changes of ascending concentration of ethanol (70%, 95% and 100%) and xylene, and then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester NY).

Frontal sections of the proximal tibia were cut at 5 Mm using a microtome (Leica RM2155; Germany) and cross sections of the tibial shaft proximal to the tibiofibular junction were cut at 40 Mm using a diamond wire Histo-Saw machine (Delaware Diamond Knives, Wilmington, DE). All sections were cover slipped with Eukitt (Calibrated Instrument Hawthorne, NY) for static and dynamic histomorphometric analysis.

Biomechanical testing

The mechanical properties of the femoral diaphysis were evaluated by three-point bending test. Load was applied midway between two supports placed 15 mm apart on the bone, the bone was positioned horizontally with the anterior surface upwards centered on supports. The pressing force was directed vertically to the midshaft of the bone. The specimens were tested in a saline bath at 37°C. Each specimen was submerged in the saline solution for 3 minutes to allow temperature equilibration. Each bone was compressed with a constant speed of 20 mm/min until failure and load displacement curves were recorded using a material testing machine (MZ500D; Maruto, Co, Ltd, Tokyo, Japan).

Histomorphometric analysis

Histomorphometric parameters of cancellous and cortical bone were measured with a digitalizing morphometry system. This consisted of an epifluorescence microscope (Olympus BH-2), a color video camera, and a digitalizing pad (Numonics 2206) coupled to an IBM computer and a morphometric program "Osteometrics" (Osteometrics, Atlanta GA).

Measured parameters in cortical bone included total tissue area, periosteal perimeter, marrow area, endocortical perimeter, periosteal and endocortical single and double labeled perimeters, inter labeled widths, and intracortical resorption area. They were used to calculate percent of cortical bone, periosteal and endocortical Bone Formation Rate (BFR) according to the standard nomenclatures as described by Jee et al [16].

Measured parameters of cancellous bone included total tissue area, cancellous bone area and perimeter, longitudinal growth rate, single and double labeled perimeters and inter labeled widths. They were then used to calculate percent of cancellous bone volume

and cancellous BFR. The region of bone measured in all groups was 1-4 mm from the growth plate in the proximal tibia. All measurements and calculations were referenced to the standard nomenclature described by Parfitt et al [17].

Statistical analysis

All data are presented as the mean and Standard Error (SE) in tables and figures. The comparison among the groups were done by Fisher's protected least significant difference test (one-way analysis variance) ANOVA. A significance level of ($p < 0.05$) was used for all comparisons.

Results

Body weight was significantly lower in ORX ($p < 0.0006$) and ANA compared to CON ($p < 0.05$). Body weight was increased in ANA compared to ORX but did not achieve statistical significance ($p < 0.4$). Femoral length was increased in CON group compared to ORX ($p < 0.001$) and compared to ANA ($p < 0.02$) however femoral length was increased in ANA compared to ORX ($p < 0.04$). Femoral dry weight was higher in CON compared to ORX and ANA ($p < 0.00001$) but the femoral dry weight was similar in ORX and ANA group (Table 1).

Longitudinal growth rate

Longitudinal Growth Rate (LGR) was reduced by 36.2% in ORX compared to CON and 31% in ANA compared to CON, however ANA LGR was 8.6% higher than ORX ($p < 0.00002$) and 127% increase in Tb.Sp compared to CON ($p < 0.00003$). In ANA there was a 44.6% reduction in trabecular area (T.Ar) ($p < 0.0001$) compared to CON and was characterized by a 29% reduction trabecular number (Tb.N) ($p < 0.005$) and 49.7% decrease in trabecular space (Tb.Sp) ($p < 0.05$) and ANA groups ($p < 0.03$) compared to CON, however there was not a statistical significant difference between ORX and ANA (Table 2).

Cortical bone structure

Cortical Area (CT.Ar) was reduced by 14% in ORX and by 7% in ANA compared to CON, however CT.Ar was 8.6% higher in the ANA group compared to ORX ($p < 0.03$). Periosteal perimeter (P.Pm) was reduced by 7.6% in ORX and by 1.3% in ANA compared to CON, however there was a 4.5% increase in P.pm in ANA compared to ORX ($p < 0.03$). Periosteal BFR was decreased by 17.8% in ORX and decreased by 5.2% in ANA compared to CON, however Periosteal BFR was increased 15.3% in ANA compared to ORX ($p < 0.02$). Endosteal BFR was increased by 7.7% in ORX and was increased by 34.8% in ANA compared to CON, however Endosteal BFR was increased 25% in ANA compared to ORX ($p < 0.008$) (Table 2).

Table 1: Bone histomorphometry in proximal tibia of CON, ORX and ANA rats.

Group	Femoral Length (mm)	Body Weight (grams)	Tb.Ar (mm^2)	Tb.N (#/mm)	Tb.Sp (mcm)	T.Th (mcm)
CON	34.99 ± 0.54	454.8 ± 36.9	1.45 ± 0.31	2.69 ± 0.46	467.07 ± 84.63	57.24 ± 9.19
ORX	33.59 ± 0.78	403.9 ± 37.6	0.59 ± 0.14	1.34 ± 0.34	1060.84 ± 304.14	53.14 ± 11.71
ANA	34.37 ± 0.57	411.9 ± 29.3	0.82 ± 0.14	1.91 ± 0.36	708.45 ± 150.51	52.45 ± 4.14
P value ANA vs ORX	P<0.04	NS	<0.01	<0.005	<0.004	NS

Values are means ± SD, P ANA VS ORX group. All differences were tested by multiple comparison with Anova when data were transformed to logarithm.

Table 2: Bone histomorphometry in proximal tibia of CON, ORX and ANA rats.

Group	CT. AR (mm^2)	P.Pm (mm)	E.BFR (mcm/day)	P.BFR (mcm/day)	LGR (um/day)
CON	4.11 ± 0.34	8.06 ± 0.27	69.33 ± 19.14	69.33 ± 19.14	58.5 ± 4.3
ORX	3.52 ±0.28	7.59 ± 0.31	74.69 ± 23.52	366.36 ± 42.55	37±2.4
ANA	3.82 ± 0.16	7.95 ± 0.17	93.45 ± 23.52	422.41± 41.41	40.2 ± 13.6
P value ANA vs ORX	< 0.03	<0.03	<0.008	<0.02	<0.00002

Values are means ± SD, P ANA VS ORX group. All differences were tested by multiple comparisons with Anova when data were transformed to logarithm

Bone mechanics

The maximal (breaking) load of the femur was 196.29 ± 18.63 for controls, 162.92 ± 5.63 for ORX and 191.47 ± 22.47 for ANA. Maximal breaking load was 19% greater in CON and 17.5% greater in ANA compared to ORX ($p < 0.001$), however maximal breaking point was only 2.25% greater in CON compared to ANA and did not achieve statistical significance ($p < 0.6$).

Discussion

Orchiectomy in rat's results in a rapid decrease of bone mass and bone volume. Similarly, to hypogonadic men, it is associated with reduced bone mineral density and increased fracture risk, despite having an elevated estradiol to testosterone ratio within circulation. This suggests that bone loss results primarily from testosterone deficiency [4,18-20]. On the other hand, defects in estrogen synthesis in men results is severe osteopenia, tall stature and delayed epiphyseal closure, indicating that estrogen not testosterone is the main hormone responsible for bone fusion [7,8].

When supraphysiological testosterone is administered to orchidectomized rats, it completely prevents bone loss without altering circulating or intra skeletal estradiol concentrations. This suggests that aromatase activity is not essential for bone maintenance in the presence of supraphysiological androgens [18,21,22]. Interestingly, when aromatase inhibitors are used in pubertal boys they exhibit higher LH and testosterone levels but have lower estradiol and IGF-1 levels [15]. It is possible that this compensatory elevation in gonadal androgen could be the reason why their bone density is preserved despite having lower IGF-1 and estrogen with no significant adverse effects or deleterious consequence on the bone [13,14].

The aromatase enzyme can be found in many tissues and in men 15% of total estradiol is derived from the gonadal aromatization of testosterone within the gonad with the remaining 85% originating from peripheral conversion [23-25]. By using anastrozole in ORX rats we created a rat model of gonadal androgen and estrogen deficiency and expected to create a more fragile bone since it has been reported that under normal physiological conditions, rat and mice don't produce adrenal androgen [26].

In our study, the body weight in ANA was similar to ORX; however, the femoral length and the longitudinal growth rate was increased in ANA compared to ORX, suggesting that estrogen limits the longitudinal growth rate and bone size in rats.

Interestingly, the ANA group showed a statistical significant increase in periosteal BFR, P.Pm and CT.Ar and Tb.Ar characterized by increase Tb.N, and decreased Tb.Sp with no difference in Tb.Th compared to ORX.

Van Weerden et al reported that adrenal glands of mice and rats do not synthetize androgens [26], however, Callewaert et al were able to detect androstenedione in pooled serum samples of anastrozole treated rats [27]. Therefore, it is possible that conversion of androstenedione to testosterone occurred at the bone level in the ANA group, since 17b-hydroxysteroid dehydrogenase is expressed in rat osteoblastic cells [28].

As expected when performing the three-point bending test, the maximal breaking load was higher in CON compared to ORX, but surprisingly there was not a statistical significant difference between ANA and CON (Table 3). The fact that the maximal breaking load was 17.5% greater in ANA compared to ORX suggests that anastrozole administration to orchidectomized rats did not decrease but rather improved their bone fragility mainly by reducing bone remodeling and increasing bone size. This effect was independent of a possible increased load since the body weight was similar in ANA and ORX [29,30].

We hypothesized that these findings can be the result of a compensatory elevation in non-gonadal androgen, increased tissue testosterone, and increased IGF-1 receptor expression at the bone level [27,28,31,32].

If our hypothesis is right, the lower IGF-1 observed in pubertal children treated with aromatase inhibitors could be secondary to increased expression of IGF-1 receptors at the bone level, leading to improve IGF-1 sensitivity.

Our findings are conflicting with the studies using letrozole and vorozole in pubertal male rats, where reduction in bone strength and impaired growth was reported [32,33]. It is unclear why the use of letrozole and vorozole led to a reduction in bone strength and impaired growth, but we speculate that these could be a result of a higher inhibition of estrogen production, or a direct negative effect of these drugs at the bone level. Additionally, the possibility arises that differences in age or species could have resulted in conflicting evidence. However, at this time we are unaware of the hormonal

differences that age and species account for, but can only assume the possibility that these factors may have an effect on the results.

Conclusion

In summary, our study demonstrated that anastrozole therapy in orchidectomized rats not only increased their bone size, but reduced their bone fragility by reducing bone modeling in the cancellous bone and increasing periosteal bone formation at the cortical level compared to ORX. Further research is needed before we can advocate for the use of anastrozole therapy in pubertal boys.

References

1. Dunkel L, Wickman S. Treatment of delayed male puberty: Efficacy of aromatase inhibition. *J Pediatr Endocrinol Metab*. 2001;14 Suppl 6:1541-1546.
2. van der Eerden BCJ, Karperien M, Wit JM. Systemic and local regulation of the growth plate. *Endocr Rev*. 2003;24(6):782-801. Doi: https://doi.org/10.1210/er.2002-0033
3. Finkelstein JS, Neer RM, Biller BMK, et al. Osteopenia in men with a history of delayed puberty. *N Engl J Med*. 1992;326(9):600-604. Doi: https://doi.org/10.1056/NEJM199202273260904
4. Gunness M, Orwoll E. Early induction of alterations in cancellous and cortical bone histology after orchiectomy in mature rats. *J Bone Miner Res*. 1995;10(11):1735-1744. Doi: https://doi.org/10.1002/jbmr.5650101117
5. Peng Z, Tuukkanen J, Zhang H, et al. The mechanical strength of bone in different rat models of experimental osteoporosis. *Bone*. 1994;15(5):523-532. Doi: https://doi.org/10.1016/8756-3282(94)90276-3
6. Turner RT, Hannon KS, Demers LM, et al. Differential effects of gonadal function on bone histomorphometry in male and female rats. *J Bone Miner Res*. 1989;4(4):557-563. Doi: https://doi.org/10.1002/jbmr.5650040415
7. Carani C, Qin K, Simoni M, et al. Effect of testosterone and estradiol in a man with aromatase deficiency. *N Engl J Med*. 1997;337(2):91-95. Doi: https://doi.org/10.1056/NEJM199707103370204
8. Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994;331(16):1056-1061. Doi: https://doi.org/10.1056/NEJM199410203311604
9. Dunkel L. Use of aromatase inhibitors to increase final height. *Mol Cell Endocrinol*. 2006;254-255:207-216. Doi: https://doi.org/10.1016/j.mce.2006.04.031
10. Miller WR, Jackson J. The therapeutic potential of aromatase inhibitors. *Expert Opin Investig Drugs*. 2003;12(3):337-351. Doi: https://doi.org/10.1517/13543784.12.3.337
11. Plourde PV, Reiter EO, Jou H-C, et al. Safety and efficacy of anastrozole for the treatment of pubertal gynecomastia: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab*. 2004;89(9):4428-4433. Doi: https://doi.org/10.1210/jc.2004-0082
12. Nilsson O, Baron J. Fundamental limits on longitudinal bone growth: growth plate senescence and epiphyseal fusion. *Trends Endocrinol Metab*. 2004;15(8):370-374. Doi: https://doi.org/10.1016/j.tem.2004.08.004
13. Mauras N, Ross JL, Gagliardi P, et al. Randomized trial of aromatase inhibitors, growth hormone, or combination in pubertal boys with idiopathic, short stature. *J Clin Endocrinol Metab*. 2016;101(12):4984-4993. Doi: https://doi.org/10.1210/jc.2016-2891
14. Hero M, Norjavaara E, Dunkel L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J Clin Endocrinol Metab*. 2005;90(12):6396-6402. Doi: https://doi.org/10.1210/jc.2005-1392
15. Neely EK, Kumar RB, Payne SL, et al. Letrozole vs anastrozole for height augmentation in short pubertal males: first year data. *J Clin Endocrinol Metab*. 2014;99(11):4086-4093. Doi: https://doi.org/10.1210/jc.2014-2432
16. Jee W, S S, Inoue J. Histomorphometric assay of the growing long bone. *Handbook of bone morphology*. 1983:101-122.
17. Parfitt AM, Drezner MK, Glorieux FH, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*. 1987;2(6):595-610. Doi: https://doi.org/10.1002/jbmr.5650020617
18. Beck DT, Yarrow JF, Beggs LA, et al. Influence of aromatase inhibition on the bone-protective effects of testosterone. *J Bone Miner Res*. 2014;29(11):2405-2413. Doi: https://doi.org/10.1002/jbmr.2265
19. Compston JE. Sex steroids and bone. *Physiol Rev*. 2001;81(1):419-447. Doi: https://doi.org/10.1152/physrev.2001.81.1.419

20. Gennari L, Merlotti D, Martini G, et al. Longitudinal association between sex hormone levels, bone loss, and bone turnover in elderly men. *J Clin Endocrinol Metab*. 2003;88(11):5327-5333. Doi: https://doi.org/10.1210/jc.2003-030736
21. Isidori AM, Giannetta E, Greco EA, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clin Endocrinol*. 2005;63(3):280-293. Doi: https://doi.org/10.1111/j.1365-2265.2005.02339.x
22. Tracz MJ, Sideras K, Boloña ER, et al. Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. *J Clin Endocrinol Metab*. 2006;91(6):2011-2016. Doi: https://doi.org/10.1210/jc.2006-0036
23. Nuti R, Martini G, Merlotti D, et al. Bone metabolism in men: role of aromatase activity. *J Endocrinol Invest*. 2007;30(6 Suppl):18-23.
24. Van Der Eerden BCJ, Van De Ven J, Lowik CWGM, et al. Sex steroid metabolism in the tibial growth plate of the rat. *Endocrinology*. 2002;143(10):4048-4055. Doi: https://doi.org/10.1210/en.2002-220093
25. Labrie F. Intracrinology. *Mol Cell Endocrinol*. 1991;78(3):113-118. Doi: https://doi.org/10.1016/0303-7207(91)90116-a
26. van Weerden WM, Bierings HG, van Steenbrugge GJ, et al. Adrenal glands of mouse and rat do not synthesize androgens. *Life Sci*. 1992;50(12):857-861. Doi: https://doi.org/10.1016/0024-3205(92)90204-3
27. Callewaert F, Venken K, Kopchick JJ, et al. Sexual dimorphism in cortical bone size and strength but not density is determined by independent and time-specific actions of sex steroids and IGF-1: evidence from pubertal mouse models. *J Bone Miner Res*. 2010;25(3):617-626. Doi: https://doi.org/10.1359/jbmr.090828
28. Ishida Y, Killinger DW, Khalil MW, et al. Expression of steroid-converting enzymes in osteoblasts derived from rat vertebrae. *Osteoporos Int*. 2002;13(3):235-240. Doi: https://doi.org/10.1007/s001980200020
29. Vanderschueren D, van Herck E, Nijs J, et al. Aromatase inhibition impairs skeletal modeling and decreases bone mineral density in growing male rats. *Endocrinology*. 1997;138(6):2301-2307. Doi: https://doi.org/10.1210/endo.138.6.5216
30. Beck DT, Yarrow JF, Beggs LA, et al. Influence of aromatase inhibition on the bone-protective effects of testosterone. *J Bone Miner Res*. 2014;29(11):2405-2413. Doi: https://doi.org/10.1002/jbmr.2265
31. Nilsson A, Isgaard J, Lindahl A, et al. Regulation by growth hormone of number of chondrocytes containing IGF-I in rat growth plate. *Science*. 1986;233(4763):571-574. Doi: https://doi.org/10.1126/science.3523759
32. Meinel L, Zoidis E, Zapf J, et al. Localized insulin-like growth factor I delivery to enhance new bone formation. *Bone*. 2003;33(4):660-672. Doi: https://doi.org/10.1016/s8756-3282(03)00207-2
33. Hero M, Toiviainen-Salo S, Wickman S, et al. Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J Bone Miner Res*. 2010;25(7):1536-1543. Doi: https://doi.org/10.1002/jbmr.56