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## Introduction

Estrogen plays a major role in the regulation of human growth plate activity in both sexes. Rising levels of estrogen at the beginning of puberty initiate the growth spurt, whereas at the end of puberty, high estrogen levels are responsible for epiphyseal fusion, thereby limiting an individual's final height. Inhibition of estrogen production is supposed to delay growth plate fusion in children with idiopathic short stature and eventually leads to an increased final height. Moreover, estrogens are of particular importance in normal bone metabolism.

In view of the application of a possible therapy to stimulate growth, the effects of inhibition of local estrogen production on growth and bone were investigated.

## Experimental design

Prepubertal female Wistar rats (n = 30, 26 days old) were either treated with aromatase inhibitor exemestane or ovariectomized (OVX). Exemestane was administered by intramuscular injections for 3 weeks (10, 30 and 100 mg/kg/week). Growth was evaluated by weekly measurements of nose-anus length.

End point measurements of tibia length and growth plate width were recorded and morphology of ovaria and uterus were examined by histology.

To confirm the presence of osteopenia, the right femurs were studied by high resolution X-ray microtomography (micro-CT). Quantitative image analysis of metaphyseal and epiphyseal cancellous bone resulted in bone parameters such as trabecular number and thickness, bone volume, calcium density, etc.

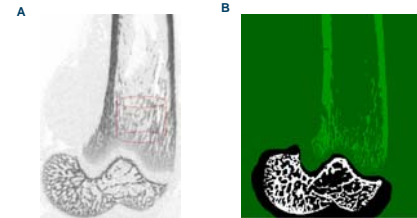
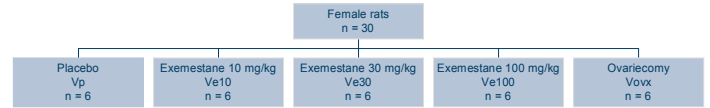


Fig. 1: Effects of estrogen inhibition on bone are evaluated by micro-CT. Right femurs were scanned *ex vivo* by a micro-CT instrument (Skyscan 1072, Skyscan, Kontich, Belgium) with a resolution of 14  $\mu\text{m}$  (80 kV, 100  $\mu\text{A}$ , 1 mm Aluminum filter). Cancellous bone was studied at 2 different locations: metaphysis on cross-sections (cylindrical volume of interest as indicated in A) and epiphysis on coronal slices (B).

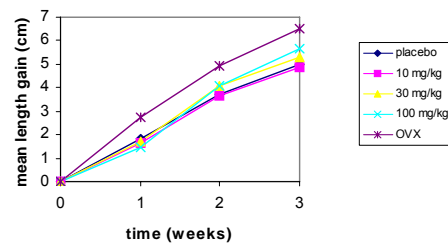


Fig. 2: Treatment with the highest dose of exemestane increased nose-anus length in an OVX-like manner. Lower doses also had a smaller effect.

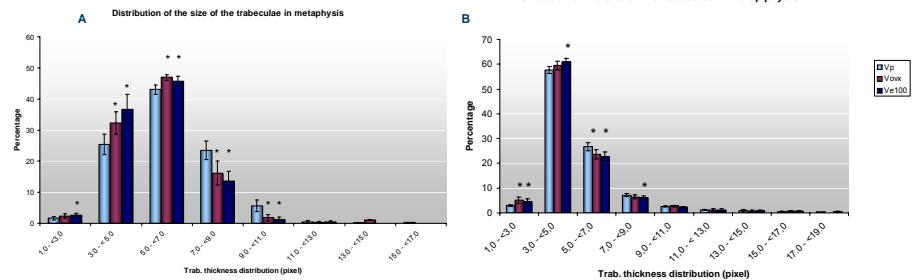


Fig. 3: Trabecular bone analysis in the femur by micro-CT. Histograms show a distribution of the size of the trabeculae in A) metaphysis and B) epiphysis. Groups with estrogen inhibition (i.e. exemestane 100 mg/kg and ovariectomy) have more and thinner trabeculae compared to the placebo treated group. \*:  $p < 0.05$  versus placebo.

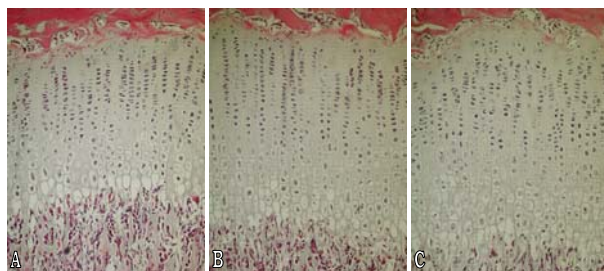


Fig. 4: Histological examination of the growth plates. Both exemestane and ovariectomy increased growth plate thickness, based on an equal thickening of both proliferative and hypertrophic zones. A) Placebo, B) Exemestane 100 mg/kg, C) Ovariectomy.

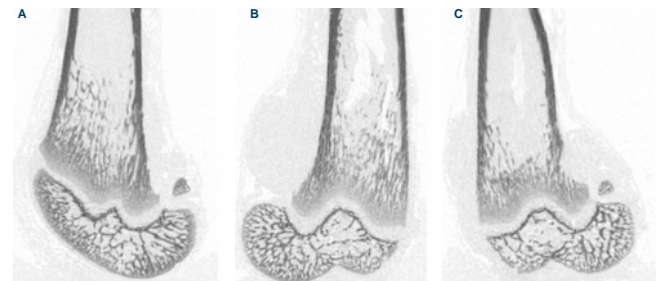


Fig. 5: Coronal slices of the femur. A) Placebo, B) Exemestane 100 mg/kg, C) Ovariectomy. Trabecular architecture is clearly affected by inhibition of estrogen production.

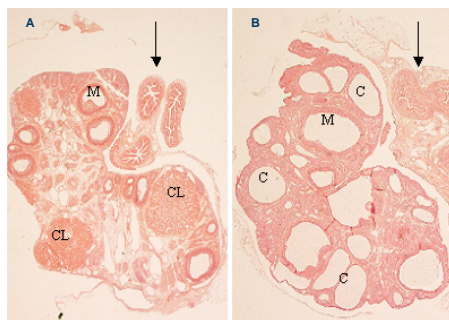


Fig. 6: Histological evaluation of the ovarian tissue. A) Placebo, B) Exemestane 100 mg/kg. Notice the presence of cysts (C) which can lead to a PCOS-like phenotype (polycystic ovary syndrome) and eventually cause infertility. CL: corpus luteum, M: mature follicle, arrow: fallopian tube tissue.

Parameter	Vp	Ve100	Vovx	
Anatomical volume $V_a$ ( $\text{mm}^3$ )	277.8	287.3	279.2	
Bone volume $V_b$ ( $\text{mm}^3$ )	95.5	97.1	90.8	
Calcium density	1	0.90	0.84	
Epiphysis	Bone volume ( $\text{mm}^3$ )	25.6	22.4 <sup>a,b</sup>	20.2 <sup>a</sup>
	Trabecular thickness ( $\mu\text{m}$ )	143.1	139.4 <sup>a</sup>	139.6 <sup>a</sup>
	Trabecular number (1/mm)	1.78	1.56 <sup>a,b</sup>	1.42 <sup>a</sup>
Meta-physis	Bone volume ( $\text{mm}^3$ )	2.49	1.85 <sup>a</sup>	1.84 <sup>a</sup>
	Trabecular thickness ( $\mu\text{m}$ )	85.1	75.9 <sup>a</sup>	78.5 <sup>a</sup>
	Trabecular number (1/mm)	3.55	2.96 <sup>a</sup>	2.85 <sup>a</sup>

Table 1: Anatomical volume, bone volume and calcium density were determined on whole femur scans (resolution 36  $\mu\text{m}$ ). Trabecular bone parameters were determined in femur epiphysis and metaphysis after scanning with 14  $\mu\text{m}$ . <sup>a</sup>  $p < 0.05$  versus placebo, <sup>b</sup>  $p < 0.05$  versus ovariectomy.

## Conclusion

Exemestane effectively blocks aromatase activity and increases longitudinal growth in female rats. Moreover, this treatment caused osteopenia since trabecular architecture in the femur was deteriorated. Despite the expected results the negative effects are unacceptable considering the PCOS-like phenotype.

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