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## Introduction and aim

Estrogen plays a major role in the regulation of growth in boys and girls. At the beginning of puberty, an increase in oestrogen levels initiates the growth spurt, whereas at the end of puberty, high estrogen levels are responsible for epiphyseal fusion, thereby limiting the final height of an individual. Inhibition of estrogen synthesis is supposed to delay growth plate fusion in children with an idiopathic short stature and with an increased final height. Estrogens are also important for normal bone metabolism. In view of the application of a possible therapy to stimulate growth, the effects of inhibition of local estrogen synthesis in bone were investigated in the present report. The cancellous bone of the femur was analysed by micro-CT to quantify the level of osteopenia.

## Experimental set-up

Young male and female Wistar rats (n=36) were treated with the irreversible aromatase inhibitor exemestane. Drug administration started before sexual maturation. The experiment lasted for 3 weeks in the female group and for 6 weeks in the male group. After sacrifice, the right knee of the animals was scanned by micro-CT to evaluate the presence of osteopenia. Quantitative image analysis resulted in bone parameters such as trabecular number and thickness, volume and calcium density. Both metaphyseal and epiphyseal cancellous bone was examined. As a reference, normal growth was examined during a weekly follow-up of young rats by micro-CT. Analysis of epiphyseal cancellous bone allows to study bone formation during growth.

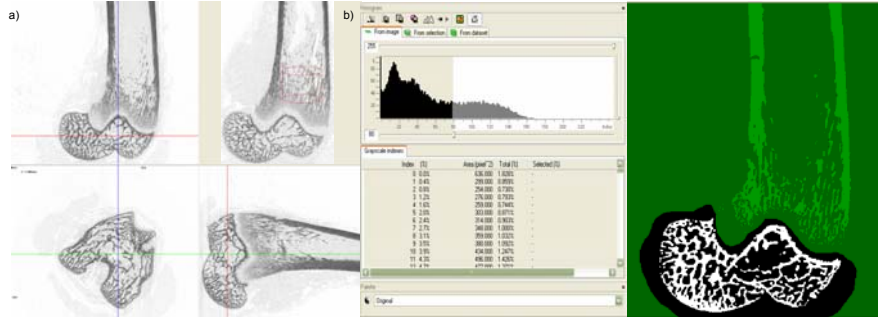
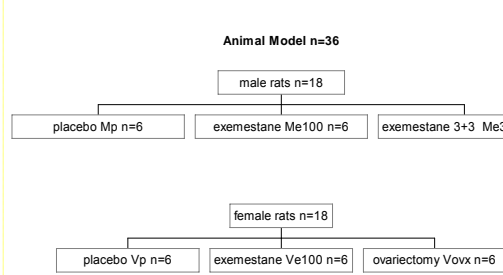


Fig. 1: Animal model. The experiment started at day 26. One male group was treated for 3 weeks + 3 weeks placebo. The female treated group was compared to a group with ovariectomy (i.e. total estrogen depletion).

Fig. 2: (a): 3 virtual cross-sections, showing greyvalues through the femur knee. The metaphysis was analysed as indicated by the red cylinder. (b): Analysis by CT-Analysing of the binary image of trabecular bone in the epiphysis. Standardisation of the thresholding procedure is required to optimize the signal to noise ratio.

## Results

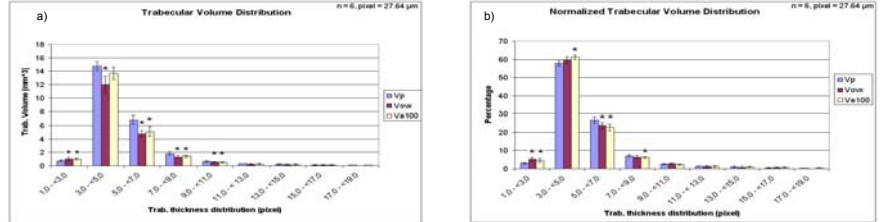
Group	Anatomical volume (V <sub>a</sub> )	Bone volume (V <sub>b</sub> )	Total Calcium	Calcium density
Vp	277.8	95.5	1	1
Vovx	279.2	90.8	0.85	0.84
Ve100	287.3	97.1	0.97	0.90
Mp	489.3	167.1	2.58	1.25
Me100	454.0	151.9	2.29	1.25
Me3+3	476.5	160.0	2.36	1.22

Fig. 3: Analysis in the whole femur (average per group). The female treated group has an increased V<sub>a</sub> opposite to the males. All treated animals show a loss of calcium: osteopenia is present. The trabecular adaptation, as a result of exemestane administration, has a sexual dimorphism.

Group	"Volume of objects" (mm <sup>3</sup> )	Trabeculaire thickness (µm)	Trabeculaire number (1/mm)
<b>Epiphysis</b>			
Vp	25,6 ± 1,4	143,1 ± 1,0	1,78 ± 0,03
Vovx	20,2 ± 1,8 *	139,6 ± 3,2 *	1,42 ± 0,07 *
Ve100	22,4 ± 1,6 *^	139,4 ± 2,3 *	1,56 ± 0,15 *^
<b>Metaphysis</b>			
Vp	2,49 ± 0,37	85,1 ± 3,3	3,55 ± 0,46
Vovx	1,84 ± 0,31 *	78,5 ± 3,1 *	2,85 ± 0,41 *
Ve100	1,85 ± 0,25 *	75,9 ± 2,9 *	2,96 ± 0,31 *

Fig. 4: Analysis in epiphysis and metaphysis in the female rats. Calcified tissue in the volume of interest (VOI) decreased due to resorption and thinning of the trabeculae. In the epiphysis the trabeculae are thicker but less in number compared to the metaphysis. \*\*\* statistical significance (p<0.05) versus control group, \*^ versus Vovx. A similar trend was observed in the male groups. Me3+3 shows a catch-up with *de novo* formation of thin trabeculae.

## Epiphysis



## Metaphysis

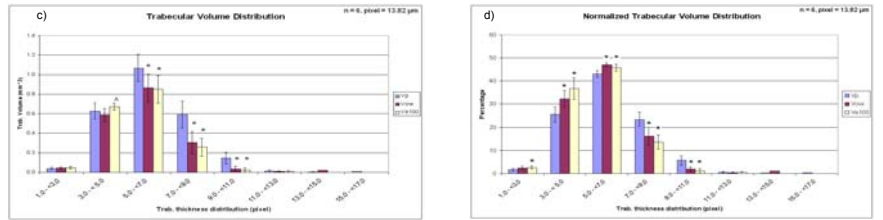


Fig. 5: Trabecular bone analysis of females. Panel (a)(b): Epiphysis. (a): The control group has a larger volume of all types of trabeculae compared to the treated and ovariectomized groups. (b): Number of trabeculae: smaller and thinner trabeculae are observed in the treated groups. Panel (c)(d): Metaphysis. (c): Control group has a larger trabecular volume. (d): Treated groups have more smaller and thinner trabeculae. \*\*\* statistical significance (p<0.05) versus control group. The males showed a similar trend, with a catch-up of Me3+3.

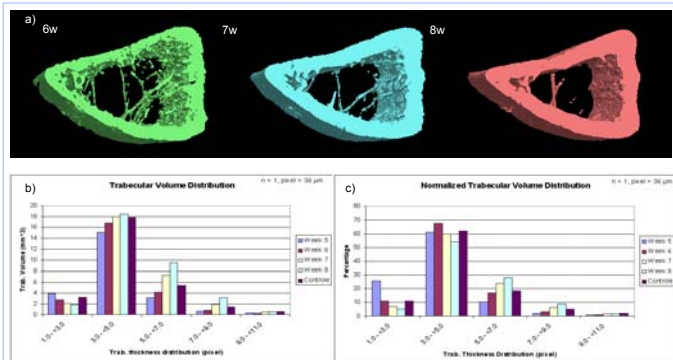


Fig. 6: Normal longitudinal growth of the rat. (a): 3D-model of the growth. Cortical bone becomes thicker and some trabeculae are resorbed, while others increase in thickness. (b): *De novo* formation of trabeculae lasted until week 6, afterwards the trabeculae became thicker. (c): The animals have more thin trabeculae when they are young; an increase in thickness is observed during aging.

## Conclusions:

As a result of exemestane administration, growth in the rats is enhanced. Moreover, in all animals administration of exemestane resulted in a reduction of calcified tissue, by thinning and loss of trabeculae, resembling the findings in the group with ovariectomy. A similar observation was made in the male rats, treated 3 weeks longer than the females. In the males treated for half of the period a remarkable catch-up occurred: *de novo* formation of trabeculae to rebuild the amount of calcified tissue was observed.

In both sexes osteopenia is present after treatment with exemestane that blocks the estrogen synthesis.

The present results clearly show that inhibition of estrogen synthesis, applied as a therapy to stimulate growth, can cause osteopenia. This requires serious consideration when starting a growth enhancing therapy in children.

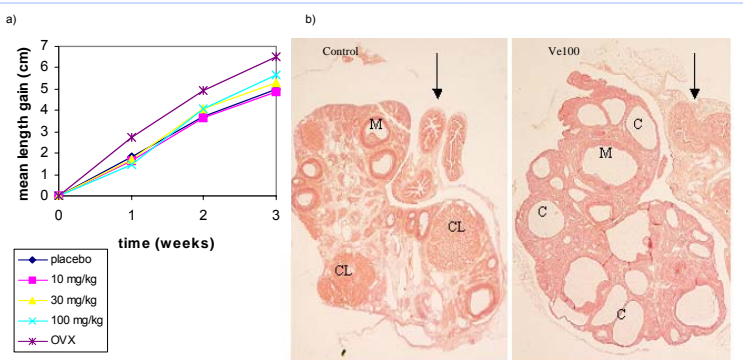


Fig. 7: (a): Growth of the females (mean increase in length). Growth enhancement in the treated rats was similar to ovx-rats. (b): Histological section of the ovaria. Notice the presence of cysts (C) which can cause infertility. CL: corpus luteum, M: mature follicle, arrow: fallopian tube tissue.