The Effect of Menopause on Osteoporosis and Bone Degeneration in Mouse Models

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Abstract

Osteoporosis is a bone loss disease affecting millions worldwide, with post-menopausal women making up 80% of diagnosed cases.[1] This increased risk is primarily due to a **decrease in estrogen** levels during menopause. Estrogen plays a key role in maintaining bone density by regulating the balance between bone resorption and bone formation. Lack of estrogen leads to a net loss in bone mass and increased susceptibility to fractures.[2]

In this project, menopause was chemically induced in mice to better understand the progression of osteoporosis in their skeleton. Analysis showed changes in structure and material properties across various ages and menopausal stages.

[1] U.S. Food and Drug Administration. (n.d.). *Osteoporosis*. FDA.
[2] Komori T. Animal models for osteoporosis. Eur J Pharmacol. 2015;759:287–294.

Introduction

Osteoporosis causes a loss of density in trabecular(spongy inner) and cortical (hard outer) bone. As bone mass declines, bones become thinner, more brittle, and more prone to fractures.

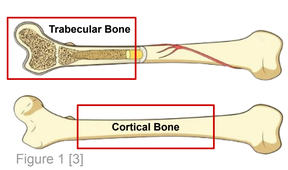


Figure 1 [3]

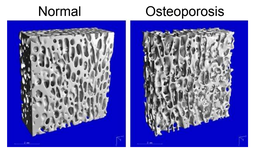


Figure 2 [4]

Osteoporosis drugs are tested on mice before they can be approved for human trials. However, mice do not experience human characteristics of menopause; there is not estrogen fluctuation and loss at the end of their reproductive cycle. (Fig. 3)

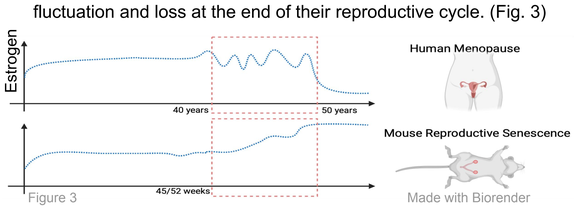


Figure 3

Current research on osteoporosis models menopause by removing the ovaries, creating a sharp drop in estrogen. Ovarian failure can also be induced chemically with injections of **4-vinylcyclohexene diepoxide (VCD)**. (Fig 4)

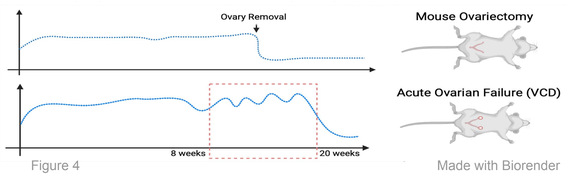


Figure 4

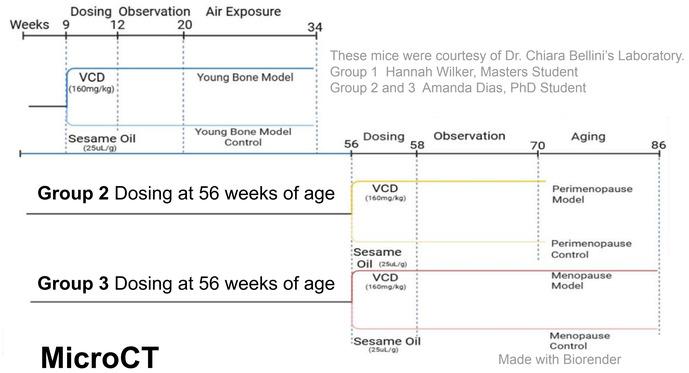
We examined the effects of VCD on bone structure and mechanics.

[4] Qin, Yi-Xian & Lin, Wei & Mittra, Erik & Xia, Yi & Cheng, Jiqi & Rubin, Clinton & Müller, Ralph. (2013).

Methods

Treatment of Mice with VCD

Group 1- Injected at the beginning of reproductive cycle at week 9	VCD Sesame Oil	Sacrificed at 36 weeks of age
Group 2-Injected at the end of reproductive cycle at 56 to 58 weeks of age	VCD Sesame Oil	Sacrificed at 71 weeks of age
Group 3-Injected at the end of reproductive cycle at 56 to 58 weeks of age	VCD Sesame Oil	Sacrificed at 86 weeks of age



These mice were courtesy of Dr. Chiara Bellini's Laboratory.
Group 1 Hannah Wilker, Masters Student
Group 2 and 3 Amanda Dias, PhD Student

MicroCT

Performed in Dr. Julia Charles' Lab
Brigham and Women's Hospital
Founding Member, Mass General Brigham

Entire bone is imaged in micro slices by X-ray (Fig 5)

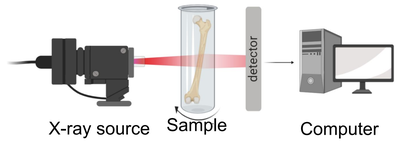


Figure 5 [5]

[5] Soha Ben Tahar Internship Report NU

Bone analyzed in ImageJ by BoneJ (Fig 6)

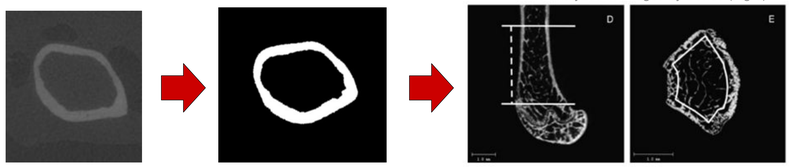


Figure 6 [5]

BoneJ calculations were used to compare bone sizes, structure, and mechanical properties.

3 Point Bend Test

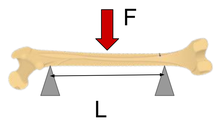


Figure 7 [3]

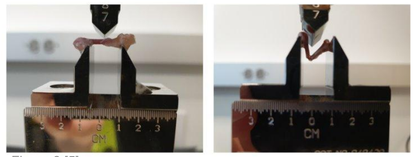


Figure 8 [5]

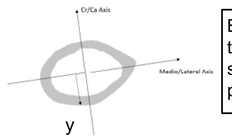


Figure 9 [5]

Beam Theory

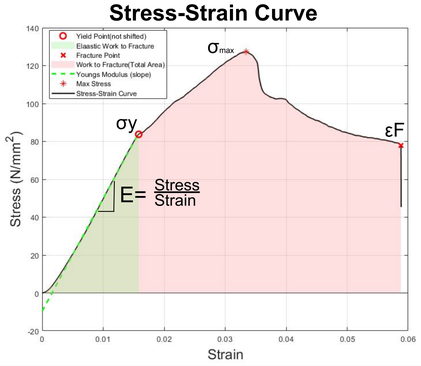
The section of bone between the supports is uniform, so beam bending equations were used to study the material's properties.

$$\sigma_{max} = \frac{-yFL}{4I_y} \qquad \epsilon_{max} = \frac{12v_{max}y}{L^2}$$

Stress- amount of force being experienced per unit area of material

Strain- measure of deformation

Stress-Strain Curve



E **Young's Modulus**-slope (stress/strain) measures stiffness and materials' resistance to deformation

σy **Yield Strength**- the stress point when the material starts to be permanently damaged plastic -> elastic

EWF **Elastic Work to Fracture**- Amount of energy required to permanently damage or deform the bone

σ_{max} **Ultimate Stress**- the maximum amount of stress the material is put under

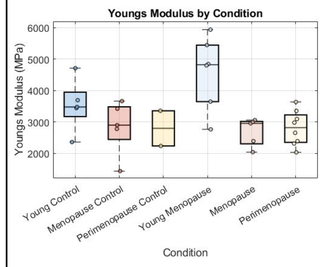
εF **Fracture point**- max stress before force being exerted on material drops by 40%

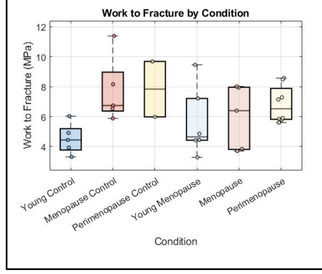
WF **Work to Fracture**- Total amount of energy required to break the bone

Converted and plotted in MATLAB

Results

Cortical analysis reveals that the cortical bones dosed with VCD at 56 weeks had more degradation and they are thinner





- Average Young's Modulus (E) was higher in the treated mice from young menopause (YM) and perimenopause (PM)
- There was a greater spread of data in YM compared to all other conditions.
- Young Control (YC) and YM has a lower Work to Fracture (WF) than other conditions
- PM and Menopause (M) shows similar mean work to fracture values, which are both lower than controls and higher than YM

Conclusions/Discussion

- YM had a low mean WF with high variability, and highest stiffness, indicating that while the bones were more rigid, their fracture resistance was less consistent compared to both the control and other experimental groups.
- Inducing menopause in mice at a week 56 compared to week 9 showed less variation in the data, suggesting that age at induction influences bone response.

Further Studies

The current study can be extended to include trabecular bone analysis, which will provide insights into changes in bone density and trabecular spacing. Additional femurs from each condition will be tested to improve the reliability of the results.

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