BMTE 08.10

Patella bone changes in a murine model for postmenopausal osteoarthritis

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Introduction

Osteoarthritis (OA) is a degenerative joint disease, characterized by cartilage destruction, subchondral bone sclerosis and osteophyte formation. The prevalence of osteoarthritis in women and men is similar till approximately the age of 50 but thereafter the disease increases dramatically in women. It has been suggested that this dominance is related to the hormonal changes occurring at the menopause [1, 4, 5, 15, 16, 19, 20]. Several studies using animal models have been performed to investigate the role of endogenous estrogens in the regulation of articular cartilage turnover and in the preservation of joint integrity. It has been observed that estrogen deficiency accelerates cartilage turnover, and increases cartilage surface erosion [1, 4, 5]. Another evidence for the implication of estrogen in OA is the presence of estrogen receptors (ERs) α and β in the articular cartilage and subchondral bone [15, 16]. These receptors are functionally active and involved in the regulation of the cartilage matrix synthesis. However, the exact roles of hormones in OA and of estrogen replacement therapy are not completely understood.

Another debating issue about the progression of OA is the relative importance of cartilage and bone changes in the development of the disease. Although OA is considered to be a cartilage disorder it also involves changes in the subchondral and periartricular bone, including sclerosis and cyst and osteophyte formation. In fact, the changes in biomechanical factors, density and architecture of the bone below the cartilage have a strong effect on cartilage degradation [3, 6, 10, 11, 12, 13, 18]. Subchondral bone resorption can create inhomogeneities in stiffness due to the loss of ability of the bone to dissipate the load and distribute the stress in the joint. This turnover can initiate or contribute to cartilage deterioration in OA [2, 3, 6, 7, 10, 18]. Therefore, it is important to describe the specific role of bone and cartilage in the development of osteoarthritis in the different compartments of the knee joint. Even if the patellofemoral joint (PFJ) is one of the most affected regions, the study on this subdivision is poorly conducted. Furthermore, the consequences, like the appearance of osteophytes, and the progress of OA in PFJ are different from the other knee compartments [9]. Patellofemoral joint osteoarthritis has clinical importance because it is related to the appearance of knee pain, decreased function and disability [14].

The aim of this study is to evaluate the role of bone changes and the effect of the lack of estrogen on the development of OA in the patella in a postmenopausal murine model by analyzing in vivo micro-CT scans.

In the previous animal experiment, postmenopausal situation was simulated by ovariectomy (OVX). In fact, OVX animals provide a useful experimental model for the evaluation of the effects of female hormone and estrogen-like substances and for the representation of postmenopausal situation. Then, osteoarthritis was induced by injection of iodoacetate. Furthermore, one mice
group received bisphosphonates, in order to inhibit bone changes, and estrogen was given to another cohort to inhibit the effect of the OVX. Therefore, the goal of this study can be divided into three main objectives. First of all, the analysis of OVX mice model aims to evaluate whether the estrogen deficiency makes the joint more susceptible to OA changes. Furthermore, the study on the effect of the replacement of the estrogen tries to estimate the consequence of estrogen treatment on the severity of OA. At last, the study on the effect of the injection of bisphosphonate intends to investigate the implication of bone changes.

**Material and methods**

**Animal model**

This analysis is based on a murine experimental model previously carried out. For that study model, 4 cohorts of 8 mice were used. At the start of the experiment they were 12 weeks old. At this baseline, the groups were subjected either to sham operation or to ovariectomy, in order to simulate postmenopausal effects. Osteoarthritis was induced in the right leg of the mice by injection of iodoacetate, which inhibits the chondrocyte metabolism and the matrix synthesis, while the left leg, where saline was administered, was used as control. Two of these groups received treatment either with bisphosphonates, in order to inhibit bone changes, or estrogen replacement, to inhibit the effect of the OVX.

In vivo microCT scans were performed in all mice just before the operation and after 3, 6, 9 and 12 weeks. X-ray images from different angles were obtained for both knees. After sacrifice, the legs were isolated and cut into sections. Each section was then stained with Safranin-O-Green for histological examination.

**Patella analysis**

Both analyses of the microCT scans (bone changes) and of the histological sections (cartilage changes) were executed in order to compare the OA effects in both structures and to investigate the role of bone in the OA patella.

**Analysis of scans**

**Bone changes**

The analysis of bone changes consists of the following steps:

**Reconstruction:** the reconstruction from the x-ray images (Fig. 1A) to a 3D datasets (with 2D cross sections (Fig. 1B)) was previously performed for each knee.

**Region of interest:** only the area with the femur and the patella was selected (Fig. 1C).
Rotation of the patella: the patella was rotated to make the major axis vertical (Fig. 1D). This operation was performed in order to facilitate the next procedures.

Segmentation: the image was segmented in order to convert it from a grey scale image to black and white one (Fig. 1E).

Isolation of patella: the patella was isolated (Fig. 1F) in order to focus the analysis on this bone structure.

Separation of bone structure: the different components of the bone (cortical, trabecular and marrow) were then separated (in Fig. 1G cortical bone in black, trabecular bone in light grey and marrow in dark grey) in order to study the specific changes in each structure.

Selection of subchondral bone: since the subchondral cortical bone is the only structure in contact with the cartilage layer, it was selected (Fig. 1H) in order to investigate whether the changes in this area are more severe.

Calculation of parameters: the bone parameters (bone volume, SMI and 3D thickness) were calculated for the entire patella (Fig. 1F), for the different structures (cortical and trabecular bones, Fig. 1G) and for subchondral cortex (Fig. 1H). SMI indicates the curvature of the structure: the relative “rod-like” or “plate-like” appearance of the bone (where the theoretical value for a perfect cylinder, convex structure, is 3 and a perfect plate, flat structure, is 0). For a structure with both plates and rods of equal thickness, the value is between 0 and 3, depending on the volume ratio between rods to plates. The negative SMI values originate from the very dense samples with a concave plate-like structure, sometimes referred to as a spherical cavity. The concave surface induces a negative value of the surface area derivative used in the calculation of SMI [8].

Additionally, for the trabecular bone, the bone volume fraction was calculated by the following formulas:

\[
BV / TV = \frac{\text{trabecular bone volume}}{\text{bone volume of trabecular bone and marrow}}
\]

The presence of osteophytes was also checked on the border of the patella (Fig. 3A). In microCT only bony osteophyte can be detected.

The detailed protocol of this analysis is shown in Appendix 1.
**Histology**

*Cartilage changes*

A histological analysis (Fig 2) of the cartilage damage was also performed to validate the presence of OA and to show the severity of the disease. For this study, the “Pritzker system” ([grading and staging](#)) [17] was used. This method is based on six grades (Fig. 4), which evaluate the depth of the lesion into the cartilage layers, and four stages, which estimate the horizontal extent of OA damage over the joint surface (Fig. 4). The standards and features of the method are presented in Appendix 2. The OA cartilage score is then a combination of grade and stage determined by the simple formula: score = grade \times stage. The presence of osteophytes was also checked in the sections (Fig.3B).
Figure 3: Presence of osteophytes detected by microCT (A) or histological analyses (B).

Figure 4: Examples of calculation of score with Pritzker method: on the left, the cartilage damage has a grade of 1 and the extent of the lesion is less than 10% (stage 1). The final score is then 1. On the right, the cartilage is severely damaged (3.5) and the lesion lies between 25 and 50% of the horizontal surface (stage 3). Then, the score is 10.5.
Statistical analysis

Statistical calculations were performed using GraphPad Prism for Windows, version 5.01 (GraphPad Software Inc.). A computer database containing all measured results was created. The data were compared using non parametric test (Kruskal-Wallis test). Groups and hypotheses about the groups were determined a priori and values were consequently analyzed by appropriate comparison.

Results

Analysis of scans

Bone changes

First of all, it is necessary to point out that some scan images were excluded due to imperfection of scanning. As previously mentioned, the calculation of the bone parameters was carried out for the entire patella, for the cortical and trabecular bones and for the subchondral cortical bone. However, for clarity, in this chapter just the entire patella (bone volume), the trabecular (bone volume fraction) and the subchondral cortical bone (cortical thickness) were taken into account. All the graphs of the bone parameters for each structure are presented in Appendix 3.

Effect of estrogen lack on the OA changes

The most significant changes of bone parameters occurred 3 weeks after operation in each bone structure. In Figure 5, entire patella is taken into consideration. An increase of bone volume was observed in all bone structure. It can be due to the aging of the mice. By considering the 3D thickness a thinning of structure was noticed in the OVX+IA group at 3 weeks.

The next figure (Fig. 6) represents the thickness of the subchondral cortical bone. In this case, it is evident that at the third week post surgery the sham+IA and both the OVX groups have a thinner cortical thickness.

However, in the trabecular bone OVX+IA led to increase of bone volume fraction after the third and sixth weeks post operation (Fig. 7). Thereafter, the values decreased again to reach no significant difference at the last week. The same effect was observed both in the bone volume and in the trabecular thickness (graphs shown in Appendix 3).

In summary, OVX+IA decreases the thickness of subchondral bone structure. This thinning is stronger at the third week post operation. Strong increase was observed in the trabecular bone parameters at the third week.
Figure 5: Estrogen depletion effect: bone volume and % change in bone volume of the entire patella.

Figure 6: Estrogen depletion effect: cortical thickness and % change of cortical thickness of the subchondral cortical bone.

Figure 7: Estrogen depletion effect: bone volume fraction and % change of bone volume fraction of the trabecular bone.
Effect of estrogen replacement treatment on the severity of OA

In Fig. 8 the effect of the replacement of estrogen in the entire patella is shown. It is clear that by adding the hormone there is an overall increase of bone volume, since both estrogen treated groups have higher values than the groups without estrogen. Also in the subchondral cortical bone the values of the estrogen-treated groups are higher than the ovariectomized ones (Fig. 9). The increase in bone volume is confirmed in the trabecular bone volume fraction (Fig. 10). However, in the trabecular bone SMI has lower values in the group treated with estrogen respectively after 6 weeks and 12 weeks (graphs shown in Appendix 3).

In summary, estrogen inhibits the effect of OVX and OVX+IA on the thinning of the subchondral structure. However, the hormone does not only inhibit bone loss but it causes an increase in bone volume.

Figure 8: Estrogen replacement therapy: bone volume and % change in bone volume of the entire patella.

Figure 9: Estrogen replacement therapy: cortical thickness and % change of cortical thickness of the subchondral cortical bone.
Role of bone in OA

Fig. 11 shows the change of bone volume in the entire patella. It was noticed that the bisphosphonate groups have higher bone volume compared to the no treated groups. This effect was also noticed in the thickness, where 3 weeks after operation the injection of bisphosphonate led to a thickening of the bone (graphs shown in Appendix 3). In the same way, by considering just the subchondral cortical bone a decrease of the thinning of the structure was experienced when the group was treated with BP (Fig12). However, in the trabecular bone the BP overcompensates the OVX+IA effect in the bone volume fraction (Fig13). In the trabecular thickness the BP inhibit the strong increase of OVX+IA at the third week post surgery.

In conclusion, bisphophonates inhibits the effect of OVX+IA on the thickness of subchondral bone structure and on the bone volume structures. However, in some cases this effect is overcompensated: bisphophonates does not only inhibit bone erosion but activates bone formation.
**Histology**

**Cartilage changes**

The histological analysis of the cartilage layer validated the presence of OA in the animal model: the cartilage was more severely damaged in the groups with induced OA compared to saline groups (Fig. 14). The highest score among the cohorts was represented by the ovariectomized groups, in particular the one treated with iodoacetate (3.59±1.24). The ovariectomized groups treated with bisphosphonate had the lowest score, in particular the one with saline (0.51±0.50). Significant changes were observed between the ovariectomized group and the ovariectomized cohort treated with bisphosphonate. Thus, the inhibitor of bone erosion leads to decreased cartilage damage. A decrease in cartilage damage was also noticed in the OVX group treated with estrogen with respect to the OVX cohort, although not significant. Thus the replacement of estrogen may lead to decreased cartilage erosion.
Osteophytes

The presence of osteophytes was checked both in the microCT and in the histological analyses. In histology it was clear that the incidence of osteophytes occurred in the cohorts treated with the osteoarthritic trigger (Fig. 15). Furthermore, in the groups treated with IA the appearance of bony osteophytes was observed. In the bisphosphonate group with iodoacetate, less bony osteophytes were present.

Figure 15: Presence of osteophytes analysed in the histology (cartilage osteophytes) and detected with the micro-CT (bony osteophytes).
Discussion

In order to evaluate the role of bone changes and the effect of the lack of estrogen on the development of OA patella in a postmenopausal murine model, micro-CT scans and histological analyses were performed. The results showed that the lack of estrogen has a severe effect on the thickness of the cortical and subchondral cortical bone, especially 3 weeks after operation. By adding bisphophonate or replacing the hormone this effect is compensated especially in the bone volume and bone thickness.

The present analysis was performed on the patellofemoral joint, one of the most affected knee structures. However, this compartment is not often studied. Moreover, the occurrence of knee pain and the development of OA are different from those observed in tibia-femoral joint OA. For this reason, an analysis of this compartment and a comparison with the results obtained from other joint areas is fundamental to interpret the progression of the disease for this specific region.

It is important to analyze the present results in relation with a study on the tibia compartment previously performed. For that study, the bone changes were analyzed by considering the epiphyseal trabecular bone and the subchondral plate, both medial and lateral. The results showed that OVX in combination with an osteoarthritic trigger (IA) leads to thinning of subchondral plate (medial and lateral) and to cartilage damage in the medial region. For this reason, it was concluded that estrogen deficiency makes the joint more susceptible to OA changes. Furthermore, it was found out that the replacement of estrogen leads to an increase of subchondral plate thickness. However, the analysis demonstrated that there is no correlation between bone and cartilage changes.

My study was conducted in a similar approach to the tibia one. The analysis of bone parameters were performed on the entire patella structure, on the trabecular bone and on the cortical bone. Additionally, a specific investigation was done by considering the subchondral cortical bone. This selection aimed to take the bone structure below the cartilage layer into account. Hence, it is possible to examine if the osteoarthritic changes are more severe in this bone structure. The patella results are comparable to those obtained in the tibia analysis. OVX+IA leads in both cases to a weakening of the joint tissues. The replacement of the hormone counteracts this effect, especially by thickening the cortical structure. Estrogen replacement also overcompensates the thinning of bone structure in the OVX+IA groups both in the tibial medial side of the subchondral plate and in patella bone structures, especially in the trabecular bone.

However, while in the tibia OVX does not have an effect on the trabecular bone, in the patella this structure responds more actively to the OVX treatment.

A research on the presence of osteophytes was carried out both in the microCT and in the histological analyses. Thus, it was possible to distinguish between bony and cartilage osteophytes.
Moreover, a histological analysis of the cartilage damage was performed. It was found that the combination of OVX with IA leads to the highest damage score. The addition of bisphosphonate or estrogen decreased the severity of the damage. This finding can be caused by indirect or direct effect of these treatments on the cartilage. On one side, the changes of the subchondral cortical bone can decrease the damage of the cartilage rising above (indirect). On the other side, the bisphosphonate and the hormone can also have an effect on the chondrocytes since the presence of receptors was also found in the cartilage tissue (direct).

There are several limitations of the present study. The most important one is the detection of a possible defect in the scanner. For this reason, some of the scan images had to be excluded from the analysis as well as some imperfect ones. Thus, the analysis could not have enough statistical power in some cohorts. Furthermore, all the parameters have also been calculated in relation to the baseline to avoid the possible errors in the results caused by biological variation. Another issue is the hormone level in the OVX+E group. It did not only inhibit bone loss, but led to an increase in bone volume. For this reason, the estrogen may have an anabolic effect or the administered estrogen may have been too high, resulting in supraphysiological levels.

Future studies evaluating the bone and cartilage changes on the side of the femur of the patella femoral joint can improve and confirm the current results of this study.

**Conclusion**

In conclusion, it was found that ovariectomy in combination with IA leads to severe changes in the bone structure and to the highest cartilage damage. Thus, OVX makes the joint tissues more susceptible to OA changes. The estrogen replacement therapy restores the bone changes, induced by OVX+IA, but the hormone also overcompensates this effect in bone structures. Furthermore, the estrogen replacement decreases the damage in cartilage. The effect is also observed in the diminished incidence of bony osteophytes in the treated groups. For these reasons, it was concluded that estrogen replacement therapy counteracts the OVX changes. At last, bisphosphonates restores bone changes, due to OVX and OVX+IA, even overcompensates. Moreover, it significantly decreases the cartilage damage induced by OVX and OVX+IA and leads to a significant decrease of bony osteophytes formation. The inhibitor of bone erosion has then an effect on the OA changes.
References


Appendix 1
Protocol of patella scan analysis

Protocol of reconstruction of in-vivo mice scans

The protocol for the reconstruction of the scans is nearly the same as the one used for the tibia analysis.

Result file: .bmp grey scale file

Protocol Rotation of the scan images

After selecting just the area with the femur and the patella it is advised to rotate the image.

- Program CTRotate

Datasets
- Source: take the datasets from the appropriate folder.
- Destination: put the datasets into a new folder.

Rotation angle
- Choose the rotation angle in order to make the head of the femur or the major axis of the patella horizontal.

Start converting
• Program 3DCalculator

Batch process viewer
  - Reslice: select mode 0.

• Program CTRotate

Datasets
  - Source: take the previous rotated datasets from the appropriate folder.
  - Destination: put the datasets into a new folder.

Rotation angle
  - Choose the rotation angle in order to make the major axis of the patella vertical.
    Also the femur can be chosen as a reference: when the patella has the biggest size, the
    femur should be vertical.

Start converting

• Program 3DCalculator

Batch process viewer
  - Reslice: select mode 1.

Result file: .bmp grey scale file

Protocol Segmentation of the rotated images

• Program 3DCalculator

Side view (cross sections)
  - Section: to scroll through the sections.
- **Upper and lower**: to limit the number of sections. Choose around 220 sections. This number of sections has to be consistent for all the datasets.

**Region of interest (ROI)**
- Make the dataset smaller by selecting the region of interest which includes the patella and the femur. Select the option *Same for all layers*.

**Save**
- Save the dataset by selecting the options *Reduce file to ROI bonds* and *Use upper and lower limit*.

**Batch process viewer**
- **Add**: add the datasets.
- **Threshold**: select
  - *Pre-threshold*: Low: 0 High: 220.
  - *Gradient cut-off factor*: Low: 0.97 High: 0.99.
  - *Gradient factor*: 4.
  - *Gauss radius*: 1.5.
  - *Loose particles*: don’t remove.
  - *Compensation for thin trabeculae*: no compensation.

Result file: .bmp black and white image

**Protocol Isolation of patella**

- **Program CT Analyzer**

**Region of interest**
- Select the patella by choosing the appropriate region of interest.
Limit
- Select 220 sections.

Save
- Save the datasets and the region of interest.

- *Program Matlab*
  - Use a Matlab code to make the size of the image a multiplier of 8.

Result file: _pat.bmp file

Protocol Separation of the cortical and trabecular bone

- *Program PrStackbot*
  - *Add*: Add the datasets.
  - Try different values of the *Upper values* and *Lower values*. Check the correctness of separation of the cortical and trabecular bone at different sections. Select *Visualize: on.*
  - For the patella insert the values: *Upper values*: 12 15
    - *Lower values*: 13 12.
  - *Visualize*: select *off*.
  - *Separate*: select *on*.
  - *Start*.

Result file: _pat_trab.bmp file
Protocol Calculation Parameters

- Program 3D Calculator

**Batch process viewer**
- *SetRes*: select this command to change the resolution.
- *Add*: add the datasets (the entire patella and the separated one).
- Insert the value of 8.88.
- *Start batch.*

➢ Entire patella:

- Program 3D Calculator

**Batch process viewer**
- *Add*: add the datasets.
- *Bone, Euler, SMI, TbTh*: select mode 0.
- *Start batch.*

- Program Extract Parameters

**Project directory**
- *Group, data*: select the folder of the results (data folder). Since all of the subfolders are included it is necessary to delete the unused folders.
- *Select parameters*: select SMI, 3D Direct Thickness (Mean and Distribution Bin Size: 25), Euler, 2D Parfitt (Total and Per slice).

➢ Separated patella:

- Program 3D Calculator

**Color tuning**
- *Binary*: select the appropriate range of color
  - Trabeculae (149): 105-155.
  - Cortex (0): 0-30.
  - Trabecular bone (149) and bone marrow (99): 80-160.
Batch process viewer

- Add: add the datasets.
- Bin+ROI.
- Bone, Euler, SMI and TbTh: select mode 0.

Program Extract Parameters

Project directory

- Group, data: select the folder of the results (data folder). Since all of the subfolders are included it is necessary to delete the unused folders.
- Select parameters: select SMI, 3D Direct Thickness (Mean and Distribution Bin Size: 25), Euler, 2D Parfitt (Total and Per slice).

➢ Subchondral cortical bone:

- Program CT Analyzer

  - Open the separated patella datasets

  Region of interest

  - Choose the rectangular selection (height of 0.75-0.80 mm) and isolate just the subchondral cortical bone area.

Limit

- Select 220 sections.

Save

- Save the datasets and the region of interest.

- Program Matlab

  - Use a Matlab code to make the size of the image a multiplier of 8.

- Program 3D Calculator

  Color tuning

  - Binary: select the appropriate range of color

    Cortex (0): 0-30.
Batch process viewer
- **Add**: add the datasets.
- **Bin+ROI**.
- **TbTh**: select mode 0.

**Program Extract Parameters**

Project directory
- **Group, data**: select the folder of the results (data folder). Since all of the subfolders are included it is necessary to delete the unused folders.
- **Select parameters**: select SMI, 3D Direct Thickness (Mean and Distribution Bin Size: 25), Euler, 2D Parfitt (Total and Per slice).

Result file: .txt file

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Compiled at : 12/11/2007
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Appendix 2
Pritzker method – grading and staging

Grades

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Stages

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<td>&lt;10%</td>
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<tr>
<td>2</td>
<td>10–25%</td>
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<td>25–50%</td>
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<td>4</td>
<td>&gt;50%</td>
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</table>

Stage = extent of joint involvement.
Appendix 3
Results graphs

ENTIRE PATELLA

Bone volume

[Graphs showing bone volume and percentage change in bone volume for different conditions: Sham + saline, Sham + IA, OVX + saline, OVX + IA, OVX + E + saline, OVX + E + IA, OVX + BP + saline, OVX + BP + IA.]
SMI

Graphs showing SMI over different conditions and time points.

1. Sham + Saline
2. Sham + IA
3. OVX + Saline
4. OVX + IA
3D thickness

![Graph 1: 3D Thickness](image1.png)

![Graph 2: % Change in 3D Thickness](image2.png)

![Graph 3: 3D Thickness](image3.png)

![Graph 4: % Change in 3D Thickness](image4.png)

![Graph 5: 3D Thickness](image5.png)

![Graph 6: % Change in 3D Thickness](image6.png)
CORTICAL BONE

Bone Volume

![Graph of Bone Volume](image1)

![Graph of % Change in Bone Volume](image2)

![Graph of Bone Volume](image3)

![Graph of % Change in Bone Volume](image4)

![Graph of Bone Volume](image5)

![Graph of % Change in Bone Volume](image6)
TRABECULAR BONE

Bone Volume

Bone Volume

% Change in Bone Volume

% Change in Bone Volume
Trabecular Thickness

% Change in Trabecular Thickness

Trabecular Thickness

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA

OVX+Saline
OVX+IA
OVX+BP+Saline
OVX+BP+IA
Bone Volume Fraction

### Bone Volume Fraction

<table>
<thead>
<tr>
<th>Time</th>
<th>Sham + Saline</th>
<th>Sham + IA</th>
<th>OVX + Saline</th>
<th>OVX + IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0</td>
<td>0.25</td>
<td>0.27</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>t3</td>
<td>0.30</td>
<td>0.31</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>t6</td>
<td>0.35</td>
<td>0.36</td>
<td>0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>t9</td>
<td>0.39</td>
<td>0.40</td>
<td>0.41</td>
<td>0.42</td>
</tr>
<tr>
<td>t12</td>
<td>0.43</td>
<td>0.44</td>
<td>0.45</td>
<td>0.46</td>
</tr>
</tbody>
</table>

### % Change in Bone Volume Fraction

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<tr>
<td>t0</td>
<td>-20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t3</td>
<td>-15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t6</td>
<td>-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>t9</td>
<td>-5</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
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SUBCHONDRAL CORTICAL BONE

Cortical thickness