Bone mineral density distribution in early osteoporotic bone

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Introduction

Osteoporosis is caused by an imbalance in the remodelling process where more bone is removed than is rebuilt through disturbed cellular activities. Such imbalance induces changes in the bone's intrinsic components, namely the mineral and organic phases, which are responsible for its strength and stiffness, consequently leading to degraded mechanical properties. One of the processes disturbed during osteoporosis is the mineralization process, which is responsible for depositing minerals in the collagen matrix of the bone tissue thus leading to localized structural defects and subsequent mechanical degradation [1].

Quantitative backscattered electron imaging (qBEI) is an *in vitro*, high-resolution technique that allows visualization and quantification of the degree of bone matrix mineralization. It determines bone mineral density distribution (BMDD), which reflects the age and size distribution of the bone packets within the bone tissue [2].

The objective of this study is to show how the onset and progress of osteoporosis affect the mineralization process by quantitatively determining the BMDD.

Methods

1. Osteoporosis model of Ovariectomized mice: (BALB/c) induces an osteoporotic phenotype, which resembles that seen in post-menopausal osteoporosis in humans. **Eight weeks old BALB/c** mice were subjected to ovariectomies (OVX) or to sham surgery (SHAM) during which the ovaries were exteriorised but replaced intact by the operator and thus used as a control group. Lumbar vertebrae (L3) were collected at the time of sacrifice and used for the qBEI study. Six experimental groups were evaluated according to the sacrifice time line, with **5 OVX and 5 SHAM** in each as shown below.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Disease duration	2 wks	1 mth	2 mth	3 mth	5 mth	7 mth

2. qBEI & BMDD analysis:
qBEI was performed using a Scanning Electron Microscope (SEM) fitted with a backscattered detector, using a voltage of 15kV and a constant magnification of 100x, leading to a resolution of 0.93µm/pixel.
The calibration procedure followed the method outlined by Roschger et al (1998) [2], using hydroxyapatite (HA), Magnesium oxide (MgO) and Silicon dioxide (SiO2) as reference materials, from which a linear relationship between gray level intensity and calcium percentage was obtained.
A custom MATLAB script was developed to analyze the qBEI images, assigning a calcium percentage to each pixel, according to the calibration procedure. BMDD histograms (resolution: 0.13% Ca) were obtained for each sample and averaged within each group. A Gaussian curve-fit was used to model the histograms and compute BMDD parameters (Ca_{MEAN}, Ca_{PEAK}, Ca_{WIDTH}, Ca_{LOW} and Ca_{HIGH}). Ca Low and Ca High represent the percentage of bone, in OVX samples, located in the 5th and 95th percentile of SHAM BMDD, for each group.

Results

Histograms of BMDD were used to calculate the BMDD parameters as shown in fig. 1. The histograms were processed based on the qBEI images as shown in fig 2. The calculated parameters of OVX and SHAM groups are revealed in table 1. Groups 1 and 2 OVX (the very early stage of Osteoporosis) show significant differences (ANOVA $p<0.05$) in Ca_{Low} and Ca_{WIDTH} demonstrating that osteoporosis causes significant changes to the bone mineralization density distribution during the very early stage of the disease. A significant change in the Ca_{Low} as a function of disease duration is also demonstrated in the OVX groups.

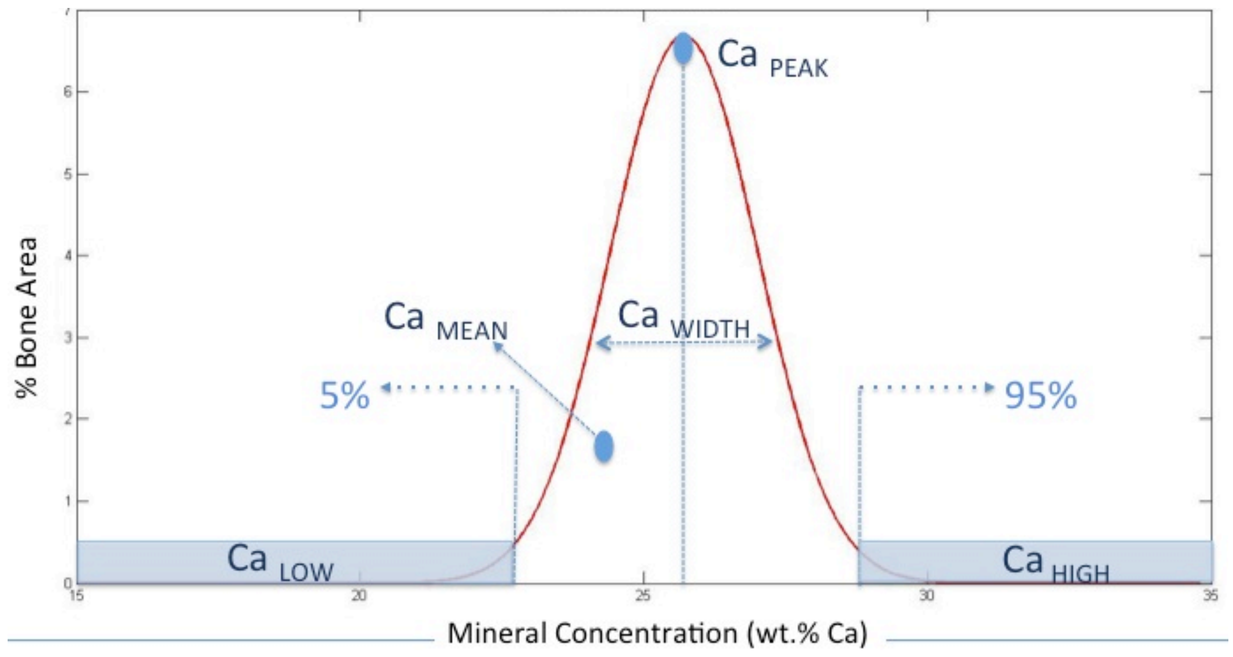

Fig. 1 BMDD histogram showning the calculations of the BMDD parameters. Ca_{MEAN} is the weighted average Ca concentration of the mineralized tissue area, obtained from the integrated area under the BMDD curve., **Ca_{PEAK}**, the peak position of the histogram, which indicates the most frequently measured calcium concentration (Ca value with the highest number of pixels) in the bone, **Ca_{WIDTH}**is the width at half-maximum of the BMDD histogram curve indicating the heterogeneity of mineralization either caused by the co-existence of differently mineralized bone matrix, **Ca_{LOW}**, the % of bone area that is mineralized below the 5% of the reference range which corresponds to the amount of bone area passing primary mineralization, **Ca_{HIGH}**, the % of bone area that is mineralized above the 95% of the reference range [2] and therefore corresponds to the amount of bone area having achieved full mineralization (reached a plateau level of mineralization).

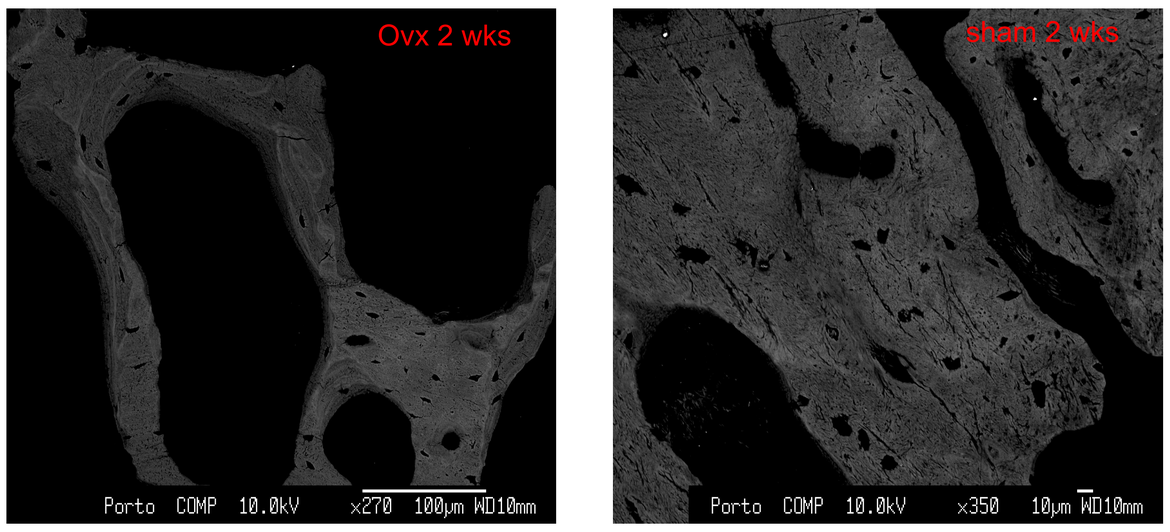

Fig.2 qBEI grey scale images obtained for OVX and SHAM groups at 2 weeks of disease duration

Table 1 BMDD calculated parameters for the OVX and SHAM groups during the onset and progress of the disease

Groups	Ca_{MEAN} [wt.% Ca]		Ca_{PEAK} [wt.% Ca]		Ca_{WIDTH} [wt.% Ca]		Ca_{LOW} [% bone area]	Ca_{HIGH} [% bone area]
	OVX(STD)	SHAM(STD)	OVX(STD)	SHAM(STD)	OVX(STD)	SHAM(STD)	OVX	OVX
1	25.08±0.95	24.81±0.86	25.79±1.02	25.62±0.86	3.76±0.30	4.16±0.65	4.04	9.47
2	24.35±0.20	24.86±0.12	25.11±0.28	25.44±0.02	3.94±0.63	3.57±0.00	7.62	5.96
3	24.57±0.45	24.94±1.00	25.17±0.63	25.61±0.90	3.76±0.61	4.23±0.30	4.94	4.10
4	24.84±0.49	25.05±0.48	25.36±0.42	25.73±0.58	3.39±0.28	3.50±0.52	4.11	4.29
5	25.48±0.24	25.33±0.10	26.00±0.31	25.96±0.17	2.90±0.08	2.95±0.19	4.41	10.55
6	25.60±0.20	25.65±0.21	26.11±0.14	26.20±0.22	3.21±0.31	3.35±0.23	5.45	7.10

Conclusions

Our results show that the % bone area that passes primary mineralization (Ca_{Low}) is significantly altered along the course of the disease as shown in OVX groups 1, 2 and 6. Although the other parameters display sever changes, they are not statistically significant and a higher sample number is therefore required for future work. The results of this study for the Ca_{MEAN} and Ca_{PEAK} are within the range reported by other researchers [1-3]. In conclusion, our study confirms that osteoporosis causes significant changes to the BMDD during the very early stage of the disease manifestation.

References

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The authors declare no conflict of interest in this study.