Published in final edited form as:

Arch Oral Biol. 2010 August ; 55(8): 599–605. doi:10.1016/j.archoralbio.2010.05.011.

Estrogen Deficiency Increases Variability of Tissue Mineral Density of Alveolar Bone Surrounding Teeth

Matthew S. Ames^a, Semi Hong^a, Hye Ri Lee^a, Henry W. Fields^a, William M. Johnston^b, and Do-Gyoon Kim^{a,*}

^a College of Dentistry, Division of Orthodontics, The Ohio State University, Columbus, Ohio, USA

^b College of Dentistry, Division of Restorative and Prosthetic Dentistry, The Ohio State University, Columbus, Ohio, USA

Abstract

Objective—Estrogen deficiency increases bone remodeling leading to increased variability of tissue mineral density (TMD). Due to the functional demands of mastication, alveolar bone around teeth is inherently a highly remodeled region of bone tissue with a highly variable distribution of TMD. This study investigated the effect of estrogen deficiency on the TMD distribution of alveolar bone.

Design—Using three-dimensional micro-computed tomography images of sham surgery (Sham) and ovariectomized (OVX) rat mandible sections, alveolar bone region (AB) and control bone region (CB) of interest were isolated. Based on histograms of gray levels equivalent to TMD values, mean (Mean), standard deviation (SD) and coefficient of variation (COV=SD/Mean) were computed. Fifth and 95th percentile gray level values were also obtained (Low₅ and High₅, respectively). Absolute value of percentage (%) differences of the gray level parameters between AB and CB regions were computed.

Results—Both SD and COV were significantly higher in AB region than those in CB region for all specimens of both Sham and OVX groups ($p < 0.001$). The mean values of % differences for SD were moderately higher ($p < 0.073$) and those for COV and Low₅ were significantly higher for the OVX group than for the Sham group ($p < 0.04$).

Conclusions—Higher variability of mineralization observed in AB of OVX group indicates that estrogen deficiency amplifies the active bone remodeling of AB already present due to mastication. These findings provide an insight that the increased variability of TMD induced by estrogen deficiency may compromise the mechanical stability of the tooth-bearing alveolar bone.

Keywords

Rat; Microcomputed tomography; Estrogen; Bone remodeling; Variability; Mineralization

*For correspondence: Do-Gyoon Kim, Ph.D, Assistant Professor, Research Program Director, Division of Orthodontics, College of Dentistry, The Ohio State University, 4088 Postle Hall, 305 W. 12th Ave, Columbus, OH 43210-1267, USA, kim.2508@osu.edu, Tel) (614) 247-8089, Fax) (614) 688-3077.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Estrogen deficiency leads to disproportionate bone remodeling by increasing bone resorption more than bone formation^{1–2}. This altered bone turnover activity can result in the net bone loss and reduction of bone quality that characterizes post-menopausal osteoporosis^{3–5}. While the effects of osteoporosis on decreased bone quality and increased fracture risk of long bones and vertebra are well established, the effects on jaw bone are less understood. Oral health status has a dramatic impact on quality of life⁶. If post-menopausal osteoporosis has significant oral consequences, then it is possible that quality of life can be compromised beyond the commonly associated skeletal fractures. Many studies have found osteoporotic effects on the onset and progression of periodontal disease and subsequent tooth loss^{7–12} and in delayed alveolar wound healing^{13–14}. Also, orthodontic tooth movement has been found to be more rapid, and subsequently more unstable, in the absence of estrogen^{15–16}. These findings suggest that estrogen deficiency has a substantial effect on oral bone properties.

As tooth-bearing bones, the maxilla and mandible are arguably more complex than other bones. The functional demands of mastication on alveolar bone surrounding teeth increase the complexity of both the structural makeup and subsequent mechanical properties. Significant regional variation in oral bone quality has been observed as the alveolar bone demonstrates higher rates of remodeling and subsequent decreased mineral density and mechanical property compared to bone more distant from teeth^{17–18}. However, the specific effects of estrogen deficiency on the regional variation of oral bone quality have not been clearly elucidated.

Bone tissue mineral density (TMD) distribution has been shown to change significantly in the presence of disease states or clinical treatments that effect bone metabolism¹⁹. Bone tissue is composed of individual cortical osteons and trabecular bone packets that are produced at different points in time. These heterogeneous compartments of bone matrix reflect bone turnover activity, mineralization kinetics and average bone matrix age^{19–21}. With untreated, disease-free bone as a reference, bone with high turnover activity has a higher proportion of less mineralized tissue and subsequent increased variability of TMD. Recently, radiographic imaging technology based studies have been able to describe the local variation in TMD using a histogram of gray level frequency distribution^{22–24}.

The objective of this study was to investigate the effect of estrogen deficiency on TMD distribution of alveolar bone. We hypothesized that estrogen deficiency would amplify the regional variation of TMD distribution in mandibular bone. This hypothesis was tested by examining 1) intra-specimen regional variation for TMD distribution parameters and 2) inter-group differences of the TMD parameters between normal and estrogen deficiency groups. The TMD of mandibular bone was assessed using three-dimensional micro-computed tomography (3D micro-CT) images of sham surgery and ovariectomized rat models.

Materials and Methods

Twenty 6-month old Sprague-Dawley female rats were utilized following experimental protocol approved by the Institutional Animal Care and Use Committee of The Ohio State University. Ten rats were bilaterally ovariectomized (OVX) and ten rats were subject to a sham surgery (Sham) at Harlan Laboratories (Harlan Laboratories Inc., Indianapolis, IN, USA). The rats received an intraperitoneal calcein (25mg/kg) injection 3 days prior to euthanization. At 2 months post-surgery, the rats were euthanized using an overdose of pentobarbital sodium (100 mg/kg). One side of the mandible was randomly chosen from each rat and tooth-bearing 5 mm mandibular bone sections were obtained using a low speed saw with two parallel diamond blades cutting under water irrigation (Fig. 1, intact). Cuts were made perpendicular to the occlusal plane of molars in the bucco-lingual direction. One cutting surface of each bone section

was polished and calcein labels on the surface of bone were observed under epifluorescence to verify turnover activity.

The mandible specimens were scanned and reconstructed using 3D micro-CT (Inveon, Siemens, Malvern, PA, USA) with a 20 μm voxel size. Bone voxels were segmented from non-bone voxels using a heuristic algorithm consisting of five successive detecting procedures: normalization, edge detection, continuity crawling, final thresholding, and connectivity testing as previously published^{25–26}. A CT attenuation value (gray level), which is equivalent to the tissue mineral density (TMD) value²⁷, for each voxel was maintained (Fig. 2). The gray levels were scaled by 16 bit. The total bone volume of mandibular bone was computed by multiplying the number of total bone voxels of the micro-CT image by a unit voxel volume ($8 \times 10^{-6} \text{ mm}^3$). The 3D images of each specimen were manipulated using imaging software (ImageJ, National Institutes of Health, USA) to isolate the regions of interest (ROI) (Fig. 1). Multiple isolating techniques were utilized to separate the teeth from the mandible. The isolated teeth image of each specimen was three-dimensionally dilated by 10 voxels (200 μm), binarized and then multiplied by the separated mandible segment. This process provided a 3D alveolar bone region (AB) within 200 μm from the tooth roots (Fig. 1b). Sebaoun et al.²⁸ found that the distance of bone turnover is up to 51 μm from periodontal ligament surface (PDL) using calcein labels for rat up to 7 weeks after *in vivo* injection. The 200 μm region we used in the current study was assigned from the tooth surface. Since the PDL width of a rat is approximately 150 μm ²⁹, the 200 μm distance from the tooth surface we used is valid to examine the bone remodeling region (200–150 = 50 μm) around teeth. A control bone region (CB) was isolated by three-dimensionally eroding the separated mandible segment 10 voxels from all internal and external bone borders. As the rat has quite limited naturally occurring cortical bone remodeling inside mandibular bone^{30–31}, the CB represented a region of mature bone isolated from probable influences of remodeling or modeling at the internal and external borders of rat mandible. The voxel count of each separated mandible segment and the volumes of each isolated region were computed for comparison.

Histograms were generated to analyze the gray level distribution parameters (Fig. 3a). Gray level mean (Mean) was computed by dividing the sum of gray levels by the total voxel count. Standard deviation (SD) of gray level distribution was obtained and coefficient of variation (COV) was calculated by dividing SD by Mean. Fifth and 95th percentile gray level values were also obtained (Low₅ and High₅, respectively). Absolute value of the percentage (%) differences of the gray level parameters between AB and CB regions were computed by a formula of $(|AB-CB|)/CB \times 100$.

To demonstrate reliability of the method, three images were manipulated a second time and the second values of gray level parameters were compared with the original values using Shrout and Fleiss intra-class correlation coefficients (ICCs). Paired t-tests were used for intra-specimen comparison between AB and CB regions for the mean values of the gray level parameters for each group. Analysis of variance (ANOVA) was performed for the inter-group comparisons. Statistical analysis was performed with SAS (Cary, NC, USA) and the significance of difference was set at $p \leq 0.05$.

Results

Calcein labels confirmed the presence of newly formed bone tissue and demonstrated remodeling in the alveolar bone region (AB). The total volume of the mandibles, after digitally removing the teeth, was significantly smaller for the OVX group ($47.312 \pm 3.109 \text{ mm}^3$) than for the Sham group ($51.235 \pm 3.829 \text{ mm}^3$) ($p < 0.022$). The AB and CB were successfully isolated using the 3D micro-CT image of each specimen (Fig. 1) and these volumes were not significantly different from each other ($p = 0.469$).

The heuristic segmentation method utilized in this study provided the normal histogram distribution of gray levels (Fig. 3a). The mean values of Mean, Low₅ and High₅ of AB were significantly lower than those of CB for both Sham and OVX groups ($p<0.001$) (Table 1 and Fig. 3b). In contrast, the mean values of the standard deviation (SD) and coefficient of variation (COV) of AB were significantly higher than those of CB for both groups ($p<0.001$). The mean values of % differences between AB and CB accounting for the magnitude of regional variation were moderately higher for SD and significantly higher for COV and Low₅ in the OVX group than those in the Sham group ($p<0.073$, $p<0.04$, and $p<0.02$, respectively) (Fig. 4). The % differences of Mean and High₅ were not significantly different between Sham and OVX groups ($p>0.093$). Shrout and Fliess ICCs confirmed reliability of the nearly fully automated method used in this study showing more than 0.99 of correlation coefficient values for all gray level parameters.

Discussion

We found that the alveolar bone region (AB) had significantly lower TMD values (Mean, Low₅ and High₅) but more variability (SD and COV) of TMD than the control bone region (CB), independent of estrogen deficiency. A state of estrogen deficiency amplified these regional differences for the variability measures and for the TMD of the less mineralized portion (Low₅) while maintaining the TMD of the mean and highly mineralized portion (Mean and High₅). These findings indicate that bone remodeling increased by estrogen deficiency likely produces a higher percentage of immature bone, resulting in increased variability of mineralization. Alveolar bone plays an important role in sustaining the mechanical stability of teeth. Masticatory forces applied to teeth are directly transferred to alveolar bone through the periodontal ligament thus stimulating bone remodeling. This subsequent remodeling alters tissue properties of the alveolar bone compared to mandibular bone more distant from the teeth. It is well known that estrogen deficiency increases bone remodeling. As such, postmenopausal alveolar bone may have more altered tissue properties than disease-free alveolar bone. The current findings suggest that the change of TMD distribution is a detectable indicator for the alteration of tissue properties resulting from bone remodeling.

Regional variation of mandibular bone properties has previously been analyzed using microscopic observation based on histological sections of specimens. This traditional histological method inherently involved a destructive sectioning process to obtain a two-dimensional slice. In contrast, micro-CT is a nondestructive 3D imaging technique that has been widely used for bone tissue mineral research^{27, 32}. Resolution of micro-CT images is substantially higher than that of conventional clinical CT³³. As such, the detailed 3D image obtained by micro-CT technique could be used to investigate a limited small region including alveolar bone. Using the micro-CT based imaging technology, we successfully isolated a uniform portion of alveolar bone immediately surrounding the teeth.

The micro-CT image of bone also provides the CT attenuation value (gray level) that is determined depending on the amount of mineral within a bone tissue region corresponding to a 3D voxel. This tissue mineral density (TMD) is different from traditional bone mineral density (BMD) measured using two-dimensional dual X-ray absorptiometry (DXA). The rough resolution of DXA images can not quantify the tissue level mineral content as accurately as that assessed by micro-CT. The reproducibility of micro-CT based morphology and TMD analyses using rat alveolar bone model were recently evaluated³⁴. This evaluation demonstrated that micro-CT images can be utilized for 3D measurement of TMD of the small alveolar bone region as used in this study. In the current study, we demonstrated that the 3D micro-CT image has a sufficient resolution to directly compare the small regional variations of TMD in the mandibular bone. Our reliability test also confirmed that the nearly automatic

digital method used in the current study to obtain the TMD parameters was repeatable and eliminated the potential error and subjectivity that comes with manual selection.

Distribution of mineral density at the tissue level reflects bone mineralization following remodeling. Bone formation initiates to lay down organic osteoid and shortly thereafter begins primary mineralization^{19, 21}. About 50% of the mineralization potential is achieved, relatively rapidly, with primary mineralization³⁵. Secondary mineralization follows as a slow and gradual maturation of the mineral component. For instance, the primary mineralization of human bone tissue takes a few days and the secondary mineralization continues for more than 5 years²¹. As bone consists of tissue packets (i.e. osteons and trabecular packets) under these different ages of mineralization, distribution of TMD varies. High turn-over activity due to amplified bone remodeling increases the amount of less mineralized, new packets of bone, resulting in increased variability of TMD¹⁹. Exposure of alveolar bone to the direct stresses and strains from masticatory forces ultimately leads to high bone remodeling²⁸. Consistent with this observation, we found calcein labels in the AB, which indicate the newly mineralizing bone tissue. Taken together, it is likely that increased bone remodeling is responsible for the lower degree of mineralization and higher variability of TMD at the alveolar region (AB) compared with the mature control bone region (CB).

It is well known that postmenopausal estrogen deficiency increases bone remodeling³⁻⁵. For alveolar bone, the combination of estrogen deficiency effects and the mechanical masticatory load may give rise to more severe alteration of the TMD distribution. We found that the difference of variability parameters (SD and COV) of TMD between AB and CB increased more for the OVX group than for the Sham group. These findings indicate that estrogen deficiency has more effects on the AB than the CB of the OVX group. An increase in the magnitude of regional variation of the less mineralized portion of bone (Low₅) for the OVX group, and no difference of the TMD of the highly mineralized portion (High₅) between the groups, together suggest that the primary mineralization of newly formed bone tissue plays a more significant role in controlling the TMD distribution than the secondary mineralization of the pre-existing mature bone tissue.

The limitation of this study was the use of an animal model for estrogen deficiency. In this study, fully matured, 6 month old rats were used comparable with the human age of postmenopause. The OVX rat is the most popularly acceptable animal model to investigate the effects of postmenopausal estrogen deficiency on bone properties³¹. However, it is still controversial whether the OVX rat model can appropriately mimic the postmenopausal condition of human bone. In the current study, we found that the total volume of mandibular bone was significantly smaller for the OVX group than for the normal group. This result is consistent with observations from previous studies³⁶⁻³⁹ and helps demonstrate that the rats did realize estrogen deficiency effects.

The use of micro-CT is currently limited to animal studies because of its high radiation dose. Thus, it remains to be verified whether the micro-CT based results of this study can be used to explain, or even applied to clinical observation of postmenopausal patients. The finest resolution of clinical 3D cone beam CT (CBCT) is about 200 μm which is 10 times coarser than the 20 μm resolution of micro-CT used in this study. As such, further evaluation is required to confirm whether the micro-CT image based analysis of TMD distribution is directly applicable for the clinical CT image based diagnosis in practice.

The absolute value of current gray levels was different from that measured from a conventional clinical CT. We scaled the gray levels by 16 bit instead of 8 bit that is used for the conventional CT image. The higher scale resolution provides more accurate values to examine the gray level distribution⁴⁰. This process caused the absolute values of current gray level to be different

from the conventional CT values. In the current study, the gray levels were compared between regions (AB vs CB) in the same specimen and the absolute percentage difference between AB to CB in the same specimen was compared between Sham and OVX groups. Therefore, the inter-specimen variation of absolute values of gray level was not necessarily taken into account.

Increasing heterogeneity of TMD has been observed for bone with a high turnover rate^{20, 41–42}. In bone tissue models based on micro-CT, it was simulated that mechanical stiffness decreases up to 24% with an increase in tissue heterogeneity from 20% to 50%^{42–44}. Thus, it is likely that loading on the top of two adjacent bone tissues with different variability of TMD produces more deformation of the bone tissue that has higher TMD variability. If the regional difference of variability increases, more deformation would develop in the bone tissue region having higher TMD variability. We found that the alveolar bone tissue adjacent to teeth had higher TMD variability than the control bone tissue and this regional variation was increased in the OVX group. This result suggests that the alveolar tissue of the OVX group would be more deformed under loading compared to that of normal group. The more deformation of alveolar tissue of OVX group may be partly responsible for the increased tooth movement observed in OVX rats under orthodontic loading^{15–16}. A decrease in the mechanical stability of alveolar bone may play a significant role in development of periodontal disease, especially as observed in postmenopausal patients⁴⁵. The results of the current study can help understand the underlying mechanism of these clinical issues.

In conclusion, the increased variability of mineralization observed in AB of OVX group indicates that estrogen deficiency amplifies the active bone remodeling of AB already present due to mastication. This increased regional variation is attributed to the greater presence of immature, less mineralized bone tissue in the AB of OVX rats. These findings provide an insight that the increased variability of TMD induced by estrogen deficiency may compromise the mechanical stability of the tooth-bearing alveolar bone.

Acknowledgments

The project described was, in part, supported by Grant Number AG033714 from National Institute on Aging (Kim, D-G). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute on Aging. We thank Ms. Michelle Carlton who scanned the specimens and Dr. Ramiro Toribio and Dr. Michael Knopp who gave us access to the micro-CT scanner.

References

1. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: An inflammatory tale. *J Clin Invest* 2006;116:1186–94. [PubMed: 16670759]
2. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. *Endocr Rev* 1994;15:275–300. [PubMed: 8076582]
3. Dervis E. Oral implications of osteoporosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100:349–56. [PubMed: 16122665]
4. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. *N Engl J Med* 2006;354:2250–61. [PubMed: 16723616]
5. McDonnell P, McHugh PE, O'Mahoney D. Vertebral osteoporosis and trabecular bone quality. *Ann Biomed Engin* 2007;35:170–89.
6. McGrath C, Bedi R. Why are we "Weighting"? An assessment of a self-weighting approach to measuring oral health-related quality of life. *Community Dent Oral Epidemiol* 2004;32:19–24. [PubMed: 14961836]
7. Payne JB, Reinhardt RA, Nummikoski PV, Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. *Osteoporos Int* 1999;10:34–40. [PubMed: 10501777]

8. Gur A, Nas K, Kayhan O, Atay MB, Akyuz G, Sindal D, et al. The relation between tooth loss and bone mass in postmenopausal osteoporotic women in turkey: A multicenter study. *J Bone Miner Metab* 2003;21:43–7. [PubMed: 12491093]
9. Taguchi A, Sanada M, Suei Y, Ohtsuka M, Nakamoto T, Lee K, et al. Effect of estrogen use on tooth retention, oral bone height, and oral bone porosity in japanese postmenopausal women. *Menopause* (New York, NY) 2004;11:556–62.
10. Krall EA, Garcia RI, Dawson-Hughes B. Increased risk of tooth loss is related to bone loss at the whole body, hip, and spine. *Calcif Tissue Int* 1996;59:433–7. [PubMed: 8939767]
11. Wactawski-Wende J, Grossi SG, Trevisan M, Genco RJ, Tezal M, Dunford RG, et al. The role of osteopenia in oral bone loss and periodontal disease. *J Periodontol* 1996;67:1076–84. [PubMed: 8910826]
12. Jeffcoat M. The association between osteoporosis and oral bone loss. *J Periodontol* 2005;76:2125–32. [PubMed: 16277585]
13. Ejiri S, Tanaka M, Watanabe N, Anwar RB, Yamashita E, Yamada K, et al. Estrogen deficiency and its effect on the jaw bones. *J Bone Miner Metab* 2008;26:409–15. [PubMed: 18758898]
14. Pereira MC, Zecchin KG, Campagnoli EB, Jorge J. Ovariectomy delays alveolar wound healing after molar extractions in rats. *J Oral Maxillofac Surg* 2007;65:2248–53. [PubMed: 17954321]
15. Arslan SG, Arslan H, Ketani A, Hamamci O. Effects of estrogen deficiency on tooth movement after force application: An experimental study in ovariectomized rats. *Acta Odontol Scand* 2007;65:319–23. [PubMed: 17917818]
16. Yamashiro T, Takano-Yamamoto T. Influences of ovariectomy on experimental tooth movement in the rat. *J Dent Res* 2001;80:1858–61. [PubMed: 11926248]
17. Huja SS, Fernandez SA, Hill KJ, Gulati P. Indentation modulus of the alveolar process in dogs. *J Dent Res* 2007;86:237–41. [PubMed: 17314255]
18. Rapoff AJ, Rinaldi RG, Hotzman JL, Daegling DJ. Elastic modulus variation in mandibular bone: A microindentation study of macaca fascicularis. *Am J Phys Anthropol* 2008;135:100–9. [PubMed: 18058791]
19. Roschger P, Paschalis EP, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. *Bone* 2008;42:456–66. [PubMed: 18096457]
20. Roschger P, Gupta HS, Berzlanovich A, Ittner G, Dempster DW, Fratzl P, Cosman F, Parisien M, Lindsay R, Nieves JW, Klaushofer K. Constant mineralization density distribution in cancellous human bone. *Bone* 2003;32:316–23. [PubMed: 12667560]
21. Ruffoni D, Fratzl P, Roschger P, Klaushofer K, Weinkamer R. The bone mineralization density distribution as a fingerprint of the mineralization process. *Bone* 2007;40:1308–19. [PubMed: 17337263]
22. Roschger P, Rinnerthaler S, Yates J, Rodan GA, Fratzl P, Klaushofer K. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. *Bone* 2001;29:185–91. [PubMed: 11502482]
23. Follet H, Boivin G, Rumelhart C, Meunier PJ. The degree of mineralization is a determinant of bone strength: A study on human calcanei. *Bone* 2004;34:783–9. [PubMed: 15121009]
24. Boivin G, Meunier PJ. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcif Tissue Int* 2002;70:503–11. [PubMed: 12019458]
25. Kim, DG.; Hunt, CA.; Zauel, R.; Fyhrie, DP.; Yeni, YN. The effect of regional variations of the trabecular bone properties on the compressive strength of human vertebral bodies. 51th Annual Meeting of the Orthopaedic Research Society; Washington DC. 2005. p. 1271
26. Kim DG, Christopherson G, Dong XN, Fyhrie D, Yeni Y. The effect of microcomputed tomography scanning and reconstruction voxel size on the accuracy of stereological measurements in human cancellous bone. *Bone* 2004;35:1375–82. [PubMed: 15589219]
27. Mulder L, Koolstra JH, Van Eijden TM. Accuracy of microct in the quantitative determination of the degree and distribution of mineralization in developing bone. *Acta Radiol* (Stockholm, Sweden : 1987) 2004;45:769–77.
28. Sebaoun JD, Kantarci A, Turner JW, Carvalho RS, Van Dyke TE, Ferguson DJ. Modeling of trabecular bone and lamina dura following selective alveolar decortication in rats. *J Periodontol* 2008;79(9):1679–1688. [PubMed: 18771369]

29. Tengku B, Joseph B, Harbrow D, Taverne A, Symons A. Effect of a static magnetic field on orthodontic tooth movement in the rat. *Eur J Orthod* October 1;2000 22(5):475–487. [PubMed: 11105404]
30. Meta IF, Fernandez SA, Gulati P, Huja SS. Alveolar process anabolic activity in c3h/hej and c57bl/6j inbred mice. *J Periodontol* 2008;79:1255–62. [PubMed: 18597609]
31. Turner RT, Maran A, Lotinun S, Hefferan T, Evans GL, Zhang M, Sibonga JD. Animal models for osteoporosis. *Rev Endocr Metabol Disorder* 2001;2:117–27.
32. van Ruijven LJ, Mulder L, van Eijden TM. Variations in mineralization affect the stress and strain distributions in cortical and trabecular bone. *J Biomech* 2007;40:1211–8. [PubMed: 16934818]
33. Ketcham RA, Carlson WD. Acquisition, optimization and interpretation of x-ray computed tomographic imagery: Applications to the geosciences. *Comput Geosci* 2001;27:381–400.
34. Park HS, Lee YJ, Jeong SH, Kwon TG. Density of the alveolar and basal bones of the maxilla and the mandible. *Am J Orthod Dentofac Orthop* 2008;133:30–7.
35. Boivin G, Farlay D, Bala Y, Doublier A, Meunier PJ, Delmas PD. Influence of remodeling on the mineralization of bone tissue. *Osteoporos Int* 2009;20:1023–6. [PubMed: 19340504]
36. Sebaoun JD, Kantarci A, Turner JW, Carvalho RS, Van Dyke TE, Ferguson DJ. Modeling of trabecular bone and lamina dura following selective alveolar decortication in rats. *J Periodontol* 2008;79:1679–88. [PubMed: 18771369]
37. Wronski TJ, Cintron M, Dann LM. Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. *Calcif Tissue Int* 1988;43:179–183. [PubMed: 3141020]
38. Jiang G, Matsumoto H, Fujii A. Mandible bone loss in osteoporosis rats. *J Bone Miner Metab* 2003;21:388–95. [PubMed: 14586795]
39. Elovic RP, Hipp JA, Hayes WC. Ovariectomy decreases the bone area fraction of the rat mandible. *Calcif Tissue Int* 1995;56:305–10. [PubMed: 7767842]
40. Kimpe T, Tuytschaever T. Increasing the Number of Gray Shades in Medical Display Systems—How Much is Enough? *Journal of Digit Imaging* 2007;20(4):422–432.
41. Kneissel M, Boyde A, Gasser JA. Bone tissue and its mineralization in aged estrogen-depleted rats after long-term intermittent treatment with parathyroid hormone (pth) analog szd pts 893 or human pth(1–34). *Bone* 2001;28:237–50. [PubMed: 11248653]
42. Hernandez CJ. How can bone turnover modify bone strength independent of bone mass? *Bone* 2008;42:1014–1020. [PubMed: 18373970]
43. Jaasma MJ, Bayraktar HH, Niebur GL, Keaveny TM. Biomechanical effects of intraspecimen variations in tissue modulus for trabecular bone. *J Biomech* 2002;35:237–46. [PubMed: 11784542]
44. Renders GA, Mulder L, Langenbach GE, van Ruijven LJ, van Eijden TM. Biomechanical effect of mineral heterogeneity in trabecular bone. *J Biomech* 2008;41:2793–8. [PubMed: 18722619]
45. Buencamino MC, Palomo L, Thacker HL. How menopause affects oral health, and what we can do about it. *Cleavel Clin J Med* 2009;76:467–75.

Fig. 1. Isolation of the AB and CB regions of a rat mandibular bone using its 3D micro-CT image. a) Bucco-lingual view and b) 3D view of the whole specimen.

Fig. 2.
Variability of gray levels based on 3D micro-CT image.

Fig. 3.

a) A typical histogram of gray level with TMD parameters examined in this study and b) histograms of AB (solid line) and CB (dotted line). % voxel counts = (voxel counts of each gray level/total voxel count) $\times$ 100.

Fig. 4.

Comparisons between Sham and OVX groups for the absolute % difference between AB to CB ($|AB-CB|/CB \times 100$). The differences were moderately for SD (*; $p < 0.073$) and significantly different for COV (**; $p < 0.04$) and Low₅ (**; $p < 0.02$) between the two groups. Mean and High₅ were not significantly different ($p > 0.093$).

Comparison of regional variations (AB vs CB) of gray levels within individual rats.

	Sham			OVX		
	AB	CB	Paired t-test	AB	CB	Paired t-test
Mean	3560.169 ±130.463	4889.503 ±146.777	p<0.001	3610.046 ±84.623	5042.313 ±117.907	p<0.001
SD	786.240 ±48.726	536.143 ±67.489	p<0.001	816.911 ±57.257	505.508 ±53.148	p<0.001
COV	0.221 ±0.012	0.110 ±0.012	p<0.001	0.226 ±0.015	0.100 ±0.011	p<0.001
Low ₅	2217.898 ±131.585	3890.571 ±109.527	p<0.001	2200.480 ±99.028	4085.465 ±122.697	p<0.001
High ₅	4801.682 ±205.132	5668.048 ±177.283	p<0.001	4883.964 ±116.643	5756.036 ±133.178	p<0.001