# Mechanical Properties of the Mandible in Postmenopausal Women Under Aledronate Treatment

**Ricardo Julio Cabrales-Salgado,[1] Jamil A. Shibli,[1] Adriano Piattelli,[2] Paula Mendes Acatauassú Carneiro,[3] Miriam Lacalle Turbino,[3] Jose Augusto Rodrigues,[1] Alessandra Cassoni[1] and Gabriela Giro[1].**

**[1]Department of Periodontology and Oral Implantology, Dental Research Division, Guarulhos University; [2]Department of Stomatology and Biotechnologies, Università degli Studi G. d'Annunzio Chieti e Pescara; [3]São Paulo University – FOUSP; Sao Paulo, Brazil.**

**Abstract**

**Aims**: This study evaluated the mechanical properties of mandibular bone tissue retrieved from postmenopausal women under alendronate treatment.

**Methods**: Twenty postmenopausal women were divided into two groups: healthy postmenopausal subjects (control group) and osteoporotic subjects treated with alendronate (alendronate group). Mandibular bone samples were retrieved with a trephine bur at the time of dental implant placement and fixed in 4% formalin. Samples were processed for hard tissue histology, and the bone surface was analyzed for nanohardness measurement. Nanohardness and elastic modulus were evaluated by using a Berkovich tip with elastic modulus of 1.016x106 MPa, Poisson coefficient of 0.3, and a load of 100 mN. Each cycle was configured with a load time of 18 seconds (speed of 1 mN/second), the discharge time of 18 seconds, and a rest time of 5 seconds during indentation at a depth of 10 μm.

**Results**: The control group presented the highest values for nanohardness and elastic modulus ($p<0.05$) in relation to the osteoporotic subjects.

**Conclusion**: Within the limitations of the study, it can be concluded that treatment with alendronate negatively influenced the mechanical properties of mandibular bone in postmenopausal women by reducing bone nanohardness and elastic modulus.

*Keywords. Dental implant; Alendronate; Osteoporosis; Nanohardness; Elastic modulus.*

## Introduction

Osteoporosis is a condition that promotes bone microarchitecture fragility and results from an imbalance in bone remodeling caused by ovarian dysfunction in postmenopausal women (Rachner *et al.,* 2011). Over the last two decades, physicians have developed various treatment options aiming to reduce fracture risk in subjects with osteoporosis. Specific treatments for the disease include antiresorptive agents (calcium, vitamin D, estrogens, calcitonin, and bisphosphonates, among others), which inhibit the process of bone resorption.

The influence of osteopenia/osteoporosis on the success rates of dental implants is currently under active investigation. The use of alendronate, a nitrogen-containing bisphosphonate that has been successfully used to reverse and prevent postmenopausal bone mass loss (Licata *et al*., 2005) is of interest on the field of oral ijmplantology. Although some studies (Holahan *et al*., 2008; Dvorak *et al*., 2011; Tallarico *et al*., 2016; Temmerman *et al*., 2017) have shown that neither the disease nor the medication used for preventing bone mass loss (alendronate) has any influence on implant survival in postmenopausal women, other studies have shown a higher rate of implant loss (Moy *et al*., 2005; de Medeiros *et al*., 2017).

Correspondence to: Gabriela Giro, Praça Tereza Cristina, 289. 07030-070, Guarulhos, SP, Brazil. Email: gabi.giro@gmail.com

Animal studies have shown that alendronate treatment improves bone/implant contact, increased bone formation between implant threads, and increases the amount of torque needed for implant removal (Frenkel *et al.*, 2001; Narai and Nagahata, 2003; Chacon *et al.*, 2006). Several studies in humans have corroborated animal findings. For example, Bell and Bell (2008) found that implant survival rate was high (~95%) for both bisphosphonate users and nonusers. In a retrospective study of postmenopausal women, Koka *et al.*, (2010) found similar implant survival rates to those reported by Bell and Bell (2008) and emphasized the absence of influence of bisphosphonate on implant success rate (95% for subjects under alendronate treatment against 96.5% that had not been treated).

On the other hand, various reports in humans have shown a direct relationship between alendronate use and an increased incidence of medication-related osteonecrosis of the jaws, especially after invasive dental procedures and inflammatory conditions of the periodontium. Therefore, the dental community needs to have a clear understanding of how bisphosphonates affect bone characteristics in the oral cavity (Lam *et al.*, 2007).

It is well documented that non-invasive procedures can prevent the occurrence of osteonecrosis of the jaws. However, this relationship is not fully understood. Studies are needed to elucidate the characteristics of peri-implant bone tissue to understand the expected tissue response and to prevent complications related to the use of this class of medications. Furthermore, peri-implant bone tissue needs to be studied in alendronate users to clarify the influence of this drug on bone tissue before and after the dental implant placement.

There are several studies regarding bone nanohardness in the literature. However, none of them have evaluated human mandible bone samples from patients undergoing alendronate treatment. Therefore, this study aimed to evaluate the mechanical characteristics of mandibular bone tissue retrieved from postmenopausal women under alendronate therapy before receiving dental implants.

## Materials and methods

### *Study design*

The study population included postmenopausal women who underwent dental implant placement at the Center for Clinical Studies at Guarulhos University (UNG), Guarulhos, SP, Brazil. Inclusion criteria for the study sample were as follows: women who had been postmenopausal for at least one year, presented a residual bone height of at least 10 mm and bone thickness of at least 5 mm for dental implant installation, had undergone routine medical check-ups and had densitometry exam results from the previous six months to evaluate bone mineral density as a function of the T-score index (World Health Organization, Switzerland, 1994). Subjects with osteoporosis had to be receiving oral treatment of 70 mg/week of alendronate (Fosamax, Merck Sharp & Dohme Pharmaceuticals Ltd, Campinas, SP). Exclusion criteria were as follows: subjects with any vascular disease, any chronic disease (e.g., rheumatoid arthritis or diabetes), smoking habit, chronic alcoholism, chronic moderate or advanced periodontal disease, use of glucocorticoids or other immunosuppressant drugs, history of radiation therapy to the head and neck area, insufficient bone tissue for insertion of dental implants, or previous regenerative therapy. Subjects provided medical and dental histories and underwent clinical intraoral tests and preoperative laboratory tests, including a complete hemogram, coagulation profile, and measurement of serum glucose, calcium, and creatinine levels.

The sample size was based on earlier studies (Gao *et al.*, 2015; Kim *et al.*, 2015; Wang *et al.*, 2016) that evaluated between 9 to 20 samples of human bone tissue.

Subjects included in the study were split into two groups based on results of the DEXA test: CONTROL GROUP: healthy subjects (T-score $\geq$ -1) using no medication for preventing bone mass loss; ALENDRONATE GROUP, subjects with the diagnosis of osteoporosis under treatment with alendronate from 1 to 4 years (T-score $\leq$ -2.5).

The Research Ethics Committee at UNG approved the protocol (protocol nº 39392914.0.0000.5506). All participants were given information about the study and signed a consent form.

### *Sample collection*

Bone tissue samples were collected during dental implant placement surgery in the posterior area of the mandible. After local anesthesia, an incision was made, and a mucoperiosteal flap was raised. During implant site preparation, a trephine (internal length: 2.0 mm; Implacil De Bortoli, São Paulo, Brasil) was used under abundant irrigation with sterile saline solution to collect a bone sample. After bone tissue retrieval, the dental implant placement followed the standard protocols using increase drills sequence (Implacil De Bortoli, São Paulo, Brasil).

### *Histological processing for nanohardness test*

All specimens were fixed in 4% formalin solution for 48 hours and transferred to a 70% alcohol solution. Samples were processed to obtain a section of non-decalcified tissue by using an automated system (Precise 1, Assing, Rome, Italy). For this purpose, specimens were dehydrated in a series of increasing concentrations of alcohol and embedded in glycol methacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After embedding and polymerizing the specimens, the blocks were sectioned. A 150-µm-thick slice was made along the longitudinal axis of the bone sample by using a high-precision diamond disc. The slices were reduced

to a thickness of approximately 30 μm (Isomet, Buehler). Only one slide per specimen was obtained.

### *Nanohardness test*

Hardness and elastic modulus were measured in the undecalcified bone sections. The cortical bone area were evaluated by using a Berkovich tip in a dynamically controlled hardness tester (DUH-W211S, Shimadzu Co., Tokyo, Japan) with diameter of approximately 100 nm. Elastic modulus of 1.016x106 MPa, Poisson ratio of 0.3 (Zysset *et al*., 1999; Hengsberger *et al*., 2002; Norman *et al*., 2008), and a load of 100 mN were the used parameters for all measurements. Each cycle was configured with a load time of 18 seconds, at a speed of 1 mN/second, the discharge time of 18 seconds, and a rest time of 5 seconds with indentation at a depth of 10 μm. Data generated by the nanoindenter were analyzed by using the TestWorks4 program (MTS Systems Corp; http://www.mts.com) and statistically evaluated by Mann Whitney test at the 5% level.

## Results

This study involved 20 subjects divided into two groups, as shown in Table 1. Table 2 shows the nanohardness results for both groups. It is observed higher nanohardness values for participants in the control groups, with a statistically significant difference in comparison to the alendronate group ($p < 0.05$). Similarly, Figure 1 shows higher elastic modulus values for subjects in the control group ($p < 0.05$).

**Table 1**. Demographic data for participants allocated to each group.

| Groups | Control | Alendronate |
|---|---|---|
| Age | 68.1 ± 6.4[a] | 69.2 ± 7.9[a] |
| Femur BMD | 1.082 ± 0.08[a] | 0.688 ± 0.172[b] |
| Lumbar vertebrae BMD | 1.144 ± 0.05[a] | 0.807 ± 0.079[b] |

Different letters indicate statistically significant difference between groups

**Table 2**. Comparison of bone tissue microhardness results between groups. Statistical analysis was performed by Mann Whitney test ($p < 0.05$).

| Groups | Control (n=10) | Alendronate (n=10) |
|---|---|---|
| Mean | 26.5[a] | 17.9[b] |
| St Dev | ± 6.61 | ± 4.36 |
| Amplitude | 16.6 - 36.7 | - 27.9 |

Different letters indicate statistically significant difference between groups

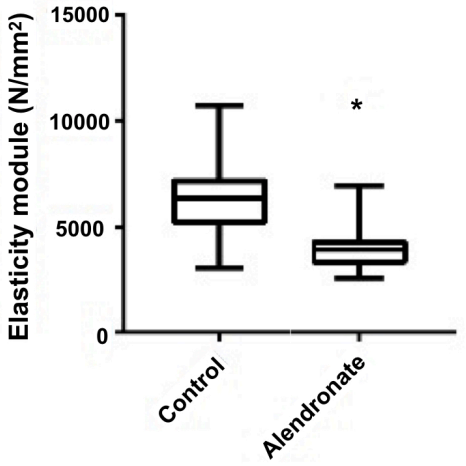


***Figure 1 Elastic modulus values for subjects in the control and Aledronate groups (*p < 0.05).***

## Discussion

This study evaluated the biomechanical characteristics of mandibular bone tissue retrieved from postmenopausal women through evaluating the nanohardness and elastic modulus of the bone tissue. The results showed a negative influence of treatment with alendronate, a nitrogen-containing bisphosphonate that has been associated with various cases of implant failure and with the occurrence of maxillary osteonecrosis. However, a direct correlation with dental implants success or clinical outcomes were not done. Further studies should correlate the nanomechanical characterization with the clinical outcomes in a long-term period.

Nanostructural changes in hardness in osteoporotic subjects are probably related to the reduction in bone mineral density associated with metabolic changes caused by estrogen deficiency (Society of Obstetrics and Gynacology Canada, 2014; Lu *et al.,* 2019; Molino *et al.,* 2019). Deformity of bone structure is inversely proportional to bone fragility. Although this study observed a lower elastic modulus for subjects in the alendronate group, the bone tissue in this group tended to be less hard, more friable, and, consequently, more fragile. Bone mineral density is highly correlated with resistance and rigidity (Fratzl-Zelman *et al*., 2009; Turner, 2006). The results of this study are in agreement with other findings for long bones, in which the aging process contributed to bone impairment since the process of hardening in the component of the organic matrix contributes to an increase of bone fragility (Fratzl-Zelman *et al*., 2009), once the collagen fibers lose the elastic properties decreasing the resistance to traction and resulting in bone friability. On the other hand, Yajima *et al.* (2017) showed that the use of alendronate treatment would increase mandibular bone cortical thickness what would be beneficial for the placement of dental implants, although no difference was found regarding cortical and trabecular bone mineral density.

The mineral portion of bone is comprised of hydroxyapatite crystals, which confer rigidity to the bone and resistance to compression. The organic fraction (collagen) allows for more considerable deformity and strength to fracture, acting as a viscoelastic component. In contrast to the results of this study, Cherkaev and Bonifasi (2011) demonstrated that the results of clinical trials of nanoindentation did not yield a significant difference for the nanohardness results in comparison with healthy, osteopenic, or osteoporotic bone. Similarly, Fratzl–Zelman *et al*., (2009) showed that although osteoporosis was associated with reduced mineral levels, there was no reduction in the rigidity or hardness of the bone material.

At the cellular level, bisphosphonates inactivate osteoclast function thus inhibiting bone resorption. Bisphosphonates regulate bone remodeling units (Rodan and Fleish, 1996) and reduce the renewal rate for bone tissue, contributing to the rigidity of collagen fibers and the aging of bone tissue. Results from Rollo and collaborators (Rollo *et al*., 2015) support these data, demonstrating that prolonged use of alendronate reduced the micromechanical properties of bone tissue because of the reduction in mineral crystallinity. Therefore, the authors suggested that although treatment with alendronate preserves bone mineral density at the structural level, it also damages the lamellar organization of the bone tissue. Thus, prolonged bisphosphonate treatment can be associated with atypical fractures. Independent of the microstructural results, clinical studies in humans found no difference in implant survival between subjects who were treated or not treated with bisphosphonates (Lam *et al*., 2007; Koka *et al*., 2010).

Overall, results in the literature suggest that osteoporotic bone exhibits organic and inorganic structural deteriorations that are reflected in the bone friability, which could affect the longevity of dental implants. In the present study, the mechanical properties of bone in the alendronate group showed lower values, suggesting that implant insertion may lead to the development of necrosis. To avoid excessive masticatory injury to the bone structure, we recommend that dental implants in osteoporotic bone involve an occlusal adjustment to reduce occlusal forces without loss of masticatory function and, thereby, increase implant survival.

Regarding implant success rate and survival in patients under alendronate treatment some studies have suggested that they did not appear to be influenced by oral bisphosphonate administration (Tallarico *et al*., 2016). However, recent studies demonstrated that although alendronate treatment increase bone mineral density and would be beneficial for marginal bone loss, the drug administration also impairs osseointegration, provoking changes in bone quality, which may be a concern for implant treatment in patients receiving bisphosphonates (Frizzera *et al*., 2019; Khojasteh *et al*., 2019).

One limitation of this study protocol is the absence of the data from subjects with osteoporosis without alendronate treatment, as well as alendronate treated subjects without osteoporosis. However, because osteoporosis is a public health problem in the elderly, and it would not be acceptable to include subjects presenting a deteriorating disease of the bone tissue microarchitecture, without acting to prevent systemic bone loss. However, data obtained from sections of bone tissue taken from human jaws yield more authentic and unique information about the mandible than data collected from other bone sites (Mullender and Huiskes 1995). Thus, the results of this study are clinically useful to understand the limitations of bone characteristics in rehabilitative treatment with implant-supported prostheses in the jaws of postmenopausal subjects who are prescribed long-term use of alendronate. This is especially important since its chronic use may induce a rare, but potentially serious side effect known as medication related osteonecrosis of the jaws (Aljohani *et al*., 2017).

Despite the limitations of this study, the results suggested that the treatment with alendronate negatively influences the biomechanical properties of bone tissue in postmenopausal women, however further long-term clinical studies should provide the effect of this feature on the peri-implant tissues.

### *Acknowledgments*

Dr. Giro would like to thank the São Paulo Research Foundation (FAPESP) for financial support (grant # 2014/25228-7).

## References

Aljohani S, Fliefel R, Ihbe J, Kühnisch J, Ehrenfeld M, Otto S. What is the effect of anti-resorptive drugs (ARDs) on the development of medication-related osteonecrosis of the jaw (MRONJ) in osteoporosis patients: A systematic review. *Journal of Craniomaxillofacial Surgery* 2017; **45**:1493-1502.

Bell BM and Bell RE. Oral bisphosphonates and dental implants: a retrospective study. *Journal of Oral and Maxillofacial Surgery* 2008; **66**:1022-1024.

Chacon GE, Stine EA, Larsen PE, Beck FM and McGlumphy EA. Effect of alendronate on endosseous implant integration: an in vivo study in rabbits. *Journal of Oral and Maxillofacial Surgery* 2006; **64**:1005-1009.

Cherkaev E and Bonifasi-Lista C. Characterization of structure and properties of bone by spectral measure method. *Journal of Biomechanics* 2011; **44**:345-351.

de Medeiros FCFL, Kudo GAH, Leme BG, *et al.* Dental implants in patients with osteoporosis: a systematic review with meta-analysis. *International Journal of Oral and Maxillofacial Surgery* 2017 pii: S0901-5027 **17:**31484-4.

Dvorak G, Arnhart C, Heuberer S, Huber CD, Watzek G and Gruber R. Peri-implantitis and late implant failures in postmenopausal women: a cross-sectional study. *Journal of Clinical Periodontology* 2011; **38**:950-955.

Fratzl-Zelman N, Roschger P, Gourrier A, *et al* Combination of nanoindentation and quantitative backscattered electron imaging revealed altered bone material properties associated with femoral neck fragility. *Calcified Tissue International* 2009; **85**:335-343.

Frenkel SR, Jaffe WL, Valle CD, *et al*. The effect of alendronate (Fosamax) and implant surface on bone integration and remodeling in a canine model. *Journal of Biomedical Materials Research* 2001; **58:**645-650.

Frizzera F, Verzola MHA, de Molon RS, *et al*. Evaluation of bone turnover after bisphosphonate withdrawal and its influence on implant osseointegration: an in vivo study in rats. *Clinical Oral Investigations* 2019; **23**:1733-1744.

Gao J, Gong H, Zhang R and Zhu D. Age-related regional deterioration patterns and changes in nanoscale characterizations of trabeculae in the femoral head. *Experimental Gerontol*ogy 2015; **62:**63–72.

Hengsberger S, Kulik A and Zysset P. Nanoindentation discriminates the elastic properties of individual human bone lamellae under dry and physiological conditions. *Bone* 2002; **30**:178-184.

Holahan CM, Koka S, Kennel KA, *et al*. Effect of osteoporotic status on the survival of titanium dental implants. *International Journal of Oral and Maxillofacial Implants* 2008; **23**:905-910.

Khojasteh A, Dehghan MM and Nazeman P. Immediate implant placement following 1-year treatment with oral versus intravenous bisphosphonates: a histomorphometric canine study on peri-implant bone. *Clinical Oral Investigation* 2019; **23**:1803-1809.

Kim DG, Jeong YH, Kosel E, *et al*. Regional variation of bone tissue properties at the human mandibular condyle. *Bone* 2015; **77:**98–106.

Koka S, Babu NMS and Norell A. Survival of dental implants in post-menopausal bisphosphonate users. *Journal of Prosthodontic Research* 2010; **54**:108-111.

Lam DK, Sándor GK, Holmes HI, Evans AW and Clokie CM. A review of bisphosphonate-associated osteonecrosis of the jaws and its management. *Journal of the Canadian Dental Association* 2007; **73:**417-422.

Licata AA. Discovery, clinical development, and therapeutic uses of BPs. *Annals of Pharmacotherapy* 2005; **39:**668-677.

Lu S, Du Z, Lin CL, Zuo Q, Huang H and Xiao Y. Effect of ovariectomy on tissue-level changes in rat maxilla. *International Journal of Oral Maxillofacial Implants*. 2019; **34**:865–872.

Moy PK, Medina D, Shetty V and Aghaloo TL. Dental implant failure rates and associated risk factors. International *Journal of Oral and Maxillofacial Implants* 2005; **20:**569-77.

Molino G, Dalpozzi A, Ciapetti G, *et al*. Osteoporosis-related variations of trabecular bone properties of proximal human humeral heads at different scale lengths. *Journal of Mechanical Behavior of Biomedical Materials* 2019; **100**:103373.

Mullender MG and Huiskes R. Proposal for the regulatory mechanism of Wolff's law. *Journal of Orthopaedic Research* 1995; **13**:503-512.

Narai S and Nagahata. Effects of alendronate on the removal torque of implants in rats with induced osteoporosis. *International Journal of Oral and Maxillofacial Implants* 2003; **18**:218-223.

Norman J, Shapter JG, Short K, Smith LJ and Fazzalari NL. Micromechanical properties of human trabecular bone: a hierarchical investigation using nanoindentation. *Journal of the Biomedical Materials Research Part A* 2008; **87**:196-202.

Rachner TD, Khosla S and Hofbauer LC. Osteoporosis: now and the future. *Lancet* 2011; **377**:1276-1287.

Rodan GA and Fleisch HA. Bisphosphonates: mechanisms of action. *Journal of Clinical Investigation* 1996; **97**:2692-2696.

Rollo J, Boffab M, Cesarc R, Schwabd DC and Leivase TP. Assessment of Trabecular Bones Microarchitectures and Crystal Structure of Hydroxyapatite in Bone Osteoporosis with Application of the Rietveld Method. *Procedia Engineering* 2015; **110:**8-14.

Society of Obstetrics and Gynecology Canada. Osteoporosis in Menopause. Clinical practice guideline. *Journal of Obstetrics and Gynaecology Canada* 2014; **36**:S1– S15.

Tallarico M, Canullo L, Xhanari E and Meloni SM. Dental implants treatment outcomes in patient under active therapy with alendronate: 3-year follow-up results of a multicenter prospective observational study. *Clinical Oral Implants Research* 2016; **27**:943-949.

Temmerman A, Rasmusson L, Kübler A, Thor A and Quirynen M. An open, prospective, non-randomized, controlled, multicentre study to evaluate the clinical outcome of implant treatment in women over 60 years of age with osteoporosis/osteopenia: 1-year results. *Clinical Oral Implants Research* 2017; **28**:95-102.

Turner CH. Bone strength: current concepts. *Annals of the New York Academy of Sciences* 2006; **1068**:429-446.

Wang C, Wang Y, Meng H, *et al*. Microstructure and nanomechanical properties of single trabecular bone in different regions of osteonecrosis of the femoral head. *Journal of Nanoscience and Nanotechnology* 2016; **16:**2264–2269.

Yajima N, Munakata M, Fuchigami K, Sanda M and Kasugai S. Influence of Bisphosphonates on Implant Failure Rates and Characteristics of Postmenopausal Woman Mandibular Jawbone. *Journal of Oral Implantology* 2017; **43:**345–349.

Zysset PK, Guo XE, Hoffler CE, Moore KE and Goldstein SA. Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur. *Journal of Biomechanics* 1999; **32**:1005-1012.