




Evaluation of bone turnover after bisphosphonate withdrawal and its influence on implant osseointegration: an in vivo study in rats

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Abstract

Objectives The aim of this study was to investigate bone turnover alterations after alendronate (ALD) withdrawal and its influence on dental implants osseointegration.

Materials and methods Seventy female Wistar rats were randomly divided in 2 groups that received on day 0 either placebo (control group—CTL; $n = 10$) or 1 mg/kg sodium alendronate (ALD; $n = 60$) once a week for 4 months. At day 120, ALD treatment was suspended for 50 animals. Then, a titanium implant was placed in the left tibia of each rat that were randomly allocated in five subgroups of ten animals each, according to the period of evaluation: day 0 (INT-0), day 7 (INT-7), day 14 (INT-14), day 28 (INT-28), and day 45 (INT-45) after ALD withdrawal. CTL group and a group that received ALD until the end of the experimental period (non-interrupted group—non-INT; $n = 10$) underwent implant placement on day 120. Animals were euthanized 28 days after implant surgery. Bone mineral density (BMD) of femur and lumbar vertebrae were evaluated by DXA, biochemical markers of bone turnover were analyzed by ELISA, and bone histomorphometry was performed to measure bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO).

Results All groups receiving ALD showed higher BMD values when compared to CTL group, which were maintained after its withdrawal. Decreased concentrations in all bone turnover markers were observed in the non-INT group, and in the groups in which ALD was discontinued compared to the CTL group. The non-INT group showed lower %BIC and notably changes in bone quality, which was persistent after drug withdrawal.

Conclusion Collectively, the findings of this study demonstrated that ALD therapy decreased bone turnover and impaired bone quality and quantity around dental implants, and that its discontinuation did not reverse these findings.

Clinical relevance The severe suppression of bone turnover caused by the prolonged use of ALD may alter the capacity of bone tissue to integrate with the implant threads impairing the osseointegration process.

Keywords Bisphosphonate · Alendronate · Rats · Osteoclasts · Dental implants · Osseointegration

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Introduction

Antiresorptive medications, such as nitrogen-containing bisphosphonates (BPs), are widely used for the management of bone diseases such as osteoporosis or bone malignancy. BPs, such as alendronate, acts by inhibiting the farnesyl diphosphate synthase in the cholesterol pathway, which prevents the prenylation of guanosine triphosphatase signaling proteins [1]. As a result, alendronate inhibits osteoclast activity by impairing differentiation, reducing intracellular transport, disrupting the cytoskeleton, and leading to cell apoptosis [2, 3]. These events translate to decreased bone turnover, improved BMD and structural bone properties, and reduced risk of fractures [4, 5].

Osteoporosis, a metabolic disease of bone, increases bone turnover leading to high bone remodeling rate. As a consequence of excessive bone resorption, osteoporosis reduces BMD and bone cancellous mass with generalized microarchitectural deterioration, and subsequent increased risk for bone fractures [6, 7]. Risk factors of osteoporosis include postmenopausal estrogen deficiency, excessive glucocorticoid intake, and gene interactions in bone metabolism [8, 9]. In this context, alendronate is among the most frequently prescribed medications for the treatment of osteopenia and osteoporosis [10].

Over the last decades, implant therapy to replace missing teeth has increased significantly. In addition, the number of patients under BPs therapy, and candidates for implants rehabilitation has been rising. The long-term clinical success of endosseous dental implants is critically related to the bone quality and to direct contact between bone and implant. In this regard, several pre-clinical studies [11–17] have shown decreased bone area, bone volume, and bone-to-implant contact (BIC) around dental implants placed in osteoporotic animals. However, BPs therapy may be able to reverse the negative effect of osteoporosis on the osseointegration of dental implants as demonstrated by increased %BIC around osseointegrated implants [16, 18–21] as well as increased torque needed for implant removal [20, 22]. On the other hand, previous studies [23, 24] in healthy rabbits and rats demonstrated that alendronate administration had no effect on dental implant torque removal values. Accordingly, lower BIC values and decreased torque for implant removal were observed in rabbits on alendronate without osteoporosis [25].

Alendronate withdrawal has been proposed as potentially beneficial in re-establishing bone turnover, which could improve bone healing [26]. However, experimental data supporting the benefit of alendronate discontinuation before implant placement are lacking. Here, we explored the effects of alendronate withdrawal in rats before implant placement by means of histomorphometric, densitometric, and biochemical analyses. Our results indicate that, within the methodology employed, alendronate therapy impaired bone quality and quantity around dental implants and these findings were sustained after its withdrawal.

Material and methods

Animal care

The experimental protocol was handled according to the guidelines of the local Ethical Committee for Animal Care and Use (protocol #12/2009), and all animals received humane care according to the recommendations of the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines for the execution and submission of studies in animals [27]. Rats were kept in the animal facilities in a humidity (55%) and temperature-controlled room (22 ± 2 °C) on a 12-h light/dark cycle (light on at 07:00 am). All rats were housed in standard cages in groups of two animals, and normal chow diet and water were provided ad libitum.

A total of 70 3-month-old female Wistar rats (*Rattus norvegicus albinus*), with average body weight between 250 and 300 g were randomly (by means of drawing lots) divided into 2 groups that received on day 0 either subcutaneously injection of endotoxin-free saline (control group—CTL; $n = 10$) or 1 mg/kg sodium alendronate (test group; $n = 60$) once a week for 4 months, following previously published protocol [20]. At day 120, alendronate treatment was suspended for 50 animals (INT-0, INT-7, INT-14, INT-28, INT-45 groups; $n = 10$ for each group). CTL group, INT-0, and a group that received alendronate until the end of the experimental period (non-interrupted group, non-INT; $n = 10$) underwent surgery for implant placement on day 120. Furthermore, a titanium implant was placed in the rats left tibiae on days 7 (INT-7), 14 (INT-14), 28 (INT-28), and 45 (INT-45) after alendronate withdrawal ($n = 10$ for each group). The dosage of 1 mg/kg of sodium alendronate was based in our previous published manuscript [20]. A flowchart comprising the experimental design is presented in Fig. 1.

Implant surgery

Experimental surgery for implant placement was performed as described [28]. Briefly, the animals were fasted for 8 h prior to surgery, and general anesthesia was done by intramuscular (*im*) injection of a combination of ketamine hydrochloride (Cetamin®; Sintec Ltda, Cotia, SP, Brazil; 0.08 ml/100 g body weight) and xylazine hydrochloride (Xilazin®; Bayer S.A., Sao Paulo, SP, Brazil; 0.04 ml/100 g body weight). Then, rats underwent local anesthesia by infiltrative injection with mepivacaine hydrochloride (0.3 ml/kg, Scandicaine 2% with epinephrine 1:100,000, Septodont, France) to assist hemostasis of the wound area [29].

To facilitate implant placement, a 10-mm-length incision was made on the proximal tibia just below the knee, running up to the tibial diaphysis. A full-thickness flap was lifted exposing the lateral tibial bone for implant placement. Then, receptor beds were prepared using a progressive sequence of

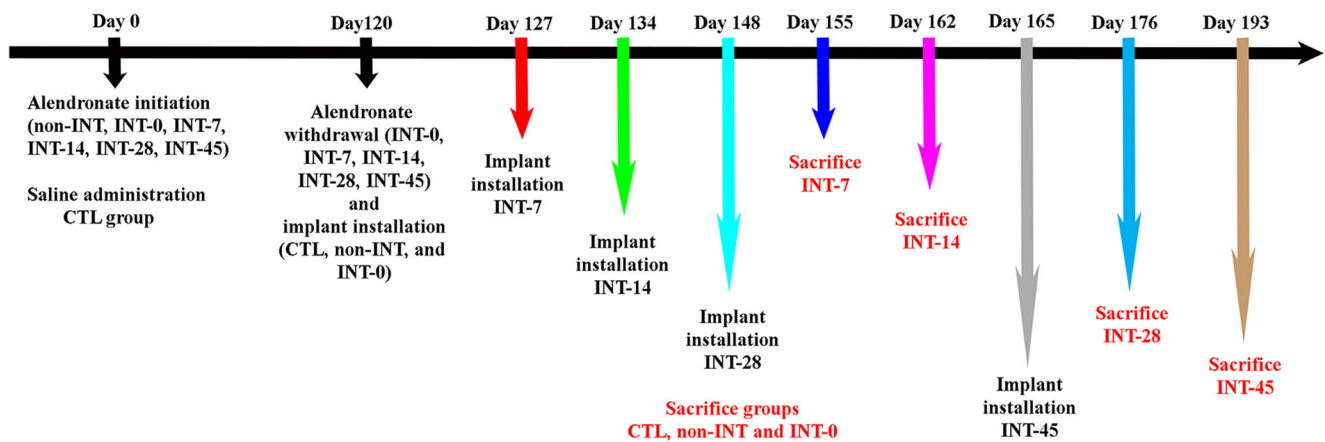


Fig. 1 Flowchart for experimental design

drills (lance drill and spiral 1.8 mm drill; Conexao Implant System, Sao Paulo, SP, Brazil) to a final diameter of 2 mm, under profuse sodium chloride irrigation (0.9% Darrow, Rio de Janeiro, Brazil). Subsequently, a titanium implant, 4 mm in length and 2.2 mm in diameter (Conexao Implant System, Sao Paulo, SP, Brazil), was installed in the tibia using both cortical bones (medial and lateral tibial metaphysis) with the aid of an electric motor (BLM 600, Driller Electric Equipment Ltda, Sao Paulo, SP, Brazil). The soft tissues were replaced and sutured in separate layers, using absorbable threads (Poligalactina 910—Vycril 4.0, Ethicon, Johnson Prod., Sao Jose dos Campos, SP, Brazil) and the skin was sutured with monofilament suture (Nylon 4.0, Ethicon, Johnson, Sao Jose dos Campos, SP, Brazil).

In the immediate postoperative period, the animals received a single intramuscular injection of pentabiotic (0.1 ml/kg, Fort Dodge Animal Health Ltd., Campinas, Sao Paulo, Brazil) and a single dose of Sodium Dipyrone (1 ml/kg/day Ariston Chemical and Pharmaceutical Industries Ltda, Sao Paulo, Brazil).

Animal euthanization and analyses

Ten rats per group were euthanized by anesthetic overdose at the end of the experimental periods, i.e., 28 days after implant placement. CTL, non-INT, and INT-0 were sacrificed at day 148. INT-7, INT-14, INT-28, and INT-45 were sacrificed at days 155, 162, 176, and 193, respectively. The vertebrae, femurs, and tibiae (bone-implant interface) from each group were removed and fixed in 10% paraformaldehyde for 72 h.

Bone mineral density evaluation

Bone mineral density (BMD) of lumbar vertebrae and femurs were analyzed by dual-energy X-ray absorptiometry (DXA) in order to evaluate the systemic effects of alendronate in each group. Using a densitometer (Discovery® A SN: 80999;

Hologic Inc., Bedford, TX, USA) operating in high-resolution mode, the samples were analyzed employing specific software for small animals (DPX, Small Animal Software Version 1.0, Lunar Radiation Corp., Madison, WI). The DXA accuracy for BMD determination was evaluated by measuring the coefficient of variation after five consecutive measurements of the same sample, and was expressed as a percentage of the mean. The coefficient of variations of 1.8% for the femur and 2.1% for the lumbar vertebrae were obtained. For lumbar vertebrae were measured the whole vertebra (Global) and four independently vertebrae, as follow: L1, L2, L3, and L4. For the femurs, the following areas were measured: Global, R1 (proximal epiphysis), R2 (distal epiphysis), and R3 (diaphyseal).

Evaluation of bone turnover biochemical markers

In the moment of implant installation (T1) and before animal euthanization (T2), blood samples were collected from the rat caudal artery in order to assess bone turnover marker serum concentrations. Markers of bone formation, such as osteocalcin (OCN) and aminoterminal propeptide of procollagen type 1 (PINP), were measured using a rat OCN enzyme-linked immunosorbent assay (ELISA) kit (OCN; RatMID Osteocalcin®; Immunodiagnostic Systems Inc., Boldon, United Kingdom) and rat PINP kit (PINP; Rat Mouse PINP®; Immunodiagnostic Systems Inc., Boldon, United Kingdom), respectively. C-terminal telopeptide of type I collagen (CTX), a marker of bone resorption, was measured using rat CTX ELISA kit (CTX; RatLaps®; Immunodiagnostic Systems Inc., Boldon, United Kingdom). A urine sample comprising the last 24 h of the experimental period for each group was collected from each animal in metabolic cages. Urinary deoxypyridinoline (DPD) was measured by ELISA kit (Metra Biosystems, Palo Alto, CA, USA). DPD results were corrected for the urinary concentration of creatinine (DPyr/creatinine; nM/mM). All ELISA tests were performed according to the manufacturer instructions.

Sample preparations and histomorphometric procedures

Tissue samples for nondecalcified sections were dehydrated in increasing concentrations of ethanol (60–100%), and were then embedded in light-cured resin (Technovit 7200 VLC®, Heraeus Kultzer GmbH & Co., Wehrheim, Germany). The block samples were cut at the center of the implant diameter with the aid of a cutting-grinding unit (EXAKT Apparatebau, Hamburg, Germany), as described [30]. A unique section was obtained from each sample and a final thickness of 30 μm was achieved after grinding and polishing. Then, samples were stained with Stevenel blue. Descriptive histologic analyses were performed by a blinded and experienced examiner (LCS). The mean values of both sides of the implant were considered.

Bone histomorphometry

The histological slides were analyzed using a light microscopy (DiaStar, Reichert & Jung products Leica, Germany) and images were captured by a digital camera Leica Microsystems DFC-300-FX (Leica Microsystems, Germany) with a resolution of eight megapixels, coupled to the optical microscope common light. The digital images of histological slides were imported and the histomorphometric analysis was performed using ImageJ analysis software (ImageJ®, Jandel Scientific, San Rafael, CA, USA) in a standardized manner by one blinded and calibrated examiner (MHAV), masked to the original treatment protocol. The results of the linear extension of BIC and BAFO between the threads occupied by bone tissue obtained from the square pixels and pixels, respectively, were converted to percentage values, as described [28, 31]. To evaluate the bone near the implant, two histomorphometric variables were analyzed as stated:

1. The implant linear extension including bone tissue in contact with the implant surface, between the initial three threads in the mesial and distal region (BIC);
2. The area between the threads, occupied by bone tissue in the first three implant threads in the mesial and distal region (BAFO). The values of BIC and BAFO for each group were compared with each other, as described previously [18, 28, 32, 33].

Statistics

Analyses were performed using GraphPad Prism Software 7.0 (GraphPad® Software, Inc., La Jolla, CA, USA). Group measures were expressed as mean \pm the standard error of the mean (SEM). The data obtained in each type of comparison were tested using the D'Agostino and Pearson normality test. For

BMD, CTX, and BIC analyses, statistical significance was assessed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons among groups. For OCN, P1NP, and BAFO analyses, statistical significance was assessed using Kruskal-Wallis followed by post-hoc Dunn test for multiple comparisons among groups. Differences were considered significant at $p < 0.05$.

Results

Clinical evaluation

Alendronate treatment did not influence implant healing and all implants presented without complication or infection. None of the rats were lost during the experiment. There were no interurrences in the follow-up periods and the rat's weight did not change during alendronate treatment or after its discontinuation (data not shown).

Bone mineral density

BMD levels of vertebrae and femurs were measured at the end of the experimental periods. For lumbar vertebrae, the whole vertebra (global, Fig. 2a), and vertebrae L1, L2, L3, and L4 were measured (Supplemental Fig. 1). For the femurs, global sub-regions (Fig. 2b), R1 (proximal epiphysis), R2 (distal epiphysis), and R3 (diaphyseal) areas were also measured (Supplemental Fig. 2). For the CTL group, BMD levels remained relatively steady in all the regions evaluated for femurs and vertebrae. On the other hand, non-INT group demonstrated increased BMD in all the regions evaluated, which was statistically significant compared to the CTL group in the femurs and vertebrae. Alendronate withdrawal did not influence BMD levels that remained unchanged in all groups evaluated compared to the non-INT group, but it was statistically significant compared to CTL group. BMD levels of R2 sub-region in the femurs after 45 days (INT-45) were statistically significantly higher compared to INT-0 group (Supplemental Fig. 2). BMD levels in the L4 vertebrae of INT-14 group was statistically significantly higher compared to INT-0, as well as between INT-45 compared to INT-0 for L3 and L4 vertebrae (Supplemental Fig. 1).

Bone turnover biochemical markers

At the moment of implant installation (T1) and before animal euthanization (T2), blood samples were collected in order to assess bone formation and resorption markers serum concentrations. On T1, the CTL group demonstrated increased concentration of P1NP and OCN compared to all other groups but the differences were statistically significant only between CTL and INT-7 for P1NP and OCN, and between CTL and

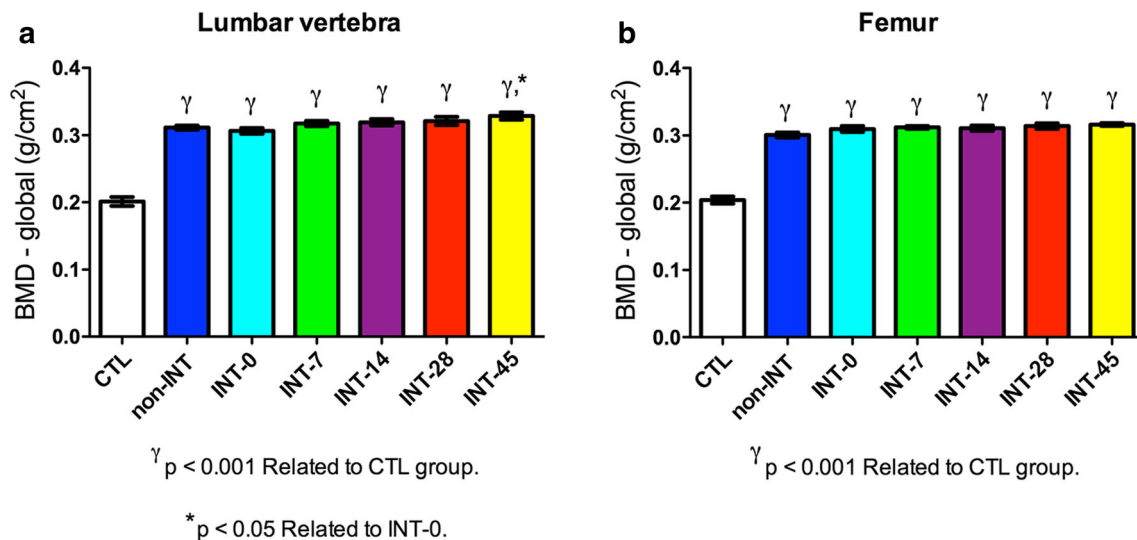


Fig. 2 **a** Quantification of BMD values in the lumbar vertebra and **b** in the femur. γ Statistically significant different compared to the CTL group ($p < 0.001$). * Statistically significant different compared to the INT-0 group ($p < 0.05$)

INT-45 group for P1NP (Fig. 3a, c). CTX and DPD in the treatment groups were decreased compared to the CTL group. However, CTX concentration was statistically significantly lower only between INT-28 group compared to non-INT and INT-0 groups. DPD concentrations were statistically significantly lower between CTL group compared to non-INT, INT-0, and INT-28 (Fig. 3e, g).

On T2, increased levels of P1NP in CTL and INT-7 groups were statistically significant compared to INT-14, INT-28, and INT-45 groups, which demonstrated lower levels of P1NP after alendronate withdrawal (Fig. 3b). Decreased concentration of OCN was observed in all the treatment groups compared to the CTL group, but the differences were statistically significant only between CTL and non-INT group and between CTL and INT-45 group (Fig. 3d). In relation to the levels of bone resorption markers, CTX concentration was significantly decreased in the non-INT and in the INT-45 groups compared to the CTL, INT-0, and INT-14 group (Fig. 3f). DPD concentration was lower in all the treatment groups compared to the CTL group, but differences were found between INT-28 and INT-45 groups when compared to the CTL group (Fig. 3h).

Histomorphometric analysis

BIC

The linear extension of bone-to-implant contact was evaluated among groups. Animals from CTL group showed more bone-to-implant contact between the implant threads compared to all the other groups. CTL group demonstrated statistical differences when compared to the all the other groups. The lowest bone fill between the implant threads was observed in the INT-45 group (Fig. 4a).

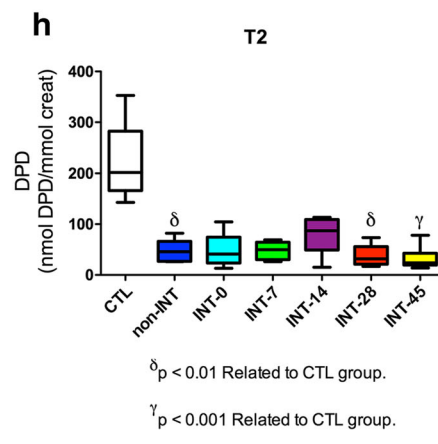
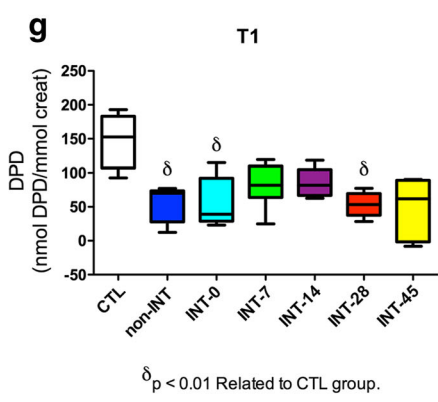
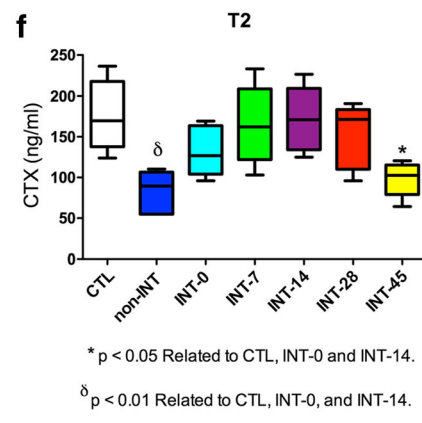
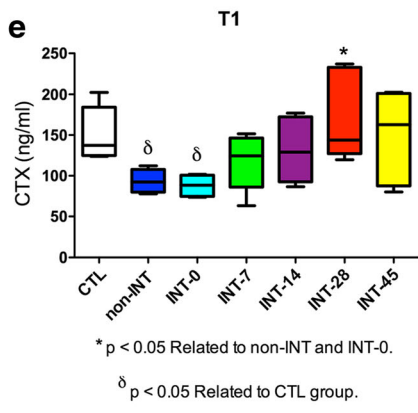
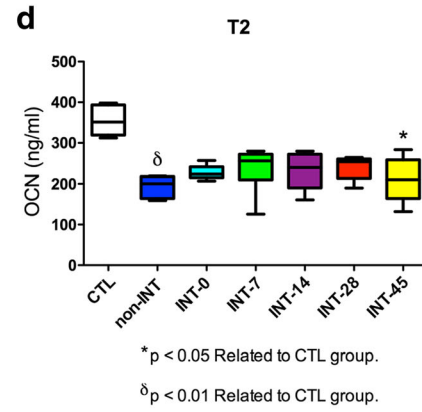
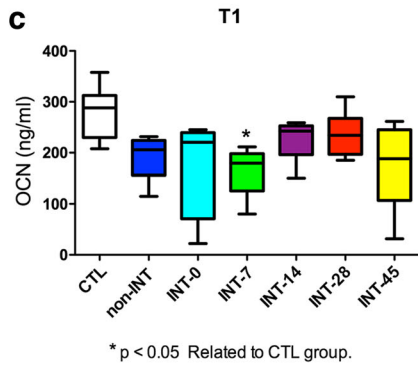
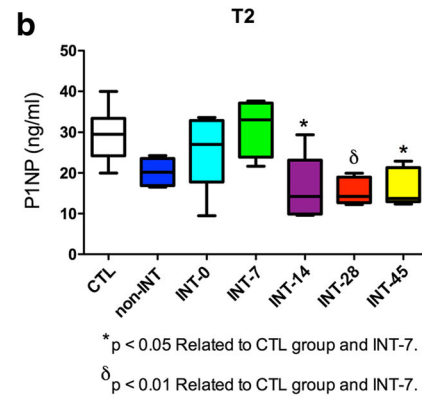
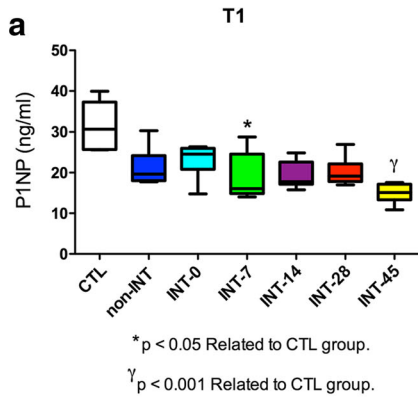
BAFO

Bone area fraction occupancy between implant threads was evaluated for all groups. The average percentages of bone area between the initial three mesial and distal threads of the implants were recorded. Non-INT and INT-0 group presented higher percentage of BAFO compared to the INT-45 group, which was statistically significant. No differences were found among CTL and alendronate treatment groups (Fig. 4b).

Histologic descriptive analysis

The effect of alendronate treatment and drug discontinuation on implant osseointegration was also assessed by descriptive histological analysis (Fig. 5a–g). All implants installed in the different groups showed successful implant osseointegration due to the observed direct contact, partial or total, between bone and implant. In the CTL group (Fig. 5), the bone tissue around osseointegrated implants showed normal and healthy characteristics with compact bone, presenting osteons with concentric lamellar organization formed in the area around the implant threads. Concentric lamellar forming haversian channels in the newly formed bone were also observed in the CTL group. Interestingly, higher percentage of bone-to-implant contact was observed in the CTL group compared to the groups that received alendronate (Fig. 5a).

In the groups treated with alendronate (non-INT), and after its discontinuation (INT-0, INT-7, INT-14, INT-28, and INT-45), the bone around the implant threads showed the presence of amorphous matrix and apparently little calcified tissue deposited between the implant threads. Loss of continuity between the resident bone tissue and the newly formed bone was also observed in these groups, suggesting the fragility of the



◀ **Fig. 3 a, c, e, g** Quantification of bone turnover markers during implant placement (T1) and **b, d, f, h** before animal euthanization (T2). * Statistically significant different ($p < 0.05$). δ Statistically significant different ($p < 0.01$; $p < 0.05$). γ Statistically significant different ($p < 0.01$; $p < 0.001$)

bone tissue, which could result in a higher rate of long-term failure of these implants. It was not possible to observe the presence of collagen fibers organized in lamellae that is characteristic of secondary bone formation. Osteocytic lacunae were absent in the bone formed around the implant threads (Figs. 5b–g).

Discussion

This study examined differences in the BMD, biochemical markers of bone formation and resorption, as well as in the percentage of BIC and BAFO in response to the discontinuation of alendronate in rats. Our data showed that BMD was significantly increased in animals under alendronate treatment and that its discontinuation did not reversed these findings. Accordingly, bone turnover markers tend to not be re-established in rats treated with alendronate. Specifically, BMD remained significantly higher in the trabecular bone of the femurs and vertebrae in all groups evaluated compared to the CTL rats. Bone formation rates (P1NP and OCN) remained lower compared to the CTL group during implant placement (T1) and before animal sacrifice (T2). Biological markers of bone resorption (CTX and DPD) also remained lower especially in the INT-45 group compared to CTL rats in T2. Moreover, BIC was lower in all groups on alendronate especially after 73 days of drug discontinuation (INT-45 group) compared to the CTL group, which might suggest the impairment of bone healing around osseointegrated implants.

The findings of this current investigation demonstrated that BPs administration increased BMD compared to the CTL animals. This expected result occurs because BPs inhibits bone resorption, allowing higher matrix deposition and mineralization, which reflects the increased bone density in the long bones and vertebrae [34]. These findings are consistent with our previous studies [18–20, 22] in normal or in ovariectomized rats, in which the increase in BMD in femurs and vertebrae after treatment with alendronate could be observed. Some clinical and pre-clinical studies [35–38] point to decreased BMD after antiresorptive discontinuation. Our findings did not reveal any change in the BMD values in both femurs and vertebrae even after 73 days of alendronate withdrawal. This fact could be explained due to the short period of follow-up in these animals, which closely resemble findings from previous reports that showed the maintenance of BMD values in patients after BPs removal [26, 39–41]. These variations in BMD indices after BP discontinuation could reflect differences in BP biodistribution, mode of administration, and bone mineral affinity.

In order to evaluate bone turnover markers after alendronate withdrawal, P1NP, OCN, CTX, and DMD were measured during implant installation (T1) and before animal's sacrifice (T2). Bone formation markers, P1NP and OCN, had their levels significantly decreased in T1 and T2 for all groups on alendronate and after its interruption compared to the CTL group. Significant differences were found for both markers in T1 and T2 especially for INT-45 group compared to the CTL group. Related to P1NP, which is a type 1 collagen marker, the results of the present study were similar to the OCN marker where BPs withdrawal did not increase its values in the later groups, which corroborates previous report [42, 43]. These results suggest that BPs has an effect on osteoblasts and in the process of bone remodeling [44]. Since OCN is released

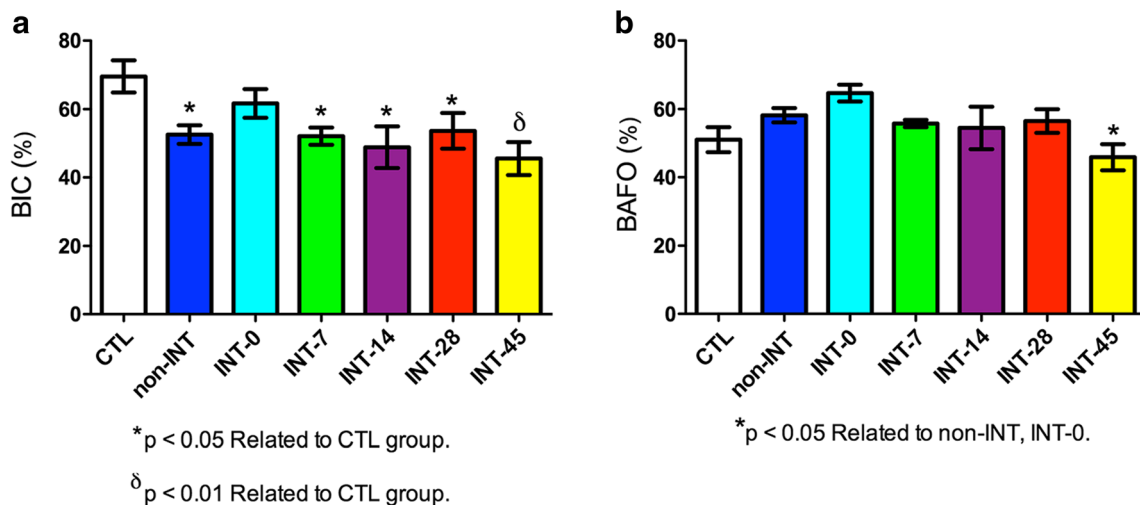
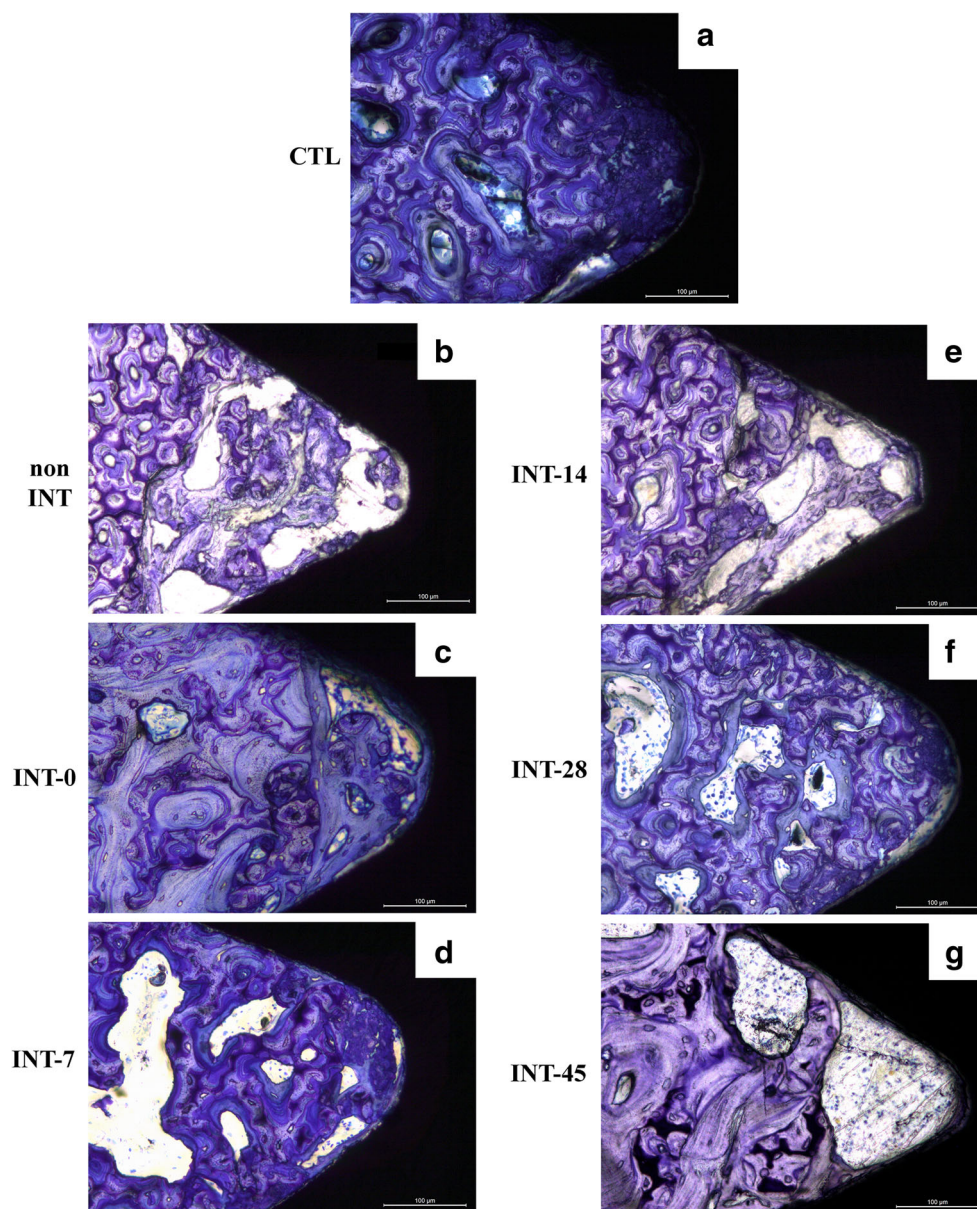


Fig. 4 a Quantification of %BIC and **b** %BAFO in the three first threads of the implants. δ Statistically significant different compared to the CTL group ($p < 0.01$). * Statistically significant different compared to the CTL group, non-INT and INT-0 groups ($p < 0.05$)

Fig. 5 a–g Representative histologic sections stained with Stevenel blue in the CTL, non-INT, INT-0, INT-7, INT-14, INT-28, and INT-45 groups, respectively. The first three threads of the implants were evaluated for descriptive histologic analysis and the first upper thread are showed



during the bone mineralization process, the lower values for OCN and P1NP in T1 and T2 might explain the deleterious effect of alendronate therapy in the histomorphometric and histomorphological findings even after its discontinuation [45, 46].

Relative to the markers of bone resorption, CTX values were significantly lower in the non-INT group when compared to the CTL group during T1 and T2. These findings indicate the effectiveness of alendronate in the inhibition of bone resorption. After drug discontinuation, CTX values were significantly higher in the INT-28 group followed by a decrease in the CTX values in the INT-45 group during T1. In T2, similar results were observed but after 73 days of drug discontinuation (INT-45 group), significantly lower CTX

values compared to the CTL group and similar with the non-INT group were noted. These findings suggest that alendronate withdrawal did not influence bone resorption markers, which result in the maintenance of osteoclastic activity inhibition, which are in agreement with previous studies [47, 48] where CTX values remained unaltered even after 3 years of BPs discontinuation. DPD values in the non-INT group were statistically significantly lower compared to the CTL group. Drug discontinuation did not influence the levels of DPD that was sustained after its removal. These findings corroborate previous studies [41, 49] where DPD values remained unaltered after BPs discontinuation.

Our findings that BIC in animals on alendronate (non-INT) and after drug withdrawal remained lower compared to the

CTL animals differ from the findings of previous study [50]. Duarte et al. [50] evaluated whether alendronate influences bone healing around endosseous implants inserted in healthy and ovariectomized (OVX) rats and also evaluated the effect of alendronate discontinuation in these animals. The results showed that alendronate therapy immediately after OVX inhibited bone loss, and that BIC and bone area presented with similar values to the sham-operated rats. Moreover, alendronate discontinuation revealed that BIC, bone area, and bone density in cancellous and cortical bone were similar to the control group. These contradictory results could potentially be attributed due to the high alendronate dose used (5 mg/kg/w), time intervals (four times/week), and period of evaluation (40 days of treatment followed by 40 days of discontinuation). It is important to note that our study used 1 mg/kg once per week for 120 days, which might have an impact on the BIC values around titanium implants. Our results parallel observations made by Mardas et al. [51], in which alendronate administration impaired bone healing around osseointegrated implants. Similar findings were encountered when alendronate gel was applied to the titanium implant and installed in the rabbit tibia [25]. Taken together, the results of the present study and others [25, 51] could be explained because alendronate has the capacity to exert their effect on osteoblasts, altering their physiology and diminishing bone formation by inhibiting their differentiation and maturation [44]. This may explain the detrimental effects of alendronate on bone remodeling for the compromised osseointegration results found in the present investigation.

BPs-suppressed remodeling allows microdamage to accumulate in the bone tissue and this fact could increase bone microdamage over time so long as bone remodeling has being suppressed [52]. According to Berglundh et al. [53], the placement of an implant in the receptor bed lead to a sequence of healing events involving bone micro-fractures and bone necrosis (due to damage caused by the drilling during implant bed preparation) followed by a subsequent resorption of the damaged bone with concomitant bone neo-formation. Due to its mechanism of action, alendronate administration strongly suppresses the osteoclast activity, which could harm the bone remodeling impairing the osseointegration process, and triggering the development of osteonecrosis after surgery for implant placement [54]. This fact could also explain the lower percentage of BIC in animals treated with alendronate and in animals after alendronate withdrawal compared to the CTL group.

The suppressing of bone remodeling that occurs after ALD treatment might impair bone-to-implant contact around osseointegrated implants [51]. Importantly, previous studies showed that the prolonged use of BPs, especially at high doses, has been clinically associated with osteonecrosis of the jaws (ONJ) after implant surgery, and increased rate of implant failures [55–58]. Periodontal and periapical diseases,

dental extraction, and surgery for dental implant placement play a pivotal role as triggers for BRONJ development [3, 59–61]. Thus, clinicians should be aware in relation to the incorporation of BPs in the bone mineral before implant installation because BPs can exert their anti-osteoclastic effects long after their administration [2]. In this regard, from a practical standpoint, clinicians should avoid dental implant placement in patients on BPs therapy to prevent ONJ development.

An interesting caveat that our studies did not address was whether a control group with geranylgeraniol would have excluded nonspecific factors. This fact should be evaluated in further pre-clinical studies to strengthen the findings of the current investigation. Another important consideration that should be mentioned is the animal model utilized. The chosen rat tibiae model for implant installation does not resemble the situation at mandible and maxilla. With the tibia being surrounded by thick and well-perfused muscles, it does not mimic to the conditions at the jaws. Additionally, experimental rat model possess some limitations compared to the humans, such as faster skeletal change and maturity, faster bone turnover, and different healing behavior, i.e., rat synthesizes ascorbic acid, which is important for the collagen synthesis. However, rats were selected as the experimental model because they are easy to handle, due to low maintenance care, and because they are the smallest animal that can accept implants in the long bones in the proximal region of the tibia, far from the growth plate. Finally, further studies utilizing different animal models, such as the minipig with commercially available implants installed in the maxilla and/or mandible, should be performed before definitive conclusions can be drawn.

In summary, we report that BMD values from femurs and vertebrae were significantly increased in rats on alendronate when compared to the CTL group, which were maintained after drug withdrawal. Moreover, alendronate therapy decreased bone turnover markers and impaired bone quality and quantity around dental implants, and that its discontinuation did not reversed these findings. Indeed, to the best of the author's knowledge, this is the first animal study to illustrate and quantify such findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

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