UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA

Aminobisphosphonates cause changes in the thickness of the different zones of the epiphyseal disk of *Wistar* rats.

Elissa Kerli Fernandes

Porto Alegre
Dezembro, 2017

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA

Aminobisphosphonates cause changes in the thickness of the different zones of the epiphyseal disk of *Wistar* rats.

Elissa Kerli Fernandes

Trabalho de conclusão de curso apresentado como pré-requisito para obtenção do Grau de Farmacêutica

Orientador: Prof. Dr. Alexandre Silva de Quevedo
Co-orientadora: Prof. Dra. Deise Ponsoni

Porto Alegre
Dezembro, 2017

“*Uma criança, um professor, um livro e um lápis podem mudar o mundo.”*

- Malala Yousafzai

APRESENTAÇÃO

Este Trabalho de Conclusão de Curso foi redigido sob a forma de artigo, o qual foi elaborado segundo as normas da revista “Osteoarthritis and Cartilage”, apresentadas em anexo.

Aminobisphosphonates cause changes in the thickness of the different zones of the epiphyseal disk of *Wistar* rats.

Elissa Kerli Fernandes[a], Mateus Müller da Silva[b], Deise Ponzoni[c], Izabel Cristina Custódio de Souza[d], *Alexandre Silva Quevedo[e]

[a]School of Pharmacy, Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, RS, Brazil. Email: elissa.kfernandes@yahoo.com.br

[b]School of Dentistry – UFRGS, Porto Alegre, RS, Brazil. E-mail: mateus18muller@gmail.com

[c]School of Dentistry – UFRGS, Hospital de Clínicas de Porto Alegre - HCPA, Porto Alegre, RS, Brazil. Email: dponzoni@yahoo.com

[d]Universidade Federal de Pelotas - UFPEL, Pelotas, RS, Brazil. Email: izzcustodio@gmail.com

[e]School of Dentistry, Department of Surgery and Orthopedics – UFRGS, Porto Alegre, RS, Brazil. E-mail: quevedoalexandre@hotmail.com

*Corresponding author:

Alexandre Silva de Quevedo, D.D.S, Ph.D.

Faculdade de Odontologia/Universidade Federal do Rio Grande do Sul

Rua Ramiro Barcelos 2492, Porto Alegre, RS, Brazil CEP 90035-004

Phone number: +55 51 3308-5010

quevedoalexandre@hotmail.com

Running title: Influence of bisphosphonates on growth

Abstract

(1) Objective: The aim of the present study was to test the hypothesis that Alendronate Sodium (AS) and Zoledronic Acid (ZA) modifies the thickness of the different zones of the epiphyseal disc of the femur of rats.

(2) Materials and methods: *Wistar* rats (n = 19) were divided into three groups: Group 01: sodium alendronate oral (3 mg / kg / day); Group 02: zoledronic acid, intraperitoneal route (0.2 mg / kg / week) and Group 03: control, without drug administration. After 21 days of treatment, the animals were euthanized and the femurs were collected for subsequent preparation of stained slides with Hematoxylin and Eosin. The images of the epiphyseal disk region were captured using Qcapture® software with a 400-fold increase. The areas analysis was performed with the Adobe Photoshop CS3 extended program.

(3) Results: AS caused reduction in the proliferative zone, while ZA caused significant decrease in most areas with cellular activity and a significant increase in the calcified zone. The total thickness of the growth plate is not caused by proportional reduction of all the zones that integrate the epiphyseal disk, because they responded differently.

(4) Conclusions: These medications caused changes in the thickness of the different zones on the femoral epiphyseal disk, showing that AS and ZA may interfere with the normal growth of the long bones.

Keywords: Bisphosphonates; Epiphyseal Cartilage; Plate, Growth;

INTRODUCTION

Bisphosphonates (BIS) are a class of drugs of pyrophosphate analogues, which bind strongly to the hydroxyapatite of bones and inhibit its resorption. The exchange of the oxygen bridge in the molecule of pyrophosphate by a carbon atom made the BIS resistant to enzymatic hydrolysis in a biological medium, making it possible to use them as medicines.[1] They have been used to treat diseases that affect bone metabolism related to high bone resorption, such as osteoporosis, Paget's disease, hypercalcemia of malignancy,[2] prevention of bone metastases, frequently observed in lung, prostate and breast cancer.[3]

Based on the presence or absence of nitrogen in its structure, the BIS may be classified in aminobisphosphonates or non-aminobisphosphonates respectively. Moreover, the aminobisphosphonates have shown to be more potent than the non-aminobisphosphonates.[4] Alendronate sodium (AS) and zoledronic acid (ZA) are examples of nitrogenated bisphosphonates.[1]

Studies have shown that the use of BIS influences the growth of long bones in rats causing changes in the epiphyseal disk.[5] This structure is responsible for postnatal linear bone growth, and is divided into different zones that have distinct morphological and biochemical stages during the process of chondrocyte differentiation.[6] Because BIS are administered to patients in different stages of growth,[7] the effects of those drugs on the epiphyseal disk should be investigated more extensively.

Therefore, more studies are necessary to understand how BIS affect the endochondral ossification and consequently modify the growing development. Considering the lack of knowledge about the events that lead to the alteration of the normal development of the long bones in animals submitted to the therapy

with BIS, the present study tested the hypothesis that aminobisphosphonates, such as AS and ZA, may cause changes in the thickness in the different zones of the epiphyseal disc of the femur of rats.

METHOD

Ethical aspects

All experiments and procedures were approved by the Institutional Animal Care and Use Committee (Hospital de Clínicas de Porto Alegre-HCPA/The Postgraduate Research Group-GPPG, protocol No. 09-366) and were compliant with Brazilian guidelines involving use of animals in research (Law No. 11,794).

Animals

The sample consisted of 19 isogenic albino rats of the *Rattus novergicus albinus* species, *Wistar*, males, with an average age of 120 days and mean weight of 300 grams, from the Unit of Animal Experimentation of the Hospital de Clinicas de Porto Alegre. The animals were distributed into different groups randomized by body weight. They were housed in the Polypropylene material cages (49x34x16cm). All animals were kept on a standard 12-hour light/dark cycle (lights on at 7.00 a.m. and lights off at 7.00 p.m.), in a temperature-controlled environment (22±2ºC), and had access to water and chow (Nuvilab, Moinhos Purina, Porto Alegre, RS - Brasil) *ad libitum.*

Treatment

The animals were randomly divided into three groups:, alendronate sodium (AS, n=7), zoledronic acid (ZA, n=7) and control group (C, n=5). The rats of the AS group were treated with alendronate sodium (Alendronato de Sódio®, Eurofarma S.A, RJ, Brasil) 3.0mg/kg by daily oral gavage. The rats of the ZA group were treated with zoledronic acid (Ácido Zoledrônico®, Eurofarma S.A, RJ,

Brasil.) 0.2mg/kg by weekly intraperitoneal injection. The animals of the control group did not receive medication intervention. After receiving the treatment for twenty-one days,[20] the animals were euthanized with overdose of anesthetics.

Histological preparation

The right and left femurs of each rat were dissected and fixed in 10% buffered neutral formalin solution. After 48 hours, the pieces were decalcified in 10% nitric acid for 7 days, dehydrated and embedded in paraffin blocks. Sagittal sections (5μm) of femurs were cut using microtome stained using haematoxylin-eosin (H&E), according to the “Hematoxylin and eosin staining of tissue and cell sections” protocol.[8]

Measurement of thickness of epiphyseal disk

Two slides were generated for each animal, corresponding to the right and left femoral sagittal cut. Three areas of distal epiphysis were selected on each slide, corresponding to the region of the medial condyle, intercondylar area and lateral condyle. Images of the epiphyseal disk in these regions were captured using Qcapture® software with a magnification of 400x. Total area of epiphyseal disk was measured with Adobe Photoshop CS3 extended program.[9]

Areas of the epiphyseal disk were classified into the resting zone, proliferative zone, hypertrophic zone and calcified zone. After identification, they were delimited and measured. The area that each zone occupies in the cartilage was calculated relatively to the total thickness of the epiphyseal disk. The analysis was performed by an experienced histologist. The area that each zone occupies in the cartilage was calculated relatively to the total thickness, according to the following formula:

$$\text{Area of each zone} = \frac{\text{thickness of each zone} * 100}{\text{thickness of the total area}}$$

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of data distribution.

Data were expressed by the means ± standard deviation of the mean (S.E.M.). The one-way analysis of variance ANOVA, followed by Fisher's LSD, was performed to compare between groups. All analysis was performed using SPSS 20.0 packages for windows. The level of probability was set at $P<0.05$ as statistically significant.

RESULTS

Medial condyle region

The figure 1 shows that the treatment with ZA caused decrease in the mean of thickness of the resting (CC vs. ZA, $F_{(2-23)}=6.15$, $p=0.04$), proliferation (CC vs. ZA, $F_{(2-23)}=24.04$, $p<0.0001$) and hypertrophic zone (CC vs. ZA, $F_{(2-23)}=10.42$, $p<0.0001$), whereas the treatment with AS caused decrease of thickness of proliferation zone (CC vs. AS, $F_{(2-23)}=24.04$, $p < 0.0001$).

----------Espaço para Figure 1----------

Intercondylar region

The figure 2 shows that the treatment with ZA caused decrease of the mean thickness of the resting (CC vs. ZA, $F_{(2-23)}=17.17$, $p<0.0001$), proliferation (CC vs. ZA, $F_{(2-23)}=20.49$, $p < 0.0001$) and hypertrophic zone (CC vs. ZA, $F_{(2-23)}=6.03$, $p =0.02$) and increase of the mean of thickness of the calcified zone (CC vs. ZA, $F_{(2-23)}=10.50$, $p<0.0001$). The treatment with AS caused decrease of the mean thickness of the proliferative zone (CC vs. AS, $F_{(2-23)}=20.49$, $p<0.0001$). The decrease in proliferative zone thickness is significantly higher in the ZA group compared to the AS group (ZA vs. AS $F_{(2-23)}=17.17$, $p <0.0001$).

----------Espaço para Figure 2 ----------

Lateral region

The figure 3 shows that the treatment with ZA caused decrease of the mean thickness of the resting (CC vs. ZA, $F_{(2-23)}$=29.60, p<0.0001) and hypertrophic (CC vs. ZA, $F_{(2-23)}$=6.50, p =0.02) and increase of the mean of thickness of the calcified zone (CC vs. ZA, $F_{(2-23)}$=6.88, p=0.04). The AS treatment caused decrease of mean of thickness of the resting (CC vs. AS, $F_{(2-23)}$=29.60, p<0.0001) and proliferation (CC vs. AS, $F_{(2-23)}$=6.51, p=0.03) zones and increase of the mean of thickness of the calcified zone (CC vs. AS, $F_{(2-23)}$=6.88, p=0.03). The decrease in resting zone thickness is significantly higher in the ZA group compared to the AS group (AS vs. ZA, $F_{(2-23)}$=29.60, p <0.0001).

----------Espaço para Figure 3----------

DISCUSSION

In general, the present study showed that AS caused a reduction in the proliferative zone while ZA caused a significant decrease in most areas with cellular activity and a significant increase in the calcified zone. The decrease in the cell zones was not compensated by the increase of the calcified zone, which explains the reduction in the total thickness of the growth plate.

There are drugs may delay, abolish or decrease the bone formation. For example, bisphosphonates, that are drugs with antiresorptive properties, inhibit bone reabsorption by interaction with clastic cells,[1] which are required for reabsorption of calcified cartilage septa and for allowing in the invasion of osteogenic cells that secrete bone matrix over the calcified cartilage during the process of endochondral ossification.[10]

Differences in anatomy, physiology and developmental biology of rats and humans should be taken into account when age is an important factor.[11] In humans, epiphysis closure is variable between individuals and between different growth plates within the body, but around the age of 20, all the epiphyses ended their growth. In rats, growth plate closure does not occur, and at about 7-8 months of life in male and female Sprague-Dawley, there is a period in which skeletal growth decreases.[12]

The structure responsible for the growth of long bones is the epiphyseal disc, which is composed of highly specialized cartilage [6] and it can be divided into different zones according to the stage of ossification: resting zone, proliferative zone, hypertrophic zone and calcified zone.[13] Chondrocytes are metabolically active cells that secrete and maintain this highly specialized matrix, and

contribute to endochondral ossification by participating in an ordered sequence of events that are reflected in their morphology.[14]

Short-term administration of ZA in rats caused a decrease in the resting zone in the three femoral condyle regions: medial, intercondylar, and lateral (fig. 1,2 and 3), and the administration of AS caused a decrease only in the lateral region (fig. 3). This decrease was more intense in both intercondylar and lateral regions in animals treated with ZA compared to AS treated animals (fig. 2 and 3, respectively). The resting zone is a narrow and irregularly contoured region, consisting of single or paired chondrocytes in a relatively quiescent state and the ratio of extracellular matrix to cell volume is high.[6; 15] The function of the resting zone is likely the endowment of chondrocytes to the proliferative zone.[16] As the chondrocytes progress on the resting zone, they gain a proliferative phenotype and adopt a flattened, oblate shape, arranging themselves into longitudinal columns.[17] Treatment with ZA caused a decrease in the thickness of this zone in the medial and intercondylar regions (fig. 1 and 2, respectively), whereas the AS treatment caused a decrease in this zone in the three regions of the femoral condyle that were observed, medial region and intercondylar region, and lateral region (fig. 1,2 and 3). In the proliferative zone, the chondrocytes undergo a rapid perpendicular division along the long axis of growth.[16] Other functions of this zone include intracellular matrix formation, proteoglycan and collagen.[17]

After a finite number of cell divisions, the chondrocytes lose the ability to divide and begin hypertrophy.[18] Cell division ceases and the chondrocytes begin to differentiate terminally.[16] The treatment with ZA caused reduction in the thickness of the zone of chondrocyte hypertrophy in the three femoral condyle regions observed, medial, intercondylar, and lateral (fig. 1, 2 and 3, respectively),

whereas the AS treatment did not cause changes. The function of this tissue is to promote calcification of the cartilage that serves as a model for bone formation.[6]

The present work shows that BIS treatment caused decrease of thickness of resting, proliferative and hypertrophic zones, which corroborates the results of Oyhanart et al, 2015. The administration of AS (0.2 mg/kg/wk) on growing animal caused a significant decrease in the total thickness of the epiphyseal cartilage, as result of a decrease in the thickness of both resting and proliferative cartilage zones and of hypertrophic cartilage, as well alteration in a number and morphology of osteoclasts.[19] The decrease of the total cartilage thickness, particularly in the proliferative zone, may be explained by the fact that AS reduces level of vascular endothelial growth factor (VEGF), that relate angiogenesis and is essential to ossification and chondrocyte survivor. The turnover of this cells in epiphyseal disk also is modulates for this growth factor.[19] However, the same did not happen when a dosage of 2.5 mg / kg for 21 days was given, which caused enlargement of cartilage zones.[20]

Other study showed that short duration ZA treatment (0.1mg/kg 3 times per week for 8 weeks) caused discontinuation in development of the growth plate of the proximal tibia of rats. They observed pathological changes in chondrocytes and cell alignement was disturbed, and there was also a decrease in physis height.[21] In previous studies of our group shows decrease in total epiphyseal disk thickness in the groups in which the animals were treated with BIS.

The differences between the response to the treatment with different bisphosphonates may perhaps be related to the difference of affinities and potency between the drugs. The strongest mineral binding is associated with

greater suppression of bone turnover, which may in turn have an influence over the pharmacological potency and clinical effects of different BIS.[22] Differences in the degree of inhibition of the farnesyl pyrophosphate synthase (FPPS) enzyme in osteoclasts should be a major contribution to the pharmacological potency of nitrogen-containing bisphosphonates[23; 24] and ZA has a higher inhibitory activity in FPPS and greater affinity for bone mineral compared to AS, which appear to contribute to its greater potency.[22]

The treatment with ZA caused an increase of the mean of the calcified zone of intercondylar region of femoral condyle (fig 2), and AS caused an increase in the same zone in the lateral region (Figure 3), showing that both bisphophonates altered the normal remodeling process of normal cartilage. Clastic cells, osteoclasts and chondroclasts, are necessary for the reabsorption of calcified cartilage septa and for allowing in the invasion of osteogenic cells that secrete bone matrix over the calcified cartilage during endochondral ossification.[16] Bisphosphonates inhibit the attachment of the clastic cells to the bone surface when internalized by these cells. Rats treated with alendronate 2.5mg/Kg for 21 days presented clastic cells with latent phenotype or with apoptotic nuclei. Although some cells were attached to the calcified cartilage matrix by short podosomes, they did not have the typical reabsorption apparatus.[20]

In conclusion, the different regions of the femoral condyle responded differently to both the same drug and the different treatments. The changes that AS and ZA cause in the thickness of the different zones of the rat epiphyseal disk appear to be dose-dependent and time-dependent of administration. The area most affected by AS treatment was chondrocyte proliferation zone. The different

zones responded non-specifically to the treatment. The reduction in the total thickness of the growth plate can be explained by the reduction of the cellular zones, that was more intense with the administration of ZA than with AS, without compensation by the increase of the zone of calcification.

ACKNOWLEDGMENTS

We would like to thank the Programa Institucional de Bolsas de Iniciação Científica (PIBIC), to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the opportunity of scientific initiation**,** and to the technicians of the Unit of Animal Experimentation of the Hospital of Clinics of Porto Alegre, where the experiments were carried out and to the Hospital de Clinics of Porto Alegre, for the allocation of resources of Fundo de Incentivo à Pesquisa e Eventos (FIPE).

AUTHOR CONTRIBUTIONS

Elissa Kerli Fernandes: The conception and design of the study, analysis and interpretation of data, drafting the article, and final approval of the version to be submitted.

Mateus Müller da Silva: Acquisition of data, drafting the article, and final approval of the version to be submitted.

Deise Ponzoni: The conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content, and final approval of the version to be submitted.

Izabel Cristina Custódio de Souza: acquisition of data, analysis and interpretation of data, and final approval of the version to be submitted

Alexandre Silva Quevedo: analysis and interpretation of data, drafting the article, revising it critically for important intellectual content, and final approval of the version to be submitted.

ROLE OF THE FUNDING SOURCE

This work was supported by Fundo de Incentivo à Pesquisa (FIPE) of Hospital de Clínicas de Porto Alegre.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1 RUSSELL, R. G. et al. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. **Osteoporos Int,** v. 19, n. 6, p. 733-59, Jun 2008. ISSN 0937-941X (Print)0937-941x.

2 SPARIDANS, R. W.; TWISS, I. M.; TALBOT, S. Bisphosphonates in bone diseases. **Pharmacy world & science,** v. 20, n. 5, p. 206-213, 1998. ISSN 0928-1231.

3 MACEDO, F. et al. Bone Metastases: An Overview. **Oncol Rev,** v. 11, n. 1, p. 321, Mar 2017. ISSN 1970-5565. Disponível em: < https://www.ncbi.nlm.nih.gov/pubmed/28584570 >.

4 LIN, J. H. Bisphosphonates: a review of their pharmacokinetic properties. **Bone,** v. 18, n. 2, p. 75-85, Feb 1996. ISSN 8756-3282 (Print)1873-2763.

5 PATAKI, A. et al. Effects of short-term treatment with the bisphosphonates zoledronate and pamidronate on rat bone: a comparative histomorphometric study on the cancellous bone formed before, during, and after treatment. **Anat Rec,** v. 249, n. 4, p. 458-68, Dec 1997. ISSN 0003-276X (Print)0003-276x.

[6] BALLOCK, R. T.; O'KEEFE, R. J. Physiology and pathophysiology of the growth plate. **Birth Defects Res C Embryo Today,** v. 69, n. 2, p. 123-43, May 2003. ISSN 1542-975X (Print)1542-975x.

[7] BIGGIN, A.; MUNNS, C. Long-Term Bisphosphonate Therapy in Osteogenesis Imperfecta. **Current Osteoporosis Reports,** v. 15, n. 5, p. 412-418, 2017. ISSN 1544-1873.

[8] FISCHER, A. H. et al. Hematoxylin and eosin staining of tissue and cell sections. **CSH Protoc,** v. 2008, p. pdb.prot4986, May 1 2008. ISSN 1559-6095.

[9] SPALLEK, H.; MOONEY, M. P. Quantitative Image Analysis using Adobe® Photoshop® CS3 Extended.

[10] LI, Z.; KONG, K.; QI, W. Osteoclast and its roles in calcium metabolism and bone development and remodeling. **Biochem Biophys Res Commun,** v. 343, n. 2, p. 345-50, May 5 2006. ISSN 0006-291X (Print) 0006-291x.

[11] ANDREOLLO, N. A. et al. Rat's age versus human's age: what is the relationship? **ABCD. Arquivos Brasileiros de Cirurgia Digestiva (São Paulo),** v. 25, n. 1, p. 49-51, 2012. ISSN 0102-6720.

[12] QUINN, R. Comparing rat's to human's age: how old is my rat in people years? **Nutrition,** v. 21, n. 6, p. 775, 2005. ISSN 0899-9007.

[13] WILSMAN, N. J. et al. Differential growth by growth plates as a function of multiple parameters of chondrocytic kinetics. **J Orthop Res,** v. 14, n. 6, p. 927-36, Nov 1996. ISSN 0736-0266 (Print) 0736-0266.

[14] MACKIE, E. J.; TATARCZUCH, L.; MIRAMS, M. The skeleton: a multi-functional complex organ: the growth plate chondrocyte and endochondral ossification. **J Endocrinol,** v. 211, n. 2, p. 109-21, Nov 2011. ISSN 0022-0795.

[15] HUNZIKER, E. B. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. **Microsc Res Tech,** v. 28, n. 6, p. 505-19, Aug 15 1994. ISSN 1059-910X (Print)1059-910x.

[16] VON PFEIL, D. J.; DECAMP, C. E. The epiphyseal plate: physiology, anatomy, and trauma. **Compend Contin Educ Vet,** v. 31, n. 8, p. E1-11; quiz E12, Aug 2009. ISSN 1940-8307.

[17] HUNZIKER, E. B.; SCHENK, R. K.; CRUZ-ORIVE, L. M. Quantitation of chondrocyte performance in growth-plate cartilage during longitudinal bone growth. **J Bone Joint Surg Am,** v. 69, n. 2, p. 162-73, Feb 1987. ISSN 0021-9355 (Print) 0021-9355.

[18] BRIGHTON, C. The growth plate. **The Orthopedic clinics of North America,** v. 15, n. 4, p. 571-595, 1984. ISSN 0030-5898.

[19] OYHANART, S. R.; ESCUDERO, N. D.; MANDALUNIS, P. M. Effect of alendronate on the mandible and long bones: an experimental study in vivo. **Pediatr Res,** v. 78, n. 6, p. 618-25, Dec 2015. ISSN 0031-3998.

[20] REZENDE, E. et al. Effects of bisphosphonates on osteogenesis and osteoclastogenesis signaling during the endochondral ossification of growing rats. **Cell Tissue Res,** v. 368, n. 2, p. 287-300, May 2017. ISSN 0302-766x.

[21] ERDOGAN, M. et al. The effect of zoledronic acid on growth plates and high turnover bones. **Bratisl Lek Listy,** v. 115, n. 3, p. 131-5, 2014. ISSN 0006-9248 (Print)0006-9248.

[22] LAWSON, M. et al. **Potential bone mineral binding differences among bisphosphonates can be demonstrated by the use of hydroxyapatite column chromatography**, 2005. p.

[23] CUMMINGS, S. R. et al. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. **Jama,** v. 280, n. 24, p. 2077-82, Dec 23-30 1998. ISSN 0098-7484 (Print) 0098-7484.

[24] HARRINGTON, J. T. et al. Risedronate rapidly reduces the risk for nonvertebral fractures in women with postmenopausal osteoporosis. **Calcif Tissue Int,** v. 74, n. 2, p. 129-35, Feb 2004. ISSN 0171-967X (Print) 0171-967x.

FIGURE LEGENDS

Figure 1. Medial condyle region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 2. Intercondylar region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 3. Lateral condyle region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 1

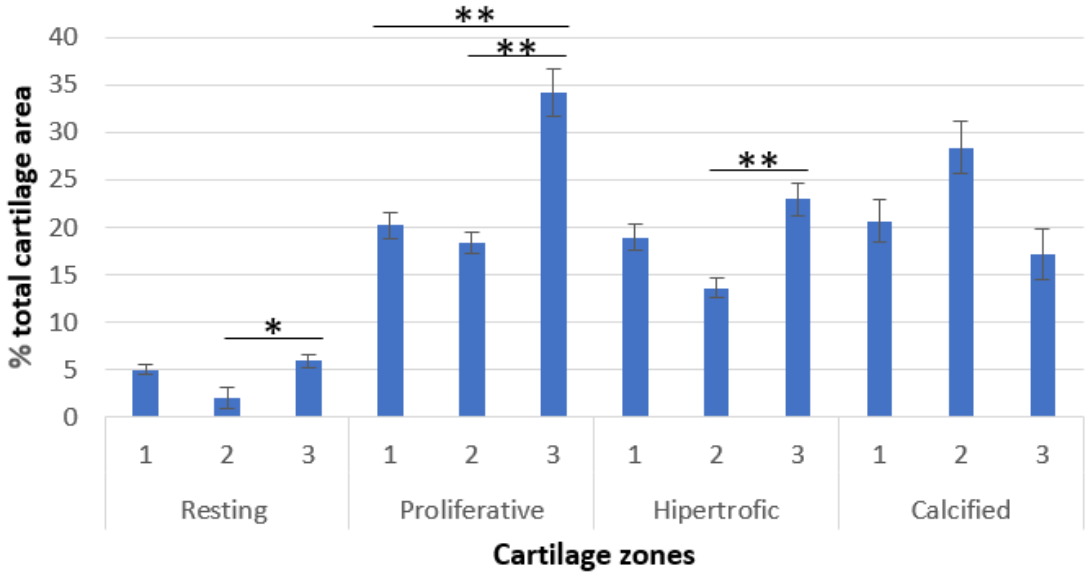

Figure 1. Medial condyle region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * $p < 0.05$ and ** $p < 0.001$. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 2

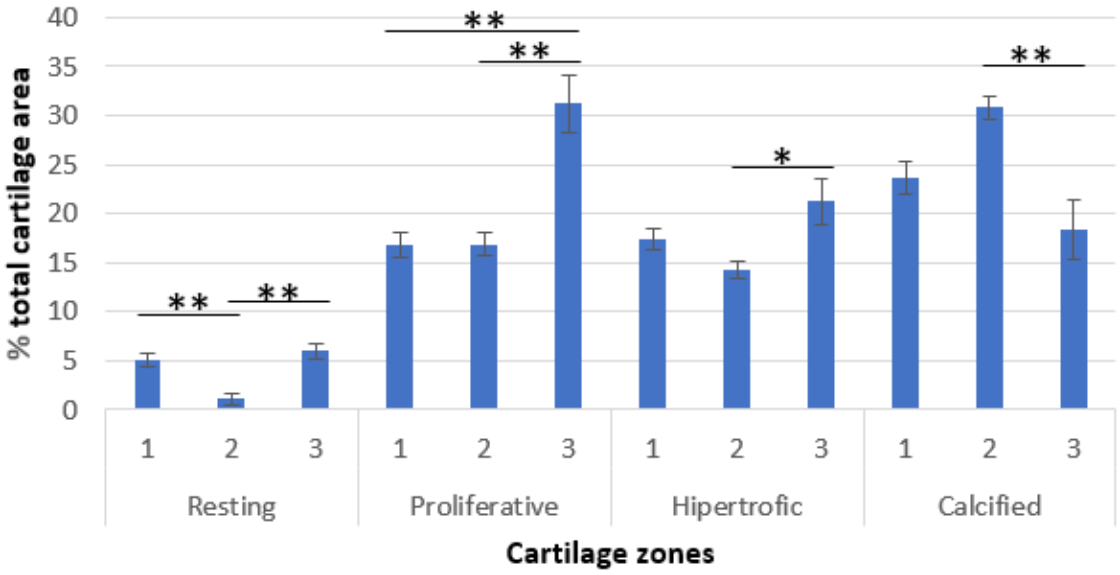

Figure 2. Intercondylar region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 3

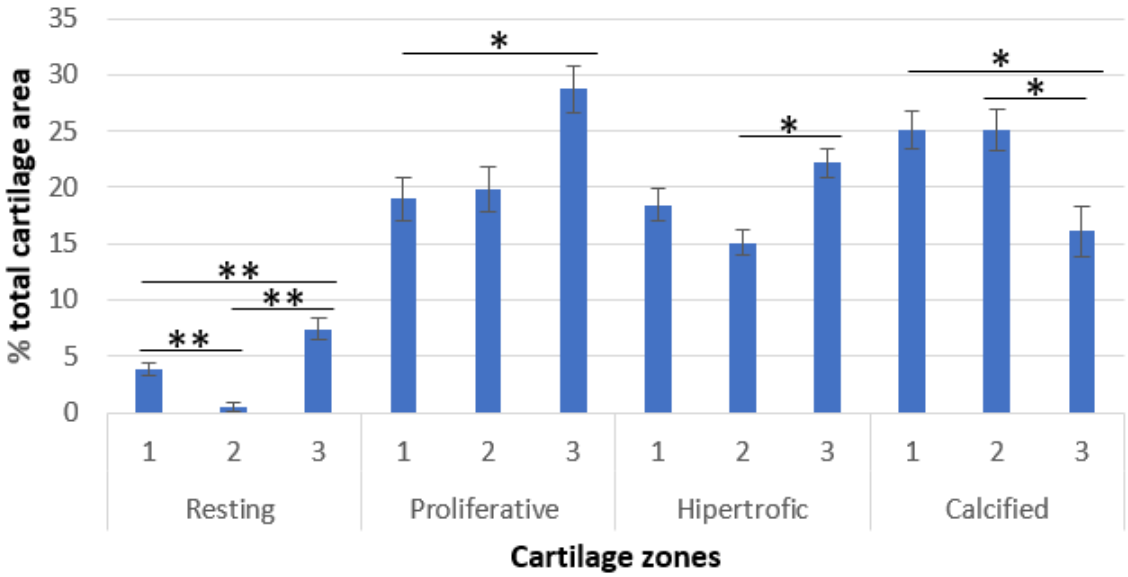

Figure 2. Intercondylar region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.

Figure 3. Lateral condyle region: Area of the epiphyseal disc zones in the medial condyle region in AS group (1) (n=7), ZA group (2) (n=7), and Control group (3) (n=5). One-way ANOVA followed by Fisher's LSD post hoc test, * p <0.05 and ** p<0.001. AS= Alendronate Sodium, ZA= Zoledronic Acid.