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Bisphosphonate treatment changes regional distribution of trabecular microstructure in human lumbar vertebrae

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ABSTRACT

Background: In osteoporosis patients, antiresorptive treatments such as alendronate reduce the resorption of trabecular bone and thus minimize vertebral fracture risk. However, fracture risk reduction efficacy of antiresorptive drugs varies between skeletal sites and is highest for vertebral bone. In human vertebrae, cancellous bone is distributed heterogeneously between regions. This microstructural heterogeneity is changing with patient age and is likely to play a major role in vertebral failure mechanisms and fracture susceptibility. Whether antiresorptive treatment affects the heterogeneity of vertebral microstructure in osteoporosis has not been unraveled.

Methods: Our aim was to assess whether antiresorptive treatment would have a region-dependent influence on vertebral trabecular bone. Therefore, we used high-resolution peripheral quantitative computed tomography (HR-pQCT), microcomputed tomography (microCT) and uniaxial compression testing to determine the structure and mechanical properties of trabecular bone cores from anterior and posterior regions of 22 lumbar vertebrae from elderly osteoporotic women. We analyzed age-matched ex vivo bone samples from bisphosphonate-treated female osteoporosis patients (age: 82 ± 7y, bisphosphonate treatment period: 4 ± 2 years) along treatment-naïve female controls (82 ± 7y).

Results: MicroCT analysis showed a significantly lower bone volume fraction ($p = 0.006$) and lower trabecular number ($p = 0.003$) for the anterior bone cores compared to posterior bone cores in the treatment-naïve group. The bisphosphonate-treated group had a more homogeneous bone volume distribution and did not show significant regional differences in bone volume, it however also displayed significantly different trabecular numbers ($p = 0.016$). In bone cores of the bisphosphonate-treated group, trabeculae were thicker in comparison to treatment-naïve controls ($p = 0.011$). Differences in bone volume further resulted in different maximum forces during compression testing between the samples. In addition, the percental difference between $BV/TV_{\mu CT}$ in anterior and posterior bone cores was lower in bisphosphonate-treated vertebrae when vertebrae with directly adjacent fractures ($n = 3$) were excluded.

Conclusion: In conclusion, regional trabecular bone microstructure in lumbar vertebrae of bisphosphonate-treated women was more homogeneous compared to treatment-naïve controls. Bisphosphonate treatment, which specifically targets resorption surfaces common in anterior vertebral bone, might have resulted in a region-specific preservation of vertebral microstructure and loading capacity. This could have positive implications for the reduction of wedge fracture risk and add to the explanation of the higher efficacy of fracture risk reduction in vertebrae in comparison to other fracture regions.

Abbreviations: BV/TV_{HRpQCT}, BV/TV derived by high-resolution quantitative computed tomography; $BV/TV_{\mu CT}$, BV/TV derived by micro-computed tomography

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1. Introduction

With high rates of fracture risk reduction, bisphosphonate treatment is a cost-effective option for the prevention of osteoporotic fractures [1], especially in postmenopausal and elderly women [2]. Different types of bisphosphonates vary in their capability to reduce fracture risk: Zoledronic acid is most effective (OR 0.61), followed by alendronate (0.64), clodronate (0.69) and risedronate (0.74) [3]. In recent years, usage of all bisphosphonates has dwindled leading to an increase in fractures and mortality, amplified by negative media attention on rare side effects such as osteonecrosis of the jaw and atypical femoral fractures [4]. The discussion about rare but severe side effects has spawned new interest into treat-to-target strategies for osteoporosis management [5]. Defining a treatment goal, e.g. a targeting of bone volume distribution or a target areal bone mineral density (aBMD), would limit exposure to medication for osteoporosis patients. Applying a treat-to-target strategy to osteoporosis management is expected to reduce treatment costs and risk for adverse side effects simultaneously [6]. Since the capabilities of BMD as a treatment target are limited [7], creating a better understanding about the underlying mechanisms of bisphosphonates' action on bone volume and structure might help to establish a reasonable treatment target and further reinstate trust in this effective treatment option.

The efficacy of most current bisphosphonates varies drastically depending on fracture site. Bisphosphonate treatments reduce fracture risk for vertebrae with an odds ratio of 0.55 and for other fracture sites with an odds ratio of 0.73 on average [3]. For alendronate specifically, the fracture incidence within the first 12 months of treatment is reduced by 57% at vertebral sites and by 28% at non-vertebral sites compared to baseline incidences [8].

The higher efficacy for vertebral fracture risk reduction might be partly explained by the higher content of trabecular bone in the vertebrae compared to other skeletal sites, e.g. the hip. The higher surface-to-volume ratio in trabecular bone favors resorption [9] and thus theoretically allows for increased binding of bisphosphonates to resorption surfaces [10]. Another important characteristic of vertebral trabecular bone is its heterogeneity: The bone volume and bone structure quality, quantified by trabecular number, thickness, and spacing, is lower in anterior vertebral regions compared to posterior regions [11–14]. A destructive compression of the anterior region is termed wedge fracture and represents the most common fracture type in men and women [15,16]. Aging-related changes in the heterogeneity of the trabecular bone in vertebrae are considered to contribute to an increasing risk of wedge fractures [17].

Whether bisphosphonates' influence varies in different regions of trabecular bone remains to be investigated. We hypothesized that aging-related changes in regional heterogeneity of trabecular bone might interact with the efficacy of bisphosphonates. Thus we analyzed trabecular bone cores from the anterior and posterior regions from lumbar vertebrae of untreated and bisphosphonate-treated osteoporotic women with high-resolution microCT imaging and mechanical testing. The goal of our study was to determine:

1. whether local differences in trabecular architecture due to bisphosphonate treatment could be detected and
2. whether these local trabecular architecture differences translated into changes in the load-bearing capacity of the bone.

2. Material and methods

We obtained second and third lumbar vertebrae post-mortem from postmenopausal women in cooperation with the Department of Forensic Medicine at the University Medical Center Hamburg-Eppendorf after approval of the City of Hamburg's Chamber of Physicians Ethics Committee (# PV3486). Exclusion criteria were bone-related metabolic diseases, bone cancer, immobility over more than one year, renal disease, and therapy with strontium, fluoride or other antiresorptive medication besides bisphosphonates [18]. All women were classified as osteoporosis patients based on a hip or spine t-score below −2.5 following WHO guidelines or based on a previous osteoporotic fracture. Since for some subjects both the second and third lumbar vertebra was used for this study, Table 1 displays the T-score of the used individual vertebrae. Eight vertebrae were excluded after osteodensitometry due to severely deflected endplates, i.e. 22 vertebrae from 17 subjects were included for further experiments in the study. Fractures in directly adjacent vertebrae were recorded. The vertebrae were divided into two groups: one osteoporotic, treatment-naïve group (OP) and one bisphosphonate-treated group (BP). The bisphosphonate-treated group received an antiresorptive treatment with either alendronate (70 mg weekly, $n = 6$), risedronate (35 mg weekly, $n = 3$) or ibandronate

Table 1
Group characteristics of vertebrae included for all steps of the study. (No significant differences between groups regarding age or T-scores.)

Group	n	Age	Treatment duration	T-score vertebra	T-score hip
Osteoporosis (OP)	11	82.0 ± 7.0 y	No treatment	−3.1 ± 1.0	−3.2 ± 0.7
Bisphosphonate (BP)	11	82.4 ± 6.5 y	4.4 ± 1.9 y	−1.9 ± 2.5	−2.7 ± 1.0

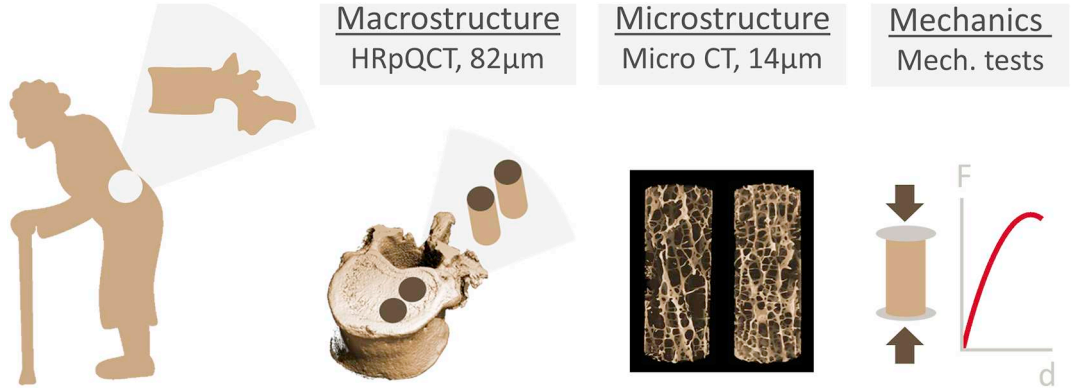

Fig. 1. Overview of methods, from left to right: Macrostructure of L3 vertebrae of elderly, osteoporotic women (treatment-naïve and bisphosphonate-treated) was assessed with HRpQCT (nominal resolution 82 µm). Microstructure of two trabecular bone cores (drilled from the same side of the vertebra) was evaluated based on microCT imaging (nominal resolution 3.5 µm). Mechanical characteristics were determined after uniaxial compressive testing.

(150 mg monthly, $n = 2$) according to the recommendation of the drug manufacturers for 4.2 ± 2.1 years. All samples were fresh frozen at −20 °C.

As a first step, all third lumbar vertebrae were scanned with a high-resolution peripheral quantitative CT to ensure the macrostructural integrity of the vertebra and check for signs of fracture (cf. Fig. 1). In a second step, bone cores from the anterior and the posterior part of one side of the vertebrae were scanned with a microCT. For the third step of our study, we performed uniaxial compression tests on the bone cores to enable a connection between microstructural and material properties.

2.1. HRpQCT imaging of whole lumbar vertebrae

Whole vertebrae imaging was performed to confirm that the macroscopic structure of the vertebrae had not been compromised by major osteophytes, deformations such as Schmorl's nodes or preexisting fractures. After soft tissue removal, all vertebrae were scanned at 82 µm resolution with a high-resolution peripheral quantitative CT scanner (XtremeCT, Scanco Medical AG, Switzerland) (cf. Fig. 1, Macrostructure). The scanning protocol was kept constant (voltage: 60 kV, current: 1 mA, filter: 0.3 mm Cu + 1 mm Al) and bone volume BV/TV_{HRpQCT} was evaluated after segmentation with a fixed threshold for a trabecular cylinder with largest possible dimensions.

2.2. MicroCT analysis of trabecular bone cores

After whole bone scanning, two trabecular bone cores were drilled from one side of third lumbar vertebrae (cf. Fig. 1, Macrostructure). To this end, vertebral processes were removed with a band saw; the remaining vertebral body was fastened in a custom-build frame. Bone cores were drilled with a low speed surgical drill (TREU Instrumente, Germany) using a core cutter bit producing cores of 9 mm in diameter. The outer boundaries of the individual cylinders were positioned 2 mm from the midsagittal plane and the midfrontal plane of the vertebral body (cf. Fig. 2). To prevent heat development and dehydration during drilling, the procedure was performed in a bath of saline solution.

Each bone core was scanned at 3.5 µm nominal resolution with a microCT (Skyscan 1272, Bruker microCT, Belgium) using the following settings: 80 kV, 125 mA, 0.5 mm Al filter. During reconstruction, values for ring artifact reduction and beam hardening correction were kept constant (NRecon, Bruker microCT, Belgium). The microstructural evaluation was conducted with CTAn (Bruker microCT, Belgium). The volume of interest was defined as a central cylinder of 7 mm diameter in the middle of the bone core (cf. Fig. 1, Microstructure), thereby excluding an outer layer of 1 mm thickness to minimize the influence of machining artifacts. The grey value dataset was coarsened to a 14-µm resolution to reduce computation time and segmented with a fixed threshold. We evaluated the following parameters, according to the ASBMR nomenclature committee: bone volume to tissue volume ratio ($BV/TV_{\mu CT}$, %), trabecular thickness (Tb.Th, µm), and trabecular number (Tb.N, 1/mm).

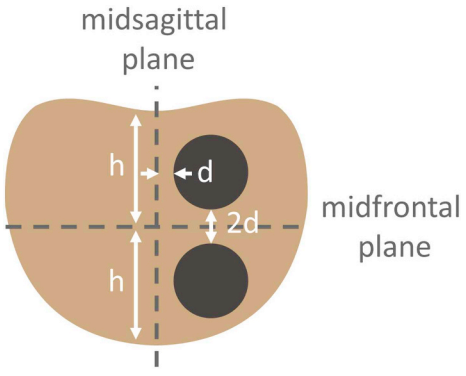

Fig. 2. Standardized position of bone cores in relation to the superior endplate. Distances: d = 2 mm, h = half of distance between vertebral foramen and anterior edge of vertebral body. Dark circles indicate position of extracted bone cores.

2.3. Compression testing of trabecular bone cores

The last step of our experimental design consisted of destructive uniaxial compression testing of the trabecular bone cores. Therefore, the bone cores were cut to 18 mm length and metal platelets were glued to the ends with cyanoacrylate to reduce end artifacts [19]. We used a uniaxial material testing machine (Z2.5N1S, Zwick/Roell GmbH, Germany) with a 2kN load cell and a recommended testing speed of 0.065%L0/s [20]. The compression test was stopped when the recorded force reached 80% of the recorded maximum force. The main outcome parameter was the maximum force F_{max} under compression.

2.4. Statistical analysis

All presented values are expressed as mean ± standard deviation. All data was evaluated using IBM SPSS Statistics 22.0 (IBM, NY, USA). Depending on normality and homogeneity of variances we employed non-parametric tests (Mann-Whitney-U test) to detect differences in whole bone characteristics.

For the comparison of regional characteristics within one group (anterior vs. posterior), we used a non-parametric paired test (Wilcoxon signed rank test). To compare the same region between two groups (OP vs. BP), we performed ANOVA with conservative Bonferroni post-hoc comparison or non-parametric tests (independent-samples Kruskal-Wallis test) depending on normality and homogeneity of variances.

Regression analysis was used to determine the relation between bone volume to tissue volume ratio and maximum force. Further, the relative influence of BV/TV, Tb.Th, and Tb.N was investigated using stepwise regression analyses for each group with Fmax as dependent variable. In addition, correlation coefficients between the trabecular parameters (BV/TV, Tb.Th, and Tb.N) were determined.

3. Results

Our micro CT data reveals differences between posterior and anterior vertebral microstructure of osteoporotic women that are not present in the same manner in the group of women treated with bisphosphonate. The observed microstructural variations go along with variations of maximum force during compression testing.

3.1. Results of high-resolution CT imaging of whole lumbar vertebrae

While all included subjects from both groups shared the same baseline characteristics − neither age, BV/TV derived from high-resolution QCT imaging, vertebra-specific T-score, nor T-score at the hip were different − the regional analysis of anterior and posterior bone cores revealed differences between the bisphosphonate-treated group and the treatment-naïve osteoporosis group.

3.2. MicroCT analysis of trabecular bone cores

Evaluation of trabecular microstructure of the extracted bone cores showed a deteriorated microstructure in the anterior bone core in comparison to the posterior one for the osteoporosis group (cf. Fig. 3). Specifically, $BV/TV_{\mu CT}$ and Tb.N were reduced in the anterior cores of the osteoporosis group ($p = 0.006$, $p = 0.003$, respectively). In contrast, the bisphosphonate-treated group did not show significant differences in $BV/TV_{\mu CT}$ but also presented a significantly lower trabecular number in anterior bone cores ($p = 0.016$). When combining anterior and posterior bone cores for each group, the bisphosphonate group

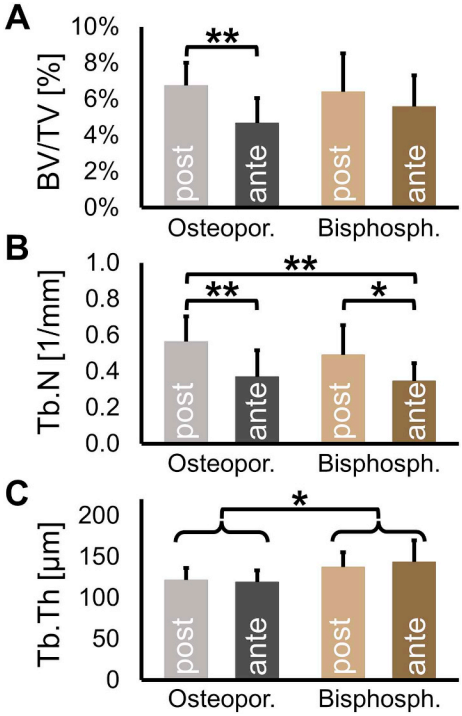

Fig. 3. High-resolution microCT imaging revealed significantly higher BV/TV and Tb.N for posterior compared to anterior bone cores in the osteoporosis group (A, B). The bisphosphonate group only displays a significantly higher Tb.N in the posterior region compared to the anterior (B). When pooling anterior and posterior bone cores, the bisphosphonate group displays a significantly higher trabecular thickness (C). $^{**}p < 0.01$ $^{*}p < 0.05$.

showed an overall higher trabecular thickness ($p = 0.011$).

Comparison of the same regions between groups showed a significant difference between Tb.N in posterior cores from osteoporotic individuals compared to anterior cores from bisphosphonate-treated ones.

Table 2 displays the results of correlation analyses between $BV/TV_{\mu CT}$ and Tb.N as well as Tb.Th. For all groups except anterior bone cores of the bisphosphonate group, a significant correlation exists between $BV/TV_{\mu CT}$ and Tb.N. No group showed a significant correlation between $BV/TV_{\mu CT}$ and Tb.Th.

To elucidate the relation between BV/TV in the different regions within each vertebrae, Fig. 4 displays the difference between $BV/TV_{\mu CT}$ in anterior and posterior normalized to the posterior $BV/TV_{\mu CT}$:

$$\text{Percental difference} = 100^{*}(BV/TV_{\mu CT\ posterior} - BV/TV_{\mu CT\ anterior})/BV/TV_{\mu CT\ posterior} \quad (1)$$

Fig. 4 displays an average percental difference between anterior and posterior bone cores of 29.8 ± 20.6% for the osteoporosis group and of 5.7 ± 36.0% for the bisphosphonate group. As vertebrae with an adjacent fracture would experience a different loading scenario, these were recorded in our study. In the osteoporosis group, one vertebrae showed a directly adjacent biconcave fracture. In the bisphosphonate group, two vertebrae showed directly adjacent wedge fractures. If vertebrae with adjacent vertebral fractures are removed from the dataset (1 from OP, 2 from BP), the percental differences change to 32.0 ± 20.4% and − 3.3 ± 33.3%, respectively for the osteoporosis and bisphosphonate group. These average percental differences between anterior and posterior bone cores from vertebrae without adjacent fractures (10 for OP, 9 for BP) are significantly different ($p = 0.010$, Mann-Whitney-U).

Table 2
Correlation analysis for $BV/TV_{\mu CT}$ and Tb.N showed significant coefficients of correlation for anterior and posterior bone cores for the osteoporosis group and posterior bone cores from the bisphosphonate group (marked bold), but not anterior bone cores from the latter. There were no significant correlations between $BV/TV_{\mu CT}$ and Tb.Th.

Trab. core position/group	Correlation $BV/TV_{\mu CT}$ & Tb.N	Correlation $BV/TV_{\mu CT}$ & Tb.Th
Anterior/osteoporosis	$\mathbf{R = 0.850, p = 0.001}$	$R = -0.099, p = 0.773$
Posterior/osteoporosis	$\mathbf{R = 0.853, p = 0.001}$	$R = -0.101, p = 0.768$
Anterior/bisphosphonate	$R = 0.240, p = 0.477$	$R = 0.508, p = 0.111$
Posterior/bisphosphonate	$\mathbf{R = 0.957, p < 0.001}$	$R = -0.004, p = 0.991$

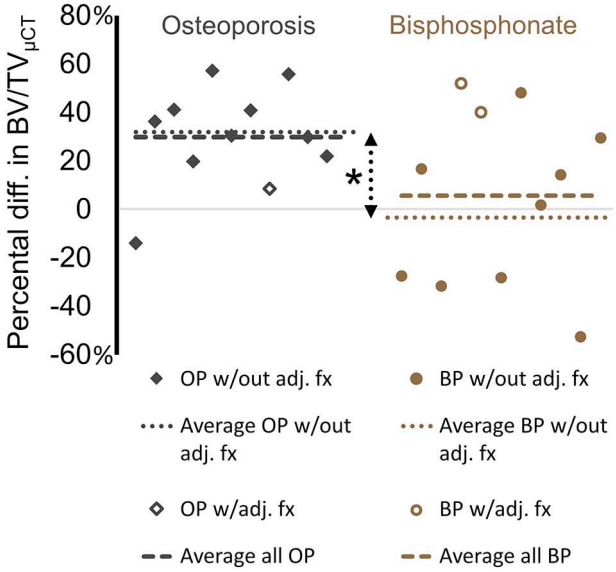

Fig. 4. Percental differences between anterior and posterior bone cores (($BV/TV_{\mu CT\ posterior}$ - $BV/TV_{\mu CT\ anterior}$)/$BV/TV_{\mu CT\ posterior}$*100). Average percental differences are not significantly different between groups with all vertebrae (dashed lines). When evaluating only vertebrae without directly adjacent vertebral fractures, average percental differences (dotted lines) are significantly lower in the bisphosphonate group ($p = 0.010$).

3.3. Compression testing of trabecular bone cores

Differences in maximum force F_{max} could be attributed to microCT-derived $BV/TV_{\mu CT}$. Statistical evaluation showed significantly lower maximum forces for anterior bone cores compared to posterior bone cores for the osteoporosis group (Fig. 5 A). Despite these differences, variations in maximum force depended mainly on $BV/TV_{\mu CT}$. When pooling results for all bone cores, the overall coefficient of determination for F_{max} as the dependent and $BV/TV_{\mu CT}$ as the predicting variable corresponds to $R^2 = 0.671$ ($p < 0.001$) (Fig. 5B).

Stepwise multi-regression analyses revealed that after inclusion of $BV/TV_{\mu CT}$ neither Tb.N nor Tb.Th could be included as significant predictors into a regression model for the prediction of F_{max}. For the posterior bone cores in the bisphosphonate group, the combination of Tb.Th and Tb.N yielded a higher coefficient of determination ($R^2_{adj} = 0.932$, Table 3) compared to $BV/TV_{\mu CT}$ alone ($R^2_{adj} = 0.588$) if only Tb.Th and Tb.N were included as predictors for the stepwise multi-regression analysis.

4. Discussion & conclusion

Our study investigated regional variations in vertebral bone of antiresorptive-treated cases, where treatment stimulated preservation of microstructure, and of age-matched osteoporotic controls. While the treatment-naïve osteoporotic women displayed an unequal distribution of bone volume within vertebrae with a structurally and mechanically

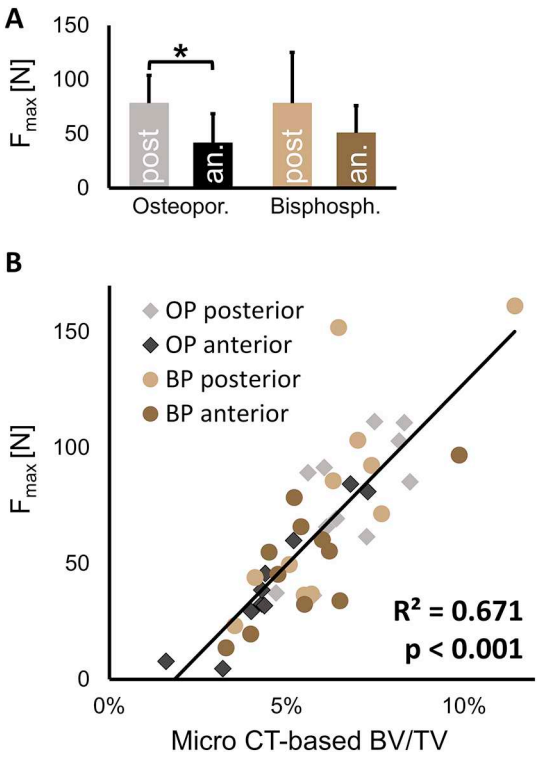

Fig. 5. A: When testing bone cores from the osteoporosis group (OP) higher maximum forces during compression were reached for posterior bone cores than for anterior bone cores, *p < 0.05. The bisphosphonate group (BP) did not display significant differences. B: Measured maximum forces were determined by $BV/TV_{\mu CT}$ ($p < 0.001$).

Table 3
Coefficients of determination from stepwise multi-regression analysis. ǂ One anterior bone core from the osteoporosis group fractured during preparation for compression testing ($BV/TV_{\mu CT}$ = 2.59%).

	Stepwise multi-regression with F_{max} as dependent variable	
Trab. core position/group	Pred: $BV/TV_{\mu CT}$, Tb.N, Tb.Th	Pred: Tb.N, Tb.Th
Anterior/osteoporosis $n = 10^{ǂ}$	R^2_{adj} = 0.932, $p < 0.001$ (incl.: $BV/TV_{\mu CT}$)	R^2_{adj} = 0.746, $p = 0.001$ (incl.: Tb.N)
Posterior/osteoporosis $n = 11$	R^2_{adj} = 0.411, $p = 0.020$ (incl.: $BV/TV_{\mu CT}$)	Not significant (both variables excluded)
Anterior/bisphosphonate $n = 11$	R^2_{adj} = 0.474, $p = 0.011$ (incl.: $BV/TV_{\mu CT}$)	Not significant (both variables excluded)
Posterior/bisphosphonate n = 11	R^2_{adj} = 0.588, $p = 0.004$ (incl.: $BV/TV_{\mu CT}$)	R^2_{adj} = 0.932, $p < 0.001$ (incl.: Tb.N, Tb.Th)

weaker anterior region compared to the posterior region, the analyzed bisphosphonate-treated group did not display the same regional differences.

In the examined osteoporotic vertebrae, the anterior trabecular bone had a lower bone volume, lower number of trabeculae and consequently a lower load-bearing capacity. This is in agreement with several microCT-based studies on the heterogeneity of the vertebral trabecular compartment [11–14]. Moreover, the lack of stiffness in the anterior part appears in the failure region of one of the most common atraumatic vertebral fracture types [21], the wedge fracture, where the anterior region collapses.

Pollintine et al. found that vertebral load distribution changes with disc degeneration during aging [22]. While the load in young lumbar spines is equally distributed between the anterior and the posterior region of the vertebra, during aging the load shifts towards the posterior regions of the vertebrae, effectively unloading the anterior region in erect spinal position [23]. Following Wolff's law, the anterior bone is thus more likely to be resorbed. This was shown by Sandor et al. for medial bone sections of lumbar vertebrae [24]. In addition, administered bisphosphonates bind to hydroxyapatite crystals in bone [25] and therefore predominantly bind to bone surfaces undergoing bone remodeling, where due to removal of the collagen matrix a high amount of hydroxyapatite is available for binding [26]. Considering the binding of bisphosphonates to sites of bone remodeling and that bone remodeling is more likely to occur in the anterior vertebra, the anterior vertebral bone is a realistic target for preferential bisphosphonate binding.

With aging, Gong et al. showed that the loss in apparent elastic modulus (69y vs. 62y) is as high as 48% for anterior vertebral bone, while it remains constant in posterior bone [27]. This loss of mechanical competence in the anterior bone of osteoporotic vertebrae is further exacerbated by higher momentary loads on the anterior bone during bending [23], ultimately increasing the risk for fracture. In contrast, in bisphosphonate-treated women, bone resorption by osteoclasts is reduced. Due to selective binding of bisphosphonates to available hydroxyapatite in resorption sites, reduction of resorption should predominantly affect the anterior bone, where without therapeutic intervention more bone loss occurs during aging than in other regions [24].

Selective binding of bisphosphonates to the anterior region could explains the apparent partial preservation of trabecular bone volume in the anterior region observed in our study. Considering the similar number of trabeculae for anterior bone cores from both treatment groups and that they are both significantly lower than in the corresponding posterior regions (Fig. 3B) may indicate that the even $BV/TV_{\mu CT}$ in anterior and posterior bone cores in the bisphosphonate group is due to thicker trabeculae in the anterior region. As our data does not show differences in trabecular thickness between regional groups, likely due to relevant regional differences along the vertical axis of the vertebra [13], we pooled results for anterior and posterior bone cores for each group. For pooled regions, we found a significantly higher trabecular thickness for the bisphosphonate group compared to the osteoporosis group. Therefore, theoretically, increased trabecular thickness in the bisphosphonate group might contribute to the observed lack of a significant difference between anterior and posterior in this group. However, further confirmation with additional experiments is necessary.

The observed lower percental difference in $BV/TV_{\mu CT}$ between anterior and posterior bone cores without adjacent fractures might influence the internal vertebral load distribution and could reduce the susceptibility to wedge fractures. Considering the likely preferential binding of bisphosphonates to the anterior region and the here observed lower percental difference in bone volume between regions, bisphosphonate treatment theoretically has the potential to preserve bone in a region-specific manner. This would point to the deposition or preservation of bone where it is most needed to avoid wedge fractures (i.e. the anterior region of the vertebra). This could contribute to reducing fracture risk more efficiently in vertebrae than in other potential fracture sites of the body.

Our study has a few limitations. First, our analysis does not account for the contribution of cortical bone to the vertebral load bearing capacity. Whether the thin cortical shell (approx. 290 µm in the lumbar spine [28]) of vertebrae is also affected by a regional variation of bisphosphonate efficacy and how this interacts with the observed differences in trabecular bone remains to be investigated. Second, while the power for regional $BV/TV_{\mu CT}$ comparisons in the osteoporosis group is good ($P = 0.95$), due to the high standard deviation of the $BV/TV_{\mu CT}$ values from the bisphosphonate group, there remains a risk of a type II error for the analysis of this group.

Our analyses showed considerable variation within our experimental groups, specifically in the bisphosphonate group. Interindividual differences that might contribute to this variation are e.g. anatomical posture, which might in turn be related to fracture status, physical activity or BMI [29], or in the case of the treatment group differences in drug response and adherence. While literature reports on the relation of BMI and lumbar curvature are conflicting [30–32], lumbar fractures are related to a decreased lumbar lordosis [33]. Therefore, with present lumbar fractures and a correspondingly decreased lumbar lordosis, the loading scenario for the remaining intact vertebrae should change. This is also reflected by our data from the bisphosphonate group, as the two vertebrae with directly adjacent wedge fractures presented a percental difference in $BV/TV_{\mu CT}$ between anterior and posterior bone cores above the 95th percentile of the vertebrae without adjacent fractures. Our retrospective study did not allow an assessment of drug adherence, thus we cannot exclude the possibility that a potential variation in adherence could be an additional factor regarding intragroup variation. Although the fracture risk reducing efficacy varies between bisphosphonates, the drugs used in this study (alendronate, risedronate, ibandronate) have a comparable fracture risk reducing effect on vertebral fractures [3,34]. Therefore, the inclusion of these three different drugs can be ruled out as a confounding factor to the intragroup variance.

In conclusion, our study points towards an additional pathway for fracture risk reduction. Here, an apparent preservation of anterior bone volume and trabecular thickness through bisphosphonates may conserve regional load-bearing capacity reducing the risk of regional overloading and thus making fracture initiation less likely. The targeting of the vulnerable anterior vertebra due to selective binding of bisphosphonates in resorption areas may be a confounding factor for explaining the higher efficacy of bisphosphonates in reducing fracture risk in the spine compared to other fracture sites. While such potential drug-specific effects on intravertebral heterogeneity should be taken into account when developing treat-to-target goals for osteoporosis treatment, further insight into other confounding factors such as changes in loading with prevalent fractures is needed.

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References

[1] I.R. Reid, Osteoporosis treatment: focus on safety, Eur. J. Intern. Med. 24 (8) (2013) 691–697 Dec.
[2] R.L. Fleurence, C.P. Iglesias, J.M. Johnson, The cost effectiveness of bisphosphonates for the prevention and treatment of osteoporosis: a structured review of the literature, Pharmacoeconomics 25 (11) (2007) 913–933.
[3] J.-H. Byun, S. Jang, S. Lee, S. Park, H.K. Yoon, B.-H. Yoon, Y.-C. Ha, The efficacy of bisphosphonates for prevention of osteoporotic fracture: an update meta-analysis, J. Bone Metab. 24 (1) (2017) 37–49 Feb.
[4] P.N. Sambrook, J.S. Chen, J.M. Simpson, L.M. March, Impact of adverse news media on prescriptions for osteoporosis: effect on fractures and mortality, Med. J. Aust. 193 (3) (2010) 154–156.
[5] Y.E. Miedany, Treat to target for osteoporosis: another step forward, Curr. Rheumatol. Rev. 10 (2) (2014) 99–105.
[6] E.M. Lewiecki, S.R. Cummings, F. Cosman, Treat-to-target for osteoporosis: is now the time? J. Clin. Endocrinol. Metab. 98 (3) (2013) 946–953.
[7] E. Michael Lewiecki, Osteoporosis: treat-to-target, Curr. Osteoporos. Rep. 15 (2) (2017) 103–109.
[8] A. Abelson, J.D. Ringe, D.T. Gold, J.L. Lange, T. Thomas, Longitudinal change in clinical fracture incidence after initiation of bisphosphonates, Osteoporos. Int. 21 (6) (2010) 1021–1029.
[9] E. Seeman, Age- and menopause-related bone loss compromise cortical and trabecular microstructure, J. Gerontol. A Biol. Sci. Med. Sci. 68 (10) (2013) 1218–1225.
[10] R.J. Tower, G.M. Campbell, M. Müller, C.C. Glüer, S. Tiwari, Utilizing time-lapse micro-CT-correlated bisphosphonate binding kinetics and soft tissue-derived input functions to differentiate site-specific changes in bone metabolism in vivo, Bone 74 (2015) 171–181.
[11] H. Chen, S. Shoumura, S. Emura, Y. Bunai, Regional variations of vertebral trabecular bone microstructure with age and gender, Osteoporos. Int. 19 (10) (2008) 1473–1483 Oct.
[12] J. Wegrzyn, J.P. Roux, M.E. Arlot, S. Boutroy, N. Vilayphiou, O. Guyen, P.D. Delmas, R. Chapurlat, M.L. Bouxsein, Role of trabecular microarchitecture and its heterogeneity parameters in the mechanical behavior of ex vivo human L3 vertebrae, J. Bone Miner. Res. 25 (11) (2010) 2324–2331.
[13] P.A. Hulme, S.K. Boyd, S.J. Ferguson, Regional variation in vertebral bone morphology and its contribution to vertebral fracture strength, Bone 41 (6) (2007) 946–957.
[14] Y. Wang, J.S. Owoc, S.K. Boyd, T. Videman, M.C. Battié, Regional variations in trabecular architecture of the lumbar vertebra: associations with age, disc degeneration and disc space narrowing, Bone 56 (2) (2013) 249–254.
[15] C. Kilincer, D. Demirbag Kabayel, B. Cagli, E. Unlu, B. Wicki, F. Ozdemir, Frequency, distribution and severity of prevalent osteoporotic vertebral fractures in postmenopausal women, Turk. Neurosurg. 23 (4) (2013) 476–483.
[16] S. Waterloo, L.A. Ahmed, J.R. Center, J.A. Eisman, B. Morseth, N.D. Nguyen, T. Nguyen, A.J. Sogaard, N. Emaus, Prevalence of vertebral fractures in women and men in the population-based Tromsø study, BMC Musculoskelet. Disord. 13 (1) (2012) 3.
[17] H. Giambini, S. Khosla, A. Nassr, C. Zhao, K.-N. An, Longitudinal changes in lumbar bone mineral density distribution may increase the risk of wedge fractures, Clin. Biomech. 28 (1) (2013) 10–14 Jan.
[18] M. Krause, O. Museyko, S. Breer, B. Wulff, C. Duckstein, E. Vettorazzi, C. Glueer, K. Püschel, K. Engelke, M. Amling, Accuracy of trabecular structure by HR-pQCT compared to gold standard ??cT in the radius and tibia of patients with osteoporosis and long-term bisphosphonate therapy, Osteoporos. Int. 25 (5) (2014) 1595–1606.
[19] T.M. Keaveny, T.P. Pinilla, R.P. Crawford, D.L. Kopperdahl, A. Lou, Systematic and random errors in compression testing of trabecular bone, J. Orthop. Res. 15 (1) (1997) 101–110.
[20] L. Rincón-Kohli, P.K. Zysset, Multi-axial mechanical properties of human trabecular bone, Biomech. Model. Mechanobiol. 8 (3) (2009) 195–208.
[21] E. Itoi, M. Sakurai, K. Mizunashi, K. Sato, F. Kasama, Long-term observations of vertebral fractures in spinal osteoporotics, Calcif. Tissue Int. 47 (4) (1990) 202–208 Oct.
[22] P. Pollintine, A.S. Przybyla, P. Dolan, M.A. Adams, Neural arch load-bearing in old and degenerated spines, J. Biomech. 37 (2) (2004) 197–204 Feb.
[23] P. Pollintine, P. Dolan, J.H. Tobias, M. Adams, Intervertebral disc degeneration can lead to 'Stress-Shielding' of the anterior vertebral body, Spine (Phila Pa 1976) 29 (7) (2004) 774–782. Apr.
[24] T. Sandor, D. Felsenberg, W.A. Kalender, E. Brown, Global and regional variations in the spinal trabecular bone: single and dual energy examinations, J. Clin. Endocrinol. Metab. 72 (5) (1991) 1157–1168.
[25] S. Cremers, S. Papapoulos, Pharmacology of bisphosphonates, Bone 49 (1) (2011) 42–49.
[26] M. Sato, W. Grasser, N. Endo, R. Akins, H. Simmons, D.D. Thompson, E. Golub, G.A. Rodan, Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure, J. Clin. Invest. 88 (6) (1991) 2095–2105 Dec.
[27] H. Gong, M. Zhang, H.Y. Yeung, L. Qin, Regional variations in microstructural properties of vertebral trabeculae with aging, J. Bone Miner. Metab. 23 (2) (2005) 174–180.
[28] H. Ritzel, M. Amling, M. Pösl, M. Hahn, G. Delling, The thickness of human vertebral cortical bone and its changes in aging and osteoporosis: a histomorphometric analysis of the complete spinal column from thirty-seven autopsy specimens, J. Bone Miner. Res. 12 (1) (1997) 89–95.
[29] E. Been, L. Kalichman, Lumbar lordosis, Spine J. 14 (1) (2014) 87–97 Jan.
[30] V.L. Murrie, A.K. Dixon, W. Hollingworth, H. Wilson, T.A.C. Doyle, Lumbar lordosis: study of patients with and without low back pain, Clin. Anat. 16 (2) (2003) 144–147 Mar.
[31] S. Romero-Vargas, B. Zárate-Kalfópulos, E. Otero-Cámara, L. Rosales-Olivarez, A. Alpízar-Aguirre, E. Morales-Hernández, A. Reyes-Sánchez, The impact of body mass index and central obesity on the spino-pelvic parameters: a correlation study, Eur. Spine J. 22 (4) (2013) 878–882. Apr.
[32] N. Onyemaechi, G. Anyanwu, E. Obikili, O. Onwuasoigwe, O. Nwankwo, Impact of overweight and obesity on the musculoskeletal system using lumbosacral angles, Patient Prefer. Adherence 10 (2016) 291 Mar.
[33] M. Krause, S. Breer, B. Mohrmann, E. Vettorazzi, R.P. Marshall, M. Amling, F. Barvencik, Influence of non-traumatic thoracic and lumbar vertebral fractures on sagittal spine alignment assessed by radiation-free spinometry, Osteoporos. Int. 24 (6) (2013) 1859–1868 Jun.
[34] J.P. Jansen, G.J.D. Bergman, J. Huels, M. Olson, Prevention of vertebral fractures in osteoporosis: mixed treatment comparison of bisphosphonate therapies, Curr. Med. Res. Opin. 25 (8) (2009) 1861–1868. Aug.