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Original Article

Baseline mineralizing surface determines the magnitude of the bisphosphonate effect on cortical bone mineralization in postmenopausal osteoporotic patients

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Abstract

Purpose: To determine the effect of short- or long-term bisphosphonate treatment on cortical bone mineralization density distribution (BMDD). **Methods:** BMDD was assessed by quantitative backscatter electron imaging in postmenopausal osteoporosis: in paired transiliac biopsy samples (n=36) at baseline and after 3 years risedronate treatment from a clinical study, in transiliac biopsy samples from patients who were treated with either risedronate (n=31) or alendronate (n=68) for 3 to 7 years from an observational study. Outcomes were related to premenopausal reference data (n=73) and to histomorphometric mineralizing surface per bone surface (MS/BS). **Results:** In the clinical study, patients with lower (below cohort median) MS/BS had normal cortical CaMean at baseline. After 3 years risedronate, their CaMean was not different versus baseline but increased versus reference (+2.9%, p=0.003). Among the groups of the observational study, CaMean did not exceed reference level, was similar for alendronate versus risedronate and similar between 3 to 5 years versus longer than 5 years treatment duration. **Conclusion:** Baseline bone mineralizing surface appears to be important for the effect of bisphosphonate on cortical bone mineralization. In patients with lower baseline MS/BS, level of mineralization after treatment can exceed reference level. Whether this is beneficial in the long-term is unknown.

Keywords: Transiliac Bone Biopsy, Cortical Bone Matrix Mineralization, Postmenopausal Osteoporosis, Bisphosphonate Treatment, Baseline Bone Turnover

Introduction

The fracture risk reduction and increase in bone mineral density (by dual X-ray absorptiometry) resulting from bisphosphonate treatment are well known[1-3]. Although high bone turnover appears to be the typical situation immediately after menopause[9,10], there is variability in bone turnover later in the postmenopausal period ranging from highly increased to even decreased bone turnover[11-13]. The effect of the level of bone turnover before treatment on outcomes in cortical bone matrix mineralization in postmenopausal patients has not been studied previously.

About 80% of the entire skeletal bone mass is cortical bone, which supports most of the mechanical load applied to bone[17]. Loss of cortical bone plays a pivotal role in bone fragility[18]. Material characteristics of cortical bone and potential treatment changes thereof are essential for the mechanical integrity of the skeleton. Bone mineralization density distribution (BMDD) is a bone material quality variable which reveals the degree and the distribution of the mineral content in bone[16] that in turn is known to influence the stiffness/elasticity of mineralized tissues[14,15].

The aim of the present work was to study the effects of 3 years treatment with risedronate on cortical BMDD measured

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Table 1. Baseline characteristics of patients from the clinical VERT-NA trial and patients from the observational study.

	Patients from the VERT-NA trial		Patients from the observational study			
	HT (n=10)	LT (n=8)	ALN 3-5(n=33)	ALN > 5 (n=35)	RIS 3-5 (n=23)	RIS > 5 (n=8)
Age (yrs)	70.1 (4.7)	64.0 (5.8)	67.8 (7.6)	71.6 (6.7)	70.8 (6.1)	68.3 (5.9)
Yrs since menopause	23.6 (5.9)	16.4 (6.4)	22.6 (13.3)	25.0 (10.2)	25.3 (9.0)	19.6 (6.9)
Treatment duration (yrs)	3	3	3.8 (0.6)	7.0 (1.8)	3.7 (0.6)	5.4 (0.7)
LS BMD before treatment	0.770 (0.094)	0.852 (0.184)	0.820 (0.101)	0.781 (0.108)	0.753 (0.083)	0.857 (0.199)
FN/hip BMD before treatment	0.611 (0.093)	0.663 (0.158)	0.733 (0.077)	0.768 (0.069)	0.761 (0.096)	0.760 (0.088)
MS/BS (%)	9.84 (1.96)	2.69 (1.65)	na	na	na	na
MAR (µm/d)	0.556 (0.113)	0.517 (0.139)	na	na	na	na
BFR/BV (%/year)	28.6 (8.9)	9.2 (4.8)	na	na	na	na
Cortical BMDD						
CaMean (wt%)	21.11 (0.48)	22.21 (0.68)	na	na	na	na
CaPeak (wt%)	21.75 (0.42)	23.00 (0.65)				
CaWidth (Δwt%)	3.97 (0.41)	3.61 (0.34)				
CaLow (%B.Ar)	7.42 (2.60)	4.66 (1.37)				
CaHigh (%B.Ar)	1.82 (0.67)	6.86 (5.46)				

Data are mean (SD). MS/BS = mineralizing surface per bone surface, MAR = mineral apposition rate, BFR/BV = bone formation rate per bone volume. na = not available (no baseline biopsy was obtained).

in paired transiliac bone biopsy samples in postmenopausal osteoporotic patients from the clinical VERT-NA (Vertebral Efficacy with Risedronate Therapy North American) trial[2] in relation to baseline bone formation by MS/BS. In addition, cortical BMDD was assessed in transiliac biopsies from postmenopausal osteoporotic patients in a separate observational study in which the patients had been treated with risedronate or alendronate for more than 3 years[19].

Materials & methods

Patients

We analyzed paired transiliac bone biopsy samples from the biopsy cohort of the clinical VERT-NA[2] trial. The patients were postmenopausal osteoporotic women who had either two prevalent vertebral fractures at baseline or one prevalent vertebral fracture and a lumbar spine mineral density T-score of -2 or less. Paired bone biopsies were obtained at baseline and after 3 years with risedronate treatment. As from these patients baseline histomorphometric indices of bone formation/turnover obtained from cancellous bone (mineralizing surface per unit bone surface MS/BS; mineral apposition rate MAR; bone formation rate per bone volume BFR/BV) were available, we studied their relationship with BMDD outcomes. Furthermore, we separated the biopsy cohort into two sub-cohorts with higher turnover (HT, n=10) and with lower turnover (LT, n=8) according to their baseline MS/BS above or below the cohort median of the original study (MS/BS of 6.25%)[20]. Considering these subgroups enabled us to study the effects on cortical BMDD dependent on baseline MS/BS. Cancellous BMDD outcomes from the present HT cohort have been published previously[4,6].

Additionally, we analyzed transiliac biopsies from an observational study[19] in which postmenopausal osteoporotic women had been treated with either alendronate (n=68) or risedronate (n=31). In this study, groups were separated by treatment duration, either 3-5 years (alendronate 3-5, risedronate 3-5) or >5 years (alendronate >5, risedronate >5). For mean duration of treatment and clinical characteristics of the groups see Table 1. In this study biopsies were taken only after treatment, not at baseline. Cortical BMDD outcomes were correlated with MS/BS (standard histomorphometry on undecalcified biopsies was performed as previously described[22,23]) and compared to previously published healthy premenopausal reference data of cortical bone (n=73)[21]. All studies were conducted according to the Declaration of Helsinki and approved by the local ethics committees or institutional review boards (IRB).

Quantitative backscatter electron imaging (qBEI)

In calibrated qBEI images of bone (as shown in Figure 1) the backscatter electron intensities/pixel grey levels are correlated with the local amount of calcium (Ca) in bone. Thus the grey level histograms derived from calibrated backscatter electron images of bone tissue provide information about the frequency distribution of pixels with a certain Ca weight% content (so called bone mineralization density distribution,

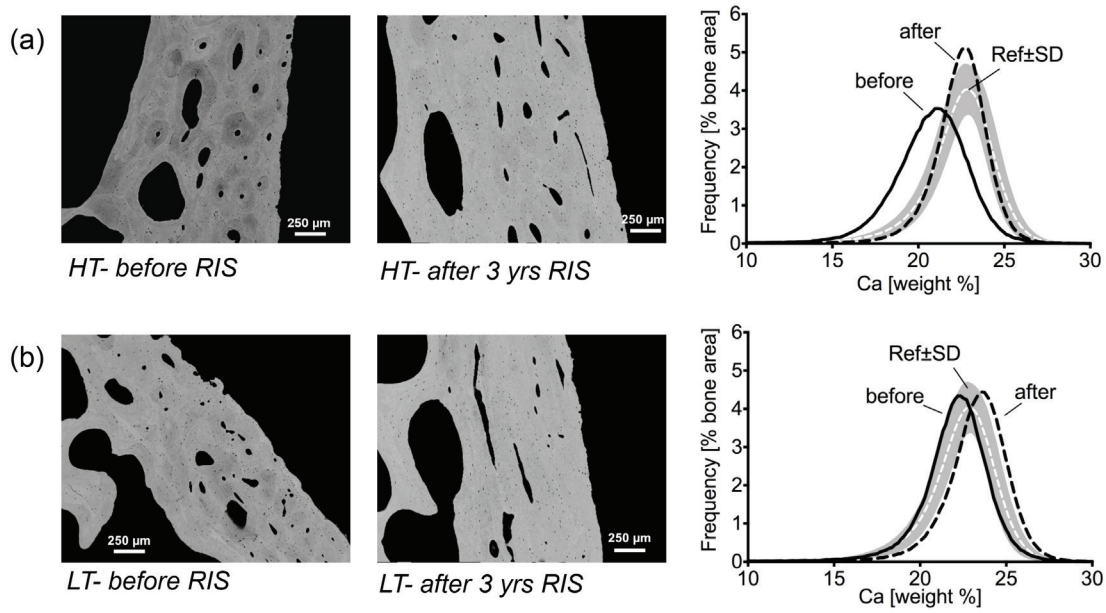

Figure 1. Examples of qBEI images of cortical bone of paired transiliac biopsy samples and corresponding cortical BMDD from (a) one higher turnover (HT) and (b) one lower turnover (LT) patient. Solid line indicates BMDD before, dashed line after treatment with risedronate. White dashed line and grey area indicate mean ± 1SD of premenopausal reference cortical BMDD.

BMDD), described in detail in a previous work[24].

Calibrated qBEI images were acquired with a DSM 962 digital scanning electron microscope (Zeiss, Oberkochen, Germany, equipped with a four-quadrant semiconductor backscatter electron detector) based on the following microscope settings: an accelerating voltage of 20 kV, a probe current of 110±0.4 pA (measured with a Faraday cup connected to a picoamperemeter), a working distance of 15 mm, and a scan speed of 100 seconds per frame. The entire bone tissue area was recorded in a series of images with a 50x nominal magnification corresponding to a scanned bone area of about 2 mm x 2.5 mm. BMDD was obtained from cancellous and cortical bone separately (Figure 1). We used five parameters to characterize the BMDD curves: CaMean= the weighted mean Ca-concentration of the bone area; CaPeak= the peak position of the histogram, indicating the most frequently measured Ca concentration; CaWidth= the full width at half maximum of the distribution, describing the heterogeneity in matrix mineralization; CaLow= the percentage of lowly mineralized bone areas (lower than 17.68 weight % Ca[25]); CaHigh= the percentage of highly mineralized bone areas (higher than 25.30 wt% Ca, corresponding to fully mineralized bone areas, mainly interstitial bone[16]). For cortical BMDD, the entire areas of the two cortical plates were analyzed separately and subsequently the arithmetic mean of the BMDD of the two plates was calculated for each sample (i.e. cortical BMDD was evaluable in intact biopsy samples), as described previously[21]. In the present work, we focussed on cortical BMDD, thus all presented BMDD outcomes are referring to cortical bone, except for the correlation between cortical CaMean versus cancellous CaMean (in this case "cortical" and "cancellous" is explicitly indicated).

Statistical analysis

The data are presented as mean (SD) (normally distributed data) or median (25^{th}; 75^{th} percentiles) (non-normally distributed data). Statistical analysis was performed using SigmaStat for Windows Version 2.03 (SPSS Inc.). Normality of the data was analyzed by the Kolmogorov-Smirnov test. Comparison between HT and LT at baseline or after 3 years risedronate treatment, as well as comparison of each HT and LT versus premenopausal reference was analyzed by t-tests. Effect of treatment with risedronate in HT and LT patients was analyzed by paired t-tests. Comparison among patients from the observation study as well as comparison of them with clinical HT and LT groups is based on ANOVA or ANOVA on ranks (Kruskal-Wallis One Way Analysis of Variance on Ranks) for non-normally distributed data. Differences among alendronate or among risedronate as well as comparison of alendronate or risedronate with premenopausal reference are based on t-tests or Mann-Whitney rank sum tests for non-normally distributed data. Correlation analyses of cortical CaMean versus cancellous CaMean and cortical Ca Mean versus MS/BS are based on Pearson correlation.

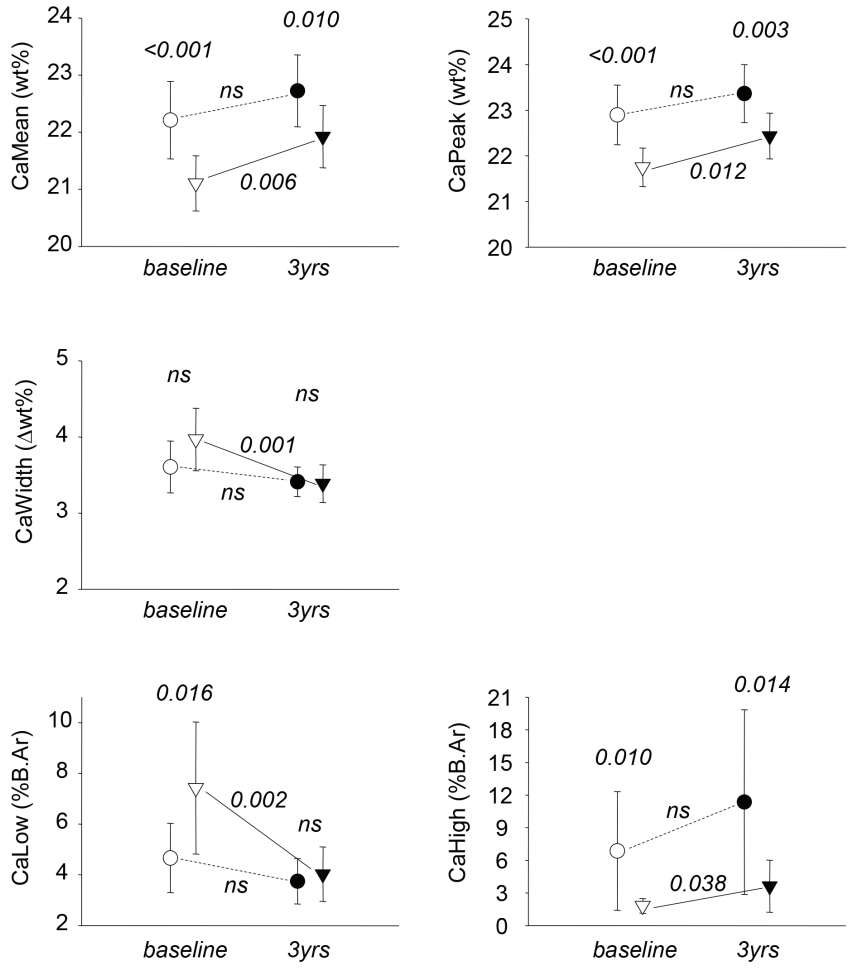

Figure 2. Cortical BMDD outcomes (Mean ± SD) of HT (triangles) and LT patients (circles) at baseline (left, white symbols) and after 3 years of risedronate treatment (right, black symbols). Statistically significant differences between LT and HT at baseline or after 3 years treatment are given by p-value at top (ns= not significant). Significantly different changes during treatment (paired comparison within HT or LT groups) are shown by solid line and corresponding p-value. Non-significant changes are indicated by dashed lines and ns.

Results

Cortical BMDD from risedronate treated clinical study patients

i) BMDD and its relation to MS/BS at baseline

At baseline, BMDD outcomes were significantly correlated with MS/BS in the total patient cohort. CaMean (R=-0.60, p=0.009), CaPeak (R=-0.62, p=0.006), and CaHigh (R=-0.45, p=0.045) were negatively related to MS/BS, while CaLow (R=0.51, p=0.03) was positively related to MS/BS. Similarly, negative correlations with CaMean (R=-0.67, p=0.005), CaPeak (R=-0.69, p=0.003), and CaHigh (R=-0.58, p=0.02), and a positive correlation of CaLow (R=0.56, p=0.02) with BFR/BV were observed. MAR was not significantly correlated to BMDD outcomes (all p>0.05).

(ii) Comparison between HT and LT subgroups at baseline

CaMean in HT was significantly lower compared to LT (-5.0%, p<0.001) and compared to reference (-4.5%, p<0.001). Similarly, CaPeak and CaHigh were significantly lower versus LT and versus reference, while CaLow was significantly higher compared to both LT and reference (comparison between HT and LT is summarized in Figure 2, comparison to reference is shown in Figure 3). CaWidth in HT was not significantly different from LT or reference. At baseline, BMDD from LT was not significantly different from reference.

iii) Effect of 3 years risedronate on BMDD in relation to baseline MS/BS

Treatment changes (paired tests) in HT and LT patients are shown in Figure 2. Risedronate treatment significantly

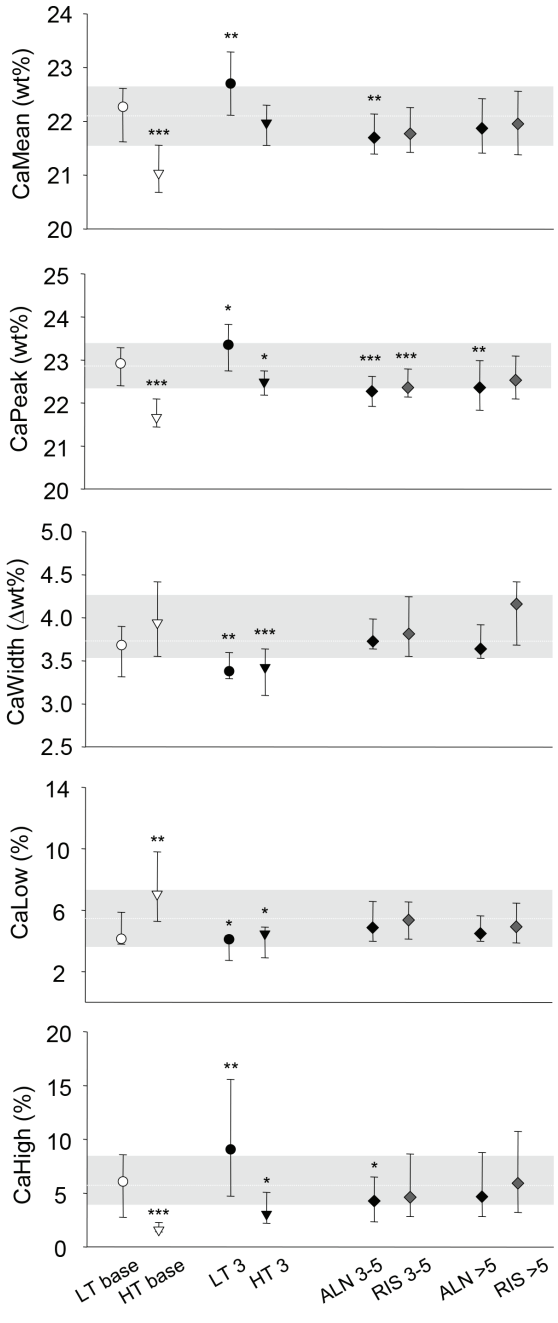

Figure 3. Cortical BMDD outcomes from HT, LT (baseline and risedronate 3 yrs) and from the observational study (alendronate and risedronate 3-5 and >5 years). White dotted lines and grey areas indicate premenopausal reference mean (or median) and reference range (±1 standard deviation or interquartile range) respectively. Reference data are from a previous work[21]. ***p<0.001, **p<0.01, *p<0.05 versus premenopausal reference. LT base= lower turnover patients baseline, HT base= higher turnover patients baseline, LT 3= lower turnover patients after 3 years with risedronate, HT 3 = higher turnover patients after 3 years with risedronate, ALN 3-5= patients from the observational study after 3-5 years with alendronate, RIS 3-5= patients from the observational study after 3-5 years with risedronate, ALN >5 = patients from the observational study after more than 5 years with alendronate, RIS >5 = patients from the observational study after more than 5 years with risedronate.

affected all BMDD parameters in HT (Figure 2): CaMean (+3.9%, p=0.006), CaPeak (+3.1%, p=0.012), and CaHigh (+100%, p=0.038) were increased, while CaWidth (-14.6%, p=0.001) and CaLow (-46%, p=0.002) were decreased. Risedronate treatment had no significant effect on any of the BMDD parameters in LT.

After 3 years risedronate treatment, CaMean in HT was still lower than in LT (-3.5%, p=0.010) but was not different from reference. CaPeak, and CaHigh from HT remained lower than in LT (Figure 2) and reference (Figure 3), while CaWidth and CaLow from HT were similar to those in LT (Figure 2) but were significantly lower than reference (Figure 3). After 3 years risedronate, BMDD in LT was significantly different from premenopausal reference. In particular, CaMean was higher (+2.9%, p=0.003), and CaPeak, and CaHigh were also higher (Figure 3), while CaWidth and CaLow were significantly lower than reference (Table 2 and Figure 3). For comparisons of 3 year risedronate treatment with reference see Table 2.

Cortical BMDD from observational study patients

i) Effect of short- or long-term risedronate or alendronate treatment on BMDD

BMDD variables were available in n=27 alendronate 3-5, n=26 alendronate >5, n=21 risedronate 3-5, and n=5 risedronate >5. Among the 4 treatment groups there were no significant differences in BMDD outcomes (Figure 3). Also, there was no significant difference due to treatment duration with either alendronate or risedronate treatment (3-5 versus >5 within drug treatment groups). Compared to premenopausal reference, alendronate 3-5 had lower CaMean, CaPeak, and CaHigh, and alendronate >5 had lower CaPeak. The only difference in risedronate from reference was lower CaPeak in risedronate 3-5 (see Table 2; Figure 3). Considering all observational study groups together, CaMean was significantly correlated with MS/BS (R=-0.31, p<0.01), showed a near to significance correlation with BFR/BV (R=-0.23, p=0.06), and it was not significantly related to MAR (p=0.74, n.s.).

(ii) Comparison of observational study groups with clinical study groups after risedronate treatment

Comparison of the observational study groups with HT and LT from the clinical study after 3 years with risedronate showed that although the patients from the observational study were treated for a longer time, they had similar CaMean and CaPeak compared to HT. Moreover, they had significantly lower CaMean and CaPeak (ANOVA p<0.01, post-hoc tests p<0.05) compared to the LT patients after 3 years with risedronate. All four treatment groups from the observational study had larger CaWidth compared to HT and LT from the clinical trial (ANOVA p<0.001, post-hoc tests <0.01). Differences were also found for CaLow (ANOVA p=0.025), which was larger in alendronate 3-5 and risedronate 3-5 compared to both LT and HT. CaHigh was similar among the groups.

Table 2. Summary of deviations in cortical BMDD from reference values after 3 or more years with bisphosphonate treatment.

	Clinical trial patients		Observational study patients			
	3yrs RIS in HT	3yrs RIS in LT	3-5yrs ALN	3-5yrs RIS	>5yrs ALN	>5yrs RIS
CaMean	normal	↑	↓	normal	normal	normal
CaPeak	↓	↑	↓	↓	↓	normal
CaWidth	↓	↓	normal	normal	normal	normal
CaLow	↓	↓	normal	normal	normal	normal
CaHigh	↓	↑	↓	normal	normal	normal

↓ = significantly lower, ↑ = significantly higher compared to premenopausal cortical reference from[21]. Normal = no significant difference to premenopausal cortical reference[21].

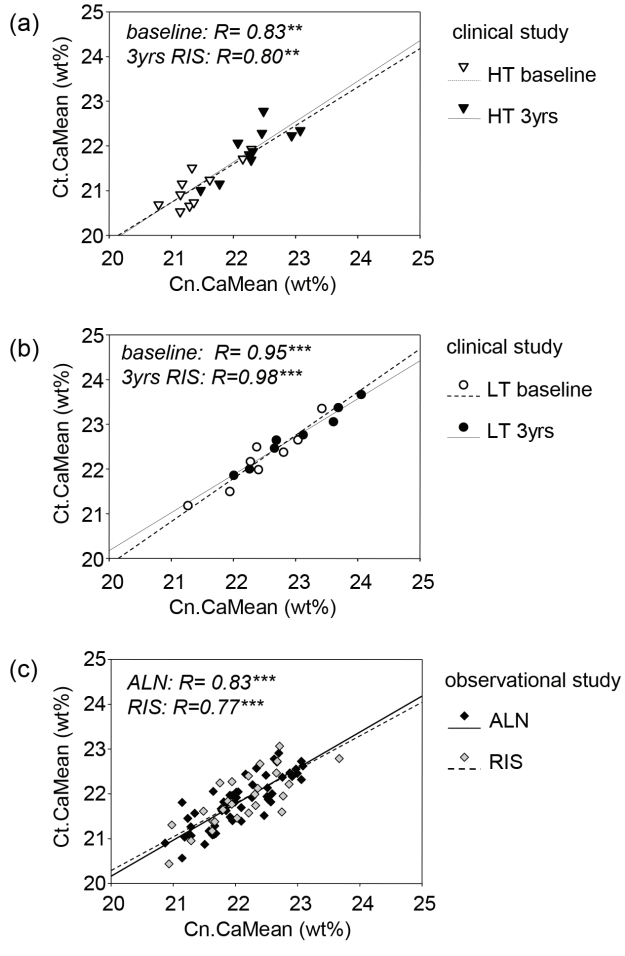

Figure 4. Correlation of cortical (Ct.) CaMean with cancellous (Cn.) CaMean. Significant correlations were found within (a) HT and (b) LT patients, and (c) patients from the observational study. Corresponding regression lines (dashed baseline, black 3 years with risedronate in (a) and (b); black alendronate, grey risedronate in (c)). Pearson correlation coefficient (R) is shown. ***p<0.001, **p<0.01, *p<0.05. Circles indicate HT, triangles LT, white symbols baseline, black symbols after 3yrs treatment, diamond shaped symbols indicate data from observational study, black alendronate, grey risedronate.

iii) Relationship between cortical CaMean and cancellous CaMean in observational and clinical study groups

Cortical CaMean was significantly correlated with cancellous CaMean in both HT and LT patients at baseline and after 3 years risedronate (clinical study) as well as in patients after 3 or more years with risedronate or alendronate (observational study). All correlations were similar (correlation coefficient and slope) and statistically significant (R ranging from 0.77 to 0.98, p ranging from 0.003 to <0.001), see Figure 4 a, b, c.

Discussion

The biopsies from the clinical study gave evidence for the dependency of the degree of bone mineralization on MS/BS and BFR/BV at baseline. Additionally, the level of MS/BS (lower or higher than cohort median) at baseline was decisive of the magnitude of the bisphosphonate treatment effect. The biopsies from the observational study (where no baseline biopsy was available), revealed the effect of short- to long-term alendronate or risedronate on cortical BMDD in a non-controlled clinical setting. We found that the degree of cortical bone mineralization remained unchanged betwen short- and long-term treatment and did not exceed reference levels even after long-term bisphosphonate treatment.

High bone turnover in osteoporosis leads to reduced average tissue age which is associated with decreased degree and increased heterogeneity of cancellous bone matrix mineralization[7,26]. This is consistent with the observed correlations of BMDD outcomes with MS/BS and BFR/BV in the present work. Calcium and vitamin D insufficiency might further contribute to reduced degree of mineralization[4,6]. Consistent with aforementioned findings in cancellous bone, HT patients revealed a clear shift of the cortical BMDD to lower calcium concentrations and an increase in mineralization heterogeneity compared to both LT patients and premenopausal reference data. In contrast, the LT patients at baseline were not different from reference cortical BMDD. At first glance this was somewhat surprising, because the MS/BS in LT was lower than previously reported MS/BS for healthy

postmenopausal women[27]. However, when compared to more recently reported other cohorts of healthy premenopausal or postmenopausal women[28,29], our LT patients seem to have bone turnover in the lower normal range. The normality of BMDD in LT suggests also that bone fragility in this patient cohort is caused by factors (e.g. microarchitecture) other than deviations in bone matrix mineralization.

It is well known that in high bone turnover patients the sudden reduction of turnover by bisphosphonates leads to an increase in the degree and a decrease in the heterogeneity of mineralization[5,7,8,30,31]. The latter is a transient effect which is observed after short term (about one to three years) therapy[6,30]. The treatment changes in cortical bone from the HT patients are in line with the aforementioned reported observations. The average degree of mineralization was even normalized in HT. The effects of further bone turnover reduction on mineralization in patients with low baseline level of bone turnover (by MS/BS) has not been previously reported. In the present study, we observed a smaller, non-significant increase in CaMean in LT. This is consistent with the observation that the increase in bone matrix mineralization after bisphosphonate treatment is proportional to the deviation of baseline level of mineralization from reference[4,32]. However, the increase in mineralization was sufficient to raise the average and typical degree of mineralization as well as the percentage of highly mineralized bone areas in LT beyond reference levels. The only previously reported observation of mineralization beyond the reference level was in a study after zoledronic acid treatment[33].

Fracture risk and bone mineral density studies have shown that bisphosphonates are beneficial to both higher and lower turnover postmenopausal patients[20,35,36], with greatest relative increases in bone mineral density in patients who had the highest bone turnover at baseline[34,36]. As bisphosphonate treatment was generally not observed to cause substantial increases in bone volume, changes in the bone material quality in addition to the increase in bone mineral density might contribute to the decrease in fracture risk[37]. A previous work, which focussed on cancellous bone, suggested that the main contribution to the increase in bone mineral density was the increase in mineralization[4]. Thus the observed increases in cortical bone matrix mineralization in HT and LT patients in the present study are in line with the previously reported increases in bone mineral density in both groups[20]. Although fracture risk reduction has been reported after bisphosphonate in patients with lower turnover before treatment[35], it remains an open question whether the moderate increase in bone mineralization above reference levels after bisphosphonate treatment in the LT patients is beneficial in the long-term or indicates unfavourable changes in the bone matrix.

In the observational study, we found that BMDD outcomes were dependent on MS/BS after 3 to 7 years antiresorptive treatment, but were not correlated with MAR. This latter finding is in agreement with the VERT-NA trial patients at baseline. There was no increase in the degree of mineralization beyond reference even after longer treatment duration. In contrast, after long-term alendronate treatment, CaPeak was below the reference level. An explanation would be a lower compliance to treatment in this non-clinical setting or alternatively, the majority of patients had high bone turnover with low degree of matrix mineralization at baseline and the treatment has shifted the degree of mineralization towards reference values. The similarity of BMDD between the 3-5 years and >5 years groups in the present data and previous observations suggests that no further substantial changes in bone mineralization and other material characteristics are expected to occur after 3-5 years of bisphosphonate treatment[6,38,41], which is the typically recommended duration of bisphosphonate treatment for patients at low or moderate risk of fragility fractures[39].

An interesting finding in the present work was the nearly 1:1 correlation of cortical CaMean versus cancellous CaMean in all study groups. This indicates a close relationship between the mineralization of the cancellous and the cortical compartments also in osteoporotic individuals similar to that found in healthy individuals[21]. The striking similarity in average degree of matrix mineralization/tissue age between cortical and cancellous bone of the iliac crest in each patient was neither influenced by the level of bone turnover (as the correlation was similar in LT and HT patients before treatment) nor by bisphosphonate treatment.

Summary: The effects of bisphosphonate treatment on cortical bone mineralization were greater in patients who had higher turnover and lower bone mineralization at baseline. After 3 years of bisphosphonate treatment BMDD parameters were either shifted toward reference or even normalized in higher turnover patients. These outcomes after 3 years bisphosphonate treatment in HT patients were similar to the BMDD outcomes in the observational study patients after 3 to 7 years bisphosphonate treatment. In contrast, in patients with lower bone turnover at baseline, the small increase in mineralization due to treatment led to cortical bone mineralization above reference levels.

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Authors' roles: Study design: EPP, RRR, KK, RP, PR. Study conduct: KK, RP, PR. Data collection: BMM, SB, SL, RP, PR. Data analysis: BMM, SB, SL, PR. Data interpretation: BMM, SB, RRR, PR, RP. Drafting manuscript: BMM, SB, RP, PR. Revising manuscript content: SL, EPP, RRR, KK. Approving final version of manuscript: BMM, SB, SL, EPP, RRR, RP, KK, PR. BMM takes responsibility for the integrity of the data analysis.

References

1. Cummings SR, Black DM, Thompson DE, Applegate WB, Barrett-Connor E, Musliner TA, Palermo L, Prineas R, Rubin SM, Scott JC, Vogt T, Wallace R, Yates AJ, LaCroix AZ. Effect of alendronate on risk of fracture in women

with low bone density but without vertebral fractures: Results from the Fracture Intervention Trial. JAMA 1998;280:2077-2082.
2. Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut CH, Brown J, Eriksen EF, Hoseyni MS, Axelrod DW, Miller PD. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis. JAMA 1999;282:1344-1352.
3. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD. Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 2003;18:1051-1056.
4. Recker R, Lappe J, Davies KM, Heaney R. Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. J Bone Miner Res 2004;19:1628-33.
5. Han Z-H, Palnitkar S, Sudhaker Rao D, Nelson D, Parfitt AM. Effects of ethnicity and age or menopause on the remodeling and turnover of iliac bone: Implications for mechanisms of bone loss. J Bone Miner Res 1997; 12:498-508.
6. Whyte MP, Bergfeld MA, Murphy WA, Avioli LV, Teitelbaum SL. Postmenopausal osteoporosis: A heterogeneous disorder as assessed by histomorphometric analysis of iliac crest bone from untreated patients. Am J Med 1982;72:193-202.
7. Arlot ME, Delmas PD, Cappard D, Meunier PJ. Trabecular and endocortical bone remodeling in postmenopausal osteoporosis: comparison with normal postmenopausal women. Osteoporosis Int 1990;1:41-49.
8. Rehman MTA, Hoyland JA, Denton J, Freemont AJ. Histomorphometric classification of postmenopausal osteoporosis: implications for the management of osteoporosis. J Clin Pathol 1995;48:229-235.
9. Parfitt AM. A structural approach to renal bone disease. J Bone Miner Res 1998;13:1213-1220.
10. Bala Y, Zebaze R, Seeman E. Role of cortical bone in bone fragility. Curr Opin Rheumatol 2015;27:406-13.
11. Roschger P, Paschalis EP, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. Bone 2008;42:456-466.
12. Landis WJ, Librizzi JJ, Dunn MG, Silver FH. A study of the relationship between mineral content and mechanical properties of turkey gastrocnemius tendon. J Bone Miner Res 1995;10(6):859-67.
13. Turner CH. Biomechanics of bone: determinants of skeletal fragility and bone quality. Osteoporos Int 2002; 13(2):97-104.
14. Hofstetter B, Gamsjaeger S, Phipps RJ, Recker RR, Ebetino FH, Klaushofer K, Paschalis EP. Effects of alendronate and risedronate on bone material properties in actively forming trabecular bone surfaces. J Bone Miner Res 2012;27:995-1003.
15. Borah B, Dufresne TE, Chmielewski PA, Johnson TD, Chines A, Manhart MD. Risedronate preserves bone architecture in postmenopausal women with osteoporosis as measured by three-dimensional microcomputed tomography. Bone 2004;34:736-746.
16. Zoehrer R, Roschger P, Fratzl P, Durchschlag E, Paschalis E, Phipps R, Klaushofer K. Effects of 3- and 5-year treatment with Risedronate on the bone mineral density distribution of cancellous bone in human iliac crest biopsies. J Bone Miner Res 2006;21:1106-1112.
17. Fratzl P, Roschger P, Fratzl-Zelman N, Paschalis EP, Phipps R, Klaushofer K. Evidence that treatment with risedronate in women with postmenopausal osteoporosis affects bone mineralization and bone volume. Calcif Tissue Int 2007;81:73-80.
18. Eriksen EF, Melsen F, Sod E, Barton I, Chines A. Effects of long-term risedronate on bone quality and bone turnover in women with postmenopausal osteoporosis. Bone 2002;31:620-5.
19. Recker RR, Ste-Marie LG, Chavassieux P, McClung MR, Lundy MW. Bone safety with risedronate: histomorphometric studies at different dose levels and exposure. Osteoporos Int 2015;26:327-37.
20. Misof BM, Dempster DW, Zhou H, Roschger P, Fratzl-Zelman N, Fratzl P, Silverberg SJ, Shane E, Cohen A, Stein E, Nickolas TL, Recker RR, Lappe J, Bilezikian JP, Klaushofer K. Relationship of bone mineralization density distribution (BMDD) in cortical and cancellous bone within the iliac crest of healthy premenopausal women. Calcif Tissue Int 2014;95:332-9.
21. Roschger P, Fratzl P, Eschberger J, Klaushofer K. Validation of quantitative backscattered electron imaging for the measurement of mineral density distribution in human bone biopsies. Bone 1998; 23:319-26.
22. Roschger P, Gupta HS, Berzlanovich A, Ittner G, Dempster DW, Fratzl P, Cosman F, Parisien M, Lindsay R, Nieves JW, Klaushofer K. Constant mineralization density distribution in cancellous human bone. Bone 2003;32:316-323.
23. Roschger P, Misof B, Paschalis E, Fratzl P, Klaushofer K. Changes in the degree of mineralization with osteoporosis and its treatment. Curr Osteoporos Rep 2014;12(3):338-50.
24. Boivin G, Farlay D, Bala Y, Doublier A, Meunier PJ, Delmas PD. Influence of remodeling on the mineralization of bone tissue. Osteoporos Int 2009;20:1023-6.
25. Recker RR, Kimmel DB, Parfitt AM, Davies KM, Keshawarz N, Hinders S. Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. J Bone Miner Res 1988; 3(2):133–44
26. Misof BM, Roschger P, Blouin S, Recker R, Klaushofer K. Bone matrix mineralization is preserved during early perimenopausal stage in healthy women: a paired biopsy study. Osteoporos Int 2016;27(5):1795-803.
27. Cohen A, Dempster DW, Recker RR, Stein EM, Lappe JM, Zhou H, Wirth AJ, van Lenthe GH, Kohler T, Zwahlen A, Müller R, Rosen CJ, Cremers S, Nickolas TL, McMahon DJ, Rogers H, Staron RB, LeMaster J, Shane

E. Abnormal bone microarchitecture and evidence of osteoblast dysfunction in premenopausal women with idiopathic osteoporosis. J Clin Endocrinol Metab 2011; 96(10):3095-105.
28. Roschger P, Rinnerthaler S, Yates J, Rodan GA, Fratzl P, Klaushofer K. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. Bone 2001;29(2):185-91.
29. Boivin GY, Chavassieux PM, Santora AC, Yates J, Meunier PJ. Alendronate increases bone strength by increasing the mean degree of mineralization of bone tissue in osteoporotic women. Bone 2000;27:687-694.
30. Ruffoni D, Fratzl P, Roschger P, Phipps R, Klaushofer K, Weinkamer R. Temporal changes in bone turnover induce complex transitions in bone mineralization density distribution: A computer simulation study. J Bone Miner Res 2008;23:1905-1914
31. Borah B, Dufresne TE, Ritman EL, Jorgensen SM, Liu S, Chmielewski PA, Phipps RJ, Zhou X, Sibonga JD, Turner RT. Long-term risedronate treatment normalizes mineralization and continues to preserve trabecular architecture: Sequential triple biopsy studies with micro-computed tomography. Bone 2006;39:345-352.
32. Misof BM, Patsch JM, Roschger P, Muschitz C, Gamsjaeger S, Paschalis EP, et al. Intravenous treatment with ibandronate normalizes bone matrix mineralization and reduces cortical porosity after two years in male osteoporosis: a paired biopsy study. J Bone Miner Res 2014;29:440-9.
33. Misof BM, Roschger P, Gabriel D, Paschalis EP, Eriksen EF, Recker RR, Gasser JA, Klaushofer K. Annual intravenous zoledronic acid for three years increased cancellous bone matrix mineralization beyond normal values in the HORIZON biopsy cohort. J Bone Miner Res 2013;28(3):442-8.
34. Seibel MJ, Naganathan V, Barton I, Grauer A. Relationship between pretreatment bone resorption and vertebral fracture incidence in postmenopausal osteoporotic women treated with risedronate. J Bone Miner Res 2004;19:323-329.
35. Gonelli S, Cepollaro C, Pondrelli C, Martini S, Montagnani A, Monaco R, Gennari C. Bone turnover and the response to alendronate treatment in postmenopausal osteoporosis. Calcif Tissue Int 1999;65:359-364.
36. Papapoulos S and Makras P. Selection of antiresorptive or anabolic treatments for postmenopausal osteoporosis. Nat Clin Practice: Endocrinology and Metabolism 2008;4:514-523.
37. Recker RR, Armas L. The effect of antiresorptives on bone quality. Clin Orthop Relat Res 2011;469(8):2207-14.
38. Roschger P, Lombardi A, Misof BM, Maier G, Fratzl-Zelman N, Fratzl P, Klaushofer K. Mineralization density distribution of postmenopausal osteoporotic bone is restored to normal after long-term alendronate treatment: qBEI and sSAXS data from the Fracture Intervention Trial Long-Term Extension (FLEX). J Bone Miner Res 2010;25(1):48-55.
39. Hassler N, Gamsjaeger S, Hofstetter B, Brozek W, Klaushofer K, Paschalis EP. Effects of long-term alendronate treatment on postmenopausal osteoporosis bone material properties. Osteoporos Int 2015;26(1):339-52.
40. Villa JC, Gianakos A, Lane JM. Bisphosphonate Treatment in Osteoporosis: Optimal Duration of Therapy and the Incorporation of a Drug Holiday. HSS J 2016;12(1):66-73.