Histomorphometric Assessment of the Long-term Effects of Alendronate on Bone Quality and Remodeling in Patients with Osteoporosis

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Abstract

Treatment effects on bone quality and remodeling was assessed in postmenopausal women with osteoporosis treated with oral alendronate. One transiliac bone biopsy was obtained from 231 women at either 24 mo (n = 11) or 36 mo (n = 120) from the start of treatment with alendronate at doses of between 5 and 20 mg/d, or placebo. 64 biopsies at 24 mo (31 from the placebo group and 33 alendronate-treated patients) and 95 biopsies at 36 mo (40 from the placebo group and 55 alendronate-treated patients) provided adequate cancellous tissue, and were analyzed by histomorphometry. Mineral apposition rate was unaffected by treatment. At 24 and 36 mo, osteoid thickness, volume, and surface significantly decreased. At each of the doses studied, mineralizing surface and activation frequency significantly decreased at each time point (e.g., −92% and −87%, respectively, for the 10 mg daily dose after 2 yr). These diminutions were of the same magnitude for each dose at 24 mo, and for the two highest doses at 36 mo. A significant increase in wall thickness accompanied by a reduction in erosion depth was detected in biopsies obtained at 24 mo. These findings confirm that mineralization is normal, and trabecular bone turnover markedly decreased in patients receiving long-term dosing with alendronate. The findings also suggest that the observed increases in bone mineral density could result both from a reduction in the remodeling space due to a decreased activation frequency and a possible trend to a positive bone balance. In addition, further studies focused on a possible increase in the degree of mineralization of bone are required. (*J. Clin. Invest.* 1997. 100:1475–1480.) Key words: alendronate • bisphosphonates • postmenopausal osteoporosis • histomorphometry • bone remodeling

Introduction

The geminal bisphosphonates are stable analogs of inorganic pyrophosphate. These agents bind to the bone mineral surface and inhibit osteoclastic bone resorption (1, 2). Alendronate sodium (4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid, sodium trihydrate), is a potent amino-bisphosphonate that has undergone extensive clinical development for the treatment of osteoporosis and other skeletal disorders (3–8). In postmenopausal women with osteoporosis, daily oral alendronate increases bone mineral density of the spine, hip and total body (6, 7, 9). As anticipated through its antiresorptive action, these increases in bone mineral density are associated with a reduction in the rate of bone turnover, as assessed by specific biochemical markers, from the high values seen after menopause to values comparable to those in healthy premenopausal women (10).

Histomorphometry and qualitative bone histology is an ideal tool for assessing the quality of bone. This use has become increasingly important, as there has been much recent interest in agents that increase bone mass, and it has become critical to evaluate whether or not the new bone thus formed is of normal bone quality, and therefore can be expected to result in greater strength and resistance to fracture. In addition, bone histomorphometry is the primary tool for elucidating at the cell and tissue levels the mechanisms and temporal sequence by which changes in bone mass occur.

In this study, histomorphometry was performed on transiliac bone biopsies taken from osteoporotic patients in two multicenter clinical trials of alendronate after 2 and 3 yr of treatment. The primary objectives of the biopsy program were to evaluate safety of alendronate, namely to exclude the presence of osteomalacia or more subtle defects in mineralization since these are known potentials of bisphosphonates, and to investigate qualitative aspects of bone. Further objectives were to assess the local effect to decrease the rate of bone turnover, and to investigate the mechanism for the observed increase in bone mineral density.

Methods

Description of clinical trials

To enter any of these studies, patients had to be at least 5 yr past menopause, and have a spine bone mineral density (BMD)[1] below 0.80 g/cm^2 using Hologic (Waltham, MA) or Norland (N. Brunswick, NJ) dual-energy x-ray absorptiometry densitometers, or 0.92 g/cm^2 using Lunar (Madison, WI) densitometers. In addition, eligible patients had to be in generally good health with absence of diseases or medications known to affect bone metabolism. Vitamin D deficiency was excluded by biochemical screening. Patients in the two Primary Phase III Studies were ages 44–84 at baseline (9, 11, 12). These stud-

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1. *Abbreviations used in this paper:* BFR, bone formation rate; BMU, basic multicellular unit; BSU, basic structure unit; BMD, bone mineral density; BS, bone surface; BV, bone volume; ES, eroded surface; EV, eroded volume; E.De, erosion depth; MAR, mineral apposition rate; MS, mineralizing surface; Oc, osteoclast; OS, osteoid surface; O.Th, osteoid thickness; OV, osteoid volume; TV, tissue volume; W.Th, wall thickness.

ies were sponsored by Merck Research Laboratories (Rahway, NJ), which also provided the alendronate tablets and matching placebo. All patients gave informed consent for the overall study and separate consent for the biopsy procedure. In all cases, the studies had prior approval from the appropriate institutional review board/ ethics committee.

Two identically designed Primary Phase III Studies were conducted; one in the United States and the other in 17 countries around the world (9, 11, 12). In both studies, patients were randomized to receive either placebo or alendronate 5, 10, or 20 mg/d in a ratio of 2:1:1:1, respectively. Those patients who received alendronate (20 mg/d) were switched to 5 mg/d for the third year (referred to as 20/5 mg) without breaking the study blinding. The other groups remained on the same treatment for all 3 yr. Out of the 516 patients enrolled in the International Primary Phase III Study, 111 patients were volunteers for a biopsy performed at the 24-mo time point. These patients came from 6 out of the 19 centers involved in this study. Out of the 478 patients enrolled in the U.S. Primary Phase III Study, 120 patients were volunteers for a biopsy performed at the 36-mo time point. These patients came from 14 out of the 18 centers involved in this study. A total of 231 biopsies were analyzed.

Histomorphometry

Biopsy procedure. Transiliac bone biopsies were obtained from consenting patients using a trephine needle (Meunier modification of Bordier trephine; Lepine, 69394, Lyon Cedex 03, France) with a minimal internal diameter of 7.5 mm. Before biopsy, patients received either 250 mg tetracycline four times daily, or 300 mg demeclocycline twice daily (for 2 d on, 12 d off, 2 d on) with the last dose timed to occur 4–6 d before the biopsy. Bone specimens were transported in 70% ethanol to the central laboratory responsible for the assessment and the analysis of biopsies (P.J. Meunier, Lyon, France).

Biopsies were serially dehydrated in graded alcohols and embedded in methylmetacrylate. Serial sections were cut at three different levels sufficiently far apart (at least 250 μm) to avoid replicate sampling of a single surface event. Some sections were stained with Goldner's trichrome, whereas others were left unstained for evaluation under ultraviolet light. Solochrome cyanin R staining was used for the assessment of wall thickness (W.Th).

Only biopsies that provided a large enough area of intact cancellous bone were assessed quantitatively, as small samples can provide misleading data due to limited sampling. For example, although mineralizing surface (MS) correlates with the rate of bone turnover, a sample containing few surfaces may either grossly underestimate or overestimate the actual rate of turnover in the bone as a whole. To limit such sampling errors, which are more likely to occur when the rate of bone turnover is low, an adequate biopsy was prospectively defined as one using all three sections combined; a minimum of either 20 mm^2 of intact cancellous tissue area for samples containing two or more tetracycline labels, or 40 mm^2 for samples with fewer than two labels. Inadequate biopsies were excluded from quantitative histomorphometric analysis, but were included in the qualitative histological assessment. 64 biopsies from the 111 specimens examined in the International Primary Phase III Study and 95 biopsies from the 120 specimens examined in the U.S. Primary Phase III Study were adequate for quantitative analysis.

Qualitative study. The appearance of the cellular components, the presence or absence of woven bone, or marrow fibrosis and any other noteworthy features were assessed qualitatively in all biopsies.

Quantitative study. The entire cancellous tissue area (including the transitional zone) of each section was analyzed (13). To confirm the predefined hypothesis (derived from preclinical studies) that alendronate at therapeutic doses would not impair mineralization in humans, the following parameters were measured: (*a*) osteoid volume/bone volume (OV/BV) was measured in cancellous bone on Goldner-stained sections, and expressed in percent of the cancellous bone volume; (*b*) osteoid surface/bone surface (OS/BS) was measured separately in cancellous and endocortical bone on Goldner-stained sections, and was expressed in percent of cancellous and/or endocortical bone surface; (*c*) osteoid thickness (O.Th) was measured on Goldner-stained sections; and (*d*) mineral apposition rate (MAR) was measured on unstained sections under ultraviolet light and was expressed in μm/d.

To estimate the effects of alendronate on bone turnover at the site of biopsy, and to investigate the mechanism of action by which alendronate induced the observed increase in bone density, the following parameters were measured: (*a*) eroded surface (ES/BS) represents the percentage of the total bone surface that consists of active (with presence of osteoclasts) or inactive (without osteoclasts) eroded surfaces; (*b*) osteoclast surface (Oc.S/BS) represents the percentage of the total bone surface that consists of active eroded surfaces; (*c*) osteoclast number (N.Oc/BS) is the number of osteoclasts per unit of bone surface; (*d*) erosion depth (E.De) was derived after rebuilding of the resorption cavity on an image analyzer. The computer automatically drew the trabecular surface. The operator rebuilt the resorption cavity by drawing a line with the help of the nonresorbed lamellae of the lacuna. Then, the analyzer automatically measured ES, the mean E.De (eight equidistant segments), and the maximum E.De (14). All resorption cavities were measured. (*e*) Eroded volume (EV/BV) represents the amount of bone eroded as a percent of cancellous bone volume, and was measured as described above (14). (*f*) The mineralizing surface was measured and expressed as a percentage of the total bone surface (MS/BS). The extent of the mineralizing surface was calculated as the length of the double-labeled surface plus half of the single-labeled surface (15). (*g*) Wall thickness (W.Th) of cancellous packets was measured under polarized light on Solochrome-stained sections. Only completed packets were measured, and measurements were performed in endocortical and cancellous bone separately. (*h*) Cancellous bone volume (BV/TV) represents the percentage of spongy bone tissue, including mineralized bone and osteoid. (*i*) Bone formation rate (BFR/BS) was calculated as MS × MAR, and expressed in $\mu m3/\mu m^2/d$. (*j*) The activation frequency that represents the probability that a new cycle of remodeling will be initiated at any point of the bone surface was calculated as (BFR/BS)/W.Th, and was expressed per yr. (*k*) Since the majority of erosion cavities were likely to have been incompletely resorbed at the time of biopsy, E.De is likely to underestimate the complete depth of erosion at the end of the resorption period. For this reason only an estimation of the bone balance (Δ) at the basic multicellular unit level could be made by substraction of E.De from W.Th.

With the exception of W.Th, these parameters were measured on Goldner-stained sections in endocortical and cancellous bone. All thickness/depth results (O.Th, MAR, E.De, W.Th) were corrected for obliquity of sections by multiplying by π/4.

All parameters were measured by using a semiautomatic (Ibas 1; Leica, Wetzlar, Germany) or an automatic (visiolab 5000; Biocom, Cedex, France) analyzer. O.Th, interlabel distance, and W.Th were measured at random according to the Kragstrup's method (16). Coefficients of variation for parameters measured were $< 6\%$ for BV/TV, OS/BS, OV/BV, ES/BS, MS (17), was 2.1% for W.Th, and was 6% for EV/BV and E.De. All histomorphometry analyses were conducted while the investigators remained blind to treatment allocation.

Statistical analyses. For the primary endpoint (assessment of mineralization) a two-sided trend test (18, 19) was used to evaluate differences among the treatment groups within each of the osteoporosis studies at a particular timepoint.

Mean and SD were computed for O.Th and MAR in each group, whereas median and SD of median were reported for OV/BV because of the nonnormal distribution of this latter parameter. Mean and SD are reported for other parameters. Significance was declared at $P \leq 0.05$ for all parameters using two-sided testing.

Strict random sampling was impossible because of the need for patient consent for biopsy separate from their consent to enter the clinical trials, and the *P* values reported are based on the (reasonable) assumption that data were similar to those that would have been obtained by a random sampling.

Table I. Qualitative Findings in Biopsies Obtained After 24 or 36 Mo of Treatment

	PBO	ALN 5 mg	ALN 10 mg	ALN 20/5 mg
	n = 98	*n = 44*	*n = 41*	*n = 48*
No. (%) with any finding	13 (13.3)	3 (6.8)	3 (7.3)	5 (10.4)
Lymphoid nodule(s)*	4 (4.1)	2 (4.5)	1 (2.4)	4 (8.3)
Very high remodeling	6 (6.1)	1 (2.3)	1 (2.4)	0
Microcallus	1 (1.0)	0	1 (2.4)	1 (2.1)
Sarcoidosis	1 (1.0)	0	0	0
Paget's disease	1 (1.0)	0	0	0

PBO, placebo; ALN, alendronate. *This finding represents a normal variant.

For the other endpoints (rate of bone turnover and mechanism of action), differences between treatment groups were tested by a nonparametric one-way analysis of variance. Comparison between two means were performed by a Mann-Whitney U test.

Results

Patients with biopsies were comparable to patients who did not undergo bone biopsy in the overall population in each study with respect to baseline characteristics (age, yr after menopause, gender, lumbar bone mineral density, data not shown). Inadequate biopsies were predominantly due to the samples either being crushed or incomplete as a result of the biopsy procedure.

Effects on bone quality. The qualitative assessment of bone did not reveal any abnormalities that could reasonably be attributed to alendronate therapy. Qualitative findings were reported in ~ 10% of the 231 biopsies at either 24 or 36 mo (Table I). There was, however, no trend for noteworthy findings to be more common in biopsies from alendronate-treated patients versus those on placebo. Microcallus was seen in biopsies from two alendronate-treated patients and one placebo-treated patient.

In all alendronate-treated patients from each of the two clinical trials, newly formed bone retained its normal lamellar structure, and there was no evidence for marrow fibrosis or cellular toxicity.

Effects on mineralization. Table II shows the results for the three predefined endpoints for assessment of mineralization. O.Th was significantly lower in alendronate-treated patients relative to those who received placebo at 5, 10, and 20 mg/d doses after 2 yr, and at 10 and 20/5 mg/d doses after 3 yr. At each timepoint, the lowest values were consistently associated with the highest current dose of alendronate. OV/BV decreased significantly with increasing dose of alendronate. No consistent trends for changes in MAR related to treatment were observed. These observations are consistent with the expected effects of a treatment-related decrease in the rate of bone turnover in the absence of any morphological or dynamic evidence of an impairment of mineralization.

Effects on bone turnover (Table III). The data from these exploratory parameters are less consistent than those from the primary study endpoints, discussed above. As shown in Table III, ES/BS and EV/BV showed no significant differences between treatment groups at either 24 or 36 mo. At 24 mo, however, mean values for E.De (either maximum or mean) and for EV/BV tended to decrease. Similarly, neither Oc.S/BS, nor N.Oc/BS showed any apparent response to treatment at either timepoint.

Alendronate at doses of 5 mg and greater was associated with significantly lower OS/BS and MS/BS than that in the placebo group at each timepoint. Osteoid surfaces were higher in endocortical than cancellous bone, in all groups (Table IV). The magnitude of the decrease of OS/BS expressed in percent of the placebo values, however, was similar in these two compartments, in alendronate-treated groups, after 2 and 3 yr.

At 24 and 36 mo, the BFR/BS and activation frequency were significantly decreased in all treated groups when compared to the placebo group, but at 36 mo, a significant decrease was also noted between patients receiving the lowest dose (5 mg/d) and those receiving one of the two highest doses.

Effects on remodeling at the BMU level (Table III). In biopsies obtained at 24 mo, values for W.Th were significantly higher in patients receiving alendronate versus those receiving placebo or the lowest dose. These data, however, need to be interpreted with caution as neither trend was apparent in analyses of biopsies obtained at 36 mo and the reduction of the daily dose from 20 mg to 5 mg between 24 and 36 mo may contribute to the loss of this effect.

At doses of 10 and 20 mg/d, alendronate induced a highly significant increase in the estimation of the bone balance (ΔBMU) at 2 yr, but not after 3 yr of 10 mg alendronate or 2 yr of 20 mg alendronate followed by 5 mg in year 3 (Table III). No change in BV/TV, however, was observed.

Discussion

Qualitative changes and effects on bone mineralization. From the qualitative assessment of bone biopsies in this study, it is evident that alendronate treatment preserves the normal lamellar architecture of bone, and did not induce woven bone, marrow fibrosis, cellular toxicity, or any other abnormality.

Table II. Effects of Alendronate on Mineralization of Bone

	Results at 24 mo (International Primary Phase III Study)				Results at 36 mo (US Primary Phase III Study)			
Treatment	Placebo	ALN 5 mg	ALN 10 mg	ALN 20 mg	Placebo	ALN 5 mg	ALN 10 mg	ALN 20/5 mg
	n = 31	*n = 9*	*n = 9*	*n = 15*	*n = 40*	*n = 19*	*n = 17*	*n = 19*
O.Th (μm)	11.10 (1.72)	8.13 (2.30)‡	7.73 (2.68)§	7.56 (2.25)§	10.71 (1.87)	9.71 (2.12)	7.32 (1.69)§	8.44 (1.84)§
OV/BV (%)	1.42 (1.27)	0.15 (0.20)§	0.10 (0.34)§	0.09 (0.07)§	1.12 (0.73)	0.46 (0.56)‡	0.12 (0.20)§	0.27 (0.43)§
MAR (μm/d)	0.61 (0.11)	0.56 (0.16)	0.56 (0.17)	0.51 (0.27)	0.59 (0.13)	0.63 (0.12)	0.63 (0.11)	0.70 (0.15)*

Results are expressed as mean (SE) for O.Th and MAR, as median (SE of the median) for OV/BV. *$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$ vs. placebo.

Table III. Effects of Alendronate on Bone Volume and Bone Turnover Mean (SEM)

	Results at 24 mo (International Primary Phase III Study)				Results at 36 mo (US Primary Phase III Study)			
Treatment	Placebo	ALN 5 mg	ALN 10 mg	ALN 20 mg	Placebo	ALN 5 mg	ALN 10 mg	ALN 20/5 mg
	n = 31	*n = 9*	*n = 9*	*n = 15*	*n = 40*	*n = 19*	*n = 17*	*n = 19*
BV/TV (%)	16.1 (0.8)	16.9 (1.6)	15.2 (1.1)	15.1 (1.1)	14.7 (0.8)	14.3 (1.6)	16.6 (1.4)	12.5 (1.1)
W.Th (μm)	32.1 (0.5)	28.8 (0.8)*	34.4 (1.0)*	34.8 (0.7)‡	31.2 (0.4)	30.1 (0.5)	32.3 (0.5)	30.2 (0.6)
OS/BS (%)	8.86 (0.89)	1.25 (0.28)§	1.02 (0.41)§	1.44 (0.79)§	8.00 (0.56)	2.26 (0.37)§	1.50 (0.32)§	2.66 (0.53)§
ES/BS (%)	3.41 (0.50)	3.14 (0.84)	2.95 (0.96)	2.72 (0.41)	1.89 (0.12)	2.38 (0.38)	1.30 (0.17)	2.05 (0.31)
EV/BV (%)	1.21 (0.29)	0.72 (0.18)	0.62 (0.21)	0.66 (0.09)	0.46 (0.04)	0.73 (0.16)*	0.30 (0.05)	0.66 (0.15)
Oc.S/BS (%)	0.31 (0.07)	0.18 (0.05)	0.43 (0.25)	0.20 (0.05)	0.15 (0.02)	0.23 (0.06)	0.13 (0.04)	0.17 (0.05)
N.Oc/BS (mm^{-1})	0.082 (0.015)	0.042 (0.013)	0.076 (0.033)	0.054 (0.014)	0.038 (0.004)	0.049 (0.012)	0.024 (0.006)	0.036 (0.010)
Max. E.De (μm)	15.79 (0.91)	13.97 (1.06)	12.92 (0.84)	13.48 (0.84)	13.50 (0.43)	14.66 (0.82)	13.96 (0.86)	14.20 (0.72)
Mean E.De (μm)	9.86 (0.60)	8.42 (0.54)	8.01 (0.55)	8.28 (0.50)	8.11 (0.24)	8.89 (0.45)	8.84 (0.56)	8.63 (0.41)
MS/BS (%)	7.57 (3.51)	0.68 (0.96)‡	0.58 (0.66)‡	0.16 (0.23)‡	6.37 (3.49)	2.30 (2.33)‡	0.25 (0.50)‡	0.62 (0.98)‡
BFR/BS ($\mu m^3/\mu m^2$/d)	0.043 (0.004)	0.004 (0.001)§	0.006 (0.002)‡	0.014 (0.010)*	0.039 (0.003)	0.019 (0.003)§	0.003 (0.001)§‖	0.006 (0.001)§‖
A.cf (/yr)	0.493 (0.049)	0.059 (0.019)§	0.065 (0.025)‡	0.142 (0.107)*	0.451 (0.030)	0.223 (0.035)§	0.035 (0.009)§‖	0.077 (0.017)§‖
ΔBMU (max.)	16.81 (1.13)	14.83 (1.41)	21.44 (1.14)*	22.28 (0.99)‡	17.72 (0.52)	15.53 (0.95)*	18.53 (0.91)	16.01 (1.14)
ΔBMU (mean)	22.42 (0.87)	20.38 (0.95)	26.35 (0.98)*	27.11 (0.84)‡	23.06 (0.41)	21.23 (0.67)	23.59 (0.65)	21.57 (0.91)

*$P < 0.05$; ‡$P < 0.001$; §$P < 0.0001$ vs. placebo; ‖$P < 0.0005$ vs. ALN 5 mg by Mann-Whitney U test.

The observed decrease in OV without any change in MAR provides evidence that alendronate decreases the rate of bone turnover without inhibition of the mineralization in long-term clinical use (20). Under steady-state conditions with normal mineralization, the proportion of bone that remains unmineralized is directly proportional to the rate of bone turnover (21). The observed small decrease in O.Th is also probably accounted for by the decrease in the rate of bone turnover, although the precise mechanism for this effect remains to be determined (2).

This lack of effect on mineralization is highly consistent with other studies in animals and man. Thus, mineralization remained normal in patients with Paget's disease of bone who received 40 mg/d alendronate for 6 mo (22, 23). In contrast, in rats, even the lowest antiresorptive dose of etidronate was associated with defective mineralization, indicating a therapeutic ratio of 1:1 for this older bisphosphonate (24). This result is consistent with clinical experience of some centers, which have observed focal osteomalacia even with low dose (400 mg/d) etidronate therapy (25–27). The lack of adverse effect of alendronate on mineralization is most likely to be related to the small amount of drug absorbed. Even after 10 yr of continuous daily treatment, the total skeletal load of alendronate is estimated to be only ~ 80–100 mg (28, 29) distributed within 2–2.5 kg of bone mineral typically found in postmenopausal women (30).

Effects on turnover at the tissue level. Reflecting the expected decrease in the rate of osteoid and mineralizing surfaces, turnover bone formation rate and activation frequency decreased. The activation frequency was reduced by 88% after 2 yr of a daily dose of 10 mg, and by 93% after 3 yr. The proportional decrease in mineralizing surface in iliac bone relative to placebo in patients treated with alendronate, however, was more marked (~ 90–95% at 2 yr) than the decrease in bone turnover in the skeleton as a whole as reflected by the biochemical markers. In the clinical studies, consistent decreases in biochemical markers of bone formation and resorption were ~ 50% of baseline values (5, 6, 10, 31). The degree of suppression of turnover, however, may vary between different skeletal sites or different types of bone.

Although this difference could potentially be accounted for by some nonspecificity of the biochemical markers, it is clear that this is not the case as, at least for *N*–telopeptide cross-links, high-dose antiresorptive treatment in healthy young men decreases the rate of bone resorption by at least 85% (32). Correspondingly, it seems likely that the relative degree of suppression is less in cortical bone, which constitutes at least 80% of the total bone mass in the body with lower remodeling

Table IV. Effects of Alendronate on Endocortical and Cancellous Bone: Mean(SEM)

	Results at 24 mo (International Primary Phase III Study)				Results at 36 mo (US Primary Phase III Study)			
Treatment	Placebo	ALN 5 mg	ALN 10 mg	ALN 20 mg	Placebo	ALN 5 mg	ALN 10 mg	ALN 20/5 mg
	n = 31	*n = 9*	*n = 9*	*n = 15*	*n = 40*	*n = 19*	*n = 17*	*n = 19*
OS/BS (%) endocortical	11.07 (1.36)	1.72 (0.49)*	1.71 (0.54)*	2.10 (1.31)*	12.74 (1.13)	3.61 (0.73)*	1.76 (0.45)*	3.23 (0.65)*
OS/BS (%) cancellous	8.32 (0.94)	1.15 (0.28)*	0.91 (0.40)*	1.25 (0.63)*	6.55 (0.51)	1.74 (0.27)*	1.43 (0.32)*	2.62 (0.55)*

$P < 0.0001$ vs. placebo by Mann-Whitney U test.

rate than in cancellous and endocortical bone (21). The response to alendronate is similar in cancellous and endocortical areas. Even in iliac cancellous bone, there was no evidence that bone turnover was suppressed completely by any dose. Absence of detectable tetracycline label in the cancellous bone, following further sectioning where necessary, was noted in only two biopsies, one of which came from a placebo-treated patient. These data are consistent with those from animal studies, in which even very high-dose, long-term alendronate treatment did not totally suppress bone turnover (33, 34).

The histological observations confirm that the bone quality is preserved in patients receiving long-term alendronate. Therefore, the increases in bone density should be associated with both increased bone strength (33–35) and a reduction in fracture incidence (36). Clinically important progressive increases in bone density of the spine, hip, and total skeleton were observed over 3 yr of treatment with alendronate, and these changes were associated with a significant (48%) reduction in the proportion of patients with an incident vertebral fracture, as well as fewer patients with fracture at nonvertebral sites (9, 12, 37). Recently, similar results were reported after 2 yr of treatment at dose range of 1–5 mg daily (38).

The other aspects of the present study were more exploratory in nature. All of the clinical studies indicate that alendronate induces marked increases in bone density that are most rapid during the first 6–12 mo, following which there is a slower, but sustained and virtually linear increase in BMD of the spine and proximal femur for at least 36 mo. The early increase is most probably explained, at least in large part, by the filling in of the remodeling space due to the early decrease in the rate of turnover at the tissue level, but a reduction of bone loss can continue during the low turnover steady state induced by alendronate (2, 39). Similarly, alendronate had surprisingly little detectable effect on bone resorption parameters, including eroded surface and volume and osteoclast number. As alendronate clearly has marked effects to inhibit bone resorption, which is evidenced by decreased urinary excretion of bone collagen breakdown products, the small changes in erosion parameters are difficult to interpret. This difficulty may result from a prolongation of the reversal phase of the remodeling cycle, a decreased erosion rate, the imprecision of histomorphometric resorption endpoints, or a higher effect in cortical than cancellous bone.

The effect of alendronate on osteoclast apoptosis remains unclear. Although previous studies suggested that alendronate may only inhibit resorption activity (40–42) (which may explain the maintenance of osteoclast number) a recent paper reported that alendronate was capable of inducing osteoclast apoptosis (43).

Effects on bone balance at the basic structure unit level. The continuing, progressive increase in BMD suggests that there is an additional effect of treatment to reverse the negative balance at the level of the individual bone remodeling unit. Such an effect could result from a decrease in erosion depth, an increase in the wall thickness, or a combination of the two. Unfortunately, erosion depth cannot be measured directly, because the preexisting surface has vanished. Attempts to estimate erosion depth, such as the method used in the current study, make assumptions about the position of the previous surface from the remaining contours (14). Such estimates are, at best, imperfect (14, 44). Alternative methods, such as counting the number of transected lamellae, also suffer from practical difficulties and are not easily replicated (45).

The data from the 24-mo biopsies did indeed suggest that, at the 10- and 20-mg doses, alendronate increases W.Th and tends to decrease E.De. These effects may have resulted in a positive bone balance at the basic structure unit (BSU) level, thereby potentially accounting for the progressive increases in bone density. Whereas W.Th was measured on complete packets (16), E.De was measured in sites where resorption was ongoing and at different stages of completion, which explains why mean W.Th was approximately threefold greater than mean E.De. Thus, although the trend towards an increase of bone balance at the tissue level seems to be the most likely explanation for the progressive gains in BMD, the data from the current study obtained at the BSU level are equivocal, and a larger number of biopsies would be required to confirm this hypothesis. A loss of the effect of alendronate at 36 mo could not be excluded.

Potential effects on the mineralization. Another hypothesis for explaining the continuing increase in BMD is the possibility of a progressive increase in the degree of mineralization of bone matrix due to the important reduction in the activation frequency. The osteons and trabecular packets stay longer before being resorbed, and can progressively increase their secondary mineralization. Confirmation of this hypothesis will require use of microradiography and back-scattered electron microscopy, or other methods for measuring the degree of bone mineralization.

In summary, the extensive histomorphometry program provides strong evidence that alendronate has no adverse effects on bone structure or mineralization, and that, as expected, alendronate dose-dependently and markedly decreases the rate of bone turnover without complete suppression, however, at any dose. At the BSU level, alendronate increased W.Th and tended to decrease erosion depth after 2 yr. This effect resulted in a possible trend towards positive bone balance at 10 and 20 mg/d. These data are entirely consistent with those from preclinical studies that have demonstrated a wide margin of safety for all measurements related to bone quality, including effects on mineralization, bone turnover, and biomechanical strength and clinical studies that demonstrate the expected reduction in fracture risk for bone of normal quality. Therefore, all of the available evidence indicates that the quality of bone formed during alendronate treatment is normal.

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