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Research Article

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Comparison of Alendronate and Sodium Fluoride Effects on Cancellous and Cortical Bone in Minipigs

A One-Year Study

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Abstract

Fluoride stimulates trabecular bone formation, whereas bisphosphonates reduce bone resorption and turnover. Fracture prevention has not been convincingly demonstrated for either treatment so far. We compared the effects of 1-yr treatment of 9-mo-old minipigs with sodium fluoride (NaF, 2 mg/kg/d p.o.) or alendronate (ALN, 4 amino-1-hydroxybutylidene bisphosphonate monosodium, 1 mg/kg/d p.o.) on the biomechanical and histomorphometric properties of pig bones. As expected, NaF increased and ALN decreased bone turnover, but in these normal animals neither changed mean bone volume. NaF reduced the strength of cancellous bone from the L4 vertebra, relative to control animals, and the stiffness (resistance to deformation) of the femora, relative to the ALN group. In the ALN-treated animals, there was a strong positive correlation between bone strength and L5 cancellous bone volume, but no such correlation was observed in the NaF group. Furthermore, the modulus (resistance to deformation of the tissue) was inversely related to NaF content and there was a relative decrease in bone strength above 0.25 mg NaF/g bone. Moreover, within the range of changes measured in this study, there was an inverse correlation between bone turnover, estimated as the percentage of osteoid surface, and modulus. These findings have relevant implications regarding the use of these agents for osteoporosis therapy. (*J. Clin. Invest.* 1995. 95:2127–2133.) Key words: bones • NaF • alendronate • minipigs • strength

Introduction

Low bone mass is a major determinant of fracture risk (1–5). Sodium fluoride (NaF) and the aminobisphosphonate alendronate (ALN)[1] increase bone mass by different mechanisms and are both being evaluated for the treatment of osteoporosis.

There are conflicting reports on the effect of NaF treatment on fracture rates in osteoporotic patients (recently reviewed in references 6 and 7): some studies found no reduction or even an increase (8–11), whereas others showed a significant reduction in fracture rate (12–16). These differences have been attributed to the NaF dose (17), which was higher in studies in which treatment did not reduce the incidence of fractures (18). A similar controversy is found in epidemiological reports on the effects of water fluoridation on the incidence of hip fractures (19–21). In animal studies, where biomechanical data are available, NaF was reported to increase (22), decrease (23–25), or have no effect on bone strength (26–28). Short-term studies in ewes showed that at a low dose NaF stimulates bone formation by increasing the recruitment and lifespan of osteoblasts; at higher doses, NaF decreased osteoblast activity (29). At the tissue level, the increase in bone mass was accompanied by a reduction in bone quality, which was attributed to nonuniformity of bone mineralization (30), to decreased bending strength at the crystal-matrix interface (31), or to inadequate crosslinks in the organic matrix (32). The biomechanical changes seem to correlate with the bone fluoride content, determined by the daily dose, the bioavailability, and the duration of treatment (33). The results from clinical trials and animal studies suggest that the therapeutic window for NaF treatment is narrow and remains to be precisely established (6, 7).

Another group of agents being developed for the treatment of osteoporosis is the bisphosphonates. The aminobisphosphonate ALN is a potent inhibitor of bone resorption through direct (34) and/or indirect (35) action on osteoclasts. ALN produces no mineralization impairment at large multiples of a pharmacologically effective dose and was shown to increase bone mass in animal models of osteoporosis (36–38) and in postmenopausal women (39, 40). Biomechanical studies have shown that 3-yr treatment with ALN at 1 mg/kg/d p.o. (about five times the human dose) did not impair bone strength in dogs (41) and that ALN treatment preserved bone strength in ovariectomized rats (36) and baboons (37).

Our objective was to compare in the same study the effects of 1-yr treatment with NaF and ALN on bone quality, estimated by mechanical testing, and to evaluate whether bone remodeling, assessed by histomorphometry, affects the mechanical properties of bone. The pig was chosen as the experimental model, because its bone structure and bone remodeling resemble those in humans (42).

Methods

Animals and diet. The study comprised 36 female Hanford 8.5- to 9-mo-old minipigs. After an acclimation period, animals were age and

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1. *Abbreviations used in this paper:* Aj AR, adjusted apposition rate; ALN, alendronate; BFR, bone formation rate; BS, bone surface; BV/TV, bone volume over tissue volume; Cs sec Ar, cross-sectional area; CtWi, cortical width; EHDP, etidronate; MAR, mineral apposition rate; Mlt, mineralization lag time; MS, mineralizing surface; Omt, osteoid maturation time; Os, osteoid surface; Oth, osteoid thickness.

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weight matched and then randomly assigned to 3 groups of 12 animals: one group was given NaF orally at 2 mg NaF/kg/d (~ 0.91 mg F^-/kg/d), one group received ALN orally at 1 mg/kg/d, and one group served as controls and received vehicle (sucrose 200 mg/ml, sodium benzoate 1 mg/ml, and corn starch 80 mg/ml). Pilot studies established the absorption of ALN given with this vehicle at 0.76% similar to that in rats and humans. Formulation for oral dosing was prepared by Bio-Serve (Frenchtown, NJ), and dosage was adjusted to weight once every 4 wk. Animals were housed in individual sties during the experimental period of 1 yr. All animals were fed with a fixed diet, including phosphorus 0.89%, calcium 1.25%, and protein 18.4%, providing 3.7 kcal/kg. Fluoride content of foodstuff was lower than 0.05%.

Before the animals were killed, fluorochrome labeling was carried out in 15 animals (5 per group) with an intramuscular injection of oxytetracyclin (Oxytet-Vedco, St. Joseph, MO, 20 mg/kg) followed 5 d later by calcein (Sigma Chemical Co., St. Louis, MO). The protocol was approved by the Institutional Animal Care and Use Committee.

Histomorphometric analysis. The fifth lumbar vertebra and the iliac crest were harvested and placed in an ethanol/formaline solution (70/30, vol/vol). The vertebra was cut longitudinally and a core biopsy was taken from the iliac crest with a 7-mm inner diameter Roboz (Rockville, MD) drill. The tibia diaphysis was transversely sawed 8.3 mm below the anterior tuberosity and sliced in 25-mm sections. Bones were placed into a Hyper Center (Shandon, Pittsburgh, PA) tissue processor and then embedded in polymethylmethacrylate.

Measurements were carried out on a Labphot 2 Nikon microscope with visible, polarized, and ultraviolet light sources, using the semiautomatic image analyzer Bioquant (REM, Biometrics, Nashville, TN), without knowledge of group assignments. A correction factor for obliquity of $\pi/4$ was applied to distance or width measurements.

Cancellous bone analysis. Quantitative histology was performed on 7.5-μm-thick sections obtained on a Polycut Reichert-Young (Leica, Deerfield, IL) microtome. Sections were stained with Solochrome Cyanin R.

Cancellous bone volume (BV/TV, %) represents the percentage of cancellous bone occupied by trabeculae, excluding the bone marrow space and including the calcified and osteoid tissue. Bone volume was measured on a 28.2-mm^2 area in the iliac crest. In the vertebra bone volume was evaluated in a 23.3-mm^2 area, taken in the midfourth of the section to avoid the primary spongiosa and trabeculae too close to the vertebral medial vessel. Osteoid surface (OS/BS, %) represents the percentage of cancellous bone perimeter covered by osteoid seams. Osteoid thickness (OTh, μm) represents the mean width of an osteoid seam, according to the Kragstrup method (43).

10-μm-thick sections were obtained for histodynamic measurements. Mineral apposition rate (MAR, μm/d) is the mean distance between the two consecutive labels divided by the time interval between the two labels (5 d). Mineralizing surface (MS/BS, %) is the sum of the double-labeled perimeter and half of single-labeled perimeter expressed as a percentage of the bone perimeter. Mineralizing surface over osteoid surface (MS/OS, %) is the ratio of mineralizing surface to osteoid surface. Adjusted apposition rate (Aj AR, μm/d) represents the product of MAR and MS/OS. Osteoid maturation time (Omt, d) is the ratio of OTh divided by MAR. Mineralization lag time (Mlt, d) expresses the mean time interval between deposition and mineralization of osteoid and is the ratio of OTh divided by Aj AR. Bone formation rate at the tissue level (BFR/BS, $\mu m^3/\mu m^2/yr$) represents the amount of mineralized bone per unit of bone perimeter per year and is the product of MS/OS and MAR multiplied by 365.

Cortical bone analysis. Embedded tibial slices from the labeled animals were cut with a low-speed saw (Isomet, Buehler, Lake Bluff, IL) at ~ 200 μm and then hand ground to obtain 60–80-μm-thick sections that were glued unstained to a glass slide.

Cross-sectional area (Cs sec Ar, cm^2) and marrow area (cm^2), mean cortical width (CtWi), number of Haversian cavities per bone area (cm^2), and mean cavity area (μm^2) were directly measured. Cortical tissue area (cm^2) represents the amount of bone tissue in the section and is calculated as the difference between Cs Sec Ar and marrow area. Cortical porosity (%) represents the percentage of a given volume of cortical bone occupied by Haversian canals.

Total Haversian labeled surface (Haversian MS/BS, %) corresponds to the percentage perimeter of Haversian canals covered with double and half of single labels.

Haversian (cortical), periosteal, and endocortical mineral apposition rates (MAR, μm/d) were measured as in cancellous bone.

Bone fluoride content. Bone fluoride content (mg/g) was measured, as previously described (44), in a calcinated rib segment and a sample of cancellous bone from the iliac crest using an Orion (Cambridge, MA) fluor ion-specific electrode. Results are expressed as percentage of bone ash.

Biomechanical parameters. The L2-L4 vertebrae and both femora were immediately harvested from each specimen and maintained at −20°C. All specimens were irrigated with saline 0.9%, individually wrapped in plastic, and maintained at 4°C before testing. All biomechanical testing was performed using a servohydraulic material testing machine (model 1331, Instron, Canton, MA). For all biomechanical measurements, a preload of 10 N was applied before testing and the actuator was displaced at a rate of 1 mm/s until failure.

Three-point bending test. To isolate the femoral diaphysis, a transverse cut was made at the level of the lesser trochanter proximally and the condyle distally. The diaphyseal extremities were then embedded in polymethylmethacrylate cement encased in aluminum tubing to provide a surface for lower supports during testing. The anterior midpoint, defined as half the distance of the femoral length, served as the loading point during testing. The lower supports were placed 35 mm from the midpoint to ensure the same bending moment for all the specimens. The failure load (N) in bending and the bending stiffness (slope of the linear portion of the load vs. displacement curve) were determined.

L2 vertebral whole body. Approximately 2–3 mm of the superior and inferior endplates of L2 were embedded in polymethylmethacrylate to ensure planoparallel surfaces for the application of the load. Compressive failure load and stiffness were determined.

L4 vertebral trabecular core. One endplate was removed from the L4 vertebral bodies to expose the trabecular surface. A 6-mm inner diameter coring tool (McMaster Corp., Rosemont, PA), mounted on a drill press, was used to obtain the trabecular cores. Radiographs of the cores showed a central region of lower density surrounded by higher density bone. After removal of the remaining endplate, a 6-mm section was taken from each end of the core. The central region was discarded. One 6-mm section was tested in compression in the cephalocaudal direction. The load at failure and stiffness were recorded. The failure load was divided by core Cs sec Ar to determine stress, and the strain at failure was calculated from the failure displacement divided by original core length. A stress versus strain curve was then plotted, and ultimate stress or "strength" (MPa) and modulus (MPa) were determined from analysis of the curve. The modulus values were corrected for compliance in the load frame evident when small high-modulus specimens are tested at low strains. The remaining 6-mm section was used to determine apparent density (mg/mm^3). The cores were subjected to mild water jetting (performed under water) to partially clean the marrow cavities. They were then placed in a mild bleach solution in an ultrasonic cleaner to further clean the cavities and centrifuged at 1,000 RPM for 15 min to remove liquid from the pores. They were then immediately weighed and apparent density was calculated as wet weight/sample volume.

Data analysis. All data were analyzed on a MacIntosh computer using the Statview II (Abacus Concepts Inc., Berkeley, CA) as statistics software. All results are expressed as means±SD. Fisher PLSD test was used for the comparison of two means after one-way ANOVA.

Results

Weight. Animals continued to grow during the study and reached the normal weight for 21-mo-old animals at the end of the study. There was no significant difference in weight between the three groups when they were killed: control, 79.0±8.6 kg; NaF, 78.3±9.5 kg; and ALN, 77.6±8.4 kg.

Table I. Static Histomorphometric Parameters in Cancellous Bone from Iliac Crest and L5 Vertebra

	Controls	NaF	ALN	P (ANOVA)
IC BV/TV (%)	25.5±4.8	25.8±4.6	28.3±5.7	NS
L5 BV/TV (%)	34.6±5.6	35.7±8.8	34.3±6.4	NS
IC OS/BS (%)	7.5±2.7	10.9±5.8*	3.1±0.87*	0.0001
L5 OS/BS (%)	4.6±2.0	7.9±4.8*	1.2±0.6*‡	0.0001
IC OTh (μm)	5.2±0.9	5.7±0.6	3.9±0.5*‡	0.0001
L5 OTh (μm)	4.7±0.6	5.4±0.8*	3.9±0.5*‡	0.0001

Values are means±SD. IC, iliac crest. * Significantly different from controls. ‡ Significantly different from NaF.

Histomorphometric data. Examination by light microscopy revealed no qualitative changes in either group; no mottled periosteocytic lacunae or signs of focal osteomalacia were seen in the NaF- or ALN-treated bones. Under polarized light, the bone displayed a normal lamellar texture in all three groups.

For cancellous bone histomorphometric findings, data are summarized in Tables I and II. There were no significant differences in mean bone volume between the three groups in the iliac crest or the lumbar vertebra. Osteoid surface was significantly higher in the NaF group by 48–75% and lower in the ALN group by 41–74%, relative to controls. Osteoid thickness was modestly increased (by 12%) in the NaF group and lower in the ALN group (by 20–30%), relative to controls.

The histodynamic measurements showed the expected changes in bone remodeling by the respective treatments. Mineral apposition rate was slightly higher in the NaF group and lower in the ALN group (by 20–30%). Mineralizing surface was higher in the NaF group (by 52–75%) and lower (by 62%) in the ALN group relative to controls. As a result, bone formation rate was significantly higher in the NaF group (by 52–80%) and lower in the ALN group (by 62–73%) relative to controls. There was no difference between the three groups

Table II. Histodynamic Parameters in Cancellous Bone from Iliac Crest and L5 Vertebra

	Controls	NaF	ALN	P (ANOVA)
IC MAR (μm)	1.31±0.13	1.27±0.18	1.06±0.15*	0.05
L5 MAR (μm)	1.10±0.15	1.17±0.07	0.91±0.05*‡	0.005
IC MS/BS (%)	24.4±7.9	37.2±4.4*	9.1±1.9*‡	0.0001
L5 MS/BS (%)	15.1±4.4	26.3±9.9*	4.8±2.5*‡	0.001
IC BRF/BS ($\mu m^3/\mu m^2/yr$)	114.3±27.6	174.0±39.7*	36.5±8.4*‡	0.001
L5 BFR/BS ($\mu m^3/\mu m^2/yr$)	62.2±25.5	112.2±42.3*	16.4±9.0*‡	0.001
IC MS/OS (%)	2.8±0.8	3.0±1.0	2.9±0.4	NS
L5 MS/OS (%)	2.8±0.8	2.6±1.2	2.8±0.9	NS
IC Aj AR (μm/d)	3.6±0.8	3.9±1.5	3.1±0.7	NS
L5 Vt Aj AR (μm/d)	3.1±1.3	3.0±1.4	2.5±0.9	NS
IC Mlt (d)	1.6±0.3	1.8±1.2	1.2±0.3	NS
L5 Mlt (d)	1.8±0.8	2.2±0.9	1.7±0.6	NS
IC Omt (d)	4.2±0.7	4.5±0.8	3.6±0.5	NS
L5 Omt (d)	4.6±0.8	5.0±0.4	4.2±0.3	NS

Values are means±SD. n = 5 per group. IC, iliac crest. * Significantly different from controls. ‡ Significantly different from NaF.

Table III. Static Parameters from Tibial Diaphysis

	Controls	NaF	ALN	P (ANOVA)
Cs sec Ar (cm^2)	2.41±0.13	2.11±0.35	2.24±0.16	NS
Ct Wi (cm)	0.42±0.07	0.43±0.08	0.43±0.05	NS
Marrow area (cm^2)	0.40±0.10	0.32±0.07	0.36±0.13	NS
Cortical tissue area (cm^2)	2.01±0.19	1.79±0.33	1.88±0.17	NS
No. Haversian cavities	234.6±39.8	180.2±47.6	207.8±36.6	NS
No. Haversian cavities per bone area ($/cm^2$)	116.3±10.2	100.4±15.2	112.0±27.5	NS
Mean cavity area (μm^2)	0.38±0.07	0.43±0.06	0.28±0.06*‡	0.02
Porosity (%)	0.45±0.09	0.43±0.10	0.31±0.05*‡	0.05

Values are means±SD. n = 5 per group. * Significantly different from controls. ‡ Significantly different from NaF.

in the parameters assessing mineralization, such as MS/OS, osteoid maturation time, or mineralization lag time.

For cortical bone, data are summarized in Tables III and IV. There was no treatment-related change in bone size or structural parameters, such as cortical width, Cs sec Ar, or cortical tissue area relative to controls. There was a significant decrease in the mean area of Haversian cavities in the ALN group (by 26%) relative to controls. As a result, porosity was lower in the ALN group (by 31%). ALN produced a similar but smaller reduction in bone remodeling (by 43%) to that seen in cancellous bone, whereas NaF increased bone remodeling by 27%, but this difference did not reach statistical significance.

Biomechanical measurements are summarized in Table V. Failure load of cortical femoral bone in a three-point bending test did not differ significantly between the treatment groups, but the stiffness of the bones from the ALN-treated group was greater than that from both the NaF and the control groups ($P < 0.05$).

In the L2 whole vertebral body compression, no significant difference was found between the three groups.

In the L4 trabecular core testing, two specimens in the ALN group were damaged during the procedure. The L4 core strength (ultimate stress) was 22% lower in the NaF group than in the control group ($P < 0.05$). Modulus showed the same trend but did not reach statistical significance.

Bone fluoride content. The bone fluoride content measured in the rib, primarily cortical bone, was 23 times higher in

Table IV. Histodynamic Parameters from Tibial Diaphysis

	Controls	NaF	ALN	P (ANOVA)
Haversian MS/BS (%)	33.0±12.5	42.1±5.4	18.8±10.6*‡	0.01
Haversian MAR (μm/d)	0.99±0.22	1.12±0.25	1.03±0.26	NS
Endocortical MAR (μM/d)	1.10±0.28	1.14±0.37	0.88±0.29	NS
Periosteal MAR (μM/d)	0.97±0.27	0.89±0.13	0.86±0.17	NS

Values are means±SD. n = 5 per group. * Significantly different from controls. ‡ Significantly different from NaF.

Table V. Biomechanical Parameters from L2, L4 Core, and Femoral Testing

	Controls	NaF	ALN	*P* (ANOVA)
L4 apparent density (mg/mm^3)	0.78±0.10	0.74±0.13	0.78±0.15	NS
L4 core modulus (MPs)	1506±549	1261±373	1717±633	NS
L4 core stress (MPa)	40.3±9.1	31.5±4.6*	35.0±11.3	0.05
L4 core stiffness (N/mm)	4075±1033	3366±758	4543±1295‡	0.04
L4 core failure load (N)	1073±284	870±125	983±329	NS
L2 stiffness (N/mm)	9553±1884	8707±2329	9420±1436	NS
L2 failure (N)	15431±1880	15102±3115	14834±4162	NS
Femoral stiffness (N/mm)	1716±374	1733±349	2040±348*‡	0.05
Femoral failure (N)	5331±1281	5321±808	5713±889	NS

Values are means±SD. * Significantly different from controls. ‡ Significantly different from NaF.

bones from NaF-treated animals relative to ALN and control groups: NaF, 0.185±0.016%; ALN, 0.08±0.005%; controls, 0.076±0.008% ($P < 0.0001$). In trabecular bone the F^- content was 0.41±0.02% in the NaF-treated animals.

Correlations. As expected, there was a strong positive correlation between the osteoid and mineralizing surfaces of the cancellous bone in the vertebrae and in the iliac crest ($r = 0.66$, $P < 0.001$). The surface-based remodeling rate was 50–100% higher in the iliac crest than in the vertebra.

For individual bones there was a strong positive correlation between bone strength, assessed by ultimate stress or failure load, and the elastic properties of bone, assessed by modulus or stiffness, both in cortical ($r = 0.65$, $P < 0.01$) and cancellous bone ($r = 0.69$, $P < 0.001$). These findings indicate that in these bones the modulus (resistance to deformation), which is a more sensitive measurement, contributes to bone strength (reflected in failure load).

In the ALN-treated group, there was a positive correlation between L4 failure load and vertebral trabecular bone volume ($r = 0.75$, $P < 0.02$). A similar trend was observed in the control group without reaching statistical significance. However, in the NaF-treated animals, failure load did not increase with trabecular bone volume (Fig. 1). Most significantly, in the NaF-treated animals, there was a negative correlation between bone fluoride content and the modulus (resistance to deformation) of vertebral cancellous bone ($r = 0.93$, $P < 0.001$; Fig. 2).

Regardless of treatment, there was a negative correlation between the level of bone remodeling, reflected in mineralizing surface, and the biomechanical properties of cancellous bone (L4 modulus; $r = 0.67$, $P < 0.01$) or of cortical bone (femoral stiffness; $r = 0.73$, $P < 0.003$) (Fig. 3). A similar relationship was observed between bone formation rate over bone surface in cancellous bone and the modulus ($r = 0.64$, $P < 0.05$). Femoral stiffness was also negatively correlated with cortical bone porosity in the tibia, regardless of treatment ($r = -0.58$, $P < 0.05$).

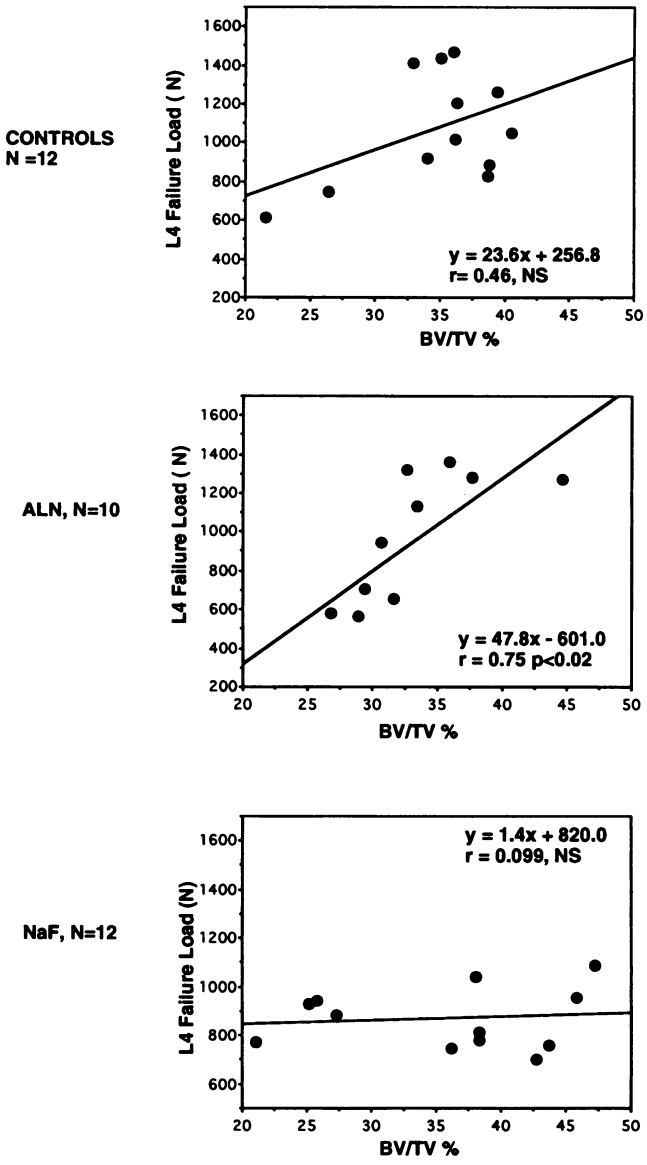

Figure 1. Linear regression analysis between trabecular bone volume (BV/TV, %) measured in the vertebrae and lumbar failure load in L4 vertebral cores.

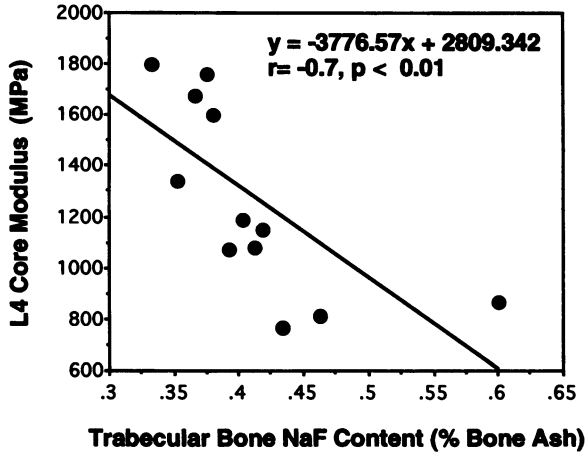

Figure 2. Linear regression analysis between bone NaF content measured in trabecular bone and L4 modulus in the NaF-treated group.

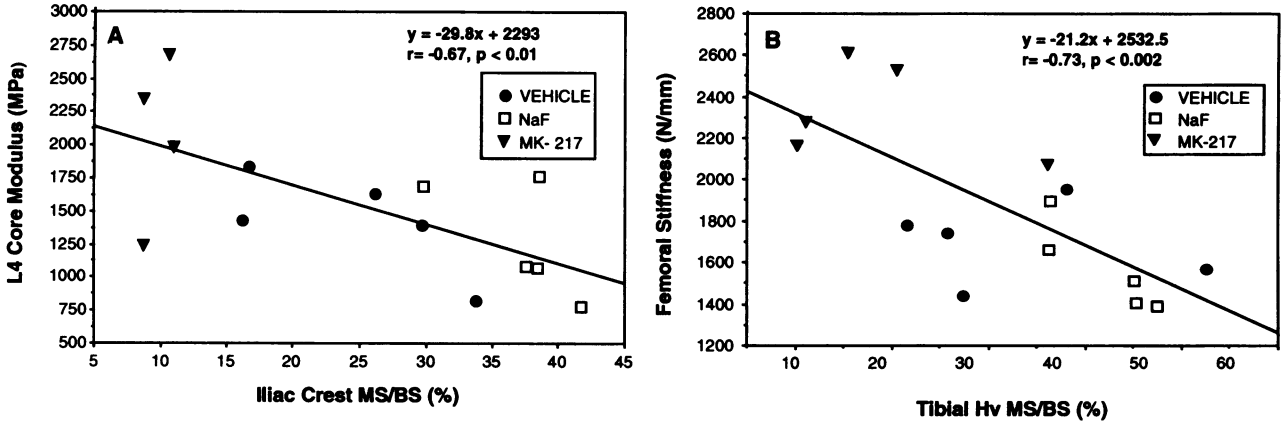

Figure 3. (*A*) Linear-regression analysis between mineralizing surface in iliac crest and L4 modulus, all treatments combined. (*B*) Linear-regression analysis between Haversian mineralizing surface in the tibia and femoral stiffness, all treatments combined. ●, controls; ▼, ALN (MK-217); □; NaF.

Discussion

NaF is so far the most potent stimulator of bone formation that has been used clinically (6–17, 45). In spite of clearcut increases in bone mass, the effect of fluoride treatment on osteoporotic fractures is equivocal. Differences between beneficial (12, 16), deleterious (9–11), or lack of effects were attributed to the administered dose or its absorption (17, 18, 46, 47), but the reason for these discrepancies is not yet clear. The effect of NaF on cortical bone remains to be established (6, 28). The accretion of bone in response to fluoride therapy has been documented in the vertebrae by bone density measurements (8, 10, 48) and in cancellous bone by iliac crest biopsies (49). Several animal studies using mechanical testing have shown a deterioration in bone "quality" with increased bone fluoride content (23–26, 33). Another therapy, which raised questions regarding the correlation of bone mass and fracture incidence, is treatment with the bisphosphonate etidronate (EHDP). Early studies in Paget's disease raised concerns about pathological fractures (50, 51). A safety study in dogs with high doses produced pathological fractures and another dog study showed impairment of fracture repair (52, 53). Most of these deleterious effects of EHDP can be attributed to inhibition of mineralization and osteomalacia. Aminobisphosphonates are a new series in this class of compounds, which are more potent than EHDP and do not inhibit mineralization at doses that are in great excess of the therapeutic dose (54). ALN is one of the potent compounds in this class (55). ALN inhibits bone resorption in vitro (34) and in vivo (36–38) by inhibiting osteoclast activity, either directly or indirectly (34, 35). ALN was shown to increase bone mass and bone strength in several animal models of human disease, including estrogen-deficiency bone loss (36–38). In clinical studies, ALN increased bone mineral density, both in the vertebrae and the proximal femur of postmenopausal osteoporotic women (39, 40).

The purpose of this study was to compare side by side the effects of NaF and ALN, administered for 1 yr to minipigs, on bone histomorphometry and bone strength. The NaF dose was chosen on the basis of previous pig studies (23, 56, 57), and it approximates the dose used in the US clinical trials (8, 10). However, the fluoride content, assessed in the rib after 1-yr treatment, was not significantly higher than that measured in patients treated with the lower dose common in Europe (50–66 mg NaF/d for 2 yr) (44). The ALN dose, given orally (1 mg/kg/d), was chosen to be 5–10 times the human osteoporosis dose. Throughout the study the animals experienced no apparent ill effects as a result of either treatment.

When the animals were killed, histomorphometry of the iliac crest and vertebrae showed, as previously reported, that NaF increased the bone formation rate of cancellous bone and ALN decreased it. These effects are consistent with the mode of action of the two agents at the tissue level (29, 37). Interestingly, the cancellous bone volume did not change and was not statistically significantly different from controls. This is an unexpected finding that supports the existence of homeostatic control for bone mass (58), evident from the constancy of this parameter in human populations. These healthy animals have obviously compensated for a 50–70% decrease or increase in bone formation produced by ALN or NaF, respectively, and maintained their "normal" bone volume. We made similar observations in normal dogs, where 3-yr treatment with 1 mg/kg/d ALN p.o., which reduced bone turnover by 80%, did not change bone volume (41). A likely controlling factor in this homeostatic feedback loop is mechanical function (59), but its mechanism and regulation by other factors require further study.

Extensive biomechanical testing carried out in this study detected better (statistically significant) elastic properties, femoral stiffness, and L4 core modulus in bones from ALN-treated animals than in NaF-treated animals. These properties have been shown to be of primary importance for preventing the breaking of bones under static or dynamic loading (60).

The most striking observation of this study emerged from the correlation of bone strength in individual bones with bone volume, estimated histomorphometrically. For vehicle-treated animals, the correlation was positive but not statistically significant, probably because the data were clustered around a mean bone volume of 35% and the strength of 1,000 N. For ALN-treated animals, where the bone volume ranged between 20 and 40% and the bone strength between 800 and 1,200 N, the positive correlation was statistically significant ($r = 0.62$, $P < 0.01$), as previously observed in baboons (37) and rats (36). For NaF, on the other hand, where bone volume had a wide range between 15 and 45%, bone strength was around 800 N and did not increase with bone volume, suggesting that for bones with higher volume, there was less strength per unit volume, that is, a deterioration in bone "quality."

A deterioration in the mechanical properties of fluoride-containing bone has been previously reported. For example, in a similar pig study, an increase in bone volume was not accompanied by an increase in bone strength (23). In a rat study, Einhorn et al. (27) found no differences in the mechanical properties of long bones after fluoride treatment. The fluoride content of these bones was not measured and no changes in histomorphometric parameters of bone turnover were observed. Most significantly, a recent study of the mechanical properties of iliac crest biopsies after 5 yr of treatment with 40–60 mg fluoride per day showed a substantial treatment-related reduction in bone quality (61). To further evaluate whether the fluoride in bone may have altered bone quality in our study, we estimated the fluoride content in the ribs and the iliac crest of individual pigs. Given the pharmacokinetics of fluoride skeletal uptake, the content in the ribs and the crest should correlate to that in the bones that were mechanically tested. In the crest, the mean value of fluoride content was 0.41% (range 0.39–0.59%). These values are not higher than those found in the iliac crest of patients treated with 50–66 mg NaF/d (44, 47), a dose commonly used in Europe. As seen in Fig. 2, there was a highly significant negative correlation between fluoride content and bone strength, which could explain the lack of increase in bone strength with bone volume in the fluoride-treated animals (Fig. 1). These findings are consistent with similar previous reports (23). Importantly, these differences are apparent when the fluoride content was not exceedingly high and the fluoride treatment did not significantly alter the mean bone volume in these animals. An increase in bone volume, produced by fluoride treatment, could possibly compensate or even exceed this decrease in quality, but the fine balance between the two effects emphasizes the difficulty associated with fluoride therapy.

The reason for the fluoride-induced changes are not known. No osteomalacia or other histological changes, including focal osteomalacia or mottled periosteocytic lacunae (47), were seen on thorough histological examination. No woven bone was seen or changes in the lamellar structure. Fluoride content could decrease the mechanical properties of bone as a result of its effects on crystal structure (30, 31, 62) or matrix properties (32) or defects that we failed to detect. Further studies are needed to examine these points.

It has been suggested that high turnover per se may decrease bone strength, and suppression of bone turnover could explain the protective effects of antiresorptive therapy in the prevention of fractures, in excess of that expected from the modest gain in bone density (63). We therefore examined whether there was a correlation between bone turnover and bone strength in this study. Interestingly, when all the bones were considered together regardless of treatment, a negative correlation, which reached statistical significance, was observed between bone turnover, estimated from bone formation parameters, and bone strength, estimated by failure load. The existence of such a correlation was recently proposed by Parfitt (64) and Riggs et al. (65). Support of this hypothesis by the data presented here is limited by the fact that different treatments were pooled, the bone turnover was altered by different mechanisms, and bone strength changes in fluoride-containing samples could be due to changes in crystal structure (62) or other factors. At the same time, these treatments affected the number of remodeling sites on the bone surface without affecting mean bone volume, the appropriate condition for testing above hypothesis. Further studies, especially in humans, are clearly required to evaluate whether there is a relationship between fracture risk and bone turnover independent of bone mass. Clinical studies on the effect of bone resorption inhibitors currently in progress should make such an analysis possible.

In summary, in ALN-treated animals there was a direct correlation between cancellous bone volume and bone strength, indicative of normal bone quality (36, 37). These findings are consistent with the conclusion that ALN treatment, under the conditions of this study, does not alter normal bone quality, estimated by biomechanical parameters. As for fluoride treatment, its effects are dependent on the delicate balance between the beneficial effects on bone mass and the deleterious effects of bone quality, which seem to be determined by the bone fluoride content.

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