Osteoporosis Int (1995) 5:115–129

Osteoporosis International

Original Article

Histomorphometric Analysis of Iliac Crest Bone Biopsies in Placebo-Treated Versus Fluoride-Treated Subjects

M. W. Lundy[1], M. Stauffer[1], J. E. Wergedal[1], D. J. Baylink[1], J. D. B. Featherstone[2], S. F. Hodgson[3] and B. L. Riggs[3]

[1]Pettis Veterans Hospital, Loma Linda, California; [2]Eastman Dental Center, Rochester, New York; and [3]Mayo Clinic, Rochester, Minnesota, USA

Abstract. In a 4-year controlled, prospective trial, histomorphometric analysis was used to compare the tissue-level skeletal effects of fluoride therapy in 43 postmenopausal women (75 mg NaF/day) with those of 35 matching placebo subjects; all subjects received 1500 mg/day elemental calcium supplement. In addition to an initial, baseline biopsy, a second biopsy was obtained after 6, 18, 30 or 48 months. Measurements were made on a third biopsy obtained from 8 subjects following at least 72 months of fluoride therapy. The change in cancellous bone volume or trabecular thickness in fluoride-treated subjects was not different from a change in placebo-treated subjects. However, paired analysis in the fluoride-treated subjects indicated that bone volume was increased between the first and second biopsies ($p < 0.005$). Both osteoid length and width were significantly increased in fluoride compared with placebo subjects; however, only the osteoid surface increased linearly ($r = 0.63$, $p < 0.001$). The mineral apposition rate and relative tetracycline-covered bone surface were not different between fluoride and placebo treatment, although they were decreased in both groups in the second biopsy. The tetracycline-covered bone surface returned to normal in the third biopsy. Definitive evidence for osteomalacia is a prolonged mineralization lag time, which following fluoride treatment was found to be increased 9-fold in the second biopsy and 4-fold in the third biopsy. Further evidence for osteomalacia was increased osteoid thickness by 6 months, evidence of focal areas of interstitial mineralization defects, and broad tetracycline labels of low fluorescence intensity. In the third biopsies, osteoclastic resorption was observed beneath osteoid seams. Fluoride therapy increased the cortical width compared with placebo treatment ($p < 0.02$), and increased the osteoid surface in Haversian canals, but did not change the osteoid width, resorption surface or cortical porosity. After an initial rise, serum fluoride levels remained constant, and the urine values fell slightly. The bone fluoride concentration rose throughout the treatment period, and was correlated with the change in osteoid-covered bone surface ($r = 0.56, p < 0.001$). Although we found definitive evidence for osteomalacia, the cause of the osteomalacia was not determined in this study. On the other hand, the presence of bone resorption beneath unmineralized osteoid and of osteocyte halos is suggestive of hyperparathyroidism. Thus, it is possible that the strong stimulus for bone formation brought about by fluoride therapy resulted in relative calcium deficiency.

Keywords: Bone; Fluoride; Histomorphometry; Osteoporosis

Correspondence and offprint requests to: Mark W. Lundy, PhD, Miami Valley Laboratories, Procter & Gamble Pharmaceuticals, PO Box 538707, Cincinnati, OH 45253, USA.

Introduction

Sodium fluoride has been used experimentally for the treatment of osteoporosis since the experiments of Rich and Ensinck [1] suggested that fluoride could improve calcium balance. Subsequently, many studies demonstrated that fluoride therapy increases cancellous bone mass [2–7]. Histomorphometric studies have shown that the increased bone mass is associated with an increase in the bone-forming surfaces, increased osteoid width,

increased osteoid volume, and increased mean wall thickness (the amount of bone formed in each cycle of bone formation) [8–11]. Studies in vitro suggested that the increased formation may be due to a direct effect of fluoride in stimulating osteoblast proliferation [12]. However, inhibition of mineralization was suggested by wide osteoid seams and decreased double tetracycline labels [8–11].

Few of these studies have followed the effect of fluoride on bone metabolism sequentially. Many studies did not include a biopsy at baseline, and few were properly controlled. The present study was designed to assess the efficacy of fluoride therapy using a placebo-controlled, double-masked design. The effect of fluoride on spinal bone mass, biochemical parameters and fracture frequency has been reported by Riggs et al. [13,14]. This report describes the effects of fluoride treatment on the sequential change in bone remodeling assessed by histomorphometry of transiliac bone biopsies.

Materials and Methods

Experimental Design

The selection of patients, design of the study, bone densitometry and fracture rate data have been previously reported by Riggs et al. [13]. The 202 postmenopausal (50–75 years of age) white women with type I (involutional) osteoporosis included in the study were randomly assigned to placebo plus calcium (as $CaCO_3$, 1500 mg elemental Ca/day), or fluoride (NaF, 75 mg/day as 30 mg tablets three times daily and two times on alternate days) plus calcium groups. The NaF was administered as non-enteric-coated tablets, which have a higher bioavailability than enteric-coated capsules [15]. Prior to starting treatment, a baseline bone biopsy was obtained from 114 subjects. A second biopsy was obtained in 82 subjects; 78 of these biopsies were technically suitable for histomorphometric analysis. Biopsies were not obtained in the remaining subjects because of dropouts, refusal of a second biopsy, or technically unsuitable biopsies.

A second bone biopsy was obtained from 35 placebo-treated subjects and from 43 fluoride-treated subjects. In both treatment groups the second biopsy was not obtained at the end of treatment but after four different intervals to determine the time course of the bone response to fluoride. The second biopsy was obtained after either 6, 18, 30 or 48 months of treatment. The distribution of subjects biopsied at each time interval is indicated in Tables A1 and A2.

Twelve subjects were maintained on fluoride therapy and biopsied a third time. Eight of these biopsies were adequate for analysis. Seven biopsies were obtained following a total of 72 months on fluoride therapy, and one biopsy after 84 months. The third biopsy was obtained 2 cm medial to the first biopsy site.

All the women gave written informed consent. The study was reviewed and approved by the Mayo Institutional Review Board.

Histomorphometric Methods

To determine the rate of and active surface involved in bone mineralization, tetracycline was administered prior to the first bone biopsy to the following schedule: tetracycline hydrochloride (250 mg q.i.d.) was given for 3 days, then 14 days later demeclocyline (150 mg q.i.d.) was given for 3 days, and the bone biopsy obtained 3–7 days following the last dose of tetracycline. For the second biopsy, the tetracycline hydrochloride was used for both labeling events, with 33 days between the start of the first and the start of the second tetracycline administration. The longer interval between the administration of the two labeling events was employed to obtain better separation between the labels, because fluoride therapy often produces wide, diffuse tetracycline labels.

Transiliac bone biopsies were obtained with a 7.5 mm internal diameter trephine, 2.5 cm below and behind the anterior superior iliac spine. The biopsies were placed in 70% ethanol, dehydrated in alcohol, and embedded in an 85% methyl-glycol methacrylate mixture [16]. Approximately one-third of the biopsy sample was removed by grinding using a plate sander. Five micrometer sections were obtained using a Reichert Autocut 1140 microtome. The sections were examined (1) unstained to visualize tetracycline labels, (2) stained with a modified Goldner's stain to obtain volume and surface measurements or (3) stained to identify osteoclasts using histochemical localization of acid phosphatase activity [17].

Quantitation of cancellous and cortical bone histomorphometry values was made with a Leitz Orthoplan microscope. Images were projected using a series of mirrors onto a Summagraphics Bit-Pad One digitizing tablet interfaced with a microcomputer. Morphometry software was obtained from BioMed Stats (Yelm, WA). Total tissue and mineralized bone areas were measured in a single Goldner's-stained section from each biopsy by projecting an image obtained with a ×1 objective onto paper and tracing the outlined bone and tissue areas on the digitizing tablet. Cancellous tissue area was defined as the area which was at least 0.25 mm from the cortical-endosteal surface. The cancellous tissue area was 30.6 ± 11.5 mm^2. Osteoid area, length and width in both cortical and cancellous bone were determined by tracing the osteoid area, projected directly onto the digitizing tablet, at a magnification of approximately ×490 using a ×10 objective. The precision of the methods ranged from 2% to 9%, similar to the 1%–8% determined by Gruber et al. [18]. The number of osteoclasts, and the number of osteoclast nuclei, were counted in the 5 μm sections stained for acid phosphatase activity at a magnification of ×250.

Several Haversian canal measurements were also made on the outer two-thirds of the cortical tables.

These included cortical porosity as a percentage of the cortical area, osteoid area as a percentage of bone area, osteoid width, Haversian canal resorbing surface length and Haversian canal osteoid surface length as a percentage of the total Haversian canal length. These measurements were made with the same digitizing tablet described above for the trabecular measurements. The number of subjects per group is different for measurements of cortical bone because (1) section orientation was not adequate for cortical examination, (2) an insufficient amount of cortical bone was present, or (3) other technical problems occurred in the processing and/or staining of the section which precluded making accurate measurements.

The nomenclature recommended by the ASBMR Nomenclature Committee was used to describe the histomorphometric parameters [19]. We have defined the mineralizing surface (%MS/BS) as the total tetracycline length per bone surface, and the osteoclast index (N.Oc/BS) as osteoclast number per 100 mm bone surface. Additional abbreviations include: % BV/TV, percentage bone volume per tissue volume; % MBV/TV, percentage mineralized bone volume per tissue volume Tb.Th, trabecular thickness (μm); %OV/BV, percentage osteoid volume per bone volume; % OV/TV, percentage osteoid volume per tissue volume; O.Th (μm), osteoid thickness; % OS/BS, percentage osteoid surface per bone surface; % DL/BS, percentage double labeled surface per bone surface; MAR (μm/day), mineral apposition rate (interlabel distance per interlabel period); Aj.AR (μm/day), adjusted apposition rate (MAR × MS/OS); Omt (days), osteoid maturation time (O.Th/MAR); BFR/BS ($\mu m^3/\mu m^2$/year), bone formation rate per bone surface; BFR/BV (%/year), bone formation rate per bone volume; BFR/TV (%/year), bone formation rate per tissue volume; Mlt (days), mineralization lag time (MAR × MS/OS); Ilt (days), interlabel time; NDl, number of double labels; Nu/Oc, nuclei per osteoclast. For cancellous bone only, the two-dimensional measurements were converted to three-dimensional terms by multiplying all perimeters by $4/\pi$, and dividing all widths by $4/\pi$. Trabecular and osteoid widths were determined by the indirect method [19]. The distance between tetracycline labels was determined by direct measurement at multiple sites. When the MAR could not be determined due to the lack of clearly defined double tetracycline labels, MAR, Omt and Mlt were specified as missing values, and bone formation rates recorded as zero.

Serum and Bone Fluoride Measurements

Serum and urine fluoride measurements were made using an ion-specific electrode (Orion) [20]. A 24-h urine specimen was collected 48 h after the last dose of fluoride. The specimen was acidified by adding 10 ml concentrated hydrochloric acid prior to taking the aliquote in order to avoid fluoride loss into precipitates.

Bone fluoride measurements were obtained on two sections (20 μm thick) of the embedded biopsy. The sections were ashed at 550 °C for 24 h and dissolved in 1 M HCl, and the fluoride separated by acid diffusion before measurement with the fluoride electrode [21]. The calcium concentration was measured using atomic absorption spectroscopy. The results are expressed as micromoles fluoride per millimoles calcium.

Statistical Analysis

The effect of fluoride treatment was compared with placebo treatment by first determining the difference between the first and second biopsy. This change between biopsies due to fluoride treatment was then compared with placebo treatment using the Kruskal–Wallis analysis of variance test.

Changes between biopsies in either the placebo group only, or fluoride treatment only, were compared using the Wilcoxon matched pairs test to determine the effect of treatment without regard to treatment time, or using the Kruskal–Wallis analysis of variance test to determine the effect of time on treatment. In subjects biopsied three times, the significance level was adjusted to 0.017 according to the Bonferroni method [22]. Logistic regression analysis, and determination of correlation coefficients, were performed on the placebo-treated subjects separately from the fluoride-treated subjects. Results for each group are reported as mean ± SEM unless specifically stated otherwise.

Results

Cancellous Bone

Bone Volume/Tissue Volume (BV/TV) and Trabecular Thickness (Tb.Th). To determine whether fluoride treatment altered histomorphometry without regard to time on treatment, the difference between the first and second biopsies was calculated for each variable. The placebo-treated group was then compared with the fluoride-treated group using the Kruskal–Wallis nonparametric analysis of variance test (Table 1). These results indicate that over the 4-year treatment period, fluoride treatment did not significantly increase bone volume or trabecular thickness compared with placebo-treated subjects. In both the placebo and fluoride-treated groups the changes in bone volume were highly variable (Fig. 1). There is an apparent increase in bone volume during the first 30 months of fluoride therapy, but at 48 months the second biopsy values are not higher than pre-treatment or placebo values.

The lack of difference in bone volume following fluoride compared with placebo treatment was unexpected on the basis of previous studies. Therefore, we used the Wilcoxon matched pairs test to evaluate pre- and post-treatment biopsies in the placebo and fluoride-treated groups separately. There was no change in BV/TV during placebo therapy ($p < 0.2$) (Fig.

Table 1. The Kruskal–Wallis test statistic (F) is a non-parametric analysis of variance test which compares the difference between the first and second biopsies in all placebo-treated (n=35) versus all fluoride-treated (n=43) subjects

	F	$p<$
BV/TV (%)	1.35	0.25
Tb.Th (μm)	0.96	0.33
OV/BV (%)	38.61	0.0001
OV/TV (%)	42.84	0.0001
OS/BS (%)	40.12	0.0001
O.Th (μm)	24.09	0.0002
MAR (μm/day)	0.48	0.49
MS/BS (%)	0.21	0.65
MS/OS (%)	14.23	0.0001
Aj.AR (μm/day)	6.41	0.01
Omt (days)	13.08	0.001
Mlt (days)	27.53	0.0001
BFR/BS ($\mu m^3/\mu m^2$/year)	0.01	0.99
BFR/BV (%/year)	0.32	0.57
N.Oc/BS (/100 mm)	0.65	0.42
Nu/Oc	0.06	0.80
Ilt (days)	4.59	0.03

For explanation of abbreviations see text.

1). However, in the fluoride-treated subjects there was a highly significant increase in BV/TV in the second compared with the first biopsies (p <0.005). Linear regression analysis indicated no relationship between changes in BV/TV and the length of time subjects were treated with fluoride (r = 0.01).

There was no change in trabecular thickness during placebo therapy when the difference between the first and second biopsies was determined (p <0.10). Fluoride therapy significanty increased trabecular thickness in the second compared with the first biopsy (p <0.001), but this change was not related to the treatment interval (r = 0.04). The mean cancellous bone volumes for the first and second biopsies for each group of placebo-treated and fluoride-treated subjects are listed in the Appendix. The additional variables discussed below are also included in the Appendix.

Oseoclast Index. The change in number of osteoclasts per bone perimeter did not differ between placebo and fluoride treatment (Table 1). The number of acid phosphatase positive osteoclasts in the post-treatment biopsy was also not significantly different from the number in the pre-treatment biopsy in either the placebo-treated or the fluoride-treated subjects. There were also no changes in the number of nuclei per osteoclast in either treatment group. The difference in osteoclast number did not change at any time point during treatment in either the placebo or fluoride-treated groups.

Osteoid: Unmineralized Bone Matrix. Fluoride treatment increased all osteoid values significantly when compared with the placebo control group (Table 1). In the placebo-treated group, osteoid volume decreased in the second biopsy compared with the first biopsy when normalized to either bone volume (p <0.001) or tissue

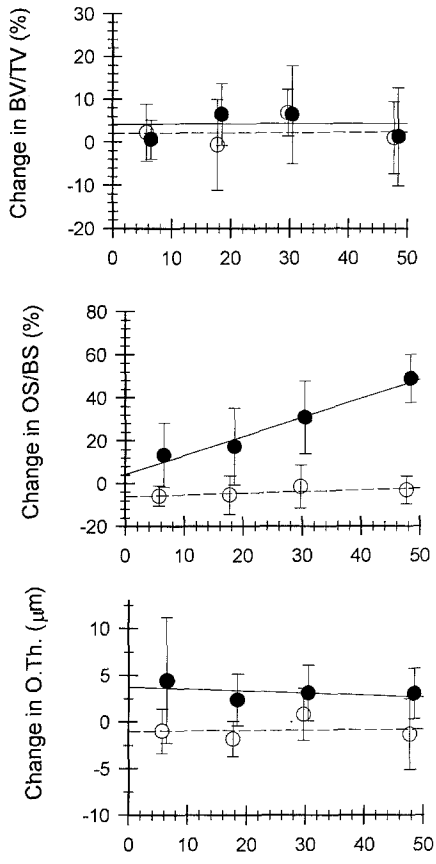


Fig. 1. Changes in cancellous bone volume (BV/TV%), osteoid surface (OS/BS%) and osteoid thickness (O.Th) are shown in placebo-treated (*open circles*) and fluoride-treated (*filled circles*) osteoporotic subjects. The differences in each individual (measurements in second biopsy minus first biopsy) were plotted against the time between the first and second biopsy. Fluoride-treated subjects were different from placebo-treated subjects by analysis of variance (p<0.001) for osteoid surface and osteoid thickness.

volume (p <0.002). The decrease was greatest at 6 months, and returned towards pre-treatment levels as time on the protocol increased. The decrease in osteoid volume during placebo and calcium treatment was a consequence of a decrease in osteoid-covered bone surface (p <0.04) (Fig. 1), with no change in osteoid thickness (p <0.02) (Fig. 1).

In the fluoride-treated group, osteoid volume increased significantly, normalized for either bone volume (p <0.0001) or tissue volume (p< 0.0001). These increases were linear with time on fluoride treatment (r = 0.50, r = 0.42, respectively) (Fig. 1). The osteoid surface was significantly increased during fluoride therapy (r = 0.63, p <0.0001); a linear increase in osteoid-covered bone surface is clearly demonstrated in Fig. 1. However, in 6 patients (14%) the osteoid surface decreased during fluoride therapy. All subjects treated with fluoride for 48 months responded with an increased osteoid surface.

The mean osteoid thickness also increased significantly in the second biopsy compared with the first (p <0.0001). However, this increase was not progressive,

in contrast to the change in osteoid surface. The increase in osteoid thickness occurred at 6 months, after which it decreased slightly (Fig. 1). Although the mean value for osteoid thickness remained constant, examination of the sections suggests that there may have been an increase in the maximum width of osteoid seams that was balanced by an increase in the length of narrow osteoid seams.

Dynamic Indices of Bone Formation. There were no significant differences in the mineral apposition rate or mineralizing surface per bone surface between the placebo-treated and fluoride-treated groups (Table 1). The number of double labels, and the bone formation rates, were not significantly different between the treatment groups.

However, because osteoid length increased, the parameters that use the length of unmineralized matrix (osteoid) as a referent were significantly different in the fluoride-treated group compared with the placebo group. The mineralization lag time was significantly longer than it was in placebo-treated subjects, as was the osteoid maturation time. Changes in the number of double tetracycline labels per section were similar in both placebo-treated and fluoride-treated groups.

The lack of significant changes in the tetracycline label length and apposition rate between placebo-treated and fluoride-treated subjects was unexpected, because both bone volume and osteoid volume increase with fluoride therapy. Therefore, we used the Wilcoxon matched pairs test to examine the changes between the first and second biopsies in the placebo subjects separately from the fluoride-treated subjects.

In fluoride-treated patients, mineral apposition rate decreased ($p < 0.01$) in the second compared with the first biopsy. There was also a reduction in the percentage of total surface, and osteoid surface, that incorporated tetracycline ($p < 0.002$, $p < 0.006$, respectively). The net effect of these changes was to decrease the tissue-level bone formation rates (BFR/BS, $p < 0.01$; BFR/BV, $p < 0.002$). There was a similar decrease in the percentage of bone surface taking up tetracycline in the placebo-treated and fluoride-treated patients ($p < 0.002$). Therefore, bone formation rates were also decreased in the placebo-treated and fluoride-treated subjects in the second compared with the first biopsies (BFR/BS, $p < 0.005$; BFR/BV, $p < 0.001$). The number of double labels per section was also decreased in both the placebo-treated ($p < 0.001$) and fluoride-treated subjects ($p < 0.003$) in the second compared with the pre-treatment biopsy.

Since the change in tetracycline labels between the first and second biopsies occurred in both placebo and fluoride groups, these changes could be due to the calcium treatment, for which there is not an adequate control, or they could be an artifact of the differences in the time between tetracycline labels in the first and second biopsies. The interlabel period was increased from 17 to 33 days to minimize smearing and overlap of tetracycline labels in the fluoride-treated subjects. Thus, the tetracycline interlabel time was significantly different between the placebo-treated and fluoride-treated groups (Table 1). The difference between the mean interlabel time in the placebo-treated subjects (first biopsy, 26.7 ± 1.4; second biopsy, 30.2 ± 1.4; $p < 0.02$) was less than in the fluoride subjects (first biopsy, 23.1 ± 1.5; second biopsy, 32.5 ± 0.23; $p < 0.0001$).

The significant decrease in bone formation rates in the fluoride-treated subjects with time is inconsistent with the increase in bone volume in the second compared with the first biopsy. The continued increase in osteoid length during fluoride therapy, coupled with the increased osteoid thickness, suggests that bone formation rates based on tetracycline uptake may not accurately reflect bone formation in fluoride-treated subjects. Therefore, we sought an alternative way to evaluate the effect of fluoride on bone formation. To determine whether fluoride increases matrix produced per forming surface, we examined the second biopsies from both placebo-treated and fluoride-treated subjects. The first set of tetracycline labels, administered prior to the basal biopsy, were buried deep within mineralized bone. We measured the distance from the first label to the bone surface, regardless of whether the surface was covered with osteoid or was fully mineralized. This distance is an index of the amount of matrix formed by osteoblasts during therapy, and is apparently independent of matrix mineralization. The distance from the initial tetracycline labels to the bone surface was significantly greater in the fluoride-treated subjects compared with those who received placebo (Table 2).

Table 2. In the second biopsy only, we measured the distance from the first, pre-treatment pair of tetracycline labels to the bone surface. We included both quiescent and osteoid-covered bone surfaces in these calculations

Months between biopsies	Placebo	Fluoride	$p<$
6	38.29±1.91 (7)	62.00±6.15 (5)	0.006
18	47.00±3.87 (5)	57.86±4.49 (7)	0.10
30	47.20±3.60 (5)	66.10±6.01 (10)	0.03
48	44.00±0.63 (5)	56.00±7.51 (3)	0.20

Values are the mean ± SEM (n).

Qualitative Evaluation of Bone Histology. In placebo-treated osteoporotic subjects and in all baseline biopsies, the osteoid seam is 4–20 μm thick, with a smooth interface between the mineralized and unmineralized matrix. Osteoid is not observed below this interface or around osteocytes within the newly formed, mineralized bone. The lamellae normally are distinct and easily identifiable, and usually are visible along the complete length of the remodeling unit.

Fluoride treatment appears to affect both the formation and mineralization of bone. Following 6 months of fluoride therapy the osteoid thickness increases, and the

interface between the mineralized and unmineralized bone is not always smooth. Small unmineralized areas are observed within the first two mineralized lamellae at the osteoid–mineral interface by 6 months. These small areas of osteoid are seen to coalesce and extend through three or more lamellae following 18 months of fluoride treatment. Mottled bone tissue with periosteocytic hypermineralized halos is apparent in patients treated for 30 months with fluoride. All patients treated with fluoride for 48 months have the irregular interface between the mineral and the osteoid seams, and occasional islands of osteoid within newly formed bone. Occasional linear formation defects are observed following 48 months of treatment. These changes in mineralization of osteoid do not appear more severe following 48 than 30 months of treatment, but they do affect all individuals. To determine whether fluoride therapy affected the pattern of matrix formation, we examined the Goldner's-stained sections using polarized light microscopy. A normal lamellar pattern was observed in both the mineralized bone and the unmineralized matrix in all fluoride-treated subjects, irrespective of the length of treatment. Fibrosis was not apparent at the bone surface in the fluoride or placebo-treated biopsies.

Serum, Urine and Bone Fluoride Values. The concentration of serum fluoride and that of either bone ($r = 0.49$) or urine ($r=-0.48$) were similar in the placebo and fluoride groups before treatment began. Serum, urine and bone fluoride concentrations remained constant in the placebo group during treatment. However, after an initial increase at 6 months, serum fluoride values remained elevated during fluoride therapy (Fig. 2). Urine fluoride output was greatest at 6 months, then declined ($r = 0.42, p < 0.006$). Bone fluoride concentrations continued to increase throughout fluoride treatment ($r = 0.74$, $p < 0.001$) (Fig. 2). There were no significant correlations between the changes in serum, urine or bone fluoride concentrations.

The correlation of histomorphometric values with fluoride concentrations revealed positive correlations between bone fluoride content and the changes in osteoid volume and in osteoid surface (Fig. 3) in fluoride-treated subjects (Table 3). There was also a positive correlation between the serum fluoride concentration and the change in bone volume in fluoride-treated subjects. There was, however, a poor correlation between the serum fluoride concentration and bone volume at the second biopsy ($r=-0.16$). Significant correlations between the changes in histomorphometric values and fluoride concentrations are shown in Table 3.

Cortical Bone

The histomorphometric changes in cortical bone were determined in a manner similar to that used for cancellous bone. To determine whether fluoride treatment

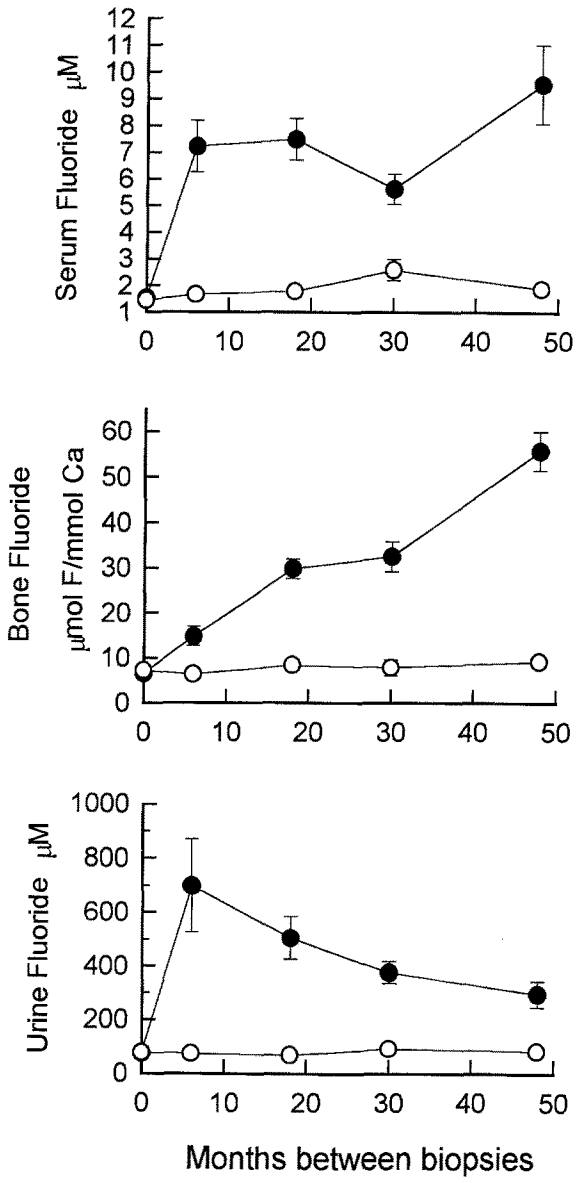


Fig. 2. The serum, urine and bone fluoride concentrations were determined at the time of the second biopsy in placebo-treated (*open circles*) and fluoride-treated (*filled circles*) subjects. Data are expressed as mean ± SEM. All fluoride-treated subjects have significantly ($p<0.01$) greater serum, urine and bone fluoride concentrations than the placebo-treated group by 6 months.

altered histomorphometry without regard to time on treatment, the difference between the first and second biopsy was calculated for each variable. The placebo-treated group was then compared with the fluoride-treated group using the Kruskal–Wallis analysis of variance test (Table 4). The cortical thickness was significantly increased in fluoride treatment compared with placebo ($p < 0.02$) (Fig. 4). Although there was a slight increase in cortical porosity following fluoride treatment compared with placebo therapy, this increase was not significant. The osteoid volume was significantly increased by fluoride therapy. As in the cancellous bone, there was a linear increase in osteoid surface in Haversian canals of cortical bone during fluoride

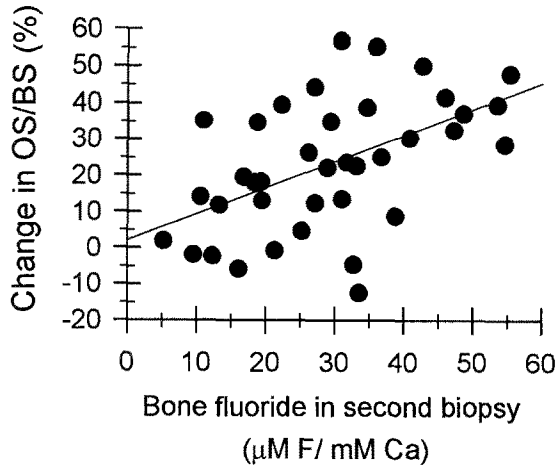


Fig. 3. The difference in osteoid-covered cancellous bone surface (second minus first biopsy) in fluoride-treated subjects compared with the bone fluoride concentration at the time of the second biopsy (r=0.55), p<0.001).

Table 3. In the fluoride-treated subjects only, the serum, bone or urine values obtained at the time of the second biopsy were correlated with the change in histomorphometric values between the first and second biopsy

	Fluoride concentration at at second biopsy	Correlation coefficient (r)	$p<$
Change between biopsies			
BV/TV (%)	Serum	0.30	0.05
OS/BS (%)	Serum	0.29	0.06
OV/TV (%)	Serum	0.43	0.005
OV/BV (%)	Bone	0.27	0.09
OS/BS (%)	Bone	0.56	0.001
OV/TV (%)	Bone	0.49	0.002
Values in second biopsy			
NDL	Urine	0.34	0.03
NU/OC	Urine	0.54	0.001
OV/BV (%)	Bone	0.41	0.01
OS/BS (%)	Bone	0.59	0.001

For explanation of abbreviations see text.

Table 4. Changes in cortical bone between placebo-treated and fluoride-treated subjects were determined using the Kruskal–Wallis analysis of variance test (F). The difference in each variable between the first and second biopsy was determined for each individual before determining the effect of placebo versus fluoride treatment

	F	$p<$
Cortical thickness (mm)	5.3	0.02
Cortical porosity (%)	0.3	0.59
Osteoid area/bone area (%)	20.0	0.0001
Osteoid width (μm)	0.9	0.35
Haversian canal resorbing surface (%)	0.01	0.9
Haversian canal osteoid surface (%)	24.3	0.0001

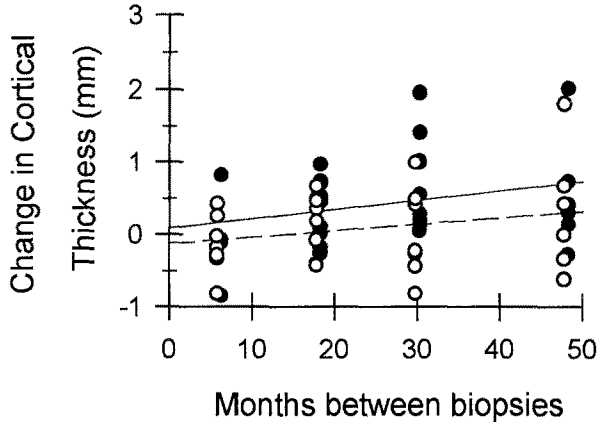


Fig. 4. The change in cortical thickness for placebo-treated (*open circles*) and fluoride-treated (*filled circles*) osteoporotic patients. The difference in cortical thickness between biopsies (second minus first) was plotted according to the time between the first and second biopsy. Fluoride treatment was greater than placebo by analysis of variance (p<0.02).

therapy (r =0.44, p <0.01). However, the osteoid width in Haversian canals was not increased at all compared with pre-treatment biopsies, or with the placebo-treated group. The Haversian canal resorbing surface was also not increased in the cortical bone during fluoride therapy. The lack of change in resorbing surface or cortical porosity suggests that bone turnover is not increased in cortical bone during fluoride therapy.

Third Biopsy

The effect of an additional 24–36 months of fluoride therapy on bone metabolism was determined in 8 subjects. There were no control subjects for comparison of the third biopsies. The bone volume continued to increase compared with that in the previous biopsies, with the third biopsy significantly greater than the first two biopsies (p <0.05; Table 5).

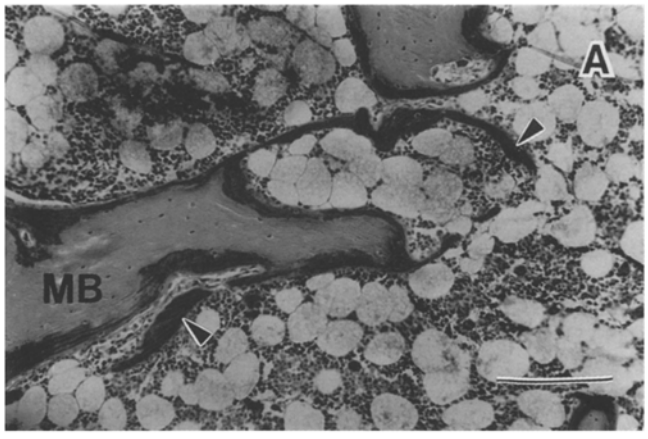
The osteoid-covered bone surface does not continue to increase further between the second and third biopsies. Osteoid thickness is significantly increased at each time of bone biopsy (each group different by Bonferroni comparison of means at p <0.05). These longitudinal results are slightly different from the cross-sectional comparisons of the change in osteoid thickness at the four different biopsy times (Fig. 1). There was no difference in the number of osteoclasts per bone surface between the three biopsies. However, there were areas of osteoid extending into the marrow from the bone surface (Fig. 5). Osteoclasts can be seen resorbing bone beneath osteoid, which appears to leave osteoid isolated in the bone marrow. These extensions of osteoid were observed in 3 of 8 subjects who had a third biopsy. Osteoclasts were observed resorbing bone beneath osteoid in only one biopsy obtained after 48 months or less.

The dynamic indices of bone formation improve between the second and third biopsies. Since the mineral apposition rate remained relatively constant

Table 5. Eight subjects were biopsied a third time following 6 years of fluoride treatment

	Baseline	Second biopsy	Third biopsy
BV/TV (%)	16.77±2.31	19.34±1.90	30.61±4.03[a,b]
Tb. Th (μm)	114.60±10.05	135.10±11.10	221.56±50.26
OV/TV (%)	0.29±0.057	1.20±0.145	1.89±0.41[a]
OV/BV (%)	1.79±0.25	6.67±0.94[a]	5.85±0.87[a]
O.Th (μm)	6.60±0.61	10.17±1.14[a]	13.52±0.88[a,b]
OS/BS (%)	15.13±1.55	43.29±5.48[a]	43.20±8.88[a]
N.Oc/BS (/100 mm)	13.79±4.44	13.30±4.12	17.25±3.84
MAR (μm/d)	0.66±0.12	0.52±0.07	0.55±0.10
MS/BS (%)	6.76±1.08	3.86±0.62	12.06±2.25[b]
MS/OS (%)	45.92±7.24	10.09±3.02[a]	37.77±10.90
Mlt (days)	24.36±1.82	423.70±137.18[a]	98.32±26.30[b]
Omt (days)	8.74±0.79	20.45±1.60	33.32±7.81[a]
BFR/BS ($\mu m^3/\mu m^2$/year)	16.38±4.43	6.80±1.73[a]	25.42±5.54
BFR/BV (%/year)	25.93±6.77	10.73±3.07[a]	29.05±6.05

For explanation of abbreviations see text.
Values are the mean ± SEM.
[a] Compared with baseline biopsy using Bonferroni adjustment, $p<0.05$.
[b] Compared with second biopsy using Bonferroni adjustment, $p<0.05$.



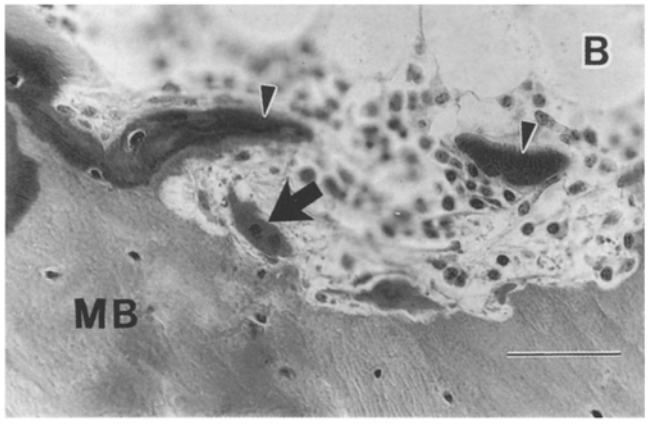


Fig. 5a,b. In three of eight biopsies obtained following 6 years of fluoride therapy, unmineralized bone matrix can be observed in the bone marrow. Multinucleated osteoclasts (*arrow*) can often be observed removing mineralized bone (*MB*) beneath osteoid (*arrowhead*). Scale bars represent 100 μm in **a** and 50 μm in **b**.

throughout this treatment period, the difference in bone formation rate is due to change in the osteoid surface incorporating tetracycline labels. The labels are predominantly observed beneath the wider osteoid seams. The osteoid maturation time increased significantly between the first and second biopsies, and then increased slightly by the third biopsy. The mineralization lag time increased significantly at 30 months, then decreased to values that remained higher than, but not significantly different from, baseline values.

Discussion

To characterize the mechanism of action of fluoride on bone, it is necessary to assess sequential changes over time. Although many non-invasive methods are available today to assess changes in bone turnover and bone mass during treatment of osteoporosis [23], only histomorphometric analysis can characterize the changes at both the cellular and tissue levels. Therefore, bone biopsies were obtained from the ilium in small groups of subjects prior to and after 6 months to 6 years of either placebo plus calcium or sodium fluoride plus calcium treatment to identify changes in bone histology and bone fluoride content.

Riggs et al. observed a linear increase in lumbar spine bone mineral density (LS-BMD) using dual photon absorptiometry during 4 years of fluoride therapy [13], which continued to increase when treatment was continued for an additional 2 years [14]. LS-BMD in the placebo-treated subjects did not change. When we determined changes in cancellous bone volume in the ilium in small groups of subjects used in the Riggs study, there was not a significant increase in bone volume in the fluoride-treated subjects compared with the control. Bone volume did increase during the 6-year fluoride treatment period when compared only with baseline

values. Increases in bone volume compared with pre-treatment biopsies have been reported previously [2,3,8–11]. Kleerekoper et al. [24] reported significant increases in total bone volume, but not mineralized bone volume, compared with pre-treatment biopsies, in subjects receiving 75 mg sodium fluoride per day for 42 months. The lack of a clear trend of increasing bone volume in the ilium following fluoride therapy compared with the continued increase in LS-BMD may be caused by a slow increase in mineralized bone volume but a faster increase in bone mineral content which is included in LS-BMD measurements. Alternatively, bone formation rates and increases in bone volume in the ilium may slow with prolonged fluoride treatment [10], while LS-BMD continues to increase. These differences observed between the ilium and vertebrae may also be a result of the high variance in bone volume determinations in the ilium, the small number of subjects in each group, and the cross-sectional nature of the comparison, compared with the repeated measures design of LS-BMD measurements which are more precise and are all measured in the same, larger numbers of subjects.

The definitive method for identification of responders to fluoride therapy is the increase in bone mass. Riggs et al. [14] found that almost one-third of the patients who had an increase in serum fluoride levels did not respond with an increase in LS-BMD, while many others have observed a lack of response in approximately 25% of subjects [8,9,25]. Neither Riggs et al. [14] nor Hodsman and Droost [26] could identify any baseline characteristic which could predict the lack of response to fluoride. The best histologic index of a response to fluoride in the bone biopsies is an increase in osteoid volume and osteoid-covered bone surface. We found in the current study that osteoid volume had not increased in 40% of the fluoride-treated subjects after 18 months of treatment, and in 15% after 30 months. All subjects biopsied at 48 months responded with an increase in osteoid volume, although bone volume changes were minimal. It is not clear from this whether all osteoporotic subjects may eventually respond with increases in bone mass, or whether the high bone fluoride content might alter mineralizations without stimulating osteoblast proliferation or activity.

Osteoclast volume and osteoid surfaces were decreased in the placebo-treated groups after 6 months. These reductions in osteoid volume may be due to restoration of positive calcium balance, and a reduction in serum parathyroid hormone concentration induced by the high daily calcium supplement of 1500 mg.

Pre-treatment bone formation rates or other histomorphometric variables were not useful predictors of a response to fluoride therapy in this or other studies [8,9]. Fluoride concentrations in serum and urine were also not useful in predicting responders. Bone fluoride concentrations were a better indicator of a response to fluoride treatment, although the osteoid-covered bone surface was not increased in all subjects with high bone fluoride concentrations. Harrison et al. [11] also found that serum and bone fluoride concentrations overlap between responders and non-responders. However, more patients respond as bone fluoride concentrations increase above 30 μmol fluoride/mmol calcium.

The increases in osteoid volume are recognizable as increases in both length and width within 6 months of treatment. Osteoid length continued to increase whether measured in different groups of subject for 4 years, or in 8 subjects receiving fluoride for 6 years. The mean osteoid width appeared to remain constant when measured in small groups of subjects at different times during the 4 years of fluoride treatment. However, osteoid width continued to increase during 6 years of fluoride treatment in the 8 subjects biopsied three times. This difference in response of osteoid width may be due to the type of comparison; the multiple measurements in the same subjects will be more reproducible than single measurements in multiple groups of individuals, so small changes are more likely to be identified. Alternatively, the minimal or slow increase in osteoid width and progressive increase in osteoid length may be a result of either the mineralization of forming areas stopping earlier than expected, or an extremely prolonged life span of the terminal osteoid. In either case, a thicker than normal layer of unmineralized matrix remains at bone surfaces which would otherwise be described as quiescent.

In addition to osteomalacia, other observations of the mineralization changes include periosteocytic halos and mottled bone, which were observed after 48 months of treatment. The bone fluoride content (measured as a function of bone calcium content) had increased to nearly 10 times pre-treatment levels in these biopsies obtained after 48 months of treatment, and were 5 times pre-treatment after 30 months of fluoride therapy. Similar calcification defects have been described by Boivin et al. [27,28] in fluoride-treated osteoporotic subjects whose bone fluoride content (measured as a percentage of bone ash) was 5–8 times pre-treatment levels. These changes in calcification were not observed in subjects whose bone fluoride content remained less than 3 times pre-treatment levels. Calcification defects may be the result of a toxic effect of fluoride on osteoblasts [29], as osteoblasts may be required for complete mineralization of matrix by removing inhibitors of crystal growth [30]. However, the problems with mineralization may not be a result of changes in osteoblast function, but rather be a direct effect of fluoride on mineral growth. A direct action of fluoride to inhibit bone mineralization has been demonstrated in vitro [31], and in rat kidneys during experimental nephrocalcinosis [32], leading Harrison et al. [31] to suggest that the physical-chemical properties of fluoride can account for the inhibition of mineral deposition without postulating direct effects of fluoride on osteoblastic metabolic processes.

The toxic effects of fluoride on osteoblasts have often been identified as reduced tetracycline incorporation into bone, or wide diffuse labels which prevent accurate measurement of mineralization rate [9,24]. The extent of tetracycline-labeled surface and mineral apposition rates are used to determine bone formation rates and adjusted mineral apposition rates, which are measure-

ments used to indicate osteoblastic function. However, if the incorporation of tetracycline is irregular due to fluoride-induced inhibition of mineralization, then measurements based on tetracycline labels may be inaccurate. Osteoblastic function appears normal on the basis of total matrix synthesis, as we found that the amount of matrix formed after labels were administered at the start of the study is increased at 6 months compared with that in placebo-treated subjects. Eriksen et al. [10] have demonstrated increases in completed mean wall thickness following 5 years of fluoride therapy. Matrix and mineral content in the vertebrae must also continue to be increasing during fluoride treatment as demonstrated in the current subjects by measurements of whole bone mass [14]. The linear increase in osteoid-covered surface would be expected if surfaces which start bone formation as a result of the normal bone remodeled process (activation, resorption formation, quiescence) do not become completely mineralized. The increases in bone mass would therefore be primarily the result of a positive bone balance at each site of bone formation, resulting in an increase in trabecular thickness [10].

Bone remodeling and bone resorption continue in the fluoride-treated subjects despite the presence of osteoid over most of the bone surface. We observed osteoclasts resorbing bone beneath osteoid seams, and fragments of osteoid isolated in the bone marrow. This type of resorption beneath unmineralized bone matrix is often observed in osteomalacia, particularly that caused by renal abnormalities and associated secondary hyperparathyroidism [33]. Although some authors have suggested that the resorption observed in skeletal fluorosis is due to secondary hyperparathyroidism in humans [34,35] and in fluoride-treated animals [36–38], others have found no effect of fluoride on parathyroid mass or serum parathyroid levels in animal studies [39,40]. There is no direct evidence for a role of parathyroid hormone in fluoride-stimulated bone formation in osteoporotic subjects.

Two recent placebo-controlled studies did not show a significant change in the vertebral fracture rate following fluoride treatment [14,41]. One study described an increase in stress fractures in the extremities in the fluoride-treated subjects, but no difference in the rate of hip fractures [14]. The other study described no changes in true peripheral fractures, although there were peripheral lesions they described as part of a "painful lower extremity syndrome" [41]. Kleerekoper and Mendlovic [42] recently reviewed the significance of peripheral lesions following fluoride therapy and also discussed reasons why the significant increase in vertebral mineral density is not reflected in a decrease in the fracture rate. One of the possibilities includes abnormal biomechanical properties of the new bone formed during fluoride treatment. In addition to the periosteocytic halos of poorly mineralized matrix, density fractionation of fluoride-treated bone found an accumulation in the highest density fractions, indicating increased mineralization [43]. Mechanical testing of fluoride-treated bone in situ found increases in compressive strength but decreases in tensile strength [44–47]. Whether the changes in bone structure and biomechanical properties are harmful remains to be determined.

Regarding stress fractures and lack of vertebral fracture efficacy with fluoride treatment, it seems possible that osteomalacia, to the extent observed in many patients in this study, could impair the mechanical performance of bone. Accordingly, it is currently thought that microdamage induces bone resorption at the site of the damage and that this is followed by replacement of the damaged bone with newly synthesized functional bone. It seems likely that osteomalacia, because of the mineralization defect, would delay the process of replacing damaged bone with new functional bone. If this were the case, osteomalacia could, in part, account for the observed increase in susceptibility to stress fractures seen in fluoride-treated patients [13]. In support of this possibility is the finding that classical osteomalacia is characterized by an increased prevalence of stress fractures [48]. Thus, the observed osteomalacia could have adverse mechanical effects on the skeleton. However, we wish to emphasize that our study was not designed to study the consequences of osteomalacia on skeletal performance, and that further work will be required to address this issue.

Although this study clearly documents in a prospective manner that fluoride therapy results in osteomalacia, our study was not designed to determine the cause of this osteomalacia. However, recent evidence suggests that fluoride may cause osteomalacia by stimulating bone formation to the extent that there is inadequate calcium absorption to support the demand for increased mineral deposition [49]. Fluoride could cause a calcium deficiency osteomalacia. Alternatively, it is possible that fluoride locally inhibits some enzymatic process which results in a direct inhibition of the mineralization process. In favor of the calcium deficiency hypothesis is that when patients with fluoride-induced calcium deficiency were treated with 1,25-dihydroxyvitamin D for several months, all evidence of calcium deficiency dissipated [49]. If this had been a toxic effect of fluoride to inhibit enzymes involved in mineralization, one would not necessary expect a correction with 1,25-dihydroxyvitamin D therapy. Thus if this osteomalacia produced by fluoride is a calcium deficiency osteomalacia, it should be possible to correct this with adequate calcium/1,25-dihydroxyvitamin D treatment. In any case, further studies will be required to determine the cause of the osteomalacia produced by fluoride therapy.

Acknowledgements. This work was supported by NIH grant AM-28576.

References

1. Rich C, Ensinck J. Effect of sodium fluoride on calcium metabolism of human beings. Nature 1961;191:184–5.
2. Jowsey J, Riggs BL, Kelly PJ, Hoffman DL. Effect of combined therapy with sodium fluoride, vitamin D and calcium in osteoporosis. Am J Med 1972;53:43–9.
3. Ringe JD, Kruse HP, Kuhlencordt F. Long term treatment of

primary osteoporosis by sodium fluoride. In: B. Courvoisier, A Donath, editors. Fluoride and bone. Bern: Hans Huber, 1978:228–32.
4. Vose GP, Keele DK, Milner AM, et al. Effect of sodium fluoride, inorganic phosphate, and oxymetholone therapies in osteoporosis: a six-year progress report. J Gerontol 1978;33:204–221.
5. Riggs BL, Seeman E, Hodgson SF, Taves DR, O'Fallon WM. Effect of the fluoride/calcium regimen on vertebral fracture occurrence in postmenopausal osteoporosis. N Eng J Med 1982;306:446–50.
6. Dambacher MA, Ittner J, Ruegsegger P. Long-term fluoride therapy of postmenopausal osteoporosis. Bone 1986;7:199–205.
7. Hansson T, Roos B. The effect of fluoride and calcium on spinal bone mineral content: a controlled, prospective (3 years) study. Calcif Tissue Int 1987; 40:315–7.
8. Briancon D, Meunier PJ. Treatment of osteoporosis with fluoride, calcium and vitamin D. Orthop Clinic North Am 1981;12:629–48.
9. Lane JM, Healey JH, Schwartz E, Vigorita VJ, Schneider R, Einhorn TA, et al. Treatment of osteoporosis with sodium fluoride and calcium: effects on vertebral fracture incidence and bone histomorphometry. Orthop Clin North Am 1984;15:729–45.
10. Eriksen EF, Mosekilde L, Melsen F. Effect of sodium fluoride, calcium, phosphate, and vitamin D_2 on trabecular bone balance and remodeling in osteoporotics. Bone 1985;6:381–9.
11. Harrison JE, Bayley TA, Josse RG, Murray TM, Sturtridge W, Williams C, et al. The relationship between fluoride effects on bone histology and on bone mass in patients with postmenopausal osteoporosis. Bone Miner 1986;1:231–33.
12. Farley JR, Wergedal JE, Baylink DJ. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone-forming cells. Science 1982;222:330–1.
13. Riggs BL, Hodgson SF, O'Fallon WM, Chao EYS, Wahner HW, Muhs JM, et al. The effect of fluoride treatment on vertebral fracture rate in osteoporotic women. N Engl J Med 1990;322:802–9.
14. Riggs BL, O'Fallon WM, Lane A, Hodgson SF, Wahner HW, Muhs J, et al. Clinical trial of fluoride therapy in postmenopausal osteoporotic women: extended observations and additional analysis. J Bone Miner Res 1994;9:265–75.
15. Nagent de Deuxchaisnes C, Devogelaer JP, Stein F. Fluoride treatment for osteoporosis. Lancet 1990;336:49–50.
16. Gruber HE, Stauffer ME, Thompson ER, Baylink DJ. Diagnosis of bone disease by core biopsies. Semin Hematol 1981;18:258–78.
17. Evans RA, Dunstan CR, Baylink DJ. Histochemical identification of osteoclasts in undecalcified sections of human bone. Miner Electrolyte Metab 1979;2:179–85.
18. Gruber HE, Ivey JL, Baylink DJ. Long-term calcitonin therapy in postmenopausal osteoporosis. Metabolism 1984;33:295–303.
19. Parfitt AM, Drezner ML, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 1987;2:595–610.
20. Fry BW, Taves DR. Serum fluoride analysis with the fluoride electrode. J Lab Clin Med 1970;75:1020–5.
21. Charen J, Taves DR, Stamm JW, Parkins FM. Bone fluoride concentrations associated with fluoridated drinking water. Calcif Tiss Int 1979;27:95–9.
22. Altman DG. Practical statistics for medical research. New York: Chapman & Hall, 1991:211.
23. Podenphant J, Riis BJ, Johansen JS, Leth A., Christiansen C. Iliac crest biopsy in longitudinal therapeutic trials of osteoporosis. Bone Miner 1988;5:77–87.
24. Kleerekoper M, Balena R, Foldes J, Shih M-S, Rao D, Parfitt AM. Histomorphometric changes in iliac bone induced by sodium fluoride (NAF) therapy depend on cumulative dose. J Bone Miner Res 1990;5(Suppl 2):S140.
25. Budden FH, Bayley TA, Harrison JE, Josse RG, Murray TM, Sturtridge WS, et al. The effect of fluoride on bone histology in postmenopausal osteoporosis depends on adequate fluoride absorption and retention. J Bone Miner Res 1988;3:127–32.
26. Hodsman AB, Droost DJ. The response of vertebral bone mineral density during the treatment of osteoporosis with sodium fluoride. J Clin Endocrinol Metab 1989;69:932–8.
27. Boivin G, Chapuy M-C, Baud CA, Meunier PJ. Fluoride content in human iliac bone: results in controls, patients with fluorosis, and osteoporotic patients treated with fluoride. J Bone Miner Res 1988;3:497–502.
28. Boivin G, Duriez J, Chapuy M-C, Flautre B, Hardouin P, Meunier PJ. Relationship between bone fluoride content and histological evidence of calcification defects in osteoporotic women treated long term with sodium fluoride. Osteoporosis Int 1993;3:204–8.
29. Meunier PJ, Briancon D, Chavassieux P, et al. Treatment with fluoride: bone histomorphometric findings. In: Christiansen C, Johansen JS, Riis BJ, editors. Osteoporosis. Viborg: Norhave A/S, 1987;824–8.
30. Glimcher MJ. The nature of the mineral component of bone and the mechanism of calcification. In: Avioli LV, Krane SM, editors. Metabolic bone disease and clinically related disorders. Philadelphia: WB Saunders, 1990:42–68.
31. Harrison JE, Hitchman AJ, Hitchman A, Hasany SA. Fluoride inhibition of bone mineralization may depend on physical/chemical properties. Bone Miner 1988;4:37–47.
32. Harrison JE, Hitchman AJW, Hasany SA, Hitchman A. Tam CS. The effect of fluoride on nephrocalcinosis in rats. Clin Biochem 1983;18:109–13.
33. Malluche HH, Faugere MC. Atlas of mineralized bone histology. Basel: Karger, 1986:60–3, 70–87.
34. Teotia SPS, Teotia M. Secondary hyperparathyroidism in patients with endemic skeletal fluorosis. BMJ 1973;1:637–40.
35. Srivastava RN, Gill DS, Moudgil A, Menon RJ, Thomas M, Dandona P. Normal ionized calcium, parathyroid hypersecretion, and elevated osteocalcin in a family with fluorosis. Metabolism 1989;38:120–4.
36. Yates C, Doty S, Talmadge RV. Effects of sodium fluoride on calcium homeostasis. Proc Soc Expv Biol Med 1964;115:1103–9.
37. Faccini JM. Inhibition of bone resportion in the rabbit by fluoride. Nature 1967;214:1269–71.
38. Ream LJ, Principato R. Fluoride stimulation of the rat parathyroid gland: an ultrastructural study. Am J Anat 1981;162:233–41.
39. Rosenquist JB, Lorentzen PR, Boquist LLV. Effect of fluoride on parathyroid of normal and calcium deficient rats. Calcif Tissue Int 1983;35:533–7.
40. Andersen L, Richards A, Care AD, Kerzel Andersen HM, Kragstrup J, Fejerskov O. Parathyroid glands, calcium, and vitamin D in experimental fluorosis in pigs. Calcif Tissue Int 1986;38:222–6.
41. Kleerekoper M, Peterson E, Nelson DA, Phillips E, Schorck MA, Tilley BC, Parfitt AM. A randomized trial of sodium fluoride as a treatment for post-menopausal osteoporosis. Osteoporosis Int 1991;1:155–62.
42. Kleerekoper M, Mendlovic DB. Sodium fluoride therapy of postmenopausal osteoporosis. Endocr Rev 1993;14:312–23.
43. Grynpas MD. Fluoride effects on bone crystals. J Bone Miner Res 1990;5(Suppl 1):S169–75.
44. Wolinsky I, Simkin A, Guggenheim K. Effects of fluoride on metabolism and mechanical properties of rat bone. Am J Physiol 1972;223:46–50.
45. Rich C, Feist E. The action of fluoride on bone. In: Vischer VL, editor. Fluoride in medicine. Bern: Hans Huber, 1970:70–87.
46. Wolinsky I, Guggenheim DY. The effect of fluoride on the mechanical strength of bone. Baroda J Nutr 1976;3:177–88.
47. Riggins RS, Rucker RC, Chan MM, Zeman F, Beljan JR. The effect of fluoride supplementation on the strength of osteopenic bone. Clin Orthop 1976;114:352–7.
48. Parfitt AM. Osteomalacia and related disorders. In: Avioli LV, Krane SM, editors. Metabolic bone disease and clinically related disorders, 2nd ed. Philadelphia: WB Saunders, 1990:329–96.
49. Dure-Smith BA, Farley SM, Linkhart SG, Baylink DJ. Fluoride treated patients become calcium deficient despite calcium supplements: correction with 1,25 vitamin D_3. In: Christiansen C, Riis B, editors. Proceedings of the Fourth International Symposium on Osteoporosis 1993. Aalborg, Denmark: Handelstrykkeriet Aalborg ApS, 1993:146–7.

Appendix

Table A1. Histomorphometric indices for bone biopsies obtained before and after placebo therapy

	Baseline	6 months	Difference	% change	*n*	Baseline	18 months	Difference	% change	*n*
BV/TV	14.69	16.88	2.19	16.47	11	17.32	16.64	−0.68	4.78	8
(%)	(1.30)	(2.38)	(1.99)	(15.22)		(1.16)	(3.26)	(3.73)	(29.27)	
MBV/TV	14.32	16.70	2.38	18.27	11	16.94	16.50	−0.44	6.73	8
(%)	(1.29)	(2.38)	(1.99)	(15.63)		(1.14)	(3.26)	(3.74)	(30.30)	
Tb.Th	100.17	118.96	17.79	23.92	11	104.74	103.13	−1.61	1.32	8
(μm)	(7.28)	(9.24)	(11.90)	(11.94)		(7.83)	(9.88)	(11.33)	(10.66)	
OV/TV	0.37	0.18	0.19[c]	−48.23	11	0.38	0.14	−0.24[a]	−59.63	8
(%)	(0.04)	(0.02)	(0.04)	(5.97)		(0.06)	(0.04)	(0.06)	(9.92)	
OV/BV	2.61	1.32	−1.28[b]	−49.00	11	2.22	1.02	−1.20	−37.20	8
(%)	(0.27)	(0.28)	(0.30)	(8.66)		(0.33)	(0.25)	(0.46)	(21.65)	
O.th	8.13	7.13	−1.00	−11.88	11	8.30	6.44	−1.85	−20.90	8
(μm)	(0.48)	(0.74)	(0.72)	(8.46)		(0.74)	(0.84)	(0.67)	(7.80)	
OS/BS	15.96	10.06	5.91[c]	−34.41	11	13.80	8.30	−5.51	−17.27	8
(%)	(1.79)	(1.23)	(1.42)	(6.40)		(1.80)	(1.94)	(3.18)	(34.68)	
MS/BS	9.77	5.75	−3.68	−23.70	10	9.34	5.37	−3.97	−8.99	8
(%)	(1.91)	(1.17)	(1.37)	(16.62)		(2.43)	(1.50)	(2.78)	(31.05)	
DL/BS	3.12	1.61	−1.37	15.92	10	4.77	1.38	−3.38	−40.44	8
(%)	(0.85)	(0.40)	(0.74)	(44.65)		(1.33)	(0.56)	(1.35)	(33.46)	
MS/OS	62.71	61.95	3.24	26.79	10	65.34	98.43	33.09	94.19	8
(%)	(10.59)	(13.52)	(13.28)	(30.32)		(13.12)	(41.56)	(44.00)	(101.27)	
N.Oc/BS	16.22	12.62	−3.60	98.57	11	18.25	12.04	−6.21	2.71	8
/100 mm	(3.19)	(2.59)	(3.91)	(119.76)		(4.14)	(2.86)	(4.09)	(33.22)	
Nu/Oc	2.09	2.64	0.56	29.50	11	2.37	1.90	−0.40	−5.90	7
(%)	(0.16)	(0.36)	(0.36)	(14.92)		(0.39)	(0.18)	(0.34)	(11.21)	
Ilt	31.91	33.00	1.09	5.64	11	20.38	32.25	11.88[a]	70.17	8
(days)	(1.19)	(0.00)	(1.19)	(5.94)		(2.46)	(0.62)	(2.31)	(14.29)	
MAR	0.47	0.54	0.02	−5.39	9	0.65	0.34	−0.31[a]	−48.60	8
(μm/day)	(0.09)	(0.08)	(0.12)	(18.79)		(0.04)	(0.08)	(0.07)	(12.43)	
Aj.AR	0.17	0.25	0.10	34.29	9	0.32	0.24	−0.08	−14.48	8
(μm/day)	(0.04)	(0.08)	(0.10)	(46.08)		(0.07)	(0.09)	(0.10)	(39.29)	
Omt	17.73	13.38	−3.10	−7.76	8	13.03	15.91	1.89	17.57	6
(days)	(2.25)	(1.53)	(2.75)	(16.42)		(1.21)	(1.89)	(2.39)	(19.09)	
Mlt	38.73	32.00	−6.81	−7.49	8	27.98	22.79	−4.67	−6.82	6
(days)	(7.48)	(8.78)	(10.93)	(21.10)		(7.20)	(7.86)	(4.42)	(21.23)	
BF/BS	15.59	11.65	−3.60	−16.54	9	22.11	9.25	−12.85[b]	−48.53	8
($\mu m^3/\mu m^2$/day)	(4.41)	(2.86)	(4.70)	(30.78)		(5.88)	(3.71)	(6.10)	(21.80)	
BFR/BV	32.33	19.77	−12.18	−39.44	9	40.07	17.30	−22.77[d]	−52.40	8
(%/year)	(7.98)	(5.08)	(8.76)	(18.49)		(8.44)	(8.58)	(9.33)	(20.94)	
BFR/TV	4.06	3.18	−0.48	12.74	9	7.08	2.84	−3.52[d]	−61.10	8
(%/year)	(0.89)	(0.78)	(0.76)	(38.45)		(1.68)	(0.97)	(1.29)	(12.99)	

Values are the mean (SEM).
Compared with baseline: [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.005$; [d]$p<0.001$.

Table A1 continued

Baseline	30 months	Difference	% change	*n*	Baseline	48 months	Difference	% change	*n*
15.60	22.39	6.79[a]	46.55	7	19.81	20.82	1.01	10.61	9
(1.25)	(2.14)	(2.07)	(15.24)		(1.62)	(2.78)	(2.79)	(15.33)	
15.34	22.07	6.73	46.84	7	19.57	20.69	1.12	11.05	9
(1.26)	(2.14)	(2.03)	(15.14)		(1.58)	(2.78)	(2.76)	(15.26)	
114.96	137.43	22.47[a]	23.51	7	116.34	135.49	19.15	15.65	9
(11.01)	(10.41)	(10.72)	(11.16)		(11.34)	(22.25)	(15.45)	(10.53)	
0.26	0.33	0.06	69.02	7	0.24	0.12	−0.12	23.17	9
(0.07)	(0.08)	(0.08)	(41.90)		(0.06)	(0.02)	(0.07)	(55.55)	
1.78	1.55	−0.23	17.51	7	1.13	0.61	−0.51[a]	−5.53	9
(0.52)	(0.45)	(0.41)	(33.96)		(0.27)	(0.11)	(0.30)	(33.14)	
7.15	7.91	0.76	20.91	7	8.17	6.81	−1.36	−3.50	8
(1.06)	(1.04)	(1.05)	(17.38)		(1.07)	(0.73)	(1.26)	(17.47)	
13.68	12.15	−1.53	29.35	7	8.79	5.65	−3.14	21.14	9
(3.64)	(2.06)	(3.80)	(40.94)		(2.30)	(1.00)	(2.17)	(44.86)	
7.49	5.67	−1.82	37.75	7	7.20	3.92	−3.07[a]	−31.19	8
(1.70)	(1.33)	(2.69)	(72.39)		(1.64)	(1.05)	(1.07)	(18.29)	
3.15	1.67	−1.48	46.93	7	2.31	0.69	−1.58	−73.85	8
(1.01)	(0.45)	(1.29)	(81.46)		(0.81)	(0.26)	(0.64)	(12.64)	
67.39	52.44	−14.94	12.32	7	76.70	67.94	−6.71	3.70	8
(11.82)	(10.65)	(14.99)	(42.36)		(10.08)	(8.73)	(13.49)	(20.78)	
22.67	20.58	−2.08	96.63	7	23.20	15.78	−7.42	1.78	8
(5.28)	(3.46)	(5.22)	(101.69)		(6.87)	(2.49)	(5.53)	(22.70)	
2.69	1.98	−0.71	−23.37	7	1.43	1.35	−0.04	−1.02	8
(0.41)	(0.23)	(0.23)	(4.60)		(0.05)	(0.06)	(0.08)	(6.26)	
26.14	33.00	6.86	40.36	7	25.22	31.33	6.11	34.64	9
(3.24)	(0.00)	(3.24)	(19.02)		(2.67)	(1.80)	(2.72)	(15.80)	
0.67	0.52	−0.15[a]	−16.09	7	0.43	0.42	−0.00	−17.28	6
(0.10)	(0.04)	(0.10)	(9.90)		(0.10)	(0.05)	(0.09)	(10.54)	
0.29	0.17	−0.12	−9.84	7	0.23	0.17	−0.05	−29.96	6
(0.05)	(0.03)	(0.07)	(34.92)		(0.06)	(0.03)	(0.07)	(20.47)	
12.14	15.01	2.87	70.71	7	15.07	17.00	1.63	27.14	6
(2.37)	(1.13)	(2.47)	(46.76)		(1.85)	(1.80)	(4.02)	(35.64)	
21.89	41.60	19.71	175.53	7	20.34	42.64	30.45	208.39	6
(5.41)	(13.97)	(15.84)	(98.46)		(3.37)	(20.33)	(31.99)	(187.08)	
18.32	10.16	−8.16	18.44	7	14.04	6.43	−7.14	−59.84	6
(4.31)	(1.67)	(5.35)	(66.86)		(3.95)	(1.78)	(2.97)	(8.87)	
32.33	15.60	−16.73	9.75	7	21.31	8.98	−11.91[a]	−65.58	6
(7.34)	(3.28)	(9.50)	(67.52)		(5.41)	(1.88)	(4.61)	(5.99)	
4.78	3.36	−1.15	36.38	7	4.59	1.85	−1.94	−69.51	6
(0.94)	(0.62)	(1.00)	(77.72)		(1.13)	(0.38)	(0.73)	(8.06)	

Table A2. Histomorphometric indices for bone biopsies obtained before and after fluoride therapy

	Baseline	6 months	Difference	% change	*n*	Baseline	18 months	Difference	% change	*n*
BV/TV	17.59	18.18	0.59	−1.46	9	14.59	20.99	6.49[bg]	32.07	12
(%)	(2.15)	(2.61)	(1.51)	(18.04)		(1.36)	(2.19)	(2.09)	(25.19)	
MBV/TV	17.27	17.30	0.03	−4.20	9	14.18	19.99	5.81[be]	29.16	12
(%)	(2.11)	(2.55)	(1.44)	(17.48)		(1.31)	(2.13)	(2.05)	(25.19)	
Tb.Th	121.52	120.93	−0.60	−3.74	9	94.24	143.30	49.06[cg]	30.57	12
(μm)	(14.57)	(11.86)	(10.07)	(12.38)		(5.09)	(11.46)	(11.61)	(18.59)	
OV/TV	0.32	0.88	0.56[ah]	885.25	9	0.41	1.00	0.59[a]	192.54	12
(%)	(0.06)	(0.16)	(0.16)	(728.71)		(0.08)	(0.17)	(0.20)	(58.63)	
OV/BV	1.74	5.07	3.33[ah]	608.30	9	2.72	5.11	2.38	167.17	12
(%)	(0.23)	(0.88)	(0.90)	(350.16)		(0.39)	(1.00)	(1.22)	(57.42)	
O.th	7.54	11.97	4.43[e]	92.68	9	8.61	10.94	2.34[e]	39.97	12
(μm)	(0.81)	(2.12)	(2.24)	(66.46)		(0.64)	(0.85)	(0.80)	(15.06)	
OS/BS	13.89	27.15	13.27[h]	149.16	9	14.11	31.25	17.14[ae]	110.10	12
(%)	(1.41)	(4.31)	(4.97)	(57.02)		(1.63)	(4.49)	(5.18)	(34.17)	
MS/BS	8.35	4.84	−3.51[e]	130.19	8	5.50	5.64	−0.09	44.64	11
(%)	(1.90)	(0.73)	(1.84)	(148.25)		(0.89)	(1.22)	(1.58)	(54.96)	
DL/BS	2.88	1.29	−1.59[c]	−36.21	7	1.98	1.47	−0.61	−37.36	9
(%)	(0.69)	(0.34)	(0.52)	(16.04)		(0.49)	(0.39)	(0.83)	(24.93)	
MS/OS	58.85	19.67	−39.19[bf]	19.64	8	40.77	26.21	−16.09	−7.75	11
(%)	(9.26)	(2.76)	(7.45)	(65.76)		(4.70)	(10.47)	(13.92)	(33.40)	
N.Oc/BS	17.23	13.22	−4.01	297.72	8	15.82	15.96	0.15[a]	60.03	12
(/100 mm)	(4.00)	(3.08)	(5.82)	(330.81)		(3.97)	(3.43)	(5.16)	(39.93)	
Nu/Oc	2.73	2.40	−0.33	56.73	8	2.30	2.04	−0.25	19.15	12
	(0.38)	(0.20)	(0.43)	(29.14)		(0.31)	(0.22)	(0.47)	(16.53)	
Ilt	33.00	32.44	−1.33	−0.26	9	18.33	33.08	14.33[ae]	62.34	11
(days)	(0.00)	(0.29)	(0.94)	(1.11)		(1.33)	(0.08)	(1.34)	(13.33)	
MAR	0.46	0.49	0.03	2.00	7	0.55	0.50	−0.06[a]	−27.19	8
(μm/day)	(0.10)	(0.09)	(0.09)	(24.01)		(0.09)	(0.07)	(0.12)	(14.15)	
Aj.AR	0.22	0.07	−0.15[a]	60.91	7	0.17	0.11	−0.07[a]	−24.20	8
(μm/day)	(0.06)	(0.02)	(0.05)	(137.37)		(0.03)	(0.05)	(0.08)	(43.52)	
Omt	19.45	22.95	9.15[e]	57.66	7	14.07	21.93	9.47[a]	85.60	7
(days)	(5.33)	(6.30)	(6.24)	(37.46)		(1.60)	(3.35)	(3.57)	(27.48)	
Mlt	50.65	194.91	169.84[ah]	385.87	7	41.63	280.46	293.52[ae]	712.24	7
(days)	(21.01)	(112.75)	(115.20)	(151.85)		(8.87)	(149.73)	(182.05)	(248.12)	
BF/BS	15.46	10.13	−5.32	6.65	7	12.27	12.08	−0.89	2.88	8
($\mu m^3/\mu m^2$/day)	(4.73)	(2.51)	(4.48)	(61.25)		(3.00)	(3.03)	(5.05)	(57.33)	
BFR/BV	23.04	16.73	−6.31	−1.05	7	25.54	17.81	−9.16	−17.54	8
(%/year)	(6.59)	(3.95)	(6.80)	(36.43)		(5.37)	(4.02)	(7.73)	(30.90)	
BFR/TV	4.38	3.04	−1.05	16.99	7	3.93	3.38	−0.84	−4.71	8
(%/year)	(1.40)	(0.77)	(0.86)	(41.96)		(1.06)	(0.77)	(1.14)	(51.04)	

Values are the mean (SEM).
Compared with baseline: [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.005$; [d]$p<0.001$.
Compared with placebo treatments at the same time after starting treatment: [e]$p<0.05$; [f]$p<0.01$; [g]$p<0.005$; [h]$p<0.001$.

Table A2 continued

Baseline	30 months	Difference	% change	*n*	Baseline	48 months	Difference	% change	*n*
16.22	22.62	6.40	48.04	14	22.45	23.73	1.27	64.92	8
(1.36)	(2.41)	(3.05)	(21.21)		(3.96)	(3.66)	(4.04)	(35.61)	
15.91	21.30	5.39	42.28	14	22.22	21.83	−0.38	58.62	8
(1.33)	(2.33)	(2.94)	(20.99)		(3.97)	(3.47)	(3.99)	(35.35)	
113.35	147.31	33.96	32.97	14	149.98	152.60	2.63	34.67	8
(7.21)	(9.62)	(14.01)	(13.01)		(17.19)	(17.57)	(21.38)	(25.49)	
0.31	1.32	1.01[ce]	821.47	14	0.24	1.89	1.66[a]	815.91	8
(0.05)	(0.16)	(0.16)	(217.50)		(0.05)	(0.35)	(0.33)	(536.90)	
1.88	6.23	4.34[ce]	594.89	14	1.30	8.47	7.17[ah]	402.88	8
(0.30)	(0.70)	(0.59)	(215.16)		(0.36)	(1.24)	(1.13)	(193.80)	
6.58	9.64	3.06[c]	60.61	14	6.92	9.91	2.99[h]	36.01	8
(0.59)	(0.75)	(0.80)	(23.12)		(0.87)	(0.76)	(0.96)	(28.15)	
15.31	46.06	30.75[ce]	360.42	14	11.48	60.24	48.76[ah]	302.55	8
(2.01)	(4.54)	(4.54)	(100.74)		(1.94)	(4.84)	(3.96)	(122.62)	
6.45	3.27	−3.19[c]	51.69	12	6.98	5.30	−2.83	31.38	7
(0.99)	(0.57)	(0.98)	(66.43)		(1.95)	(1.68)	(3.49)	(55.42)	
2.70	1.44	−1.12[b]	55.00	10	2.36	0.64	−2.05	−32.32	7
(0.64)	(0.43)	(0.84)	(109.56)		(0.71)	(0.38)	(0.94)	(56.88)	
41.15	8.66	−34.32[c]	−74.49	12	62.52	9.34	−58.70[af]	−23.38	7
(5.84)	(2.25)	(6.30)	(7.69)		(11.38)	(3.06)	(14.46)	(46.66)	
15.97	12.81	−3.16	102.27	14	21.08	18.06	−3.02	360.66	8
(3.83)	(3.08)	(3.76)	(108.59)		(4.58)	(4.13)	(7.04)	(312.15)	
2.10	1.82	−0.30	−5.78	12	1.39	1.62	0.24	−27.46	8
(0.24)	(0.20)	(0.31)	(21.10)		(0.09)	(0.13)	(0.19)	(12.06)	
21.43	32.43	11.64[c]	44.52	14	27.38	32.38	5.13	10.23	7
(1.91)	(0.57)	(1.96)	(13.85)		(2.98)	(0.56)	(3.11)	(12.92)	
0.59	0.48	−0.16[a]	38.24	10	0.52	0.17	−0.39[a]	−60.98	7
(0.09)	(0.06)	(0.14)	(73.50)		(0.10)	(0.08)	(0.11)	(26.37)	
0.19	0.03	−0.17[e]	−63.43	10	0.25	0.01	−0.26[bf]	−79.37	7
(0.04)	(0.00)	(0.04)	(22.55)		(0.06)	(0.00)	(0.06)	(18.98)	
9.48	19.61	11.89[be]	84.55	9	12.50	22.51	14.57	94.23	3
(1.12)	(1.62)	(1.89)	(27.95)		(1.84)	(2.04)	(4.94)	(53.79)	
23.16	427.25	460.70[ce]	2092.30	9	22.25	478.22	618.20[ah]	1312.93	3
(2.69)	(113.88)	(132.85)	(655.88)		(6.80)	(308.99)	(457.48)	(845.10)	
14.92	6.04	−9.14[a]	50.19	10	13.83	3.73	−12.97	12.73	7
(3.09)	(1.41)	(4.07)	(85.26)		(3.49)	(2.51)	(4.61)	(104.35)	
24.70	8.22	−17.24[a]	−2.28	10	17.82	6.04	−16.56	8.74	7
(4.71)	(1.97)	(6.08)	(49.06)		(5.16)	(4.42)	(6.73)	(102.28)	
4.17	1.81	−1.90[a]	42.45	10	4.40	1.01	−2.85	5.85	7
(0.89)	(0.41)	(0.94)	(75.73)		(1.26)	(0.65)	(1.03)	(95.92)	

Received for publication 22 August 1994
Accepted in revised form 17 October 1994