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Original Article

A Randomized Trial of Sodium Fluoride (60 mg) ± Estrogen in Postmenopausal Osteoporotic Vertebral Fractures: Increased Vertebral Fractures and Peripheral Bone Loss with Sodium Fluoride; Concurrent Estrogen Prevents Peripheral Loss, But Not Vertebral Fractures

D. H. Gutteridge[1,5], G. O. Stewart[2], R. L. Prince[1,5], R. I. Price[7], R. W. Retallack[1], S. S. Dhaliwal[1], B. G. A. Stuckey[1], P. Drury[3], C. E. Jones[1], D. L. Faulkner[1], G. N. Kent[4], C. I. Bhagat[4], G. C. Nicholson[2,5,*] and K. Jamrozik[6,†]

Departments of Endocrinology, [1]Sir Charles Gairdner Hospital (SCGH) and [2]Fremantle Hospital; [3]Department of Radiology, SCGH; [4]The Western Australian Centre for Pathology and Medical Research; [5]Department of Medicine and [6]Department of Public Health, University of Western Australia; and [7]Medical Technology & Physics, SCGH, Australia

Abstract. Postmenopausal Caucasian women aged less than 80 years (n = 99) with one or more atraumatic vertebral fracture and no hip fractures, were treated by *cyclical* administration of *enteric coated* sodium fluoride (NaF) or no NaF for 27 months, with precautions to prevent excessive stimulation of bone turnover. In the first study 65 women, unexposed to estrogen (–E study), age 70.8 ± 0.8 years (mean ± SEM) were all treated with calcium (Ca) 1.0–1.2 g daily and ergocalciferol (D) 0.25 mg per 25 kg once weekly and were randomly assigned to cyclical NaF (6 months on, 3 months off, initial dose 60 mg/day; group F CaD, n = 34) or no NaF (group CaD, n = 31). In the second study 34 patients, age 65.5 ± 1.2 years, on hormone replacement therapy (E) at baseline, had this standardized, and were all treated with Ca and D and similarly randomized (FE CaD, n = 17; E CaD, n = 17) (+E study). The patients were stratified according to E status and subsequently assigned randomly to ± NaF. Seventy-five patients completed the trial. Both groups treated with NaF showed an increase in lumbar spinal density (by DXA) above baseline by 27 months: FE CaD + 16.2% and F CaD +9.3% (both p = 0.0001). In neither group CaD nor E CaD did lumbar spinal density increase. Peripheral bone loss occurred at most sites in the F CaD group at 27 months: tibia/fibula shaft –7.3% (p = 0.005); femoral shaft –7.1% (p = 0.004); distal forearm –4.0% (p = 0.004); total hip –4.1% (p = 0.003); and femoral neck –3.5% (p = 0.006). No significant loss occurred in group FE CaD. Differences between the two NaF groups were greatest at the total hip at 27 months but were not significant [$p < 0.05$; in view of the multiple bone mineral density (BMD) sites, an alpha of 0.01 was employed to denote significance in BMD changes throughout this paper]. Using Cox's proportional hazards model, in the –E study there were significantly more patients with first fresh vertebral fractures in those treated with NaF than in those not so treated (RR = 24.2, p = 0.008, 95% CI 2.3–255). Patients developing first fresh fractures in the first 9 months were markedly different between groups: –23% of F CaD, 0 of CaD, 29% of FE CaD and 0 of E CaD. The incidence of incomplete (stress) fractures was similar in the two NaF-treated groups. Complete nonvertebral fractures did not occur in the two +E groups; there were no differences between groups F CaD and CaD. Baseline BMD (spine and femoral neck) was related to incident vertebral fractures in the control groups (no NaF), but not in the two NaF groups. Our results and a literature review indicate that fluoride salts, if used, should be at low dosage, with pretreatment and co-treatment with a bone resorption inhibitor.

*Present address: Department of Medicine, Geelong Hospital, Geelong, VIC, Australia.
†Present address: Department of Primary Health Care, Imperial College, London, UK.
Correspondence and offprint requests to: D. H. Gutteridge, Department of Endocrinology, Sir Charles Gairdner Hospital, Nedlands, WA 6009, Australia. Fax: 61-8-9346 3221.

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Introduction

Postmenopausal osteoporosis (PM-OP) is the major cause of atraumatic vertebral fractures worldwide. The commonest treatments (calcium, estrogen, bisphosphonates, calcitonin) are chiefly antiresorptive and are capable of either maintaining bone mass or increasing it moderately [1]. Thus great interest has been focused on stimulators of bone formation (fluoride salts, parathyroid hormone) which are capable of increasing vertebral bone mass in a continuous fashion.

Three randomized controlled studies of patients with vertebral fractures due to PM-OP, using sodium fluoride (NaF), have reached divergent conclusions concerning its efficacy in preventing further vertebral fractures. In two studies [2,3] using plain NaF 75 mg daily, plus calcium, there was no difference in vertebral fracture rates compared with controls treated with calcium; gastrointestinal (GI) intolerance and lower limb stress fractures were problems in the groups receiving NaF. In the third study, cyclic administration of slow-release NaF 50 mg daily, given simultaneously with calcium and combined with estrogen in 29% of patients, produced a significant reduction in vertebral fractures, with no difference in GI symptoms between the active and control (calcium-only) groups, and no stress fractures [4].

The present study used an intermediate dose of NaF (60 mg), as enteric-coated tablets. Cyclic administration was used to prevent prolonged stimulation of bone turnover and an associated risk of hip fractures [5]. Like the above three studies, all patients received concurrent calcium 1–1.2 g/day, but unlike them, all patients also received a vitamin D supplement. (Unlike the US, in Australia milk and bread are not routinely fortified with vitamin D). In view of the potential for NaF to increase bone resorption concurrently with formation in some patients with stress fractures [6], a subgroup of patients, on treatment with estrogen at baseline, was also investigated to assess the role of simultaneous antiresorptive treatment in the acquisition of bone mass and prevention of fractures. Bone density studies included a novel site, the lower tibia/fibula (a common site of stress fractures). The primary end-point of this study was the proportion of patients sustaining a first fresh vertebral fracture.

There are at least two possible mechanisms by which fluoride increases vertebral bone mass. The first is redistribution of bone from other sites – suggested by radial cortical bone loss [2] and increased cortical porosity in post-treatment biopsies in two studies [7,8]. The second is a fluoride-induced increase in absorption of calcium by the gut. To attempt to answer the latter possibility, which has not been considered previously, we measured oral stable strontium absorption (an indirect measure of calcium absorption) at baseline and after 6 months of treatment.

Materials and Methods

Patients

All 99 patients were postmenopausal Caucasian women under 80 years with at least one vertebral fracture. Exclusion criteria included past hip fracture, renal impairment (plasma creatinine >130 μmol/l), prior treatment with NaF (>5 mg/day), past treatment with oral corticosteroids, anti-epileptics, or high-dose vitamin D (over 1000 IU/day for >1 month in the past year), and other medications or diseases associated with altered calcium metabolism. Patients on calcium supplements were not excluded but the information was used in adaptive assignment (see below). Years since menopause (YSM) was tabulated, wherever obtainable. This was based on the date of the last menstrual period or, in premenopausally hysterectomized patients, the year of onset of hot flushes. The Perth, West Australian water supply has been fluoridated for almost 50 years (F^- content 0.80–0.89 p.p.m.).

Treatment

All patients were treated with calcium (Ca) 1.0–1.2 g daily [either Sandocal (calcium galactogluconate, Novartis) 1 g nocte or 0.5 g b.d.; or Caltrate (calcium carbonate, Whitehall) 1.2 g nocte or 0.6 g b.d.]; and with ergocalciferol (D) 0.25 mg per 25 kg body weight once weekly. Patients were randomly allocated to cyclical fluoride (F) treatment, or no fluoride (open design), using adaptive assignment by computer program [9] with three relevant criteria: (a) previous calcium treatment (yes = 1 g/day or more for the last 6 months or longer; no = otherwise); (b) number of vertebral fractures (1–2; 3–5; or greater than 5) and (c) age (<65 years; or >65 years).

Patients currently on oral estrogen ± progestogen treatment had this continued, but standardized, in all patients regardless of hysterectomy status, to piperazine estrone sulfate (Ogen, Upjohn) 0.625 mg daily continuously and medroxyprogesterone acetate (Provera, Upjohn) 2.5 mg daily continuously, increasing to 5 mg daily if bleeding became a problem.

Patients on estrogens (E) were similarly randomly assigned to two groups. Thus the four groups were:

F CaD	fluoride, Ca, vitamin D
CaD	Ca, vitamin D
FE CaD	fluoride, estrogen, Ca and vitamin D
E CaD	estrogen, Ca and vitamin D

There was no significant difference in the average duration of estrogen before starting NaF: 25 ± 7 months (mean ± SEM) in group E CaD (65% $\geqslant$6 months, 23%

between 1 and 6 months, and 12% <1 month) and 11 ± 3 months in group FE CaD (41% $\geqslant 6$ months, 47% between 1 and 6 months, and 12% <1 month).

Fluoride treatment utilized enteric-coated tablets of NaF. Tablets purchased from the Protea Co., each containing 20 mg NaF, were crushed to a powder, repunched into new tablets, then enteric-coated. The new enteric-coated tablets underwent dissolution/disintegration testing in line with BP standards, and were assayed for F^- content. The code of good manufacturing practice was followed with respect to batch-size testing. All patients were treated initially with 20 mg t.d.s. (with meals), and treatment followed a cycle of 6 months on and 3 months off. Calcium and ergocalciferol were continued throughout. Patients were seen at 3-monthly intervals and, when on NaF treatment, fluoride dosage was adjusted downwards (to 40 or 50 mg daily) if bone formation indices [plasma total alkaline phosphatase (ALP) or serum osteocalcin (OC)] showed an increase $>$ 150% above baseline; and adjusted upwards (to 70 or 80 mg daily) if there was no ALP or OC response. The mean NaF dose in group F CaD cycle 1 was 59 ± 1 mg/day (mean ± SEM); cycle 2, 54 ± 3 mg; cycle 3, 49 ± 3 mg, and in group FE CaD cycle 1, 59 ± 2 mg; cycle 2, 63 ± 6 mg; cycle 3, 53 ± 2 mg. None of these differences was significant.

This study was designed with a power of 80% to detect a 40% reduction in the vertebral fracture rate as a result of using cyclical NaF treatment at the 5% level of significance. These power calculations were based on data from a preliminary study comparing 14 patients treated with cyclical NaF and continuous calcium + vitamin D with 15 patients treated with continuous calcium + vitamin D over 2 years.

At entry, 99 patients were randomized (Table 1). Withdrawals (17), deaths (4) and physician advice (3) resulted in the removal of 24 patients from the trial. Seventeen patients (18%) withdrew from the study before completion (7 in CaD, 6 in F CaD, 1 in E CaD and 3 in FE CaD). Four withdrew due to the inconvenience of visits, 4 due to illness unrelated to osteoporosis, 5 due to side-effects of medication and 4 due to other reasons (unrelated to trial or health). Of the 5 who withdrew due to side-effects of medication, 1 was due to side-effects of hormone replacement therapy (HRT) (vaginal bleeding), 1 was due to calcium (indigestion), and 3 were due to side-effects of NaF (nausea, shin pains). Four deaths occurred while on treatment; none was related to osteoporosis or treatment (1 due to myocardial infarction, 1 due to a stroke, 1 following eye surgery and 1 due to lung cancer). Three patients were removed from the trial. One was removed at 4 months (group F CaD) due to a stress fracture of the femoral neck. Another patient (group CaD) was removed at 20 months (during cycle 3) because of multiple vertebral fractures and was started on treatment with HRT. One further patient (group F CaD) was removed from the trial at 18 months after multiple peripheral stress fractures and was also started on treatment with HRT. Seventy-five patients completed the trial.

Bone Densitometry

Bone mineral density (BMD) of the lumbar spine (L1–L4), left proximal femur at standard sites [10], two femoral shaft sites in the proximal femur, and distal lower limb was measured at baseline and every 9 months using a Hologic QDR 1000 dual energy X-ray absorptiometer (Hologic, Waltham, MA).

Femoral Shaft Sites. BMD of the proximal shaft of the femur was measured at a site 20 mm in length starting immediately distal to the lower margin of the lesser trochanter (proximal shaft of femur) and at a site 10 mm in length immediately distal to this region (shaft of femur). These measurements were made using the Hologic QDR 1000 software 'spinal analysis of hip data' on the hip image taken for the usual hip analysis.

Tibia/Fibula Sites. The lower left tibia and fibula (tib/fib) were scanned (anteroposteriorly) with the patient's foot positioned in a customized frame, and analyzed using the Hologic forearm software. The tib/fib was measured at three sites: the *ultradistal site* was 20 mm in length, the distal margin being 6 mm proximal to the talocrural joint space; the *mid* tib/fib site was 44 mm in length, immediately proximal to the ultradistal site; and the *shaft* site was 20 mm in length, immediately proximal to the mid tib/fib site. The *total* measurements included all three above sites. BMD was calculated at each visit from bone mineral content (BMC) and *baseline* area to allow for differences in positioning between visits (likewise for forearm measurements and for precision studies at each site: see below).

Forearm Sites. The nondominant forearm (or, if previously fractured, the dominant forearm) was measured by ^{125}I single photon absorptiometry (SPA) using a Molsgaard BMA 1100 bone mineral analyzer. Three sites were measured: ultradistal (60% trabecular), distal (84% cortical) and shaft (95% cortical), as previously described [11]. Duplicate measurements were performed at baseline and 27 months, with a single measurement at 18 months.

Measurement Precision. The precision of the femoral shaft sites was established on 10 18-year-old female volunteers scanned twice on the same day on the Hologic QDR1000. The coefficient of variation (CV) for BMD was 1.1% (1.6% for BMC) [10]. At the ultradistal lower limb, studies in 8 healthy subjects gave a CV for BMD of 0.5% [12] using the Hologic QDR1000. The ultradistal forearm CV (using SPA) in the same 8 subjects was 1.6% for BMD [12].

Biochemistry

Fasting blood and second morning urine samples were taken 3-monthly for biochemical assessment. Included were the bone markers ALP and OC [10] (CV for the OC assay is 22% at 6.5 μg/ml and 16.5% at 16 μg/ml), serum

intact parathyroid hormone (iPTH) [10], serum 25-hydroxyvitamin D (25OHD) [13], plasma total calcium, albumin and phosphate, and urinary calcium, phosphate, creatinine (Cr) and hydroxyproline (Hyp). Fasting urinary calcium excretion (CaE) was calculated by multiplying the urinary calcium/creatinine ratio by plasma creatinine, thus relating calcium excretion to glomerular filtration rate [14]. Hydroxyproline excretion (HypE) was calculated in a manner analogous to CaE and urinary Hyp/Cr was also calculated. Serum ionized calcium was measured on a Radiometer ICA1 analyzer and was corrected to a pH of 7.4. Serum fluoride levels were collected, as part of the fasting test, at 3-monthly intervals in all patients at least 12 h after the last fluoride dose, and analyzed using an ion-specific electrode .

Serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$, calcitriol) [13] and intestinal strontium absorption were measured at baseline and following 6 months of treatment to assess the effects of each treatment regimen on tests related to absorption of calcium by the gut. Vitamin D2 supplementation was monitored and varied downwards dependent on 25OHD and CaE. Intestinal calcium absorption was measured indirectly using a stable strontium absorption test, based on that described by Milsom et al. [15] using 2.5 mmol of strontium chloride hexahydrate with orange juice and a standard breakfast [13]. Serum strontium concentration 4 h after the dose was measured by atomic absorption spectrophotometry [13].

Fractional strontium absorption was calculated as follows:

$$\text{Fractional Sr absorption} = \frac{\text{Serum Sr conc } (\mu\text{M}) \times 0.15 \times \text{body weight}}{\text{Sr dose (mmol)}}$$

Calcium Intake

Baseline dietary calcium intake was assessed on 67 patients using a self-administered food frequency questionnaire based on that designed by Angus et al. [16].

Bone Scan

^{99m}Tc-labelled bisphosphonate bone scans were performed in all patients at baseline to exclude Paget's disease, to help establish the timing of baseline vertebral fractures, and to compare with a later scan performed in some patients when stress fractures were suspected.

Radiology

At baseline, anteroposterior and lateral radiographs were taken of the thoracic and lumbosacral spine (with a constant focus-to-film distance of 110 cm). Lateral spinal radiographs were repeated at the end of each 9 month cycle. Quantitative morphometry on lateral spinal radiographs of 67 normal West Australian premenopausal women was used to establish reference data [17].

Morphometry (performed by one operator, D.L.F.) was restricted to baseline lateral radiographs. Vertical height at the anterior (Ha), middle (Hm) and posterior (Hp) locations of vertebrae T3–L5 were measured. Ratios of Ha/Hp, Hm/Hp and Hp/Hp above or Hp/Hp below were derived to define wedge, concavity or compression deformities. Baseline (prevalent) fracture was defined as a ratio more than 3 SD from the mean of the reference population [17], with the final decision (fracture/nonfracture) being made by an experienced radiologist (P.D.) with access to the morphometry data and in the presence of the morphometrist. Incident fractures were determined by the same radiologist, at the end of the trial, examining all radiographs serially (in conjunction with the morphometrist), masked to the treatment group, and utilizing needle-tipped calipers to determine height ratios (as for baseline studies) in cases of uncertainty.

Safety Variables

At each 3-monthly visit patients answered a questionnaire administered by the research nurse (C.E.J.) concerning tablet consumption, skeletal pain (back, limbs and joints), GI symptoms and other causes of discomfort. Lower limb or rib pain, if not present at baseline, and if persistent (>2 weeks), constant and localized, was assessed by radiography and usually by bone scan also. A diagnosis of incomplete (stress) fracture was made only if symptoms were confirmed by radiography and/or bone scan (the latter compared with baseline scans).

Height

Standing height was measured using a stadiometer with a right-angled block, taking the mean of three measurements. Height was measured at baseline and thereafter at 9-monthly intervals.

Statistical Analysis

The bone density, biochemistry and fracture data from each study (F CaD v CaD; FE CaD v E CaD) were analyzed separately to reflect the method for the random allocation of patients to fluoride treatment. The *bone density* data for each site were expressed as the percent change from baseline at each time point (9, 18 and 27 months). The data for each site were then analyzed using repeated measures ANOVA with baseline covariates age, YSM, height, weight and baseline BMD at the same site. Since the treatment groups were compared at multiple sites, to protect against type 1 error, only differences between treatment groups at $p<0.01$ were considered significant in the analysis of BMD data. The

biochemistry data were also analyzed using repeated measures ANOVA with baseline values as covariates.
The *vertebral fracture* data were analyzed using Cox's proportional hazards model (i.e., percent of patients free of first fresh fractures at each time point [18]) and the effect of treatment was assessed after adjustment for baseline covariates: baseline height, weight, age, YSM, number of vertebral fractures, and baseline BMD at lumbar spine, femoral neck and total hip. Confidence intervals (95%) were calculated for significant treatment effects.

Results

Baseline Characteristics (Table 1)

Age, YSM, weight, height, vertebral fractures, BMD at three sites, dietary Ca, and biochemistry in the four groups were examined. The only significant differences were in (a) age at entry, the CaD and F CaD groups being significantly older than the FE CaD group; and (b) Ca_E, the two +E groups differing from each other.

Standing Height

The total group showed a significant fall in height with time but in each treatment group there was no significant height loss from baseline, no significant differences between groups and no overall effect of NaF or estrogen on height. Overall, height loss in those with incident vertebral fractures [1.27 ± 0.37 (SEM) cm, $n = 23$] was significantly greater than in those with no such fractures (0.23 ± 0.21 cm, $n = 56$) ($p = 0.022$).

Bone Densitometry

Lumbar Spine (Fig 1A). At this site the effects of fluoride, with or without estrogen, were profound. There was an overall 9.3% increase from baseline in group F CaD and a 16.2% increase in group FE CaD at 27 months. Both changes were significant ($p = 0.0001$). The response of group F CaD was significantly greater than that of CaD ($p < 0.002$), as was the FE CaD/E CaD contrast ($p < 0.002$).

A markedly different picture was obtained at the other skeletal sites.

Femoral Neck (Fig. 1B). Significant bone loss was present at 9, 18 and 27 months only in the F CaD group (difference from baseline $p < 0.01$ at each time point, −3.5% at 27 months). Group FE CaD showed no significant bone loss.

Intertrochanteric. Significant loss of bone occurred in the F CaD group (mean change at 27 months, –4.4%) and again there was no significant change in the FE CaD group (+1.2%).

Table 1. Baseline characteristics of women in the four treatment groups

	Group			
	F CaD	CaD	FE CaD	E CaD
n (at entry)	34	31	17	17
n (at 9 months)	30	25	14	16
n (at 27 months)	24	22	14	15
Age at entry (years)	70.9 ± 5.7[a]	70.7 ± 6.8[a]	64.5 ± 7.7[b]	66.5 ± 6.2[a,b]
Years since menopause	24.6 ± 5.3	24.6 ± 8.6	19.3 ± 9.2	19.3 ± 8.5
Weight (kg)	60.2 ± 8.3	58.4 ± 13.5	60.7 ± 10.0	57.6 ± 10.0
Height (cm)	156.1 ± 7.1	155.2 ± 7.6	158.3 ± 6.5	157.6 ± 7.4
Vertebral fractures (no. per patient)	3.6 ± 2.5	4.3 ± 3.0	4.3 ± 3.4	4.6 ± 3.2
Lumbar BMD (mg/cm^2)	0.69 ± 0.11	0.69 ± 0.12	0.70 ± 0.11	0.69 ± 0.17
Total hip BMD (mg/cm^2)	0.70 ± 0.10	0.69 ± 0.11	0.71 ± 0.11	0.68 ± 0.13
Femoral neck BMD (mg/cm^2)	0.59 ± 0.07	0.58 ± 0.10	0.59 ± 0.10	0.57 ± 0.10
Dietary Ca, excluding supps. (mg)	696 ± 304	764 ± 368	848 ±300	624 ± 376
25OHD (nmol/l) [30–160]	87.8 ± 56.9	100.9 ± 71.7	84.0 ± 38.4	98.2 ± 46.6
1,25-$(OH)_2$D (pmol/l) [50–155]	131 ± 50	128 ± 55	107 ± 39	110 ± 46
Strontium absorption (% dose)	7.2 ± 3.4	7.3 ± 2.8	8.0 ± 3.8	8.3 ± 2.8
iPTH (pmol/l) [0.8–5.5]	3.02 ± 1.43	2.44 ± 0.99	2.54 ± 0.93	2.66 ± 1.02
Ca_E (μmol/LGF)	29.1 ± 17.7[a]	28.4 ± 18.1[a]	15.8 ± 10.6[b]	27.1 ± 17.2a
Ca/Cr (mol/mol) [0.10–0.58]	0.39 ± 0.24	0.38 ± 0.24	0.24 ± 0.19	0.35 ± 0.23
Total ALP (U/l) [35–135]	91.9 ± 24.0	100.5 ± 25.6	79.9 ± 21.8	78.2 ± 27.5
Hyp_E (μmol/l) [0.4–1.9P]	1.53 ± 0.53	1.63 ± 0.85	1.13 ± 0.34	1.34 ± 0.72
Hyp/Cr (mmol/mol) [6–27][†]	20.0 ± 4.8	21.1 ± 8.0	17.0 ± 6.1	16.7 ± 7.7

Values are mean ± SD.
Values in square brackets are the reference range. [†]Premenopausal reference range.
Means with different superscripts (a or b) are significantly different ($p < 0.05$). There were no other significant differences in baseline characteristics.

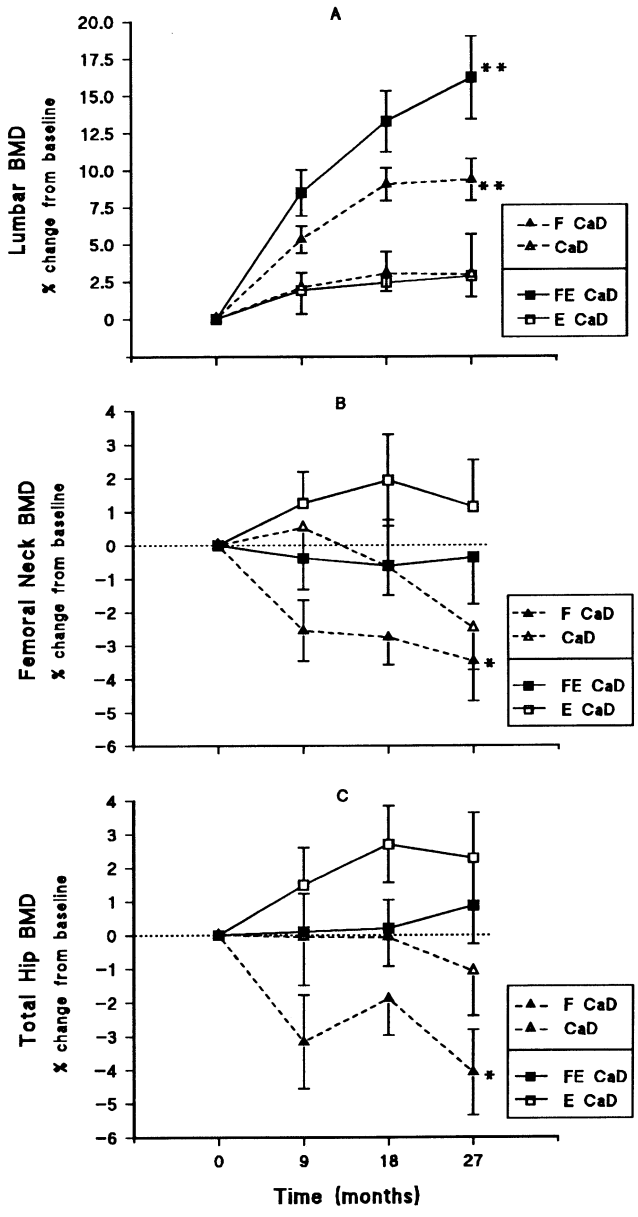

Fig. 1. Change in lumbar spine density (**A**), femoral neck density (**B**), and total hip density (**C**) over the 27 month study period [mean percentage change (± SEM) from baseline] in the –E study (*dashed lines*) and the +E study (*continuous lines*). **Significant change from baseline, $p = 0.001$. *Significant change from baseline, $p < 0.01$.

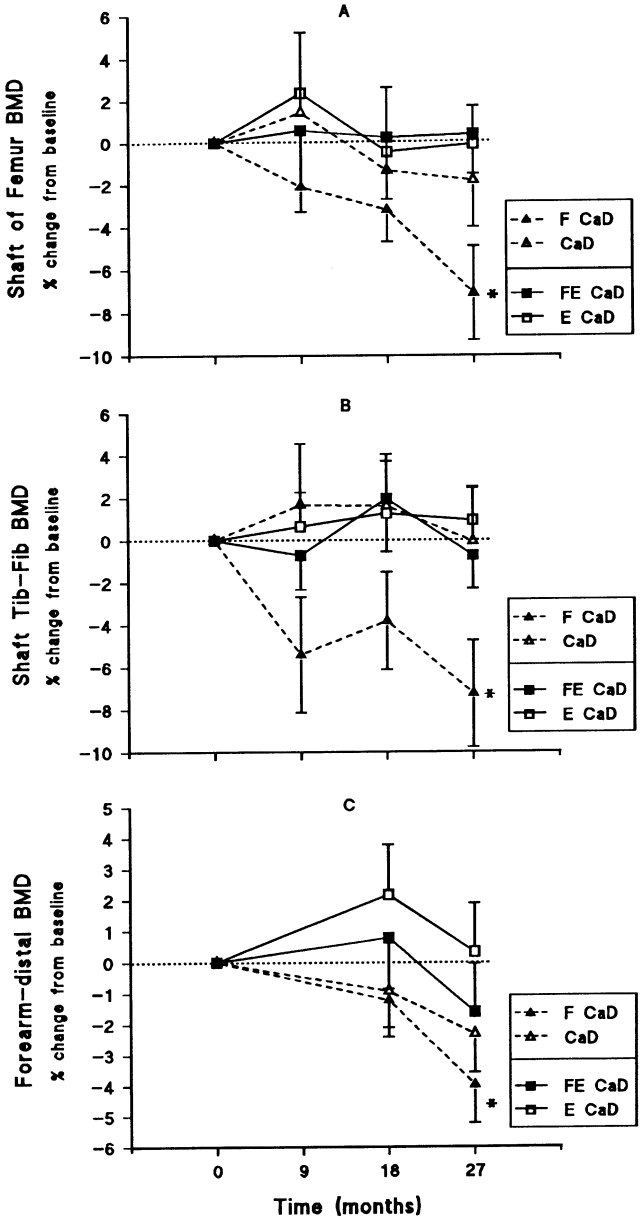

Fig. 2. Change in shaft of femur density (**A**), shaft tibia/fibula density (**B**) and distal forearm density (C) over the 27 month study period [mean percentage change (± SEM) from baseline] in the –E study (*dashed lines*) and the +E study (*continuous lines*). *Significant change from baseline, $p < 0.01$.

Total Hip (Fig. 1C). At this site the percentage changes mirrored the intertrochanteric percentage changes, with group F CaD showing significant loss at 27 months (−4.1%) but no loss in group FE CaD (+0.9%).

Shaft of Femur (Fig. 2A). There was significant bone loss (–7.1%) in group F CaD at 27 months ($p = 0.004$) with no loss in group FE CaD (+0.4%). At the *proximal shaft*, the 6.8% loss at 27 months in group F CaD was not significant ($p = 0.015$).

Tibia/Fibula Sites. At each site (ultradistal, mid, shaft and total) there was loss at 27 months in group F CaD. Mean losses (and significances) were: ultradistal, –9.7% ($p = 0.015$); mid, –9.3% (p = 0.02); shaft, –7.3% ($p = 0.005$) (Fig. 2B); and total, –8.9% ($p = 0.012$). Again there was no loss at any site or any time point in group FE CaD.

Forearm. Significant bone loss occurred in group F CaD only at the predominantly cortical distal site at 27 months (4.0%; $p = 0.004$; Fig. 2C). There was no significant change in any group at the other two sites.

Summary. There was a marked dissociation between the effects of NaF at the spine and at most limb sites. At the spine, significant percentage gain was restricted to groups F CaD and FE CaD. At most limb sites, significant bone loss occurred in group F CaD. The greatest percentage loss (7.3%) was at the tibia/fibula shaft ($p = 0.005$). The combination of NaF with estrogen (FE CaD) was not associated with significant bone loss at any site.

Is there any relationship between spinal BMD gain and hip BMD loss in group F CaD? There were no significant correlations (change in spine BMD from baseline versus change in BMD at each hip site from baseline, at 0–9 months and 0–27 months).

Fractures

Vertebral Fractures. Using Cox's proportional hazards model, with adjustment for covariates, on comparing group CaD with F CaD, a deleterious influence of NaF on fracture risk ratio was found: RR 24.2 ($p = 0.008$), 95% CI 2.3–255. Significant covariates were baseline spine density, height and weight (Fig. 3A). There was a marked difference in the data for the first 9 months, with 23.3% of group F CaD patients (7/30) and none of group CaD (0/27) developing fresh fractures.

On comparing groups FE CaD and E CaD (i.e., the two estrogen-treated groups) with adjustment for covariates (Fig. 3B), no significant difference was found ($p = 0.097$). Again there was a difference in the first 9 months, with 29% of group FE CaD (4/14) and none of group E CaD (0/16) developing first fresh fractures. The number of vertebral fractures per treatment cycle, and number per 100-patient years, are presented in Table 2.

Did the treatment in group FE CaD provide any amelioration in vertebral fracture rates over that in group F CaD? The trial was not designed to answer this question. However, the percent who fractured in the first cycle appear comparable (29% vs 23%) whereas group FE CaD appears to have had an advantage in the next 2 cycles (0 vs 23%). In cycle 1, multiple fractures were a feature of group F CaD (7 patients, 17 fractures, range 1–7 per patient), whereas in FE CaD the 4 patients who fractured had only one fracture each (Table 2).

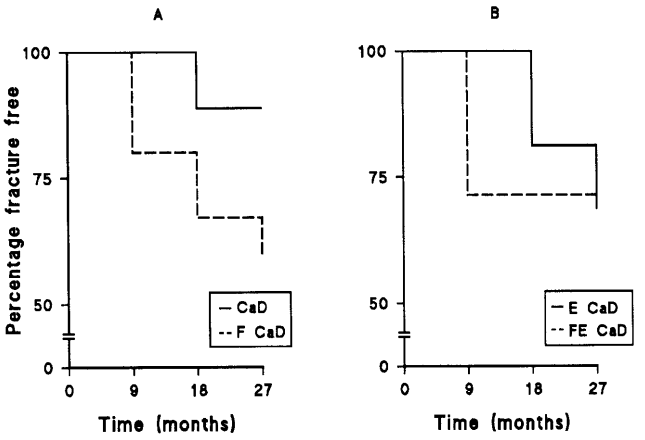

Fig. 3A,B. Percentage of women in the four treatment groups who did not develop new fractures during the 27month period. **A** F CaD vs CaD (RR = 24.2, 95% CI 2.3-255, $p = 0.008$). **B** FE CaD vs E CaD ($p = 0.097$, NS).

Nonvertebral Fractures: *Incomplete (Stress) Fractures.* These occurred only in patients treated with NaF:

(F CaD): 7 patients, 7 episodes, 11 sites.
(FE CaD): 3 patients, 4 episodes, 5 sites.

Most of the 11 stress fracture episodes occurred in the first two cycles: 4 in the first cycle, 6 in the second cycle and only 1 in the third cycle. The feet were the commonest location (9/16; calcaneus 4, talus 1, cuboid 1, metatarsal 1, diffuse 2); next the lower tibia (3/16, followed by ribs (2/16), femoral condyle (1/16) and femoral neck (1/16). Was fluoride dosage relevant? In 8 episodes, the current dosage was 60–80 mg; and only 3 episodes occurred on 48–52 mg. Repeat bone scans were performed once in each of the 10 patients at the time of clinical stress fracture (10/11 episodes) and were useful, in conjunction with radiology performed in 9 episodes (scan positive in 10/10; radiology positive in 5/9), in confirming the diagnosis and accurately localizing the site.

In each of the 11 episodes in 10 patients, the NaF was stopped for periods of at least a month (in 1 case with an

Table 2. Incident vertebral fractures in each treatment group during each 9 month period

	0–9 months				9–18 months				18–27 months				Overall	
Group	*n*	Frs.	Pts.	Rate	*n*	Frs.	Pts.	Rate	*n*	Frs.	Pts.	Rate	Pts.	Rate
F CaD	30	17α	7	75.6	26	4	4[a]	20.5	25	3	2	16	11	39.5
CaD	27	0	0	0	24	9β	3	50	22	2	1b	12.1	3	20.1
FE CaD	14	4	4	38.1	14	0	0	0	14	0	0	0	4	12.7
E CaD	16	0	0	0	16	6γ	4	50	15	3	2b	26.7	5	25.5

Frs., fractures; Pts., number of patients with fresh fractures in the time period; Rate, number of vertebral fractures per 100 patient-years.
[a]Two patients fractured in the previous cycle also.
[b]One patient fractured in the previous cycle also.
Patients (fractures per patient): α 3(1) 2(2) 1(3) 1(7); β 1(1) 1(3) 1(5); γ 3(1) 1(3).
Deteriorations in pre-existing or earlier fractures were noted but not included in any analyses.

early femoral neck stress fracture it was not restarted). In all other episodes (n = 10) the NaF was restarted 1–7 months later – in 7 of 10 episodes at a lower dose. In 2 of 11 episodes symptoms did not settle completely due to other pathology; in the remainder, symptomatic recovery was complete.

Nonvertebral Fractures: Complete Fractures. These occurred after minimal trauma (usually a fall from standing height). None occurred in estrogen-treated patients (FE CaD or E CaD). In group F CaD, 5 patients had 6 fractures: 2 neck of femur (requiring internal fixation), 1 Colles', 1 humerus, 1 ribs and 1 pubic ramus. In group CaD 6 patients had 6 fractures: 2 Colles', 1 humerus, 2 foot, 1 lateral malleolus and no femoral neck. There was no significant difference between these two groups ($\chi 2$).

One patient in group CaD developed unexplained aseptic necrosis of the left medial femoral condyle soon after entry with residual deformity and arthritis.

Biochemistry

Strontium Absorption Tests. The four treatment groups were not different in baseline fractional strontium absorption (Table 1). There was no significant change in strontium absorption from baseline, or group difference, after 6 months of treatment (Table 3).

There was no correlation between baseline serum $1,25(OH)_2D$ levels and baseline strontium absorption. Multiple regression analysis was performed with baseline fractional strontium absorption as the dependent variable, and age, YSM, body mass index (BMI), dietary calcium intake, baseline neck of femur BMD, trochanteric BMD, intertrochanteric BMD, total hip BMD, Ward's triangle BMD and total lumbar BMD as the independent variables. Baseline total lumbar BMD was the only significant correlate of baseline strontium absorption (r = 0.3. p = 0.03); i.e., the lower the baseline BMD, the lower the strontium absorption.

Vitamin D Metabolites. There were no significant differences between the groups at baseline in either 25OHD or $1,25(OH)_2D$ (Table 1). Three patients had 25OHD levels below the reference range (27, 21 and 17 nmol/l; only the last of these had an elevated iPTH level at baseline = 6.7 pmol/l); all three normalized their 25OHD on treatment.

There were no significant changes in $1,25(OH)_2D$ in any group between baseline and 6 months, and no differences between the groups at 6 months. In the combined groups (n = 69), the decline in $1,25(OH)_2D$ from baseline to 6 months was not significant (p = 0.053).

Serum Fluoride. The data in the four treatment groups are shown in Fig. 4. Mean fasting F^- levels, while on NaF treatment, in each cycle, in group F CaD were not associated with BMD loss at any hip site in each cycle, by simple regression.

Ca_E, Ca/Cr, PTH Data. Fluoride treatment did not have a significant effect on the levels of iPTH, Ca/Cr or Ca_E in either study (F CaD and CaD; FE CaD and E CaD).

Markers of Bone Turnover (Fig. 4). The average serum fluoride, ALP, OC and HypE data for each patient were determined by averaging each parameter over the four 'on-fluoride' times (3, 6, 12 and 15 months) in the two fluoride-treated groups (F CaD and FE CaD). There was no correlation between the average patient serum fluoride and either the average ALP or the average HypE. However, there was a significant correlation between average fluoride and average OC ($p < 0.05$).

Interactions: Bone Density, Bone Resorption, Vertebral Fractures

Association of Baseline Spine and Femoral Neck Density with Incident Vertebral Fractures in Patients Treated With and Without NaF. In view of the incidence of early

Table 3. Strontium absorption and vitamin D metabolities

	Group								
	F CaD	*n*	CaD	*n*	FE CaD	*n*	E CaD	*n*	Total *n*
Strontium Absorption (% dose)									
Baseline	7.2 ± 3.4	23	7.3 ± 2.8	22	8.0 ± 3.8	10	8.3 ± 2.8	15	70
6 months	7.1 ± 2.9		7.2 ± 2.3		7.4 ± 3.0		8.3 ± 2.6		
1.25(OH)$_2$D (pmol/l)									
Baseline	131 ± 50	23	128 ± 55	22	107 ± 39	10	110 ± 46	14	69
6 months	117 ± 43		120 ± 47		91 ± 39		99 ± 40		
25OHD (nmol/l)									
Baseline	85 ± 57	25	97 ± 68	23	90 ± 45	14	97 ± 45	16	78
6 months	137 ± 37		159 ± 74		121 ± 37		138 ± 42		

Values are mean ± SD

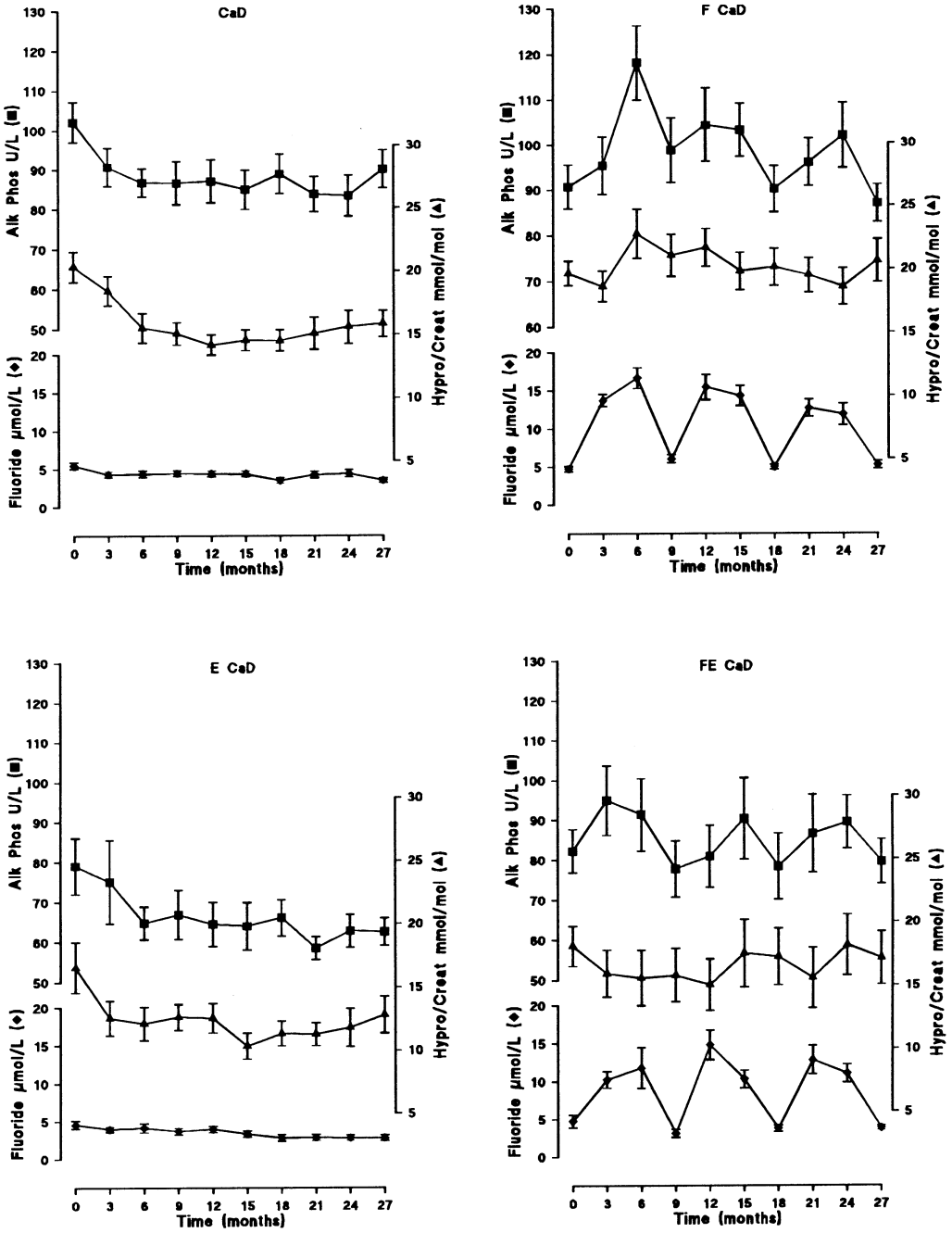

Fig. 4. Mean (± SEM) serum fluoride, plasma alkaline phosphatase and urinary hydroxyproline/creatinine levels over the 27 month study period. Patients in the two NaF groups were receiving NaF at 3, 6, 12, 15, 21 and 24 months.

vertebral fractures in the two NaF groups we questioned whether baseline BMD influenced incident vertebral fractures to the same degree in the NaF and non-NaF groups. In post-hoc analyses we found that baseline BMD at the spine and femoral neck appeared to have predictive value for incident vertebral fractures in the non-NaF groups but not in the two NaF groups. In the two non-NaF groups combined (n = 39), baseline femoral neck BMD and spine BMD were significantly lower in those 8 patients who developed incident vertebral fractures than in those who did not ($p < 0.001$) [femoral neck BMD: incident fracture patients, 0.49 ± 0.02 (SEM); intact, 0.61 ± 0.02 g/cm^2]. The mean baseline femoral neck BMD (but not spine BMD) in the 14 of 39 NaF patients with incident vertebral fractures (0.58 ± 0.02 g/cm^2) was significantly

higher than in the 8 of 39 above in the non-NaF groups ($p < 0.01$), but was not significantly different from the 25 of 39, on NaF, with no incident fractures (0.59 ± 0.02 g/cm^2).

Discussion

This randomized controlled trial was designed to maximize the potential benefits of NaF on bone, and minimize the side effects. The primary end-point was the proportion of patients sustaining a first fresh vertebral fracture. We used a lower initial dose (60 mg) than the 75 mg used in the two NIH trials [2,3], a calcium supplement to reduce bone resorption and limit impairment of mineralization, and cyclical administration to limit prolonged stimulation of bone formation and turnover. Finally we used a flexible dose to avoid excessive stimulation of bone turnover (by reducing dosage in response to excessive increases in ALP increases) and, less commonly, to reduce the likelihood of nonresponders (by increasing dosage when no ALP response occurred). We have reported [5] an association between NaF-induced ALP increase and spontaneous hip fracture. Enteric-coated tablets were used to minimize dyspepsia. Vitamin D (ergocalciferol) supplements were used to exclude vitamin D deficiency during treatment. Daily calcium intake (including supplements) in the two NaF groups was a mean of 1700–1900 mg (F CaD) and 1850–2050 mg (FE CaD) (from Table 1, plus a supplement of either 1000 or 1200 mg). Dure-Smith et al. [34] reported calcium deficiency, based on calcium infusion data, in good fluoride responders with a mean total calcium intake of 2000 mg/day. In view of the need to monitor NaF dosage 3-monthly, dependent on indices of bone formation, the study was not double-masked.

Vertebral fracture rates and peripheral bone density changes were surprising – and demonstrate that NaF administration is capable of *increasing* vertebral fracture rates and of increasing peripheral (nonspinal) bone loss. Thus our study demonstrates the potential for an anti-osteoporosis agent, under certain circumstances, to worsen a patient's clinical state. It is possible that the initially relatively high vitamin D dosage might have been a contributing factor to NaF-related peripheral bone loss. Evidence against this possibility includes: (a) the absence of significant loss in group CaD, (b) the lack of a significant change in serum $1,25(OH)_2D$ levels from baseline to 6 months, (c) the fact that regular biochemical assessment (3-monthly) resulted in vitamin D dosage reductions in 40% of patients.

Why Are There Early Fluoride-Associated Vertebral Fractures?

In the –E study the vertebral fracture RR (F/no F) was 24.2 (95% CI 2.3–255). In both NaF studies (i.e., F CaD/CaD and FE CaD/E CaD) there were no fresh fractures in the control groups in the first 9 months – the time when fractures in the NaF groups were maximal (11/44 patients). The initial NaF dosage in this study was 60 mg = 27 mg F. In a number of other studies in PM-OP [2,3,20,21] the vertebral fracture rates in the first 6–12 months were higher in the fluoride than the control group. In only one of these studies however [20], was the difference statistically significant. This was a non-randomized study comparing NaF 80 mg/day against no treatment, with no calcium supplements in either group. In the first of two NIH-funded studies [2] the NaF/placebo fracture rates per 100 person-years in the first year were 63.1/42.8, giving a RR of 1.47 (95% CI = 0.04–5.5). In the second NIH study [3] the rates in the first 6 months were 123.3/60.3 (RR 2.04) and in the first year 78.7/50.7 (RR 1.55), with neither being significant. A third randomized study compared two groups: NaF (50 mg/day) plus calcium (1 g/day) and vitamin D_2 (800 IU/day), with calcium + vitamin D supplementation alone [21]. In the first year, 24% of patients in the NaF group developed new vertebral fractures compared with 17% in the control group – a nonsignificant difference. In the second year the percentages were identical: 15.3% and 15%. A meta-analysis of the two NIH studies, in combination with the present study, using N100 methodology suggested a greater risk of fluoride – associated vertebral fractures in the first 9–12 months of treatment (RR 1.8, 95% CI 1.2–2.7).

The clustering of NaF-associated fractures in the first 9 months of our study and the absence of fractures in the two control groups at this time, suggested that low bone density was not the basis of all the NaF-associated fractures, and that other factors may have been responsible in some. We found that low mean baseline spine and femoral neck BMD was associated with incident vertebral fractures in the two control groups, but not in the two NaF-treated groups. The evidence suggests that NaF-induced BMD increases may be associated with increased fracture risk, particularly in the first 9 months of treatment, even with relatively high baseline BMD. Studies on bones of osteopenic rats and birds supplemented with fluoride [22] using torsional tests to failure have shown a significant loss of strength. Other workers [6] have noted increased bone resorption (in iliac biopsies) at the time of periarticular stress fractures during fluoride treatment.

Potential Benefits of HRT in Fluoride Treatment

Did pretreatment with HRT before NaF have any beneficial effect on incident vertebral fractures? The trial was not designed to answer this question, since those on HRT and those not so treated were randomized separately. However, on comparing the two studies (Table 2, Fig. 3), in each case there was a marked difference in incident vertebral fractures in the first 9 months, in favor of the control group. Thus, pretreatment and co-treatment with HRT did not appear to reduce the number of patients with incident fractures in group FE CaD in the first 9 months in comparison with group F

CaD. Thereafter, however, no more fractures occurred in the FE CaD group whereas in F CaD further fractures were noted at 18 and 27 months. There were more early multiple fractures in F CaD than FE CaD (see Results and Table 2). Thus there was a limited protective effect of HRT co-treatment in the first 9 months, but at later time points there appeared to be an advantage. Addition of estrogen resulted in prevention of complete non-vertebral fractures, prevention of vertebral fractures after the first 9 months, prevention of nonspinal bone loss, but no apparent reduction in incomplete (stress) fractures.

We found that the mean percentage change in spinal densitometry at 27 months in group FE CaD was 16.2% and in group F CaD was 9.3%. In a study of 100 healthy postmenopausal women [23], combined (estrogen + progestogen) continuous HRT combined with mono-fluorophosphate (MFP) (F^- = 20 mg/day) plus calcium produced a significantly greater lumbar BMD response per year at 2 years (11.8 ± 1.7%) than HRT plus calcium (4.0 ± 0.5%) ($p < 0.05$), but not MFP plus calcium (2.4 ± 0.6%) (mean ± SEM)

Effect of Fluoride (± Estrogen) on Cortical Bone

A surprising finding in our study was in the changes in BMD at nonspinal sites in patients in group F CaD – where significant bone loss occurred by 27 months at all nonspinal sites examined. The greatest loss occurred in the lower tibia/fibula, where the loss at the shaft site was 7.3%. The lower tibia/fibula is a common site of fluoride-related stress fractures and these BMD results help to explain the mechanism of this common complication of treatment with NaF. It is noteworthy that no significant loss at any site occurred in group FE CaD, suggesting a protective effect of HRT at nonspinal sites. One other study of changes in BMD using fluoride [24] in a daily dose of 26.4 mg F^-, as MFP (equivalent to 58 mg NaF) reported a nonsignificant loss of BMD at the femoral neck (–2%) and other hip sites. The authors found a significant inverse relationship between trough fluoride concentration and change in BMD at the femoral neck using DXA methodology, which was not found in the present study. Alexanderson et al. [23] found mild loss of bone at the total hip, femoral neck and forearm in the fluoride plus calcium group (not significantly different from placebo). At these sites, plus the total body and spine, the responses in the group receiving fluoride plus HRT were significantly better than for placebo, suggesting a preventive effect of HRT on the fluoride-associated negative effect on cortical bone sites. Significant radial shaft bone loss using NaF has been described in a number of studies [2,25]. Pak et al. [4] used slow-release NaF 50 mg, given cyclically (equivalent to 19.5 mg F^- daily) taken concomitantly with calcium citrate (an inhibitor of F^- absorption). No significant difference was found in radial shaft BMD between the active and calcium-only groups over 4 years. Concurrent estrogen was given to 29 of their total 99 patients, but the effect of estrogen on the BMD responses was not assessed.

At the distal radius and tibia NaF has been shown, using peripheral computed tomography, to be associated with cortical bone loss and cancellous bone gain [26]. Using an early method of hip BMD measurement (dual photon absorptiometry, DPA) a mild but significant gain in BMD at both the femoral neck and the intertrochanteric sites was found [2], compared with the calcium control group. Using a mixture of DPA and DXA methodologies Pak et al. [4] found a mild significant mean increase in femoral neck BMD (2.4 ± 3.3%/year) (± SD) over 4 years in the NaF group. In a 4 year study of low spinal BMD patients treated with MFP (F^- 20 mg) plus calcium, the BMD response at the total hip was not significantly different from a calcium control group [27].

The serial BMD changes in the present study are strongly suggestive of an anabolic action of fluoride at the spine (a chiefly trabecular site) with a catabolic action at many other sites, chiefly cortical. All other similar studies have shown spinal gain; those with cortical loss (forearm and hip) are documented above. Major spinal gain could theoretically only be achieved by an associated increase in gut calcium absorption (not found in this study) or by a transfer of bone from nonspinal, chiefly cortical sites. Two groups have found evidence of a fluoride-associated increase in cortical porosity [7,8]; this was not found in two other studies [28,29]. The capacity of estrogen pretreatment and co-treatment (with fluoride) in this study to prevent peripheral bone loss while possibly enhancing spinal gain without increasing gut calcium absorption is difficult to explain. Estrogen-associated renal calcium conservation is probably relevant, as is an association between NaF stimulated bone formation (at trabecular or axial sites) and estrogen-suppressed bone resorption (at peripheral, chiefly cortical sites). An early non-randomized study of a similar population [19] found a significant advantage of FECa (±D) over FCa, ECa or no treatment – in terms of incident vertebral fractures.

Is Fluoride Dosage Relevant to Vertebral Fracture Rates?

A recent similar study [30] of a PM-OP vertebral fracture population (n = 134) randomly compared 1 g Ca/day (group C) with two groups (A and B) treated with MFP and calcium for 3 years. In group A the average daily fluoride (F^-) dose was 11.2 mg/day given cyclically (3 months on and 1 month off) and in group B, 20 mg/day, given continuously. The best overall result was in group A, with the lowest N100 vertebral fracture rate and a lesser spine BMD response than group B, but with a significantly better response at the femoral neck and radial shaft than group B where bone loss occurred. By comparison our starting dose of NaF was 60 mg = 27.3 mg F^-, higher than either of their groups A or B.

Future Recommendations

The results of the present study plus the recent literature [23,30] suggest that if fluoride salts are to be used in treatment of PM-OP with vertebral fractures, certain precautions are necessary to prevent fluoride-associated peripheral bone loss and spinal and peripheral fractures. Firstly the average daily fluoride dose should be low – between 11.5 mg [30] and 20 mg [4,23,30]. The lower of these two dose ranges appears to offer the greater safety and efficacy. Secondly, treatment with fluoride should be used in combination with an antiresorptive agent [31], either estrogen as in this study and that of Alexandersen et al. [23], or a bisphosphonate [32]. To limit increased bone turnover associated with the initiation of fluoride treatment, pretreatment with an antiresorptive for some months, to fill the resorption space, seems desirable. Thirdly, fluoride should be avoided in patients with renal impairment due to the risk of fluoride-induced hip fractures [33], and used with caution, if at all, in those with hip fractures [5]. Finally, to avoid dyspepsia, either enteric-coated formulations (as in this study) or slow-release compounds [4] are recommended.

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