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Monofluorophosphate Combined with Hormone Replacement Therapy Induces a Synergistic Effect on Bone Mass by Dissociating Bone Formation and Resorption in Postmenopausal Women: A Randomized Study*

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ABSTRACT

Sodium fluoride stimulates bone formation and has been used to treat osteoporosis for decades despite debate about the antifracture efficacy. Hormone replacement therapy (HRT) results in only modest increases in bone mineral density (BMD). However, for women with low bone mass, the ideal therapy should not only inhibit bone resorption but simultaneously stimulate bone formation to increase bone mass above the fracture threshold. We thus performed a randomized, double-blind, placebo-controlled intervention study to prospectively investigate the effect of a low dose of fluoride, in combination with HRT, on BMD and biochemical markers of bone turnover.

One hundred healthy postmenopausal women (60–70 yr old) were thus randomly assigned to: 1) HRT [transdermal 17β-estradiol, releasing 50 μg/day; plus oral norethisterone acetate (NETA), 1 mg/day]; or 2) oral monofluorophosphate (MFP; equivalent to fluoride, 20 mg/day); or 3) HRT+MFP; or 4) placebo, for 96 weeks. All participants received a calcium supplement of 1000 mg/day. Sixty-eight women completed the study.

We found a pronounced, linear increase in spinal BMD during treatment with HRT+MFP [11.8% (1.7% SEM)], which was significantly greater than the increase in the HRT group [4.0% (0.5% per yr); $P < 0.05$]. MFP produced a smaller increase [2.4% (0.6% per yr)], whereas there was no change in the placebo group [0.0% (0.5% SEM)]. Similar changes were found at the other skeletal sites (distal forearm, hip, and total body). Markers of bone formation showed a fall in the HRT group, which was significantly more pronounced than in the combined HRT+MFP group. A nonsignificant increase was found in the MFP group, whereas the placebo group showed a decrease caused by calcium treatment. The marker of bone resorption decreased significantly more in the HRT and the HRT+MFP groups than in the placebo group but tended to increase in the MFP group.

In conclusion, this study shows, by use of biochemical markers of bone turnover, that bone resorption and formation may be dissociated, as a result of actions of two compounds with diverging effects on bone turnover. Furthermore, the synergistic effects of relatively low doses of the compounds suggested statistically and clinically significant increases in trabecular and probably also cortical bone. Adverse effects were relatively rare and mild. (*J Clin Endocrinol Metab* **84:** 3013–3020, 1999)

THE PAST few years have seen a positive development in the options for osteoporosis prevention and treatment. Hormone replacement therapy (HRT), calcitonin, and bisphosphonates are now approved in several countries. These compounds are all antiresorptive and thus primarily prevent further bone loss, although modest increases in bone mineral density (BMD) may occur. For the woman with a (serious) low bone mass, the ideal therapy should not only inhibit bone resorption but also stimulate bone formation, to increase bone mass continuously and by several percent per year. Sodium fluoride stimulates bone formation and has therefore been used to treat osteoporosis for decades (1–7) despite debate about the antifracture efficacy of this therapy (8, 9). The problems are related to the sodium fluoride dose and its pharmaceutical formulation. A dose that would markedly increase spinal BMD (75 mg/day, by a highly bioavailable preparation) has been associated with fluorosis and related adverse effects (4). On the other hand, a relatively low dose of fluoride (equivalent to 15–25 mg of fluoride ion per day), combined with an adequate calcium supplement, would be acceptable in terms of adverse advents, but the bone effects would be accordingly smaller (10, 11). In addition, the formulation of sodium fluoride (6) or the use of other fluoride salts with a better bioavailability and a suitable pharmacokinetic profile, such as monofluorophosphate (MFP) (12), may also be important.

In 1982, Riggs *et al.* (13) hypothesized that the combination of calcium, fluoride, and estrogen would be an effective treatment for osteoporosis, but the suggestion was never pursued. The scientific theory behind the success of such a combination is that it might dissociate bone formation and bone resorption, with a relative increase in formation and decrease in resorption as the outcome. The aim of the present study was to confirm this theory.

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Materials and Methods

Study design

One hundred healthy Danish postmenopausal women were enrolled in this prospective, placebo-controlled, and double-blind monocenter study. They were 60–70 yr old, had all passed a natural menopause, and

had a BMD of the distal third of the nondominant forearm of at least 1 SD below the premenopausal mean (T-score). To obtain this, 852 women were invited to information meetings about the study, and 236 came to a meeting. The women who met the inclusion criteria were assigned to one of the following double-blind (and double-dummy) treatments by means of randomization in blocks of 8 subjects: sealed sequential and identical boxes containing either combined continuous HRT [17β-estradiol (matrix patch), applied twice weekly and releasing 50 μg/day, continuously combined with oral NETA, 1 mg/day (n = 26)]; oral MFP [L-glutamine MFP, equivalent to a total of 20 mg/day fluoride (n = 25)]; combined HRT+MFP (n = 25); or placebo (n = 24). All participants were given an oral calcium supplement of 1000 mg/day. The drugs used were all supplied by Rotta Research Laboratorium, Monza, Italy. The participants were not taking any medication known to influence bone metabolism, and they had not taken any such medication for at least 1 yr before enrollment. No one had more than three vertebral crush fractures or had a history of femoral fracture at enrollment. None of the participants had a body mass index (BMI) 30% above the ideal weight or had renal insufficiency, hepatic failure, or malignancy, or smoked more than 10 cigarettes/day. All participants gave their written informed consent, and the study was approved by the Danish Health Authorities and Ethics Committee and was conducted in accordance with the guidelines proposed in The Declaration of Helsinki and with good clinical practice.

The duration of the study was 96 weeks (approximately 2 yr). Bone mass measurements of the spine, the nondominant forearm, and the hip, and samples of blood and urine, were taken at baseline (at enrollment) and every 6 months throughout the study period. Bone mass measurement of the total body was determined annually. Blood and urine (as the second void) samples were collected in the morning, always after an overnight fast and tobacco abstinence. Mammography was performed before enrollment and after 2 yr. Gynecological examination (including cervical smear) was offered at baseline, at the end of the study, and if indicated during the study.

Bone density

BMD of the distal third of the nondominant forearm was determined by single x-ray absorptiometry using DTX-100 (Osteometer Meditech A/S, Hoersholm, Denmark), whereas BMD of the lumbar spine (vertebrae L2-L4, including intervertebral disks, postero-anterior projection), the left hip, and the total body was measured by dual-x-ray absorptiometry using QDR-2000 (Hologic, Inc., Waltham, MA). The long-term *in vivo* imprecision of the single-x-ray absorptiometry is 1.0% (14) and of the dual-x-ray absorptiometry, 1.0% (15).

Spinal fracture

Lateral thoracolumbar x-rays were taken at baseline and at the end of the study, under standardized conditions, with a fixed film-focus distance, and they were evaluated blindly for fractures by the same technician. Each participant's x-rays were displayed simultaneously in chronological order. Quantitative assessment applied for diagnosis of vertebral fractures required measurement of the anterior, middle, and posterior heights of each vertebra of the thoracolumbar spine using a ruler (to the nearest millimeter). A vertebral fracture was defined as more than 20% reduction in any of these heights, as defined by Genant (16).

Markers of bone formation

Serum osteocalcin was measured by a newly developed enzyme-linked immunosorbent assay (ELISA) method, detecting the N-terminal midfragment of the molecule (N-mid OC) (Osteometer Biotech A/S, Herlev, Denmark) (17). This fragment has been demonstrated to be more stable in serum than total serum osteocalcin; and the interassay and intraassay variations are, respectively, 6.5% and 6% (17). Serum bone-specific alkaline phosphatase (B-AP) was determined by immunoradiometric assay (Tandem-R, Ostase, Hybritech, San Diego, CA) with interassay and intraassay variations of 7–8% and 4–7%, respectively (18). Specimens were stored at −20 C immediately after sampling, and all measurements were performed using the same batch of assay reagent for each individual.

Marker of bone resorption

Urinary CrossLaps (CrossLaps, Osteometer Biotech A/S), determined by ELISA adjusted for urinary creatinine (CrossLaps/Cr), has been shown to be a sensitive and specific marker of bone resorption, because it measures a C-terminal telopeptide (8 amino acids) of the α-1 chain of type 1 collagen (19). Urine samples were stored at −20 C immediately after sampling, and all measurements were performed with the same batch of assay for each individual. The interassay and intraassay variations of CrossLaps are, respectively, 6.6% and 5.3% (19).

Serum lipids and lipoproteins

Total serum cholesterol (TC), high-density lipoprotein (HDL) cholesterol (HDL-C), and serum triglycerides (TG) were measured enzymatically by the Cobas Mira Plus (Roche Diagnostic Systems, Inc., F. Hoffmann-La Roche, Basel, Switzerland), whereas the cholesterol content of the HDL was measured after precipitation with phosphotungstate-magnesium chloride of apolipoprotein B-containing lipoproteins (20). Low-density lipoprotein cholesterol (LDL-C) was then calculated according to the formula of Friedewald *et al.* (21): LDL-C = TC − HDL-C − (0.45 × TG), requiring a TG concentration below 4.5 mmol/L .

Statistical analysis

All statistical analyses were performed with the Statistical Analysis System (SAS) using a level of significance of 5% (22). Baseline comparisons (age, years since menopause, BMI, bone mass measurements, biochemical markers of bone turnover, and serum lipids) between the groups were made by two-way ANOVA. The change in BMD (in percent; termed αBMD) was calculated by linear regression analysis for each woman from a total of five (forearm, spine, and hip) or three (total body) bone measurements. If a statistical significance was revealed by ANOVA, comparisons with placebo were done using Dunnett's test, whereas further *post hoc* comparisons were done by Scheffe's test. The response to treatment in the biochemical markers was calculated as the individual average change in percent during the study period. ANOVA was also applied to changes in BMD (αBMD), response to treatment of the biochemical markers of bone turnover, and lipids. The biochemical markers of bone turnover and lipoproteins were logarithmically transformed before analysis, to obtain homogeneity and normality of the data. Based on an expected postmenopausal decrease in BMD_{spine} of about 4% over 2 yr, and 1 SD of about 6% for the BMD_{spine}, with 100 subjects, the power of the study was thus about 91% at a 5% significance level, dropping to 80% with 68 subjects (23).

Results

The baseline characteristics of the four treatment groups are shown in Table 1. According to the World Health Organization operational definition, 52% of subjects of the whole population were osteopenic ($-2.5 \leq$ T-score of the forearm < -1), whereas 48% were osteoporotic (T-score < -2.5), equally distributed in the four groups. The baseline characteristics of the initial population were similar to those of the subjects who completed the 96 weeks of treatment (Table 1).

BMD

Treatment with HRT+MFP resulted in a pronounced and almost linear increase in spinal BMD [mean (SEM); 11.8% (1.7% per yr)], which was statistically significantly greater than the increase in the HRT group [4.0% (0.5% per yr)] ($P < 0.05$). Treatment with MFP alone induced a smaller increase [2.4% (0.6% per yr)] in spinal BMD, whereas there was no change over time in the placebo group [0.0% (0.5% per yr)] (Fig. 1). A similar pattern was found in the forearm, (Fig. 2), although less pronounced than in the spine, with a change

TABLE 1. Baseline values

	HRT (n = 26,17)[a]	MFP (n = 25,17)	HRT + MFP (n = 25,15)	Placebo (n = 24,19)
Age (yr)[b]	65.1 ± 2.2	64.2 ± 2.8	66.0 ± 2.5	65.4 ± 2.1
	64.9 ± 2.1	64.7 ± 3.1	66.6 ± 2.4	65.4 ± 1.9
Menopause (yr)[b]	50.0 ± 3.1	50.0 ± 3.7	49.7 ± 4.6	49.3 ± 4.4
	50.2 ± 2.8	49.1 ± 5.3	50.3 ± 4.2	50.4 ± 3.8
BMI (kg/m^2)[b]	25.7 ± 4.0	25.3 ± 4.3	25.3 ± 3.7	25.0 ± 3.5
	25.8 ± 3.9	25.8 ± 3.8	25.5 ± 4.9	25.0 ± 3.4
BMD_{spine} (g/cm^2)[b]	0.92 ± 0.14	0.92 ± 0.17	0.91 ± 0.14	0.89 ± 0.15
	0.93 ± 0.15	0.95 ± 0.16	0.85 ± 0.13	0.90 ± 0.15
T-score (spine)	−1.14 ± 1.31	−1.13 ± 1.51	−1.27 ± 1.31	−1.43 ± 1.37
	−1.04 ± 1.33	−0.89 ± 1.43	−1.82 ± 1.14	−1.32 ± 1.34
BMD_{hip} (g/cm^2)[b]	0.79 ± 0.11	0.81 ± 0.15	0.74 ± 0.08	0.77 ± 0.13
	0.82 ± 0.12	0.84 ± 0.15	0.70 ± 0.08	0.79 ± 0.13
T-score (hip)	−1.54 ± 0.95	−1.40 ± 1.27	−1.94 ± 0.67	−1.74 ± 1.12
	−1.26 ± 0.97	−1.13 ± 1.28	−2.26 ± 0.63	−1.64 ± 1.14
BMD_{neck} (g/cm^2)[b]	0.68 ± 0.10	0.69 ± 0.14	0.63 ± 0.09	0.65 ± 0.12
	0.71 ± 0.11	0.72 ± 0.14	0.58 ± 0.07	0.66 ± 0.12
T-score (neck)	−2.05 ± 0.99	−1.99 ± 1.36	−2.56 ± 0.86	−2.41 ± 1.15
	−1.77 ± 1.05	−1.64 ± 1.40	−3.02 ± 0.71	−2.31 ± 1.19
$BMD_{whole\ body}$ (g/cm^2)[b]	0.98 ± 0.09	0.97 ± 0.10	0.93 ± 0.08	0.94 ± 0.10
	1.00 ± 0.10	0.98 ± 0.11	0.90 ± 0.08	0.95 ± 0.09
T-score (whole body)	−1.46 ± 0.92	−1.54 ± 1.06	−1.97 ± 0.86	−1.94 ± 1.01
	−1.25 ± 1.02	−1.47 ± 1.13	−2.25 ± 0.88	−1.78 ± 0.90
N-mid OC (ng/mL)[c]	28.4 (22.2–36.4)	31.7 (23.5–42.8)	29.1 (23.7–35.7)	29.7 (23.5–37.6)
	29.3 (23.7–36.3)	30.7 (22.2–42.4)	29.2 (23.4–36.3)	29.4 (23.8–36.3)
B-AP (ng/mL)[c]	8.7 (5.3–14.2)	9.2 (5.6–15.1)	10.1 (6.6–15.5)	9.8 (6.9–14.1)
	8.2 (5.2–12.9)	10.5 (7.1–15.4)	10.5 (6.9–15.9)	9.6 (6.9–13.4)
CrossLaps/Cr (mg/mol)[c]	224 (120–419)	282 (158–503)	222 (136–365)	255 (158–413)
	217 (118–401)	269 (145–500)	220 (131–371)	251 (162–387)
TC (mmol/L)[c]	6.51 (5.57–7.60)	7.03 (5.93–8.34)	6.62 (5.79–7.56)	7.19 (6.32–8.17)
	6.54 (5.54–7.73)	7.06 (5.96–8.36)	6.69 (5.97–7.51)	7.36 (6.50–8.32)
TG (mmol/L)[c]	1.11 (0.78–1.57)	1.10 (0.78–1.56)	1.08 (0.78–1.50)	1.15 (0.73–1.81)
	1.11 (0.80–1.54)	1.14 (0.77–1.67)	1.15 (0.83–1.60)	1.10 (0.68–1.79)
HDL-C (mmol/L)[c]	1.62 (1.28–2.05)	1.74 (1.29–2.35)	1.85 (1.36–2.50)	1.68 (1.36–2.07)
	1.63 (1.50–2.04)	1.73 (1.29–2.32)	1.79 (1.33–2.40)	1.72 (1.40–2.12)
LDL-C (mmol/L)[c]	4.29 (3.46–5.33)	4.69 (3.81–5.78)	4.15 (3.38–5.08)	4.91 (4.21–5.72)
	4.33 (3.41–5.49)	4.70 (3.81–5.79)	4.28 (3.67–4.98)	5.05 (4.37–5.83)

For each variable given, the first row represents data for all subjects, whereas the second line represents data for subjects completing the study only.

[a] First number in the bracket indicates the initial number of subjects, the last number indicates the number of subjects completing the study.

[b] Mean ± SD.

[c] Geometric mean and in parentheses, respectively mean − SD and mean + SD.

of +1.4% (0.6% per yr) in the HRT+MFP group, +1.2% (0.6% per yr) in the HRT group, −0.2% (0.4% per yr) in the MFP alone group, and −0.9% (0.3% per yr) in the placebo group (ANOVA, $P < 0.05$). Treatment with HRT+MFP produced the greatest increase in the annual BMD of the total hip [3.0% (0.7% per yr) *vs.* 0.1% (0.3% per yr) for placebo, $P < 0.05$], the femoral neck [3.4% (1.5% per yr) *vs.* −0.1% (0.3% per yr) for placebo, $P < 0.05$], and the total body [2.8% (0.4% per yr) *vs.* 0.3% (0.3% per yr) for placebo, $P < 0.05$] (Fig. 3).

Biochemical markers of bone turnover

The response to treatment in biochemical markers of bone turnover is illustrated in Fig. 4. For N-mid OC (bone formation) (Fig. 4, *top*), the fall in serum concentration was more pronounced in the HRT group [−32.6% (3.0% per yr)] than in the HRT+MFP group [−12.2% (4.7% per yr), $P < 0.05$]. For the MFP group, the serum concentration of N-mid OC tended to increase [3.4% (5.4% per yr)], although this increase was not statistically different from zero. The placebo group decreased by [−13.0% (2.9)%, $P < 0.05$]. Similar changes in the response to treatment were found for serum B-AP (Fig. 4, center). Urinary CrossLaps/Cr (bone resorption) decreased by −71.1% (6.1)% ($P < 0.05$ *vs.* placebo) in the HRT group and by −53.8% (6.2)% in the HRT+MFP group ($P = 0.05$ *vs.* placebo). In the placebo group, the decrease was −24.5% (5.5)% during the 2 yr. The urinary CrossLaps/Cr tended to increase in the MFP group [+15.7% (14.0)%], although this was not significantly different from zero (Fig. 4, *bottom*). For each of the treatment groups, there was no significant difference in results in bone density and those bone markers when calculated for women who completed the study or when calculated as intention-to-treat (data not shown).

Compliance and safety

Sixty-eight women completed the 96 weeks: 17 in the HRT, 17 in the MFP, 15 in the HRT+MFP, and 19 in the placebo group. Data on those completing the study were used for all analyses presented. Compliance was considered low if a participant had taken less than 70% of the trial medication. Five subjects completing the study had low compliance according to this definition. One subject (placebo) left the study because of leg pain, and 1 (HRT+MFP) because of endometrial bleeding; 2 subjects (MFP and placebo) because of er-

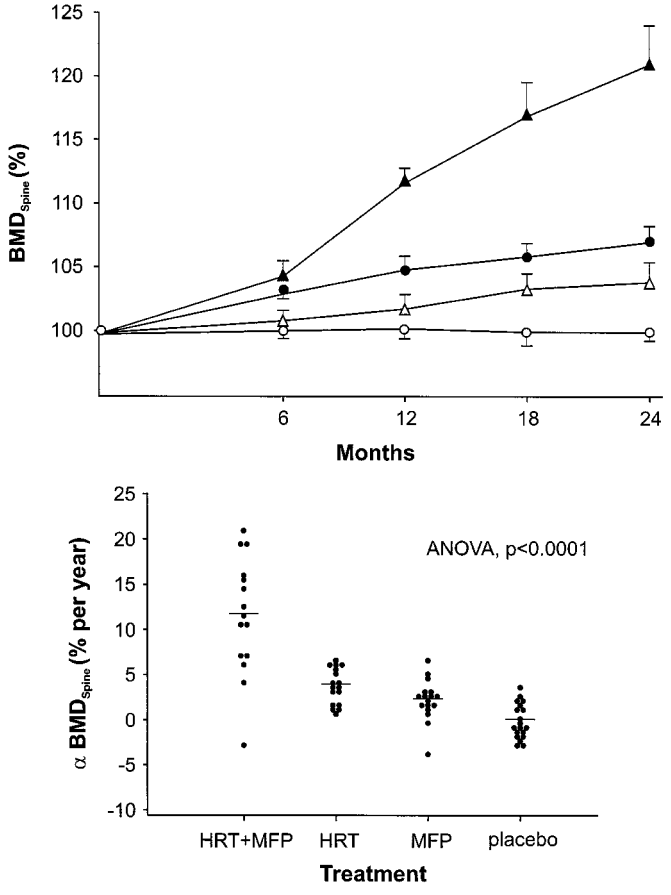

FIG. 1. The time-related changes in percent for BMD of the lumbar spine (*top*). Values are mean ± SEM. αBMD is also shown (*bottom*). *Horizontal lines* indicate the mean. ANOVA, $P < 0.0001$ applies for both sites. ▲, HRT+MFP; ●, HRT; △, MFP; ○, placebo.

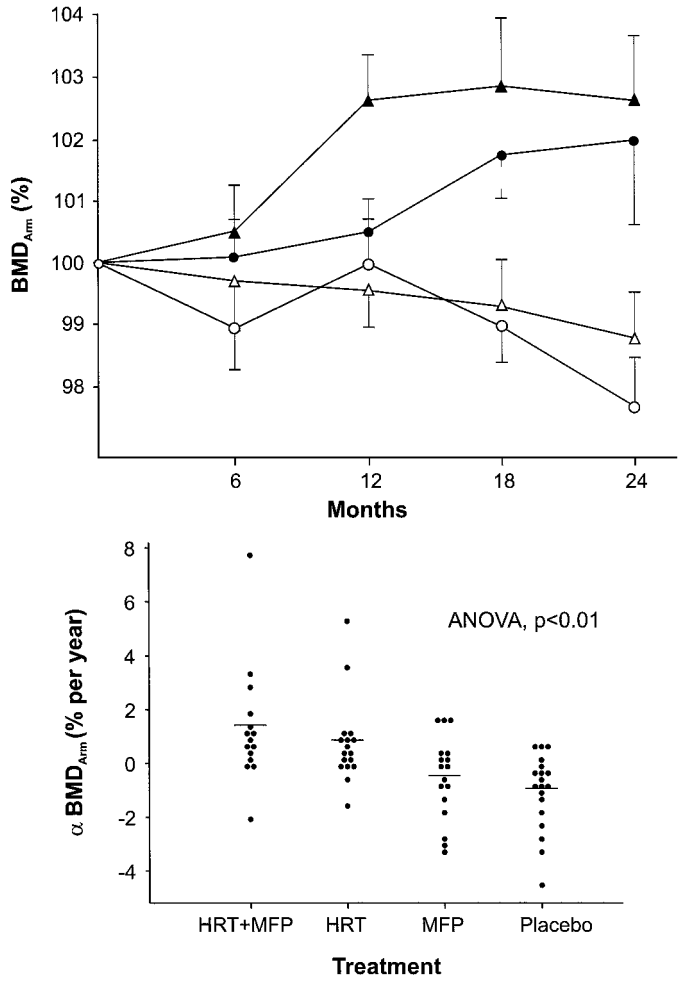

FIG. 2. The time-related changes in percent for BMD of the nondominant forearm (*top*). Values are mean ± SEM. αBMD is also shown (*bottom*). *Horizontal lines* indicate the mean. ANOVA, $P < 0.0001$ applies for both sites. Symbols are as defined in Fig. 1.

ythema (patch) (Table 2). With respect to joint pain and pain in extremities, there was virtually the same number of adverse events in all groups (Table 3). However, endometrial bleeding, tenderness of the breasts, weight gain, mood change, and nausea were more frequent in the HRT-treated groups than in the MFP or the placebo groups (Table 3). Sixty percent of the subjects classified the bleeding as mild and 30% as moderate (60% had bleeding episodes for less than 12 weeks, 13% for 12–24 weeks, and 27% for longer than 24 weeks). For other HRT-related adverse events of interest mentioned above, 65.5% of the subjects classified these as mild, whereas 30.9% reported them as moderate, and 3.6% as severe (35% had events for less than 12 weeks, 37% had events for 12–24 weeks, and 28% for longer than 24 weeks). Regarding adverse events of special interest related to MFP (joint pain and pain in extremities), 65% and 35% of the subjects, respectively, reported these to be mild or moderate in severity (21.1% had events less than 12 weeks, 26.3% for 12–24 weeks, and 52.6% for longer than 24 weeks).

Fractures

We do not report any antifracture efficacy data. During the study, 2 new vertebral fractures occurred: 1 in the HRT group and another in the combined HRT+MFP group. There were 10 patients experiencing appendicular fractures, mostly of clear traumatic origin: 1 patient in the HRT group (metatarsal fracture after 24 weeks), 3 patients in the MFP group (1 patient experienced fractures of the patella and wrist after 12 weeks, 1 had a fracture of the wrist after 48 weeks, and 1 experienced a fracture of the thumb after 4 weeks), 1 in the combined HRT+MFP group (finger fracture after 12 weeks) and 3 in the placebo group (foot fracture after 72 weeks, and 2 arm fractures within the first 12 weeks). There were no cases of breast or gynecological cancer in any of the subjects during the study.

Serum lipids and lipoproteins

TC was (borderline) significantly different among groups (ANOVA, $P = 0.058$), with a decrease in the HRT and the HRT+MFP groups [respectively, +7.8% (2.9)% and +10.4% (2.5%)], compared with placebo), and a small increase in the MFP group [0.8% (3.1%)]. For LDL-C, similar changes were found in the HRT and HRT+MFP groups [respectively, −20.2% (6.7%) and −29.5% (5.9%)], whereas no change occurred in the MFP group [+1.9% (6.1%), all values placebo

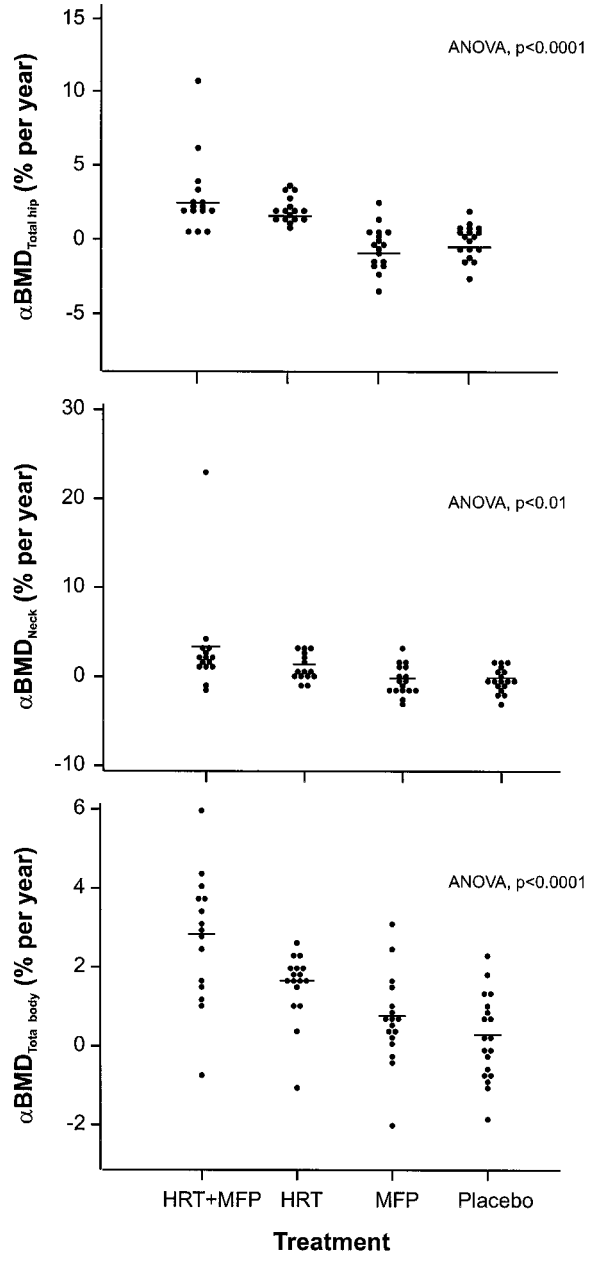

FIG. 3. The individual annual change in BMD for the total hip (A), the femoral neck (B), and the total body (C). *Horizontal lines* denote the mean. For the total hip: ANOVA, $P < 0.0001$; for the femoral neck: ANOVA, $P < 0.01$; and for the total body: ANOVA, $P < 0.0001$.

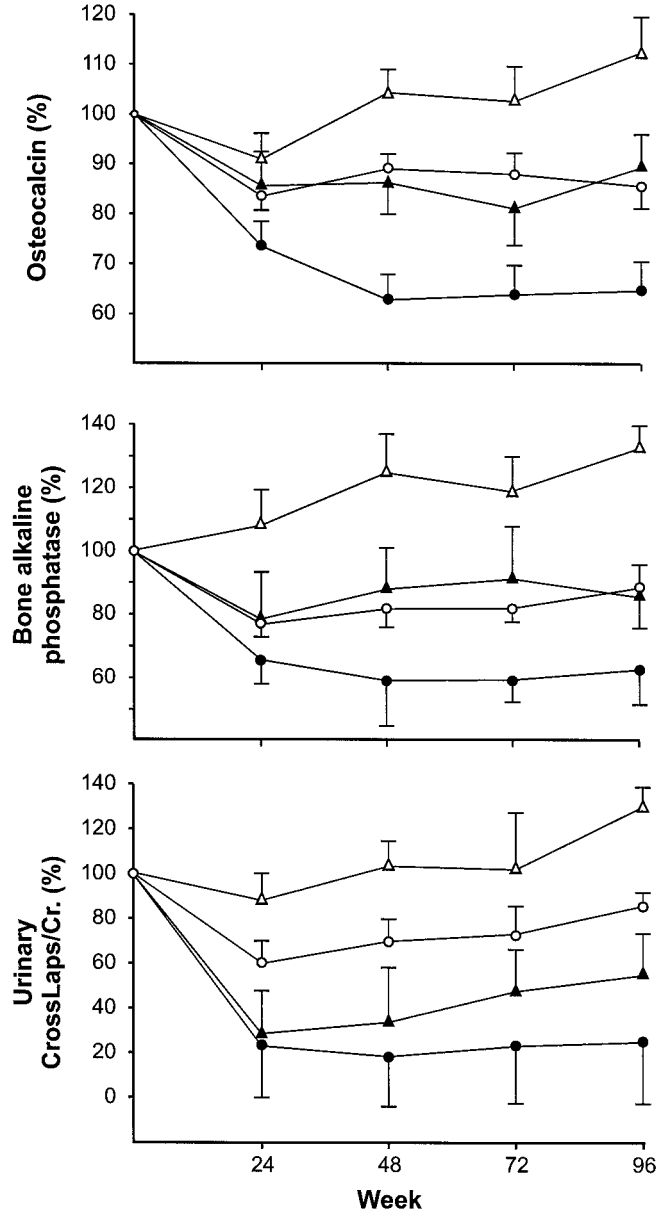

FIG. 4. The time-related change in percent of biochemical markers of bone formation [serum N-mid OC (*top*), and serum B-AP (*center*)], and bone resorption [urinary CrossLaps adjusted by urinary creatinine (*bottom*)]. Symbols are as in Fig. 1.

corrected] (ANOVA, not significant); for very-low-density lipoprotein cholesterol, the corresponding changes were: −7.9% (3.6)%, −10.8% (3.0)%, and +1.0% (4.2)% (ANOVA, not significant). TGs tended to decrease in the HRT and HRT+MFP groups [respectively, +19.7% (6.7%) and +29.3% (5.9%)], whereas showing a slight increase in the MFP group [+1.8% (6.1%)] (ANOVA, not significant; all values placebo corrected). For HDL-C, the two hormone groups induced a significant decrease [HRT: −15.2% (3.4%); HRT+MFP: −13.2% (3.4%)], whereas there was no net change in the MFP group [+1.2% (3.6%)] (ANOVA, not significant; all values placebo corrected).

Discussion

Sodium fluoride has, for several decades, been used to treat patients with osteoporosis. Despite its widespread and growing use, there is debate about its adverse effects and antifracture efficacy. The incidence of side effects is relatively high, particularly those relating to gastric irritation and pain in the lower extremities (4, 7). Moreover, bone formed in association with fluoride may be structurally abnormal, because of defective mineralization of the newly synthesized bone (8, 9), and thus not possess increased strength and antifracture properties. Indeed, in some uncontrolled studies, fluoride therapy increased the occurrence of hip fractures (24), although this has not been confirmed in controlled stud-

TABLE 2. Number of dropouts and the primary reason for dropout according to treatment groups

	HRT (n = 9)	MFP (n = 8)	HRT+MFP (n = 10)	Placebo (n = 5)
Bleeding	0	0	1	0
Breast tenderness, oedema, headache, nausea, weight gain, mood change	2	2	4	1
Pain in joints or extremities	0	0	0	1
Local erythema (patch)	0	1	0	1
Other reasons[a]	7	5	5	2

[a] Includes personal reasons, moving away, poor compliance (intake <70%), failure to return, or reasons not related to treatment (*e.g.* vertigo).

ies (2, 3, 6). In a 4-yr randomized study by Pak and coworkers (6), using slow-release sodium fluoride (50 mg/day) in combination with calcium, spinal and hip BMD (but not arm BMD) increased, compared with calcium alone (spinal BMD, about 5% per yr; and hip BMD, about 1.4% per yr), and prevented new vertebral fractures (but not recurrent fractures). Eventually, two double-blind studies were carried out to further examine these issues in depth (4, 7). The Mayo Clinic study (4) comprised 202 postmenopausal women and used 75 mg/day sodium fluoride (equivalent to 33.9 mg fluoride ion per day) and a calcium supplement of 1500 mg/day. One hundred thirty-five women completed the 4 yr of treatment. The French study (7) comprised 354 osteoporotic women who received either fluoride, as sodium fluoride (50 mg/day, equivalent to 22.6 mg fluoride ion per day) or as MFP (150 mg or 200 mg, equivalent to 19.8 mg or 26.4 mg fluoride ion per day, respectively), or placebo, for 2 yr. In this study, all the women received 1000 mg calcium plus 800 IU vitamin D per day. In the Mayo Clinic study, bone mass increased by 35% in the spine and 12% in the femoral neck over 4 yr (about 8–9% and 3% per yr, respectively), but decreased by 4% in the radius. The trend for a decrease in vertebral fractures with fluoride was not significantly different from placebo, and the number of nonvertebral fractures even tended to be higher in the fluoride group. It was argued that the seemingly negative results of the Mayo Clinic study were attributable to the dose. A daily dose of 75 mg was said to be so high that it would result in fluorosis. Consequently, expectations based on the French study, using lower doses of fluoride, were high. Nevertheless, the results were almost as disappointing. BMD of the lumbar spine increased significantly, by 10.8% (or about 5% per yr) in the women treated with fluoride, but there was no reduction in the percentage of new vertebral fractures (assessed semiquantitatively) during the 2 yr. In fact, the incidence of women with 1 or more vertebral fractures was similar in the 2 groups (33% in the fluoride group and 27% in the placebo group). The incidence of nonvertebral fractures was also similar between the groups (7). Recent data in postmenopausal women with low bone mass but no baseline fracture [in contrast to the 2 studies (4, 7)] indicate that a low dose of MFP (similar dose as in our study) combined with calcium prevents vertebral fracture incidence, as compared with calcium alone (25). In that study, however, spinal BMD had increased by 8% after 2 yr of MFP treatment (and 10% after 4 yr) (25). This is about double the amount experienced in our study; however, the women in the present study generally had about 13% higher BMD in the spine than in that study (but were comparable with respect to age and menopausal age) (25), and this may, in part, account for the differences in the 2 studies, regarding change in spinal BMD. In the French study (7), the increase in spinal BMD, after 2 yr of treatment with 150 mg of MFP (comparable with the MFP dose in our study), was about 10%, which was more than in the present study (about 5%, after 2 yr). The reason for this difference is unclear, but it could be that the population in the FAVOS study generally was more osteoporotic (all having 1–4 vertebral fractures at enrollment) than in our study (one third having 1–3 vertebral fractures) and, therefore, could have a better response in BMD to fluoride treatment, although this is a matter of debate (7).

In 1982, Riggs *et al.* (13) reported a placebo-controlled study that included a combined fluoride-estrogen-calcium group. Although not conclusive, the data suggested that this combination was very efficacious. The present paper is the first to report a prospective, randomized, double-blind, and placebo-controlled study of this combination. As a positive control group, we used the combination of estradiol and NETA because earlier studies have demonstrated that this combination, to a certain extent, is able to uncouple bone formation and resorption (26) and has a potent increasing effect on bone mass (+12% in spinal BMD over 2 yr) in this type of patient, although generally more osteoporotic than in this study (27). Thus, the next logical step would be to add a pure bone stimulatory agent to the hormone replacement, in the hypothesis that this combination would lead to a synergism on bone density. The mechanisms of action of the two regimens are obviously different, as confirmed by the present study, but it remains presently unknown just how the synergistic activity would be accomplished. However, fracture data are clearly needed.

The present study is the first on fluoride to include the new sensitive markers of bone turnover (17, 19), and the bone markers demonstrated that the HRT+MFP therapy was able to uncouple bone turnover, *i.e.* bone formation was kept at a relatively high level whereas bone resorption decreased. This separation was reflected in a large increase (>10% per yr) in spinal BMD, which was almost double that of the HRT-alone group and larger than that seen with other currently available therapies. Furthermore, the effect was present in trabecular (and seemed to be so also in cortical) bone areas, and it may be appreciable in osteopenic and osteoporotic women, because both were included in the study population.

In the Mayo Clinic study (4), the women had one or more vertebral fractures, and their average age was 68 yr. In the French study (7), the women had one to four vertebral fractures, and their average age was 65.7 yr. The proportion of women in the Mayo Clinic study with new vertebral fractures was about 30%. In our study, the women were about 65 yr old and had a forearm T-score of −1 SD or less (Table 1), and 30% had a prevalent vertebral fracture at enrollment.

TABLE 3. Number of adverse events (AEs) in those completing the study during the 2-yr period according to treatment groups. Numbers in brackets indicate the number of subjects with an AE. AEs occurring at several time points in a subject were recorded once

	HRT	MFP	HRT+MFP	Placebo
Endometrial bleeding	6 (6)	2 (2)	5 (5)	2 (2)
Breast tenderness, oedema, headache, nausea, weight gain, mood change	24 (17)	13 (10)	24 (19)	3 (3)
Joint pain, pain in extremities, heartburn	5 (4)	7 (7)	8 (7)	7 (7)
Exanthema (patch)	8 (8)	3 (3)	2 (2)	8 (8)
Fracture (vertebral and appendicular)	2 (2)	4 (3)	2 (2)	3 (3)
Other AEs[a]	28 (16)	30 (15)	35 (17)	36 (16)
All AEs	73 (24)	59 (23)	76 (24)	59 (21)

[a] Includes infection, flu, cough, bronchitis, pneumonia, cold, hay fever, cystitis, polakisuria, crural ulcer, salpingitis, paradontosis, abdominal pain, constipation, meteorism, borborygmia, diarrhea, altered taste of sachets, restlessness, vertigo, fainting, erysipelas, leukemia (one participant in the HRT group), hypertension, cataract, ischias, back pain, insomnia, varices, increased diuresis, hypokalemia, alopecia.

From the new large intervention studies recently completed, it is now known that the vertebral fracture incidence in osteoporotic women without prevalent fracture is about 3% per yr (28). The present study was not designed to answer the question of whether the combination HRT+MFP prevents osteoporotic fractures. However, the pronounced decrease in bone resorption, as well as increase in bone mass, should predict antifracture efficacy (29).

Although there was one single dropout in the HRT+MFP group (as compared with the HRT group) or 2 more than in the MFP group, the reasons for leaving the study were not the typical adverse events of fluoride. Moreover, there was virtually no difference in the occurrence of HRT-related adverse events whether HRT was given alone or in combination with MFP.

The changes in serum lipids and lipoproteins were those expected to occur when given this HRT. The decrease in serum HDL-C is regarded as an adverse effect and has been shown by others (30, 31) using estradiol in combination with NETA (a 19-nortestosterone derivative). However, epidemiological data indicate that the risk of developing myocardial infarction may be even lower in postmenopausal women treated with estrogens in combination with a 19-nortestosterone derivative, compared with those receiving estrogen monotherapy (32).

In conclusion, the present study, using biochemical markers of bone turnover, demonstrates that the combination of relatively low doses of antiresorptive and bone-stimulating agents may dissociate bone resorption and bone formation and thus, by a synergistic effect, induce a pronounced increase in bone mass throughout the skeleton.

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