Calcif Tissue Int (1994) 55:417–419

Calcified
Tissue
International

Hormone Replacement Therapy Increases Serum 1,25-Dihydroxyvitamin D: A 2-Year Prospective Study

H. J. C. van Hoof,[1] M. J. van der Mooren,[2] L. M. J. W. Swinkels,[1] R. Rolland,[2] Th. J. Benraad[1]

[1]Department of Experimental and Chemical Endocrinology, University Hospital Nijmegen St. Radboud, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
[2]Department of Obstetrics and Gynecology, University Hospital Nijmegen St. Radboud, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Received: 2 March 1994 / Accepted: 11 July 1994

Abstract. Osteoporosis is a common disorder in postmenopausal women, which is probably due to decreased ovarian function. Currently, hormone replacement therapy (HRT), involving administration of estrogen and progestogen, is successfully applied to reduce bone resorption. We studied the effect of HRT on 23 postmenopausal women. This consisted of a combination of 17β-estradiol and dydrogesterone, on the serum level of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) after 0, 6, 12, and 24 months. We found mean serum concentrations (±SD) of $1,25(OH)_2D$ of 130.5 pmol/liter (46.1), 152.7 pmol/liter (45.1), 170.8 pmol/liter (64.0), and 155.2 pmol/liter (59.7), respectively. The baseline values in these women were found to be significantly lower than those during therapy ($P \leq 0.005$). No statistically significant differences were observed when comparing the estrogen-only phase with the combined estrogen-progestogen phase. It is concluded that HRT results in an increase in the serum $1,25(OH)_2D$ concentration which lasts for at least 2 years. This increase may partly explain the preventive effect of HRT on osteoporosis. Furthermore, these results suggest that dydrogesterone does not influence the estrogen-induced changes in serum $1,25(OH)_2D$ concentration.

Key words: Vitamin D — Cholecalciferol — Estrogen — Dydrogesterone — Postmenopause.

Osteoporosis is a common bone disorder, affecting millions of older people in the Western world, especially women after menopause [1]. With the menopause, a significant decrease in bone density occurs [2], the exact causes of which are still unknown. Many therapies are used to try to halt or even to reverse this phenomenon, such as supplementation with fluoride [3], as well as administration of calcitonin [4], biphosphonates [5], or 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) [6]. Probably the most effective therapy currently available is estrogen therapy [7]. The strong decline in estrogen concentrations after menopause suggests a direct relationship between estrogen concentration and bone density.

During estrogen therapy a rise in serum $1,25(OH)_2D$ has been observed [8–10]. The latter hormone is capable of stimulating the intestinal absorption of calcium. However, literature on this issue is contradictory [11, 12], and most studies describe short treatment intervals of no more than 6 months. Estrogen administration is often combined with progestogen to reduce the hyperplastic effects of estrogen on the endometrium. It has been postulated that progestogen not estrogen [13], is the cause of the enhanced bone mineralization.

To gain a better insight into the effect of estrogen on serum $1,25(OH)_2D$ levels, we have studied 23 healthy postmenopausal women during a sequential 17β-estradiol-dydrogesterone treatment for up to 2 years. This treatment consisted of 24 cycles of 4 weeks, including a 2-week phase of 17β-estradiol-dydrogesterone administration and a 2-week phase of only 17β-estradiol administration. We assessed the serum $1,25(OH)_2D$ concentration at several time intervals during the 17β-estradiol-dydrogesterone phase. Furthermore, to determine any additional effects of the progestogen on serum $1,25(OH)_2D$ concentration, we compared the estrogen-only with the combined estrogen-progestogen phase in the last cycle studied.

Materials and Methods

Experimental Subjects

In this study, 23 healthy, nonhysterectomized, postmenopausal women, aged 49–59 years, were included as described elsewhere [14]. All women suffered climacteric symptoms and complaints and were amenorrhoeic for at least 6 months. They were screened to have follicle-stimulating hormone (FSH) concentrations within the range characteristic for the postmenopausal phase (≥ 36 IU/liter). Excluded were women using hormonal therapy for the previous 2 weeks. Characteristics of the study population are given in Table 1.

All women were continuously treated with 2 mg daily of micronized 17β-estradiol (Zumenon®), and with dydrogesterone (Duphaston®), 10 mg daily for only the first half of each 28-day treatment cycle. Both analogs were administered orally (Solvay Duphar B.V., Weesp, The Netherlands).

Fasting venous blood sampling was performed before study entry and on days 12, 13, or 14 of the combined 17β-estradiol-dydrogesterone intake of cycles 6 ± 1, 12 ± 1, and 24 ± 1. Blood sampling took place after a 12-hour fast. To evaluate the possible differences in serum $1,25(OH)_2D$ between the combined estradiol-dydrogesterone phase and the estradiol-only phase blood was also sampled on cycle days 26, 27, or 28 of cycle 23 or 24. Serum samples were stored at −80°C until measurement of $1,25(OH)_2D$.

Extraction and paper chromatography were performed as described elsewhere [15]. In short, to 1.0 ml of serum 25 μl (10,000 dpm) ^3H $1,25(OH)_2D_3$ (166.4 Ci/mmol, 26,27-^3H) (NEN Products, Dreieich, Germany) in ethanol was added. The samples were incubated and extracted with diethylether. The solvent was removed by

Correspondence to: H. J. C. van Hoof

Table 1. Characteristics of the study population[a]

Age (years)	54.3 ± 3.5
Amenorrhoea (months)	56 ± 40
FSH (IU/liter)	84 ± 26

[a] Mean ± SD (n = 23)

drying under a stream of dry nitrogen, and the samples were chromatographed in a descending paper-chromatography system [16]. After locating the tracer, the appropriate paper area was cut out and the $1,25(OH)_2D$ was eluted with ethanol. The ethanol was then evaporated under a stream of dry nitrogen and the residue was dissolved in 200 µl of 25% ethanol in phosphate buffer in order to concentrate the sample.

The radioreceptor assay was performed as described by Hollis [17], with some modifications. A standard curve (0–1200 pmol/liter) was set up in duplicate. To 50 µl of standard, control or unknown, 50 µl of ^3H-$1,25(OH)_2D_3$ (10,000 dpm) in 25% ethanol in phosphate buffer, and 500 µl receptor preparation from calf thymus in phosphate buffer was added. Following a 3-hour incubation at room temperature, receptor-bound and unbound $1,25(OH)_2D$ were separated using 150 µl dextran-coated charcoal to adsorb the unbound fraction. After 3 minutes, the samples were centrifuged at 2000 × *g* for 10 minutes at room temperature. The supernatants were decanted simultaneously into counting vials using the device described by Vecsei and Gless [18]. After addition of 4 ml of scintillation liquid, the radioactivity was determined. The intra- and interassay CV were 10% (n = 15 at 93.4 pmol/liter) and 11% (n = 8 at 103.3 pmol/liter), respectively.

The standard curve was fitted to a four-parameter model as described by Healey [19], using a nonlinear fit algorithm according to Marquardt [20]. The concentration of hormone present in the sample eluate was read from the standard curve after correction of the total counts for the contribution of the radioactivity by the recovery tracer. The concentration was then corrected for mass-contribution of recovery tracer and reagent blank. Finally, a correction for procedural losses was performed.

Paired Student's *t*-tests were performed using the SAS program on a VAX/VMS computer.

Results

The basal mean serum concentration (±SD) of $1,25(OH)_2D$ was 130.5 pmol/liter ± 46.1 pmol/liter. At cycles 6, 12, and 24 the concentrations were 152.7 pmol/liter ± 45.1 pmol/liter, 170.8 pmol/liter ± 64.0 pmol/liter, and 155.2 pmol/liter ± 59.7 pmol/liter, respectively (Fig. 1). The concentrations during therapy were significantly elevated compared with the baseline level ($P \leq 0.005$). There was no significant difference between the serum concentrations during therapy. Mean serum $1,25(OH)_2D$ in the 17β-estradiol-only phase of cycle 23 or 24 was 154.2 ± 55.3 pmol/liter. This was significantly higher than the baseline level ($P \leq 0.005$), but not significantly different from the concentration during the combined 17β-estradiol-dydrogesterone phase of the same cycle.

Discussion

The present study discloses a significant rise in serum $1,25(OH)_2D$ during 2 years of hormone replacement therapy (HRT) in postmenopausal women. This is in accordance with other reports [9, 10], which, however, did not exceed study periods of more than 6 months. Civitelli et al. [21] have reported elevated serum levels of $1,25(OH)_2D$ during estrogen therapy in postmenopausal osteoporosis after 1 year.

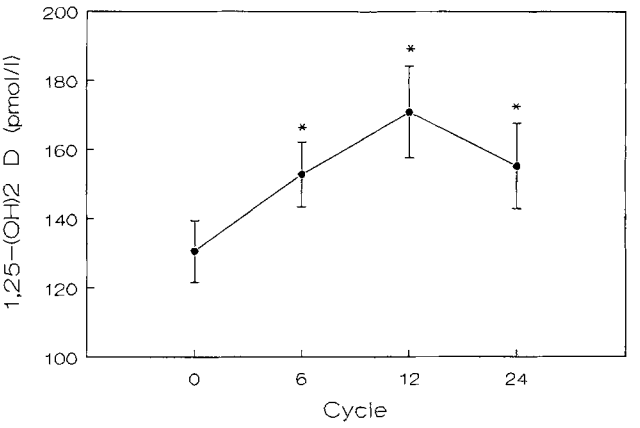

Fig. 1. Mean concentration (±SEM) of $1,25(OH)_2D$ during hormone replacement therapy in postmenopausal women (n = 23) (*$P \leq 0.005$ vs baseline).

Prince et al. [8] also studied the effect of HRT on the serum levels of $1,25(OH)_2D$ in postmenopausal women during a 2-year period (n = 40). These authors, however, did not find a significant increase in serum $1,25(OH)_2D$ concentration after 2 years, although they reported changes in concentrations similar to those we have found. The underlying reason for the lack of any significance may well be the result of their intra- and interassay CV of 21% and 24%, respectively.

Literature data on the effects of HRT on the serum level of $1,25(OH)_2D$ are, however, unequivocal. When estrogen was applied transdermally, no change in serum $1,25(OH)_2D$ was observed [11], even though the serum estrogen concentration was significantly elevated. Further, a decline in the $1,25(OH)_2D$ serum concentration during estrogen therapy has been found in rats [12].

These findings raise the question of whether estrogen exerts its effect on bone density directly by inhibition of bone resorption only or also via $1,25(OH)_2D$. It is generally believed that the estrogen-induced elevation of the serum $1,25(OH)_2D$ is a secondary effect due to a stimulation of vitamin D binding protein (DBP) synthesis in the liver [22–24]. A higher concentration of DBP would result in an increase of the total serum $1,25(OH)_2D$ concentration but would leave the free $1,25(OH)_2D$ index constant. This free index is derived by dividing the concentration of total serum $1,25(OH)_2D$ by the concentration of DBP, and is thought to reflect the biological active moiety. However, reported results of total serum $1,25(OH)_2D$ measurements and of the free $1,25(OH)_2D$ index during HRT have not been equivocal. In some studies, a rise was found not only in DBP and total serum $1,25(OH)_2D$, but also in the free $1,25(OH)_2D$ index [10, 25]. These results indicate that estrogen exerts its effect on bone density also via $1,25(OH)_2D$, and that the rise of serum $1,25(OH)_2D$ should not be ascribed only to an increase in DBP synthesis.

Progestogen is often combined with estrogen administration to reduce the hyperplastic effects of estrogen on the endometrium. Lee [13] suggests that progestogen and not estrogen is responsible for the increased bone density during HRT. This hypothesis cannot be dismissed on the basis of our results. However, we have found no difference between the serum $1,25(OH)_2D$ concentration during the combined estrogen-dydrogesterone phase and during the estrogen-only phase. Dydrogesterone is completely excreted in 72 hours [26]. Any residual effects of dydrogesterone on the serum

$1,25(OH)_2D$ after 2 weeks are therefore, although not completely impossible, highly unlikely. This indicates that dydrogesterone does not induce the change in serum $1,25(OH)_2D$ concentration and therefore also not a $1,25(OH)_2D$-induced change in bone density.

Trémollières et al. [27] have reported estrogen to be a stronger inhibitor of postmenopausal bone loss than promegestone, a progestogen which also has no androgenic effects, similar to dydrogesterone. The results with medroxyprogesterone have been contradictory [22, 25]. Scheven et al. [28] have reported that natural progestogen is a more potent stimulator of osteoblastic growth than estrogen, and Lee [13] also used natural progesterone. It seems likely therefore that the effects of progesterone on bone density results from the androgenic characteristics of progesterone.

Estrogen has also been found to induce the synthesis of a cell/matrix surface-associated inhibitory factor of osteoclastic bone resorption from osteoblastic cells [29]. This finding may indicate that the rise in serum $1,25(OH)_2D$ has two causes. First, as a result of a higher concentration of DBP due to stimulation of its synthesis in the liver by estrogen, and secondly as a result of a lower level of blood calcium (Ca) due to lower bone resorption. This may explain the contradictory results in assessing changes of serum $1,25(OH)_2D$ concentrations after estrogen therapy. The higher $1,25(OH)_2D$ level will generate a higher level of blood Ca through higher Ca uptake in the intestine, which subsequently can suppress the $1,25(OH)_2D$ synthesis via a feedback mechanism.

In conclusion, HRT induces a rise in serum $1,25(OH)_2D$ concentration, which lasts for at least 2 years; this rise appears to be a result of the estrogen alone. Literature data indicate that progestogen seems able to improve bone density only as a result of its androgenic characteristics whereas analogs with no androgenic effects such as dydrogesterone apparently have no effects on the serum $1,25(OH)_2D$ concentration and consequently on the bone density.

References

1. Riggs BL, Melton III LJ (1986) Involutional osteoporosis. N Engl J Med 314:1676–1686
2. Albright F, Bloomberg F, Smith PH (1940) Postmenopausal osteoporosis. Tran Assoc Am Physicians 55:298–305
3. Riggs BL, Hodgson SF, O'Fallon WM, Chao EYS, Wahner HW, Muhs JM, Cedel SL, Melton III LJ (1990) Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. N Engl J Med 322:802–809
4. Civitelli R, Gonnelli S, Zacchei F, Bigazzi S, Vattimo A, Avioli LV, Gennari C (1988) Bone turnover in postmenopausal osteoporosis. Effect of calcitonin treatment. J Clin Invest 82:1268–1274
5. Reginster JY, Lecart MP, Deroisy R, Sarlet N, Denis D, Ethgen D, Collette J, Franchimont P (1989) Prevention of postmenopausal bone loss by tiludronate. Lancet 2:1469–1471
6. Tilyard MW, Spears GFS, Thomson J, Dovey S (1992) Treatment of postmenopausal osteoporosis with calcitriol or calcium. N Engl J Med 326:357–362
7. Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clarke AC (1976) Long-term prevention of postmenopausal osteoporosis by oestrogen: evidence for an increased bone mass after delayed onset of oestrogen treatment. Lancet 1:1038–1041
8. Prince RL, Smith M, Dick IM, Price RI, Webb PG, Henderson NK, Harris MM (1991) Prevention of postmenopausal osteoporosis. N Engl J Med 325:1189–1195
9. Lund B, Sørensen OH, Lund B, Agner E (1982) Serum 1,25-dihydroxyvitamin D in normal subjects and in patients with postmenopausal osteopenia. Influence of age, renal function and oestrogen therapy. Horm Metabol Res 14:271–274
10. Aloia JF, Vaswani A, Yeh JK, McGowan DM, Ross P (1991) Biochemical short-term changes produced by hormonal replacement therapy. J Endocrinol Invest 14:927–934
11. Selby PL, Peacock M (1986) The effect of transdermal oestrogen on bone, calcium-regulating hormones and liver in postmenopausal women. Clin Endocrinol 25:543–547
12. Kalu DN, Salerno E, Liu CC, Echon R, Ray M, Garza-Zapata M, Hollis BW (1991) A comparative study of the actions of tamoxifen, estrogen and progesterone in the ovariectomized rat. Bone Miner 15:109–124
13. Lee JR (1991) Is natural progesterone the missing link in osteoporosis prevention and treatment. Med Hypothesis 35:316–318
14. Van der Mooren MJ, Demacker PNM, Thomas CMG, Rolland R (1993) A 2-year study on the beneficial effects of 17β-oestradiol-dydrogesterone therapy on serum lipoproteins and Lp(a) in postmenopausal women: no additional unfavourable effects of dydrogesterone. Eur J Obstet Gynecol Reprod Biol 52:117–123
15. Van Hoof HJC, Swinkels LMJW, van Stevenhagen JJ, van den Berg H, Ross HA, Benraad ThJ (1993) Advantages of paper chromatography as a preparative step in the assay of 1,25-dihydroxyvitamin D. J Chromatgr 621:33–39
16. Bush IE (1961) The chromatography of steroids. Pergamon Press, Oxford, London, New York, Paris
17. Hollis BW (1986) Assay of circulating 1,25-dihydroxyvitamin D involving a novel single-cartridge extraction and purification procedure. Clin Chem 32:2060–2063
18. Vecsei P, Gless KH (1975) Aldosteron-radioimmunoassay. Ferdinand Enke Verlag, Stuttgard
19. Healey MJR (1972) Statistical analysis of radioimmunoassay data. Biochem J 130:207–210
20. Marquardt DW (1963) An algorithm for least-squares estimation of nonlinear parameters. J Soc Ind Appl Math 11:431–441
21. Civitelli R, Agnusdei D, Nardi P, Zacchei F, Avioli LV, Gennari C (1988) Effects of one-year treatment with estrogens on bone mass, intestinal calcium absorbtion, and 25-hydroxyvitamin D-1α-hydroxylase reserve in postmenopausal osteoporosis. Calcif Tissue Int 42:77–86
22. Bikle DD, Halloran BP, Harris ST, Portale AA (1992) Progestin antagonism of estrogen stimulated 1,25-dihydroxyvitamin D levels. J Clin Endocrinol Metab 75:519–523
23. Hagenfeldt Y, Carlström K, Berlin T, Stege R (1991) Effects of orchidectomy and different modes of high dose estrogen treatment on circulating "free" and total 1,25-dihydroxyvitamin D in patients with prostatic cancer. J Steroid Biochem Mol Biol 39: 155–159
24. Bouillon R, Van Assche FA, Van Baelen H (1981) Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D_3. J Clin Invest 67:589–596
25. Cheema C, Grant BF, Marcus R (1989) Effects of estrogen on circulating "free" and total 1,25-dihydroxyvitamin D and on the parathyroid-vitamin D axis in postmenopausal women. J Clin Invest 83:537–542
26. Kuhl H (1990) Pharmacokinetics of oestrogens and progestogens. Maturitas 12:171–197
27. Trésmollières F, Pouilles JM, Ribot C (1993) Effect of long-term administration of progestogen on post-menopausal bone loss: result of a two-year, controlled randomized study. Clin Endocrinol 38:627–631
28. Scheven BAA, Damen CA, Hamilton NJ, Verhaar HJJ, Duursma SA (1992) Stimulatory effects of estrogen and progesterone on proliferation and differentiation of normal human osteoblast-like cells in vitro. Biochem Biophys Res Comm 186:54–60
29. Ishii T, Saito T, Morimoto K, Takeuchi Y, Asano S, Kumegawa M, Ogata E, Matsumoto T (1993) Estrogen stimulates the elaboration of cell/matrix surface-associated inhibitory factor of osteoclastic bone resorption from osteoblastic cells. Biochem Biophys Res Comm 191:495–502.