Metabolic and Hormonal Effects of 25-mg and 50-mg 17β-Estradiol Implants in Surgically Menopausal Women

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A prospective study involving 12 surgically menopausal women was undertaken to determine whether 17β-estradiol pellets could maintain bone mineral content without inducing adverse cardiovascular side effects. Surgically menopausal women were randomly selected to have either 25-mg or 50-mg pellets implanted subcutaneously. The bone mineral content of the midshaft of the nondominant radius in the combined group—measured by single photon absorptiometry—increased by 1.8% over the two-year period of observation ($P < .03$); the distal bone mineral content of the radius was maintained at 0.8% per annum. No adverse effects were noted in the coagulation profiles or in the coagulation inhibition and fibrinolysis assays of both groups. Serum high-density lipoprotein cholesterol and triglycerides were unaltered, but serum cholesterol values decreased during the six-month period of observation by 14 mg/dL ($P < .05$) and 11 mg/dL in the 25- and 50-mg groups, respectively. Carbohydrate and insulin metabolism was unaffected, as was the systolic and diastolic blood pressure. There were no significant intergroup differences in any of the parameters measured. The serum estradiol/estrone ratios of 1.45 and 1.59 reflected a physiologic estrogen milieu at the 25- and 50-mg dosages. Subcutaneous 17β-estradiol pellets can effectively maintain the bone mineral content of surgically menopausal women without inducing adverse cardiovascular side effects. (Obstet Gynecol 70:749, 1987)

Women who experience a premature surgical menopause are at increased risk of developing osteoporosis and atherosclerotic cardiovascular disease. Long-term estrogen replacement therapy has been shown to lessen the risk of menopause-related osteoporosis[1] and, according to some, cardiovascular disease.[2,3] The beneficial effect on the conservation of bone mass is lost rapidly in surgically menopausal women once estrogen therapy is stopped.[4] In addition, orally administered estrogen is associated with alterations in biologic parameters, such as plasma renin substrate and coagulation factors, that may predispose some women to hypertension and other cardiovascular-related complications.[5] The need for a method to ensure long-term compliance and safety is thus self-evident. Subcutaneous estradiol implants have the potential advantage of achieving patient compliance because they must be administered by a physician, usually at four- to six-month intervals. Irregular hormonal usage or noncompliance can thus be monitored easily. Furthermore, this form of therapy bypasses the enterohepatic circulation, thus avoiding the induction of hepatic factors that may have a negative effect on the cardiovascular system.

Subcutaneous 17β-estradiol pellets have proved effective in the management of the symptomatic menopause.[6] However, there are very few data to document that they are as effective for the preservation of bone mass, the most important indication for long-term estrogen therapy in the menopause. The following study posed four questions: 1) Is parenterally administered 17β-estradiol effective in maintaining the bone mineral content of surgically menopausal women? 2) What effect has this route of administration on cardiovascular-related parameters: blood pressure, lipids and lipoproteins, coagulation and anticoagulation factors, and glucose and insulin metabolism? 3) Do the therapeutic and potential side effects of 25 mg of 17β-estradiol vary significantly from those associated with a 50-mg dosage? 4) Are the hormonal levels obtained by this method compatible with a "physiologic" approach to estrogen replacement therapy?

Materials and Methods

Twelve women who had each had a total hysterectomy and bilateral salpingo-oophorectomy for benign dis-

ease were admitted into the study and observed for two years. They were randomly selected to receive either 25 or 50 mg of 17β-estradiol by subcutaneous implant. Because of the remote possibility of an adverse reaction, we routinely administer a test dose of 10 mg 17β-estradiol by intramuscular injection and monitor the patient's response. No side effects were noted in the study subjects. Pellets were inserted eight or more weeks after this test injection. There were six women in the 25- and 50-mg dosage groups, closely matched for age (38.3 $\pm$ 6.9 and 35.7 $\pm$ 5.9 years), height (164 $\pm$ 6.6 and 160 $\pm$ 3.0 cm), weight (64.7 $\pm$ 16.8 and 61.2 $\pm$ 7.6 kg), and years since surgical menopause (2.5 $\pm$ 1.1 and 2.7 $\pm$ 2.0 years, respectively). Both groups had previously taken oral contraceptives for similar periods of time, 2.5 $\pm$ 1.1 and 2.7 $\pm$ 2.0 years, respectively.

We performed tests for the coagulation and anticoagulation profiles and lipid and carbohydrate metabolism at approximately 8:00 AM, after a 12-hour overnight fast. Sampling was performed before treatment (baseline) and repeated three and six months after pellet insertion. Sex steroid levels were measured at monthly intervals for six months. Bone mineral content measurements were taken and blood pressure and weight recorded at six-month intervals for two years.

Glucose tolerance was evaluated by the serum glucose and insulin response to 100 g of orally administered Glucola. Blood samples were taken before and at 30-minute intervals for two hours after the glucose stimulus. Glucose was measured by the hexokinase[7] method, and insulin by the technique of Horowitz et al.[8]

We also tested an aliquot of the first sample of blood for serum cholesterol, triglycerides, and high-density lipoprotein cholesterol, as described.[9] Coagulation (prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen antigen and activity: α_1-antitrypsin, α_2-macroglobulin, α_2-antiplasmin) studies were performed on platelet-free plasma obtained from the first sample of blood and prepared by centrifugation of 9 mL of blood mixed with 1 mL of 3.8% sodium citrate solution.[10] Hormones (follicle-stimulating hormone [FSH], luteinizing hormone [LH], estrone, estradiol, and total and free testosterone) were measured in serum from three batched samples obtained at 15-minute intervals, starting with the first blood specimen.[10]

The bone mineral content of the nondominant radius was measured by single photon absorptiometry (I-125) using the Norland Cameron Model 278 densitometer (Ft. Atkinson, WI). The midshaft and distal portions of the radius were measured at the standard one-third length and 1.5 cm proximal to the

tip of the styloid process, respectively. The midshaft represents almost entirely cortical bone, whereas the distal portion contains a mixture of cortical and trabecular bone. Three measurements were recorded at each point.

After the baseline studies, we implanted one or two 25-mg pellets of crystalline 17β-estradiol (Barter Corp.) in the subcutaneous tissues of the lower abdomen, just above the groin. The pellets were inserted after local infiltration of the skin with 5 mL 2% xylocaine. Using an aseptic technique, we made a small stab incision in the skin and inserted the pellets with a Kearn's pellet implanter. Firm pressure for a few minutes was all that was required for hemostasis. The implantation site was covered with a Band-aid.

The data were examined statistically with Student's t test and linear regression analysis.

Results

In the analysis and interpretation of the results, we used subjects as their own controls and evaluated the effect of treatment by changes over a six-month or two-year period. Comparisons between each group were also made.

Both doses of estrogen maintained or increased the bone mineral content of the midshaft and distal radius. The distal bone density (bone mineral content divided by bone width) of the 25-mg group changed slightly from a baseline value of 0.436 $\pm$ 0.024 to 0.441 $\pm$ 0.023 mg/cm^2 at 24 months. The respective values for patients given 50 mg were 0.423 $\pm$ 0.043 and 0.432 $\pm$ 0.045 mg/cm^2. When these results were pooled, the mean percentage increase in the distal radial density over the two-year period was 0.8%, a change that was not statistically significant. A better response was noted at the midshaft: The first detectable increase occurred after 12–18 months of treatment. The combined improvement of the midshaft bone mineral density over two years was 1.8% ($P < .03$). The bone density measurements did not differ significantly between the 25- and 50-mg dosages.

Because of the known loss of bone in surgically menopausal women, we considered it inappropriate to include a comparative placebo group. Using single photon absorptiometry in an untreated surgically menopausal population, Lindsay et al[4] noted a 0.95% loss of bone mineral content per year. Linear regression analysis of the bone mineral content of 56 untreated surgically menopausal women who had attended the Climacteric Center's Osteoporosis Clinic, but who did not participate in the study, revealed an annual bone loss that increased with age and years since menopause from 0.4–0.8% per annum.

Table 1. Effect of 25-mg and 50-mg 17β-Estradiol Implants on Coagulation and Lipids/Lipoproteins

Parameter	Group	Baseline	3 mo	6 mo
Coagulation				
Prothrombin time(s) (normal 9.5–12.0)	25 mg	12.2 ± 0.24	12.2 ± 0.26	12.2 ± 0.24
	50 mg	12.9 ± 0.22	12.9 ± 0.12	12.9 ± 0.17
Activated partial thromboplastin time(s) (normal 35)	25 mg	26.0 ± 1.3	26.0 ± 1.5	27.0 ± 0.79
	50 mg	32.0 ± 1.38	32.0 ± 0.96	32.0 ± 1.38
Antithrombin III activity (normal 80–120%)	25 mg	112.0 ± 2.1	108.0 ± 2.9	105.0 ± 2.5
	50 mg	108.0 ± 4.1	106.0 ± 1.4	106.0 ± 2.8
Plasminogen activity (CTA U/mL) (normal 2.4–3.8)	25 mg	4.2 ± 2.6	3.8 ± 0.2	3.8 ± 0.2
	50 mg	3.1 ± 0.3	3.4 ± 0.2	3.2 ± 0.2
α_2-antiplasmin activity (normal 80–120%)	25 mg	112.0 ± 12.1	107.0 ± 10.6	97.0 ± 7.4
	50 mg	92.0 ± 10.7	96.0 ± 5.2	95.0 ± 7.1
Lipids/liproproteins				
Cholesterol (normal 130–250 mg/dL)	25 mg	180 ± 14	167 ± 7	166 ± 12*
	50 mg	195 ± 15	182 ± 13	185 ± 16
Triglycerides (normal 40–170 mg/dL)	25 mg	68 ± 14	72 ± 18	82 ± 21
	50 mg	113 ± 26	95 ± 25	110 ± 31
HDL cholesterol (normal 36–80 mg/dL)	25 mg	51 ± 4	49 ± 3	48 ± 2
	50 mg	46 ± 5	41 ± 3	48 ± 7

CTA = cyano-trimethyl-androsterone; HDL = high-density lipoprotein.
* $P < .05$.

The lipid and lipoprotein profile of the two groups responded favorably to both the 25-mg and the 50-mg doses. The serum cholesterol decreased from 180 ± 14 to 166 ± 12 mg/dL ($P < .05$) and from 195 ± 15 to 185 ± 16 mg/dL in the 25- and 50-mg groups, respectively. The latter difference was not statistically significant. The serum triglyceride and high-density lipoprotein cholesterol values were maintained throughout the study (Table 1).

No differences in prothrombin times, activated partial thromboplastin times, or thrombin times were observed between the 25- and 50-mg groups. The mean fibrinogen activity decreased in the women in the 25-mg group over the six-month period of observation, from 434 ± 58.6 to 390 ± 30.8 to 377 ± 32.9 mg/dL, but this trend was both statistically nonsignificant and well within the range of normal for our laboratory. We noted no changes in factors associated with inhibition of coagulation either between or within the groups. The antithrombin III activity in the 25-mg group subjects decreased slightly from 112% of normal to 105% of normal, but this change was not significant. Fibrinolysis was assessed by plasminogen antigen and activity and by α_2-antiplasmin activity. No significant changes occurred.

Glucose tolerance was normal in both groups. One subject in the 25-mg group was an insulin "hypersecretor," resulting in insulin values in these subjects significantly greater than in the 50-mg group ($P < .01$). Carbohydrate metabolism was assessed by the glucose tolerance and insulin curves, by the areas under the curve for glucose and insulin, and by the cumulative

insulinogenic index. The latter is calculated by dividing the area of insulin under the curve by the area of glucose under the curve. Absolute values at all of the measure points—baseline and 30, 60, 90, and 120 minutes after a glucose load—were within normal limits. The shape of the curve improved in the 25-mg group as treatment progressed, with both the glucose and insulin values approaching fasting values more closely. The insulinogenic index, however, remained the same at 0.6. The 50-mg group maintained normal glucose tolerance more efficiently, with an insulinogenic index of only 0.4. Their insulin pattern also appeared to improve qualitatively with estrogen therapy (eg, the two-hour mean insulin level decreased from the baseline tests of 50 to 34 μU/mL at the six-month test interval). The fasting values were identical at 14 μU/mL (Figure 1).

The serum estradiol values increased significantly in both groups ($P < .0005$). This resulted in a reversal of the original 25-mg group estradiol/estrone ratio of 0.71 to a six-month value of 1.45. The baseline estradiol/estrone ratio in the 50-mg group (1.19) also improved to 1.59 at six months. Figure 2 reflects these changes and illustrates stable postimplantation blood levels. Serum testosterone values were suppressed by approximately 25% from the baseline. This was also reflected in decreased free testosterone values: a mean decrease of 0.9 ng/mL in the 25-mg group ($P < .05$) and a lesser and statistically nonsignificant drop of 0.4 ng/mL in the 50-mg group. Dihydrotestosterone values were unchanged. Expected decreases occurred in both
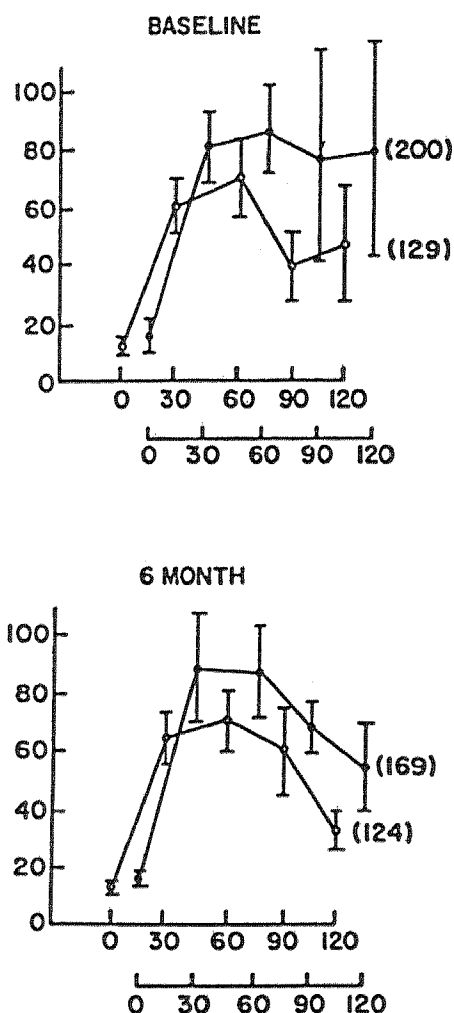
BASELINE

6 MONTH

Figure 1. The plasma insulin response to a glucose load before and six months after the insertion of either 25-mg (*closed circles*) or 50-mg (*open circles*) pellets in surgically menopausal women. Values are measured in μU/mL (mean $\pm$ SD); numbers in parentheses represent the total insulin area under the curve (μU/mL/minute) for the respective groups.
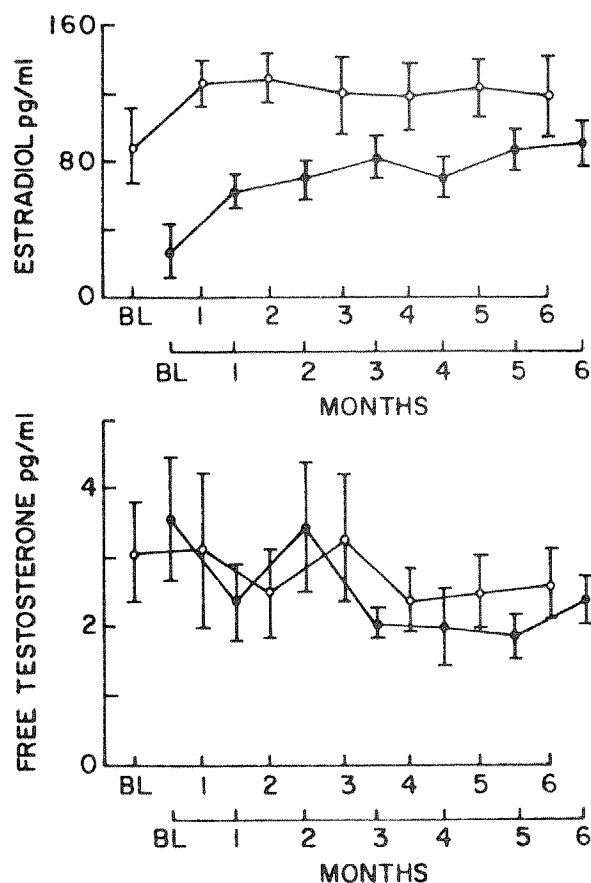

Figure 2. Monthly plasma estradiol and free testosterone levels after the injection of either 25-mg (*closed circles*) or 50-mg (*open circles*) 17β-estradiol estrogen pellets. Values are mean $\pm$ SD.

FSH and LH, LH by 56 and 51% in the 25-mg and 50-mg groups, respectively.

The systolic and diastolic blood pressures of the women in both groups were unaffected, but we noted an average increase of 1.35 kg in weight over a two-year period ($P < .09$).

Discussion

Using single photon absorptiometry, investigators have demonstrated that the bone mineral density of the distal radius decreases by 1.01% and that of the midshaft by 1.04% per year in women between the ages of 50–65 years.[11] This "normal" bone loss pattern is exaggerated in women experiencing a surgical menopause; thus patients who have had an oophorectomy before the menopause have a greater risk of osteoporosis. The exogenous use of estrogens maintains the bone mineral content and may even result in an accrual of bone.[12] However, withdrawal of estrogen therapy results in a rapid loss of bone mineral content, with values four years after cessation of therapy indistinguishable from those in women who had never received estrogen therapy.[4]

The present study has confirmed the bone-conserving effects of 17β-estradiol implants. The overall 1.8% gain in midshaft bone mineral content and the 0.8% maintenance of the distal shaft areas is compatible with other studies that have used single photon absorptiometry. For example, Christiansen et al,[13] using rectilinear scanning, showed a bone gain of 1.2% in women treated with an estrogen/progestogen combination and a loss of 1.9% in those receiving a placebo over a three-year period. In the present study, we observed an improvement in bone mineral content with both dosage schedules—25 mg and 50 mg of

17β-estradiol. These doses resulted in serum estradiol values between 67–92 and 120–131 pg/mL, respectively—levels equivalent to those in the early, mid-follicular, and late luteal phases of normal premenstrual women.[14] The estradiol/estrone ratios of 1.42 for the lower dosage and 1.59 for the 50-mg dosage reflect the physiologic estrogen milieu achieved with the implants.

The menopause, whether induced surgically at an early age or achieved biologically during the middle years, is associated with a dramatic change in the lipoprotein moeity; high-density lipoprotein cholesterol concentrations decrease and low-density lipoprotein cholesterol increases.[9] Fahraeus et al,[15] in a study of postmenopausal women receiving either percutaneous estradiol cream or oral micronized estradiol, found that high-density lipoprotein cholesterol was increased after the oral estrogen but unaffected by the estrogen cream. Using 50 mg of 17β-estradiol implants, Farish et al[16] found only minimal alterations in the lipoprotein metabolism, with a slight rise in high-density lipoprotein due primarily to an increase in the nonantiatherogenic HDL_3 subfraction. This result is consistent with our findings. Some studies have found that estrogen raises serum triglycerides and lowers cholesterol. Our results showed a modest decrease in serum cholesterol—14 mg in patients receiving 25 mg of estradiol and 11 mg in those given 50 mg of estradiol—and a maintenance of the serum triglycerides. Burger et al[17] treated 17 patients with a combination of 50 mg of estradiol and 100 mg of testosterone pellets, and observed no changes in triglycerides, total cholesterol, or their subfractions.

Exogenous estrogens exert a biphasic effect on carbohydrate metabolism; a deterioration in glucose tolerance is found in about 40% of menopausal women on estrogen therapy.[18] Although the insulin levels are usually normal, the response to oral estrogen is frequently delayed, with the peak insulin value shifted to the right. The mechanism for this is not known. The qualitative response to a glucose stimulus, as assessed by the shape of the curve for both glucose and insulin, improved in both groups of subjects. The six-month results reflect this in the return to the fasting levels of the two-hour blood glucose levels, the shift in the peak insulin values to 30 minutes in the 25-mg group subjects, and the lowering of the two-hour plasma insulin values in the 50-mg group. The insulinogenic index was unaffected by the pellet implants at all three time intervals. In view of the progressive improvement in the glucose tolerance curve and the normal insulinogenic indices, one may conclude that estrogen implants are unlikely to have either glucogenic poten-

tial (reversible elevation in glucose) or diabetogenic potential (permanent alteration in glucose tolerance).

Despite the recognized association between oral contraceptive usage and myocardial infarction and stroke in older premenopausal women, a similar relationship has not been noted in postmenopausal women on hormone replacement therapy.[19] It was reassuring to note that both the 25- and 50-mg dosages of 17β-estradiol had an imperceptible effect on our subjects' coagulation-anticoagulation profile. This is consistent with earlier work from our laboratory and may be explained by the following: 1) The menopause per se appears to induce a relative anticoagulant/fibrinolytic state[10]; 2) the type of estrogen used is "natural" and is much less potent than that used in oral contraceptives; 3) parenterally administered estrogens are less likely to stimulate factors synthesized by the liver; and 4) the blood levels of estrogen obtained were within the range expected for premenopausal women.

Although orally administered estrogens are known to stimulate renin substrate, prospective studies have shown oral estrogens to cause, variously, no effect or a slight increase in blood pressure, or even moderate hypotension.[20] The blood pressure response in both of our study groups was unaffected throughout the period of observation. A slight increase in weight ($\pm$ 1.35 kg) was noted with both implant dosages; although this conflicts with the experience with oral estrogen usage, an increase in weight has been reported with combination hormone therapy. The cause for the weight increase in our subjects was not established, and did not require specific therapy.

Both the 25- and the 50-mg estradiol implants resulted in plasma levels of 17β-estradiol equivalent to those of normal premenopausal women.[14] The total and free testosterone values were significantly reduced, but both alterations were within our laboratory's range of normal. Furthermore, all subjects exhibited an excellent symptomatic response, with 42% actually reporting an improvement in libido. Because decreased libido is a frequent menopausal complaint, some authors routinely combine estrogen with testosterone pellets.[6] Our subsequent clinical experience has confirmed that 17β-estradiol pellets alone improve libido and generally enhance sexuality and sexual enjoyment.

The main indication for the long-term usage of estrogen replacement therapy is to prevent osteoporosis. It would be inaccurate and inappropriate to extrapolate the experience of 12 women treated with estrogen implants over a two-year period. Nevertheless, this detailed prospective study has shown that estrogen replacement with subcutaneous implants maintains the bone mineral content of the radius over

a limited period and is free from the potential metabolic disturbances and cardiovascular complications sometimes associated with oral estrogen therapy.

References

1. Aitken JM, Hart DM, Lindsay JR: Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. Br Med J 3:515, 1980
2. Bush TL, Cowan LD, Barrett-Conner E: Estrogen use and all-cause mortality. Preliminary results from the Lipid Research Clinic's program follow-up study. JAMA 249:903, 1983
3. Henderson BE, Ross RK, Paganini-Hill A, et al: Estrogen use and cardiovascular disease. Am J Obstet Gynecol 154:1181, 1986
4. Lindsay R, MacLean A, Kraszewski A: Bone response to termination of oestrogen treatment. Lancet i:1325, 1978
5. Sitruk-Ware R, Basdevant A, de Lignieres B, et al: Percutaneous oestrogen therapy, The Climacteric: An Update. Edited by H van Herendael, B van Herendael, FE Riphagen, et al. Lancaster, MTP Press, 1984, pp 127–139
6. Brincat M, Magos A, Studd JWW, et al: Subcutaneous hormone implants for the control of climacteric symptoms. Lancet i:16, 1984
7. Richterich R, Dauwalder H: Vergleichenbe Bestimmungen der Uringlucosekonzentration hit der Polarimetric und ener Enzymatis Schen Methode Hexokinase/Glucose-6-Phosphate-Dehydrogenase. Schwiez Med Wochenschr 101:860, 1971
8. Horowitz DL, Starr JI, Mako MC, et al: Pro-insulin, insulin and C-peptide concentrations in human portal and peripheral blood. J Clin Invest 55:1275, 1975
9. Notelovitz M, Gudat JC, Ware MD, et al: Lipids and lipoproteins in women after oophorectomy and the response to estrogen therapy. Br J Obstet Gynaecol 90:171, 1983
10. Notelovitz M, Kitchens CS, Rappaport V, et al: Menopausal status is associated with increased inhibition of blood coagulation. Am J Obstet Gynecol 141:149, 1981
11. De Deuxchaisnes N: The pathogenesis and treatment of involutional osteoporosis, Osteoporosis: A Multidisciplinary Problem. Edited by AAJ Dixon, RGG Russell, TCB Stamp. London, Academic Press, 1983, pp 291–333
12. Aitken JM, Hart DM, Anderson JB, et al: Osteoporosis after oophorectomy for non-malignant disease in premenopausal women. Br Med J 2:325, 1973
13. Christiansen C, Christensen MS, Transbol I: Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. Lancet i:459, 1981
14. Nichols KC, Schenkel L, Benson H: 17β-estradiol for postmenopausal estrogen replacement therapy. Obstet Gynecol Surv 39:230, 1984
15. Fahraeus L, Larsson-Cohn U, Wallentin L: Lipoproteins during oral and cutaneous administration of 17β-estradiol to menopausal women. Acta Endocrinol 101:597, 1982
16. Farrish E, Fletcher CD, Hart DM, et al: The effects of hormone implants on serum lipoproteins and steroid hormones in bilaterally oophorectomised women. Acta Endocrinol 106:116, 1984
17. Burger HG, Hailes J, Menelaus M, et al: The management of persistent menopausal symptoms with oestradiol-testosterone implants: Clinical, lipid and hormonal results. Maturitas 6:351, 1984
18. Notelovitz M: Carbohydrate metabolism in relation to hormonal replacement therapy. Acta Obstet Gynecol Scand Suppl 106:51, 1982
19. Notelovitz M, Ware M: Coagulation risks with postmenopausal oestrogen therapy, Progress in Obstetrics and Gynecology. Vol 2. Edited by J Studd. Edinburgh, Churchill Livingstone, 1982, pp 228–240
20. Wren BG, Routledge AD: The effect of type and dose of oestrogen on the blood pressure of postmenopausal women. Maturitas 5:135, 1983

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