Estrogen and Metabolic Bone Disease

SOUTHWESTERN MEDICAL SCHOOL - MEDICAL GRAND ROUNDS

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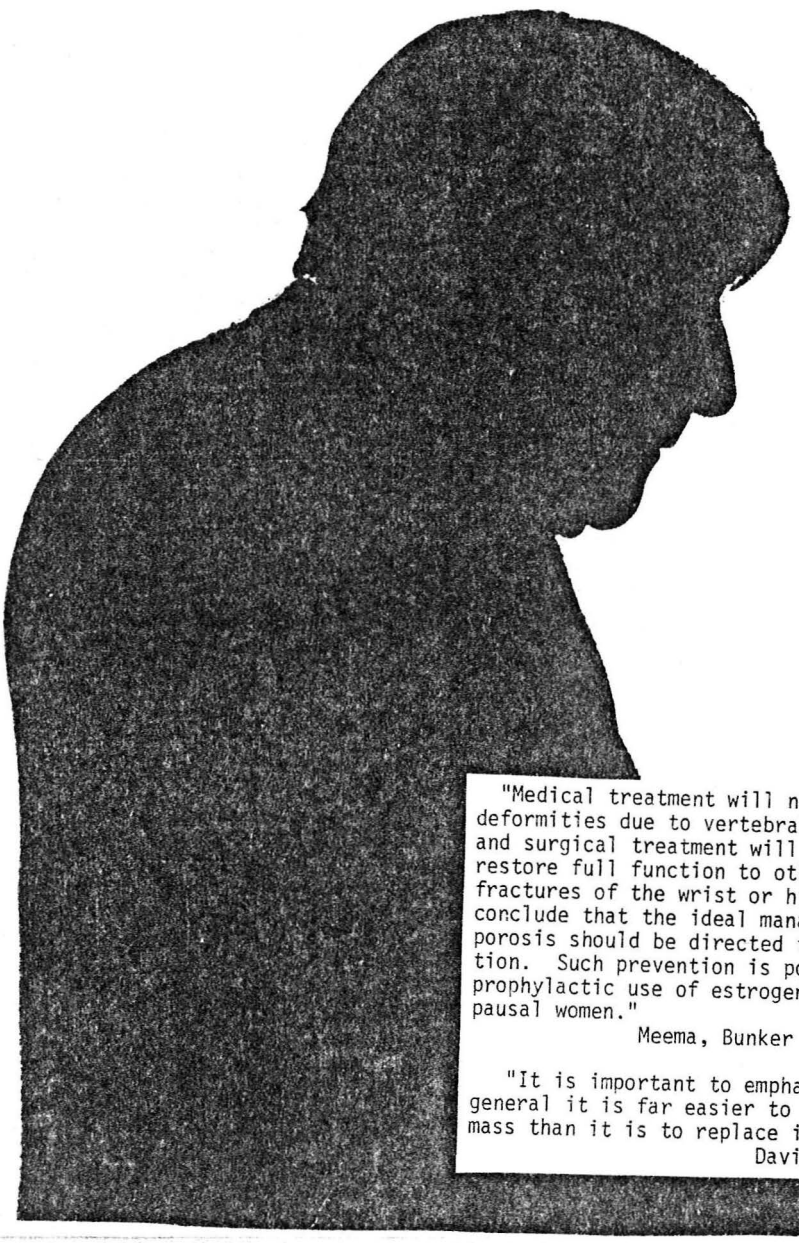
August 25, 1983



"Medical treatment will not correct the deformities due to vertebral compressions, and surgical treatment will not always restore full function to other osteoporotic fractures of the wrist or hip. One must conclude that the ideal management of osteoporosis should be directed to its prevention. Such prevention is possible by the prophylactic use of estrogen in postmenopausal women."

Meema, Bunker and Meema, 1975

"It is important to emphasize that in general it is far easier to maintain bone mass than it is to replace it."

David Baylink, 1983

Table of Contents

WHY SHOULD ESTROGEN BE USEFUL IN THE MANAGEMENT OF METABOLIC BONE DISEASE I.E. WHAT ARE THE PROPERTIES OF ESTROGEN WITH RESPECT TO CA HOMEOSTASIS?

A. BLOCKS BONE RESORPTION. There is considerable evidence which suggests that estrogen might modify PTH-induced bone resorption. Administration of PTH for 3 weeks caused greater reduction in bone content of calcium and hydroxyproline in ovariectomized rats than in intact ones (1). In postmenopausal women with hypoparathyroidism, the age-related loss of bone does not develop, whereas this loss is accentuated in women with hyperparathyroidism (2, Fig. 1). CA/TA in the figure refers to cortical area/total area of the second metacarpal.

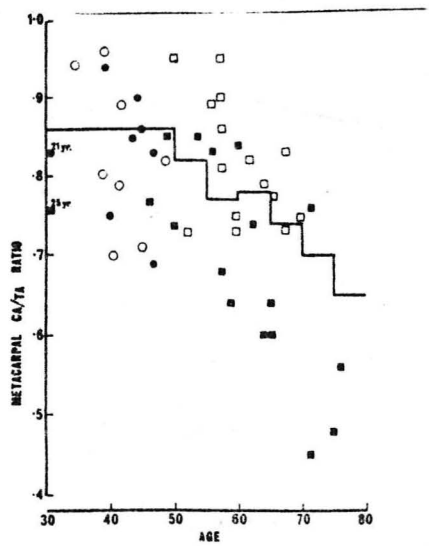


Fig. 1.

Metacarpal C.A./T.A. ratios in premenopausal and postmenopausal hypoparathyroid and hyperparathyroid women.
The solid lines denote the normal means.
○ = Premenopausal hypoparathyroid.
□ = Postmenopausal hypoparathyroid.
● = Premenopausal hyperparathyroid.
■ = Postmenopausal hyperparathyroid.

Studies of bone density, as measured by ^{125}I-photon absorption of the distal third of the radius, also support this observation (3). Bone density is significantly reduced in the majority of white postmenopausal women with primary hyperparathyroidism, unlike in male patients of comparable age with this condition. These results support the conclusion that estrogen deficiency may contribute to the development of bone disease by sensitizing bone to the action of PTH.

When estrogens are given to patients with postmenopausal osteoporosis or primary hyperparathyroidism, the following changes usually occur (4-10): retention of Ca and P; decreases in serum and urinary Ca and P,

urinary hydroxyproline, fasting urinary Ca and bone resorption (by histomorphometry); and an increase in serum PTH. Thus, despite stimulation of parathyroid function (presumably from the decline in serum Ca), bone resorption is inhibited. These findings support the concept that estrogens decrease the responsiveness of bone to endogenous PTH.

Despite the abundant clinical evidence for a "protective" effect of estrogen on bone, in vitro data have been conflicting. In one study, estrogen (particularly ethinyl estradiol) was shown to inhibit the PTH-induced release of Ca from mouse calvaria in a dose-related manner (11). Histologically, the PTH-treated bone showed extensive resorption cavities compared with the untreated control tissue, while bone treated with PTH and estrogen appeared similar to untreated control bone. Although this study has been criticized for using non-physiologic doses of estrogen, the authors argue that (1) inhibitory effects were observed at concentrations which did not adversely affect bone metabolism and (2) the dose of PTH needed to produce resorption in this model was itself well above the physiologic range.

Another study confirmed inhibition of PTH-induced ^{45}Ca release from embryonic rat bone by 17β-estradiol or testosterone, but again at supra-physiologic doses ($>10^{-5}M$) (12). Larry Raisz's group has not been able to confirm an inhibitory effect of 17β-estradiol at physiologic concentrations in fetal rat long bone shafts (13). At the concentrations required to demonstrate an inhibitory effect, even 17α-estradiol, the biologically inactive epimer, or cholesterol itself, had inhibitory effects. Therefore, Raisz's group concluded that PTH-inhibitory effects of steroids at high concentrations were non-specific and possibly related to bone toxicity. It should also be noted that estrogen receptors have not been detected in mammalian bone (13,14,15).

All agree that sex hormones clearly affect bone metabolism in vivo, and the difficulties in demonstrating the inhibitory effects in vitro may simply indicate we have not yet developed the appropriate models. Alternatively, the protective affect of estrogen on bone may be indirect. For example, the effect may be mediated partly through calcitonin secretion (16). Calcitonin, which is produced by thyroid parafollicular C-cells, has specific high affinity receptors in bone, where it acts via the adenylate cyclase enzyme system (17). It inhibits osteoclastic resorption and has been shown in humans to inhibit both PTH and vitamin D-induced bone resorption (18). Calcitonin secretion is lower in women than in men and declines progressively with age (19,20). According to some investigators, it is lower in postmenopausal osteoporotic females than in age-matched controls (21,22), but others disagree (23). Estrogen therapy given to postmenopausal women has been shown to increase basal (Fig. 2, 24) and calcium-stimulated (25) serum calcitonin levels.

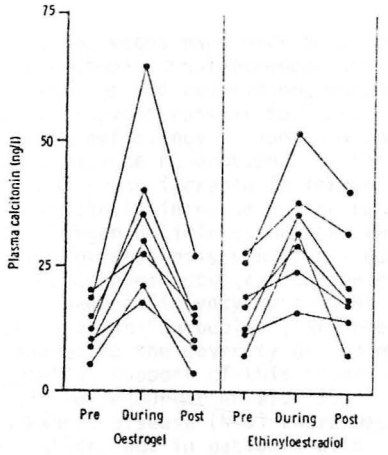


Fig. 2.

Midday plasma-calcitonin levels in postmenopausal women before, during, and after treatment with either percutaneous oestradiol (oestrogel) or oral ethinyloestradiol.

The observation that oophrectomy diminishes and estradiol increases the plasma concentration of calcitonin was confirmed in rats (16). Although it has been difficult to demonstrate a decline in basal calcitonin levels immediately after the menopause (because serum concentrations are already at lower limit of detection), it has been suggested that estrogen lack at the menopause further accelerates the decline of calcitonin secretion, thus sensitizing bone to PTH-induced resorption (24). That calcitonin deficiency may result in osteoporosis has been supported by the finding of reduced bone mineral content in both sexes following total thyroidectomy (17). Studies of the effects of calcitonin administration to osteoporotic subjects have been either poorly controlled (26) or produced variable results (27,28).

B. STIMULATES BONE FORMATION OR IMPROVES "COUPLING".

In vitro studies in the rat (29) and in vivo studies in the guinea pig (30) have demonstrated that 17β-estradiol stimulates collagen biosynthesis in bone (as assessed by incorporation of radio-labelled proline). Neither testosterone nor dihydrotestoserone had any effects on bone collagen synthesis (29). The stimulatory effect of estrogen on endosteal bone formation is best demonstrated in the mouse model (31). The stimulus is so potent that if treatment is prolonged, the marrow elements may be completely displaced by the centripetal growth of bone trabeculae. Autoradiographic studies with tritiated thymidine in mice have shown that estrogen treatment increases the rate at which metaphyseal and endosteal osteoblasts are formed from undifferentiated precursor cells (31). Although estrogen increased the number of osteoblasts and thereby promoted increased collagen synthesis, the rate of collagen synthesis by individual osteoblasts did not change.

Normally bone formation keeps pace with bone resorption, but Ivey and Baylink propose that in postmenopausal osteoporosis there is a perturbation of the "coupling" of formation and resorption, which may be due to estrogen deficiency (32). Their argument runs as follows. It has been suggested that bone loss in estrogen deficiency is due to a corresponding increase in sensitivity to PTH-mediated bone resorption. In this case, a normal coupling response should lead to an increase in formation, resulting in an increase in turnover with little increase in the rate of bone loss. Thus, if the only effect of estrogen deficiency on bone were to indirectly increase resorption, then osteoporosis should not occur following ovariectomy or menopause. Since it does occur,and can be prevented by estrogen replacement therapy, estrogen deficiency must cause an impairment of the coupling response, acting as an "uncoupler", and presumably the extent of uncoupling would be related to the severity of the estrogen deficiency. The only evidence in partial support of this theory derives from the calcium kinetic data of Lauffenburger et al. (33). These data revealed that in patients with Pagets disease (N=6) there was complete coupling of bone formation to resorption, but in patients with postmenopausal osteoporosis (N=15) only half as much formation as resorption occurred. No evidence has been provided that estrogen treatment improves coupling. It is conceivable that the coupling of formation and resorption becomes impaired apart from estrogen deficiency e.g. osteoblast activity may decrease with aging.

C. STIMULATES 1,25-$(OH)_2D$ SYNTHESIS AND INCREASES INTESTINAL CA ABSORPTION.

Kidney homogenates from adult male Japanese quail or chickens demonstrate hydroxylase activity predominantly for the 24- rather than the 1-position of 25-(OH)-D (Fig. 3, 34,35). In an egg-laying female bird, 1,25-$(OH)_2D$ is the predominant product (34,35). A single injection of 17β-estradiol into a male bird completely suppresses the 24-hydroxylase and greatly increases the 1-hydroxylase activity within 24 hr of injection (35).

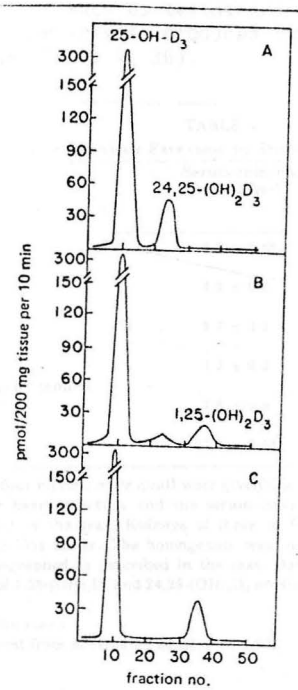


FIG. 3 Stimulation of 25-OH-D_3-1-hydroxylase by estrogen. Homogenates were prepared from pooled kidneys from three quail in each group. The homogenates were incubated with 25-OH-[26,27-^{3}H]D_3 as described in the *text*, in duplicate, and the lipid extracts of the incubation mixtures were chromatographed on an 8 g Sephadex LH-20 column prepared and eluted with 65% chloroform–35% Skellysolve B. In each case, 2.8 ml fractions were collected. The ^{3}H in each fraction was determined by liquid scintillation spectrometry. The identity of each metabolite was determined by high-pressure liquid cochromatography as described in the *text*. This experiment has been repeated four times with identical results. (A) Homogenate from mature male Japanese quail, (B) homogenate from laying quail, and (C) homogenate from mature male quail given estradiol (5 mg per quail) 24 hr prior to sacrifice.

As little as 0.1 mg estradiol/quail was found effective in stimulating the 1-hydroxylase and suppressing the 24-hydroxylase. Estradiol caused a 15-fold stimulation of 1-hydroxylase which was maximum at 1 day post-injection, and no longer present by the 8th to 12th day post-injection (Table 1). Other hormones such as testosterone, progesterone, cortisone and FSH, even at high dose levels, produced little or no change in 25-(OH)-D 1-hydroxylase (Table 1, 36).

TABLE I

FAILURE OF HORMONES OTHER THAN ESTRADIOL TO STIMULATE THE 25-OH-D_3-1-HYDROXYLASE

Hormone given	Serum calcium (mg/100 ml)	24,25-$(OH)_2D_3$ produced (pmol/200 mg/10 min)	1,25-$(OH)_2D$ duced (pmol/200 min)
None	6.8 ± 0.5[b]	277	17
Testosterone (5 mg/quail)	6.8 ± 0.2	176	18
Progesterone (1 mg/quail)	6.7 ± 0.3	207	40
Cortisone (5 mg/quail)	7.2 ± 0.3	316	14
Follicle stimulating hormone (30 units/quail)	7.8 ± 0.4	280	24
Estradiol (5 mg/quail)	17.1 ± 0.5*	38	268

[a] Groups of three to four mature male quail were given one of the hormones 24 h before sacrifice; blood was collected by heart puncture and the serum calcium concentration of individual quail determined as described in the text. Kidneys of three to four quail in each group were pooled, homogenized in sucrose-Tris buffer. The homogenate was incubated with 25-OH-[26,27-^{3}H]D_3 and extracted and chromatographed as described in the text. Data from duplicate incubations were identical. The amount of 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ produced was calculated from the specific of the 25-OH-[^{3}H]D_3.

[b] Standard error of the mean.

* Significantly different from nontreated quail, $P < 0.001$.

The stimulation of 1-hydroxylase by PTH was of smaller magnitude than that of estradiol, and the effects of the 2 hormones were additive (Table 2, 36).

TABLE II

THE STIMULATION OF THE 25-OH-D_3-1-HYDROXYLASE BY PARATHYROID EXTRACT AND ESTRADIOL[a]

Hormone given	Serum calcium (mg/100 ml)	24,25-$(OH)_2D_3$ produced (pmol/200 mg/10 min)	1,25-$(OH)_2D_3$ produced (pmol/200 mg/10 min)
None	7.8 ± 0.2[b]	108	16
Estradiol	20.3 ± 1.3	90	157
PTE[c]	13.9 ± 1.1	88	34
PTE + estradiol	25.5 ± 0.6	74	206

[a] Four groups of four to six mature male quail were given either a single dose of estradiol 24 h prior to sacrifice, 25 units of parathyroid extract subcutaneously every 8 h beginning 32 h prior to sacrifice, or both. The blood was collected by heart puncture and the serum calcium was determined as described in the text. Kidneys from the quail in each group were pooled and homogenized in the buffer. The homogenate was incubated with 25-OH-[26,27-^{3}H]D_3, extracted, and chromatographed as described in the text. The identification of the compounds produced was carried out by high-pressure liquid chromatography as described in the text, and the amount of the product was calculated from the specific activity

The mechanism whereby estradiol stimulates the 1-hydroxylase in not certain. Since the estrogen preparations were injected into intact birds prior to preparation of the kidney homogenates, it was not certain whether the stimulation of the 1-hydroxylase was a direct effect of estrogen or mediated by another hormone e.g. PTH. Unfortunately, it was not possible to remove the parathyroids of the Japanese quail prior to estrogen administration, and serum PTH could not be measured. Other groups have confirmed that single injections of estrogen into intact chickens increased conversion of 25-(OH)-D to 1,25-$(OH)_2$D in kidney homogenates, and that indeed the circulating level of 1,25-$(OH)_2$D was enhanced (37). However, there has never been in vitro kidney cell culture confirmation of a direct effect of estrogen in stimulating the 1-hydroxylase. There is some recent evidence that estrogen may "up regulate" receptors for PTH in chick kidney (38). The density of receptors for PTH and PTH-dependent adenylate cyclase activity of renal plasma membranes is increased 2-3 fold in egg-laying hens compared to age-matched male birds. When 14-week old cockerels were treated with estrogen for 2 weeks, the renal membranes showed a 3-fold increase in PTH receptor density and enhanced adenylate cyclase activity in response to PTH. Therefore, estrogen may regulate the renal synthesis of 1,25-$(OH)_2$D indirectly by enhancing the response of the kidney to PTH.

The ability of estrogens to stimulate the renal 1-hydroxylase in egg-laying birds is considered a hormonal adaptation to meet the requirement of $CaCO_3$ shell formation. The responses of mammals to the sex hormones may differ quantitatively if not qualitatively. A direct assessment of the role of estrogens in the regulation of vitamin D metabolism in mammals in

general, and humans in particular is needed. Available evidence to date includes: (1) Studies of mammalian pregnancy, another Ca requiring state during which estrogen has been implicated as a potential stimulus for the enhanced renal 1,25-$(OH)_2D$ synthesis (39,40); (2) postmenopausal osteoporotic women often have low serum 1,25-$(OH)_2D$ and reduced intestinal Ca absorption, which may improve following estrogen replacement (41,42). It is not clear, however, whether the rise in 1,25-$(OH)_2D$ is a direct effect of estrogen, or the result of secondary hyperparathyroidism (and associated serum P reduction) due to a decline in serum Ca arising from the inhibition of bone resorption by estrogen (42,43). We have planned studies of the effect of estrogen on 1,25-$(OH)_2D$ synthesis and intestinal Ca absorption in patients with proven PTH-deficient hypoparathyroidism to help resolve this issue. In the first such patient evaluated, estrogen treatment did not affect basal 1,25-$(OH)_2D$ levels, but caused a 4-fold increment in the response of 1,25-$(OH)_2D$ to PTH injections. (3) Finally, we and others (44) have noted an increase in serum 1,25-$(OH)_2D$ concentration and intestinal Ca absorption in postmenopausal women with primary hyperparathyroidism who were treated with estrogen. This rise in 1,25-$(OH)_2D$ was associated with an increase in urinary cyclic AMP, but no change in serum PTH. Perhaps estrogen treatment does increase renal receptors for PTH (38).

Another mechanism by which estrogen may improve intestinal Ca absorption has been recently noted (45). The decline in Ca absorption with age has been attributed to a decrease in the renal production of 1,25-$(OH)_2D$, but resistance to the action of this sterol has also been postulated. To investigate the latter, Birge and his colleagues measured intestinal absorption of ^{45}Ca and intestinal content of 1,25-$(OH)_2D$ receptors as a function of age and estrogen status in the rat (45, Table 3).

Table 3.

1,25-$(OH)_2D$ RECEPTOR CONTENT (fmoles/mg prot)	AGE 6 wks	15 wks	40 wks	Sham	OX
	155±21	91±12	70±9	73±12	48±8

Sham and ovarectomized (OX) rats were studied at 26 weeks of age, 4 weeks following surgery. These data demonstrate a progressive decline in the 1,25-$(OH)_2D$ receptor content of the aging and estrogen deficient rat associated with a parallel reduction in the ^{45}Ca absorption. The decline in Ca absorption and apparent resistance to vitamin D associated with age and menopause may be due to a loss of intestinal 1,25-$(OH)_2D$ receptors. Birge's group was also able to show that estrogen treatment of the ovarectomized rats "up regulated" the intestinal 1,25-$(OH)_2D$ receptors.

To summarize then, estrogen blocks bone resorption, stimulates bone collagen formation by recruitment of osteoblasts, and augments 1,25-$(OH)_2D$ synthesis and intestinal Ca absorption. Clearly, by reducing the amount of calcium removed from bone and increasing the amount of calcium absorbed from the intestine, estrogen administration should result in a more positive Ca balance.

ESTROGEN DEFICIENCY MODEL OF POSTMENOPAUSAL OSTEOPOROSIS.

Osteoporosis is not a single disease with a single cause but, rather, the cumulative response of bone over time to a variety of noxious factors (i.e. genetic, hormonal, nutritional, physical and perhaps others). Nevertheless, it has been known since the time of Albright that menopause has a major adverse effect on Ca homeostasis (4). The qualitative changes in estrogen production ensuing from menopause have been reviewed by Drs. Wilson and Pak at previous Grand Rounds (46,47). During the premenopausal state the predominant estrogen is estradiol secreted from the ovarian follicle. Of less physiologic importance in the premenopausal years is estrone derived from "extraglandular" aromatization of ovarian and adrenal androstenedione. During the postmenopausal state, estrogen production can be totally accounted for by estrone produced from circulating androstenedione; essentially no estradiol is secreted. Besides the qualitative difference in the type of estrogen produced, the total amount of estrogen produced is substantially reduced in the postmenopausal state (48,49,50). Some investigators have found that estrogen deficiency was more severe in postmenopausal women with bone disease than in comparable women without bone disease (51,52), but others have been unable to confirm this (50,53,54). More recently, decreased levels of free estradiol and testosterone were found in a group of postmenopausal women presumed to have osteoporosis on the basis of hip fractures (55).

The relationship between estrogen deficiency and postmenopausal osteoporosis, which has been only recently convincingly documented, is perhaps best described in the model of Riggs et al. of the Mayo Clinic (56, Fig. 4).

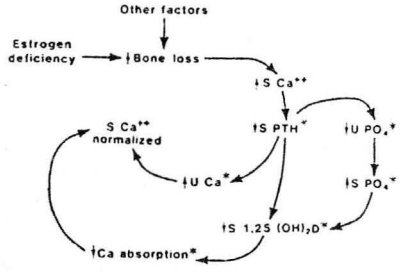


Fig. 4. Hypothetical sequence for deranged calcium homeostasis in postmenopausal osteoporosis, which is consistent with observed measurements. Increased bone loss results from estrogen deficiency (which increases the responsiveness of bone to PTH) and from other factors. The increased release of calcium from bone into the extracellular pool transiently increases the serum ionic calcium (Ca^{++}) concentration. This decreases PTH secretion. Both decreased serum PTH and the resultant increase in serum phosphate decrease the rate of $1,25(OH)_2D$ production and, thus, decrease calcium absorption. Decreased serum PTH also decreases tubular calcium reabsorption. Both decreased calcium absorption and increased renal calcium loss normalize the serum calcium concentration. Thus, the serum calcium concentration is maintained but, compared with the process in normal subjects, more by bone loss than by absorbed dietary calcium. * Documented by direct measurements.

According to the Riggs' model, the development of bone disease in the postmenopausal state is associated at least in part with the loss of the protective effect of estrogens against parathyroid hormone action. Indeed, percentage of bone resorbing surfaces have been found to be increased in patients with postmenopausal osteoporosis and this abnormality may be corrected by short-term estrogen therapy (9,10, Fig. 5).

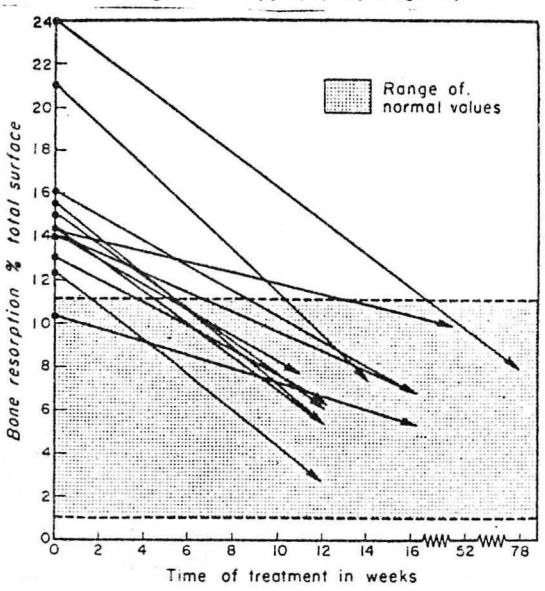


FIGURE 5 Effect of sex hormone on bone resorption.

Because of the enhanced mobilization of Ca from bone, parathyroid function may be suppressed in postmenopausal osteoporosis. Reduced serum PTH has been reported in the majority of patients with this disease (57,58). Serum P may rise from an increased mobilization from bone and from reduced renal excretion of P (associated with parathyroid suppression). Thus, three factors contribute to impaired $1,25\text{-}(OH)_2D$ production during estrogen lack: PTH, serum P, estradiol. The impaired $1,25\text{-}(OH)_2D$ results in reduced intestinal Ca absorption, and inability to adapt to low Ca intake. Thus, bone resorption maintains the serum Ca level instead of absorbed Ca. The validity of this scheme has been supported by the findings of reduced circulating concentrations of $1,25\text{-}(OH)_2D$ and of low intestinal Ca absorption in patients with postmenopausal osteoporosis (41). Moreover, a short-term treatment with estrogen was found to restore these values towards normal (42, Table 4, Figs. 6,7).

Table 4. Effect of estrogen treatment on metabolic parameters in postmenopausal osteoporotic women (N=12)

	Before	After Estrogen	Age and Sex Matched Normal Values
Frac Int Ca Abs	.53±.01	.65±.04*	.61±.02
Serum 25-(OH)-D, ng/ml	17.8±2.0	21.1±2.2	15.9±1.3
Serum 1,25-$(OH)_2D$, pg/ml	24±3	33±4*	33±2
Serum PTH, µl-eq/ml	23±4	33±5*	26±2
Serum Ca, mg/dl	9.5±.1	9.2±.1*	9.3±.1
Serum P, mg/dl	3.6±.1	3.1±.1*	3.5±.1
Urine hydroxyproline, mg/d	26±3	18±3*	-

*p<.05

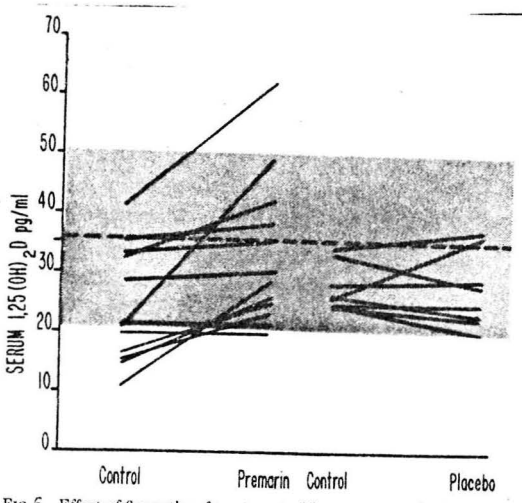


FIG. 6 Effect of 6 months of treatment with estrogen or placebo. The mean and 95% confidence interval for age- age sex-matched normal subjects are given by *horizontal lines* and *shaded areas*, respectively. Treatment with estrogen, but not placebo, resulted in a highly significant ($P < 0.001$) increase in the mean serum 1,25-$(OH)_2D$ level.

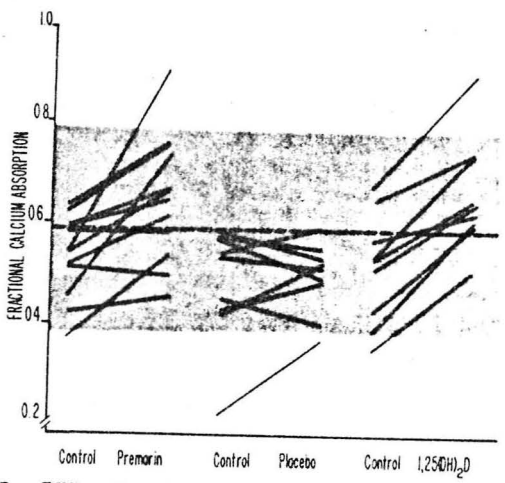


FIG. 7 Effect of 6 months of treatment with placebo, 1,25-$(OH)_2D$, and estrogen on calcium absorption. The mean and 95% confidence interval for age- and sex-matched normal subjects are given by *horizontal lines* and *shaded areas*, respectively. Note that treatment with either 1,25-$(OH)_2D$ or estrogen, but not placebo, increased calcium absorption.

The essence of the Mayo Clinic model is that estrogen deficiency not only sensitizes bone to increased resorption, but also reduces the usual stimuli to the kidney to make 1,25-$(OH)_2D$. Implicit is that the renal 1-hydroxylase remains intact, and that given the appropriate stimulus e.g. estrogen, the kidney can once again synthesize 1,25-$(OH)_2D$. This model has been challenged by the Boston group (59) which believes that a primary renal impairment in the synthesis of 1,25-$(OH)_2D$ occurs with aging. It is expected that parathyroid function should be stimulated if the defective 1,25-$(OH)_2D$ synthesis were primary, and there are some reports of an

increased circulating concentration of PTH with advancing age (60), although it has been argued that at any age it is lower in osteoporotic subjects (56,61). To support their hypothesis that the decline in 1,25-$(OH)_2D$ production with aging is a primary renal defect, the Boston group showed a blunted increment in serum 1,25-$(OH)_2D$ following exogenous PTH administration in elderly osteoporotic patients (N=5, mean age 58) compared to young normal subjects (N=6, mean age 29) (59, Fig. 8).

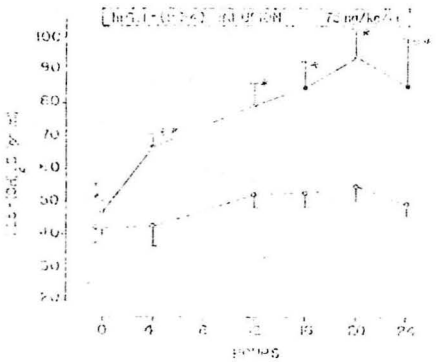


FIGURE 8 Effect of Synthetic Parathyroid Hormone (bPTH-(1-34)) on Levels of 1,25-$(OH)_2D$ in Normal Subjects (Solid Circles) and Patients with Untreated Osteoporosis (Open Circles).

However, two other groups have shown that postmenopausal osteoporotic subjects had a normal increment in 1,25-$(OH)_2D$ in response to PTH when compared to age matched controls (62,63, Fig. 9).

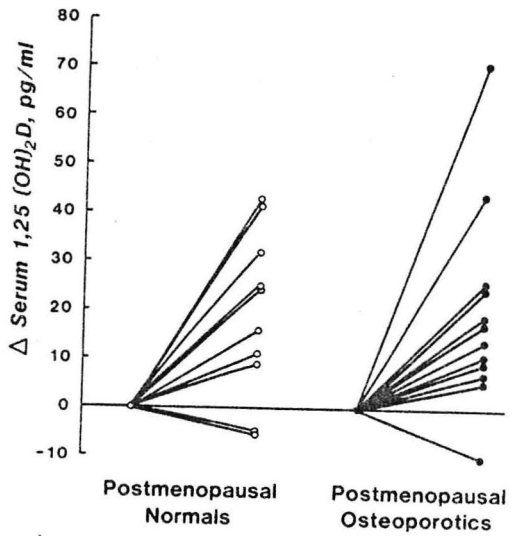


FIG. 9 Response of serum 1,25-$(OH)_2D$ to 72 h of im PTE administration (200 U, twice daily).

The possibility that very elderly persons may have a primary decrease in conversion of 25-(OH)-D to 1,25-$(OH)_2$D, analogous to the defect observed in aging rats (64) cannot be excluded.

Relevant to the controversy over extent of 1-hydroxylase reserve in the aging kidney are several observations made here in Dallas. Lawoyin et al. observed that short-term treatment of 6 postmenopausal women (mean age 62) with 25-(OH)-D (20 mcg/day) increased serum 25-(OH)D from 9 to 30 ng/ml, serum 1,25-$(OH)_2$D from 21 to 43 pg/ml and fractional intestinal Ca absorption from .38 to .49 (65). Thus, "25-(OH)D loading" may be viewed as another way to examine renal 1-hydroxylase reserve. Subsequently, when the number of osteoporotic patients treated in this fashion expanded to 12, 7 were responders in terms of enhanced 1,25-$(OH)_2$D and intestinal Ca absorption, but 5 were not (66). Another way we attempted to evaluate 1-hydroxylase reserve in postmenopausal women was to study 76 women with primary hyperparathyroidism, ranging in age from 29 to 75 years (67). We reasoned that if a sustained increase in PTH were present because of parathyroid autonomy, then measurement of serum 1,25-$(OH)_2$D and intestinal Ca absorption at various ages should indicate the extent of any primary decline in the renal 1-hydroxylase with aging. Results are shown in Table 5 and Fig. 10.

Table 5. Changes in 1,25-$(OH)_2$D and Intes Ca Abs With Age in 76 Women with Primary Hyperparathyroidism

	<50-yr-old (N=21)	>50-yr-old (N=55)
S_{Ca}, mg/dl	10.9±0.7	11.0±0.6
PTH, µl-eq/ml	40±22	38±22
1,25-$(OH)_2$D, pg/ml	84±44	65±32
Intes Ca Abs, frac.	.72±.14	.63±.15*
C_{Cr}	111±33	78±22*

Data shown as mean ± SD, *p<.05

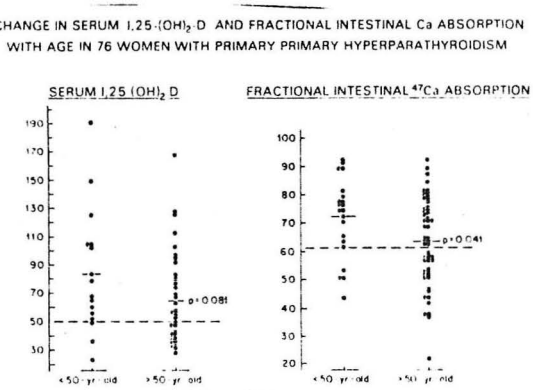


Fig. 10

Our data revealed that although there was a modest decline of $1,25\text{-}(OH)_2D$ and intestinal Ca absorption with age, the mean values for the over 50 yr-old group remained above the upper limits of normal (50 pg/ml and .61 respectively). Furthermore, the mean values for $1,25\text{-}(OH)_2D$ and intestinal Ca absorption for 7 patients in the 71-75 age group were 70 pg/ml and .65 respectively. These data would indicate that the majority of elderly women maintained considerable 1-hydroxylating ability given the appropriate stimulus, consistent with the Mayo Clinic Model. The persistence of an active 1-hydroxylase beyond the menopause, provides an important basis for the use of estrogen in stimulating this system.

CAUTIONARY NOTE ON THE ESTROGEN DEFICIENCY MODEL OF OSTEOPOROSIS AND ON ESTROGEN TREATMENT.

Despite its attractivenesss, the estrogen lack theory has certain drawbacks. Although the increased sensitivity of bone to PTH could explain bone loss, it cannot adequately explain the low urinary Ca excretion and low bone remodelling (57) frequently seen in postmenopausal osteoporotic patients (before treatment). Furthermore, although most patients given estrogen initially develop a strongly positive calcium balance, it is clear from longer term studies that the degree of positive balance diminishes with time (68,69). This is probably because of the long-term decrease in bone formation which accompanies inhibition of bone resorption (10, Table 6).

Table 6. Results of microradiographic studies of trans-iliac biopsies in 9 women with postmenopausal osteoporosis

	Bone Resorbing Surfaces (%)	Bone Forming Surfaces (%)
Before estrogen	15.0±.8	4.8±.3
After 2 1/2-4 mos.	6.4±.7*	4.0±.7
After 26-42 mos.	10.6±.8*	0.6±.2*

*$p<.01$ compared to before treatment. Normal bone-resorbing surfaces 1.3-11.0 %; normal bone-forming surfaces 1.0-8.8 %.

This would explain why estrogen therapy has a preventive action on further bone loss but does not substantially increase bone mass in osteoporotic patients.

THE BONE-LOSING EFFECT OF ESTROGEN DEFICIENCY.

We have reviewed the physiologic properties of estrogen and a model wherein estrogen deficiency causes the serum Ca to be maintained more by bone resorption than by intestinal Ca absorption. The pathogenetic role of estrogen lack in osteoporosis is further supported by the finding of accelerated bone loss with the onset of menopause (70,71). Recent studies have shown that women lose peripheral cortical bone mineral at 0.5 to 3.0% per year initially after menopause or oophorectomy (72-79). Magnification radiographs of the proximal phalanx of a placebo-treated woman show the development of marked intracortical resorptive tunneling or striation 2 years after oophorectomy (72, Fig. 11).

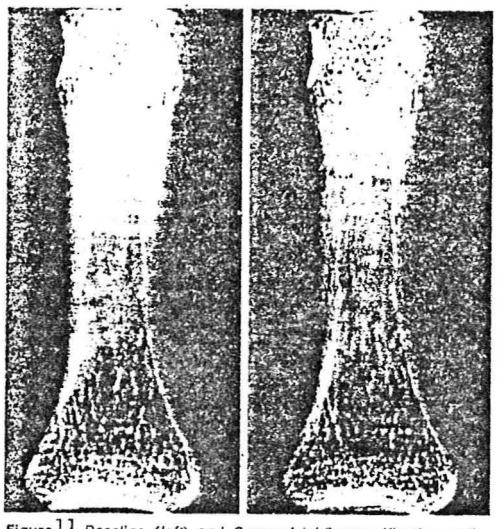


Figure 11 Baseline (*left*) and 2-year (*right*) magnification radiographs of the proximal phalanx of a placebo-treated woman show the development of marked intracortical resorptive tunneling or striation.

Using the highly sensitive tool of quantitative computed tomography, Genant et al. found mean annual bone mineral losses of 7-9% from the vertebral spongiosum (trabecular bone) following oophorectomy (72). The correlation between axial and appendicular loss was weak (r=.581) precluding a reliable estimate of spinal loss from peripheral measurements. Bone mineral loss may be 5-7 fold greater from the spinal spongiosum than from the appendicular cortex (72). The computed tomography technique may be more sensitive than another recently developed technique--dual photon absorptiometry--because it measures only the vertebral centrum (mostly trabecular) and not integrated spine: 2/3 trabecular and 1/3 cortical. Cortical bone is less responsive to metaboli stimuli than trabecular bone. Some have criticized computed tomography for measuring changes in marrow fat rather than bone. However, using dual photon absorptiometry in a

longitudinal study, Krolner and Pors Nielsen confirmed an accelerated vertebral bone loss immediately after the menopause amounting to nearly 6% per year (80). In a cross-sectional study of 70 normal women, the same authors were able to show a drop-off in lumbar bone mineral content beyond the menopause at age 50 (80, Fig. 12).

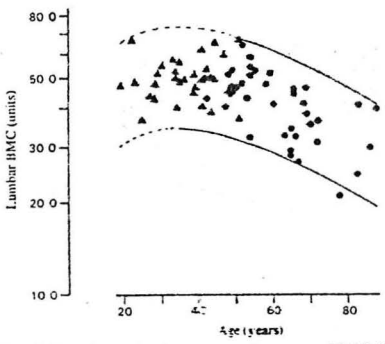


FIG. 12 Lumbar spine bone mineral content (BMC) in 70 normal women, in relation to age: ▲, premenopausal women; ●, postmenopausal women. The stippled area denotes the normal range for the premenopausal women. The curves indicate limits of the age-related normal range (i.e. 95% confidence intervals).

Another group using the same technique found a linear decline in bone density of the axial skeleton occurring before menopause (81). In the Krolner and Pors Nielsen study, mean lumbar bone mineral content of 72 women with postmenopausal osteoporosis was 41% lower than that of normal premenopausal women and 18% lower than that of age-matched controls (80, Fig. 13).

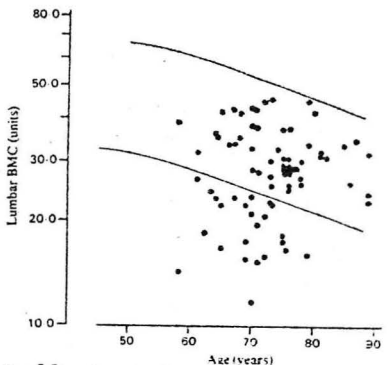


FIG. 13 Lumbar spine bone mineral content (BMC) in 72 women with symptoms and signs of primary osteoporosis, in relation to age. The women had on average three vertebral compression fractures. The stippled area denotes the normal range for premenopausal women. The curves indicate limits of the age-related normal range (i.e. 95% confidence intervals).

An inverse relationship between lumbar bone mineral content and the number of compression fractures was found. A 30-year follow-up study of women who had undergone oophorectomy at ages 15-30 also revealed a striking increase in clinical bone fractures (82).

Other causes of estrogen deficiency have been linked to a reduction in bone mass with varying degrees of probability:

1. Prolactinoma: hyperprolactinemia is a relatively common clinical problem, occurring in more than 25% of women who present with secondary amenorrhea. Because of pituitary gonadotropin suppression by the high prolactin levels, hypogonadism occurs in many of these women and is evidenced by serum estrdiol levels comparable to those of postmenopausal women. A recent report suggests that these women have reduced bone density which is most severe in those with the lowest serum levels of estradiol, and may be at risk for development of osteoporosis (83, Fig. 14). The decrease in bone density was strongly correlated with the relative or absolute estrogen deficiency and not with the prolactin level, although others have challenged this (84). The estrogen deficiency of hyperprolactinemic women is not usually caused by pituitary destruction and may be reversible by restoration of normal prolactin levels by surgical removal of the prolactinoma or medical therapy with bromocryptine. Recently, a 52 year-old woman presented to Barnes Hospital for evaluation of a pituitary tumor, mental changes, and diffuse osteopenia with vertebral compression fractures (85). The patient did not have Cushing's syndrome, but rather a serum prolactin >750 ng/ml (normal <15 ng/ml) and very low serum estradiol. History revealed that she had irregular periods as a teenager, and stopped menstruating entirely after pregnancy at age 26 suggesting long-standing prolactinoma.

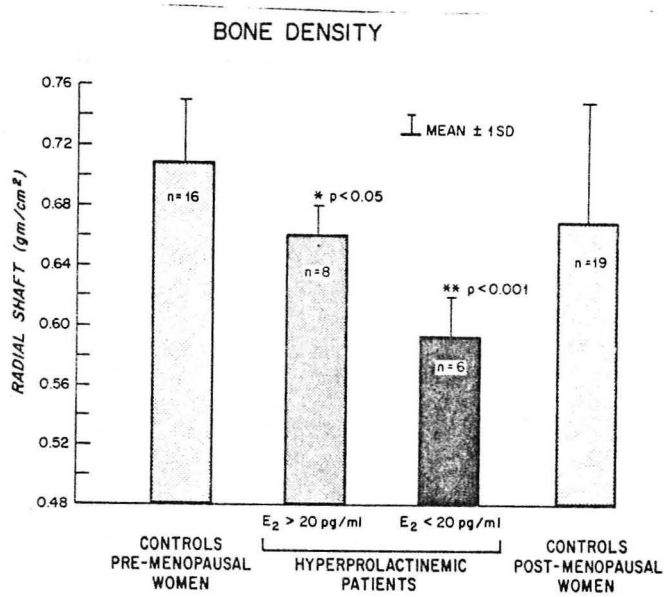


Figure 4 Bone Density in Hyperprolactinemic Women with Detectable and Undetectable Serum Estradiol (E_2) Concentrations and in Premenopausal and Postmenopausal Controls.

2. Smoking: An increased risk of osteoporosis and osteoporotic fractures has been reported among women who smoke (86). In urine specimens collected during the luteal phase of the menstrual cycle current smokers had total estrogen levels about 30% below those of nonsmokers or exsmokers (87). The differences were significant for each of the estrogens measured (estrone, estradiol and estriol) and for the total estrogens. The authors suggested that the mechanism whereby smoking increases the risk of fractures is through a reduction of endogenous estrogens.

3. Thinness vs. Obesity: It has been recognized that obese women have better preserved bone mass than would be expected for chronologic age and years after menopause (88). In these women, increased peripheral conversion of adrenal precursors (androstenedione) to estrone has been described (88).

4. Vegetarian Diet: Although I am unaware of current reports linking a vegetarian diet with increased risk of osteoporosis, if the "estrogen lack" theory holds, women who adhere to such a diet may be at increased risk. It has been reported that a vegetarian diet causes increased fecal excretion of estrogen and decreased plasma estrogen levels (89). Also the high fiber content of the diet may impair intestinal Ca absorption.

THE BONE-PROTECTING EFFECT OF ESTROGEN REPLACEMENT.

Estrogen replacement would seem rational in postmenopausal osteoporosis since it should inhibit PTH-induced bone resorption, possibly improve osteoblast function, raise intestinal Ca absorption and restore Ca balance. In fact, it is now well-established that estrogen therapy prevents bone loss in oophorectomized or postmenopausal women. Lindsay's group has performed a series of prospective, double-blind, placebo-controlled studies, in which they have demonstrated that institution of estrogen therapy shortly after oophorectomy prevents bone loss (73,74,90,91; Fig. 15).

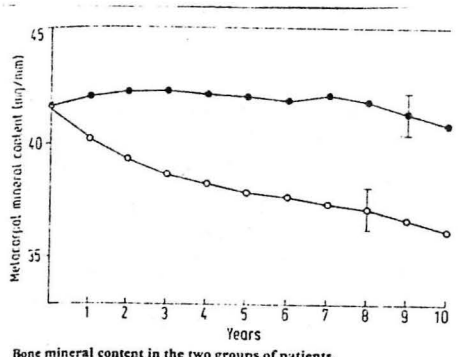


Fig. 15

Bone mineral content in the two groups of patients.
Maximum SE shown. Upper line represents those treated with oestrogen; lower line those treated with placebo.

The estrogen used in Lindsay's studies was mestranol 23 mcg/day, and the site of bone density measurement for follow-up was the mid-point of the third metacarpal of the right hand. Initially, it was believed that if estrogen replacement were delayed for more than 6 years post-oophorectomy, it would no longer afford protective effects against bone mineral loss (90). Nordin has argued that estrogen therapy would still be effective in preventing bone loss even if instituted that late, but the decline in bone density may be less steep at that time, and therefore a beneficial effect more difficult to measure (92,93). Other groups have confirmed the beneficial effect of estrogen even 15 years after natural menopause (79), but it should be emphasized that early estrogen replacement is wise since bone loss of 2.6% per year may occur the first 4 years after oophorectomy and then gradually taper to 0.75% per year (91). Another important point is that cessation of estrogen administration may be followed by a loss of bone mass that is at least as rapid as that occurring after oophorectomy (91, Fig. 16). The loss may negate the therapeutic benefit within a 2-4 year period.

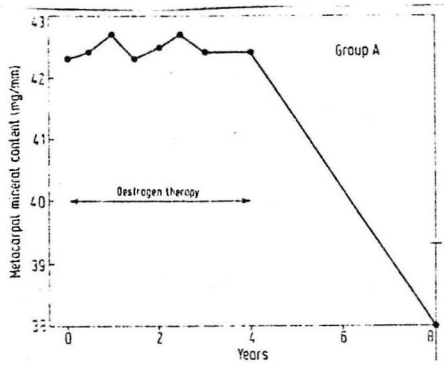


Fig. 16

—Effects of withdrawal of œstrogen therapy on bone mineral content after 4 yr of active treatment.
Results are in a similar form to figs. 1 and 2.

Lindsay et al. have also accummulated evidence for a protective effect of estrogen replacement on the spine as well as cortical bone in women who have had oophorectomy (74). During a 10 year follow-up period of 100 oophorectomized women divided evenly into placebo and estrogen groups, there was a significant reduction in height among the placebo group (38% lost height), but not the group treated with mestranol (4% lost height). Furthermore, lateral x-rays of thoracic and lumbar spine revealed the placebo group had considerably more wedging and crushing of the vertebrae. Using the sensitive technique of quantitative computed tomography, estrogen replacement has been shown to reduce vertebral mineral losses from 7 to 9% down to less than 0.5% in the first year following oophorectomy (72).

The aforementioned studies have dealt primarily with oophorectomized women. Lindsay has argued that there is no evidence that oophorectomized women differ from those undergoing natural menopause (91). However, these

women may have slightly less circulating estrone, estradiol and androstenedione and therefore have a slightly greater risk for bone loss and fractures than postmenopausal women. Nevertheless, the beneficial effect of estrogen in postmenopausal women is just as well-substantiated. In a prospective study of 60 postmenopausal nuns, Recker et al. found that sex hormone therapy (premarin .625 mg plus methyltestosterone 5 mg daily for 21 days each month) reduced loss of radial bone density from 2.88%/year (control group) to .73%/year (estrogen group) (76). Ca balance and kinetic studies performed one year into the study showed that sex hormone replacement had decreased bone loss by suppressing bone turnover, resorption more than accretion. In another study, 82 postmenopausal women were followed 4-10 years by bone density measurements of the radius (79). This was a mixed group including some ovarectomized women, and some as much as 15 years post-natural menopause. The results are shown in Table 7.

Table 7. Effect of Estrogen Replacement on Bone Loss in Artificial and Natural Menopause

	Artificial Menopause (Castrate)	Natural Menopause	Untreated	Estrogen
Bone Mineral Loss Per Year (mg/cm^2)	-9.1	-6.9	-7.99	+3.25*

*Estrogen treated differed from untreated by $p<.01$

A Danish study was performed in 92 normal female volunteers 2.5 to 5.0 years post-menopausal (78). This was a randomized, placebo-controlled, prospective study comparing effectiveness of 3 different doses of natural estrogens (17β estradiol and estriol: 4/2, 2/1, 1/0.5 mg respectively). A progestational agent was added for 10 days to each cycle (norethisterone acetate). Bone mineral content (estimated in forearm by photon absorptiometry) declined by 2% ($p<.001$) in the placebo group, remained constant in the low hormone group, and increased by 0.8% ($p<.05$) and 1.5% ($p<.01$), respectively in the medium and high hormone group (Fig. 17). All participants received 500 mg Ca/day.

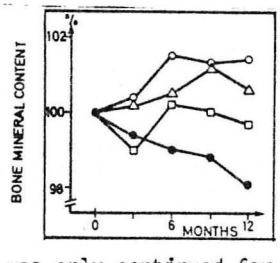


Fig. 17. Sequential changes in forearm BMC during 1 year of treatment of postmenopausal women with 3 different doses of estrogens or placebo. O-high dose, △-medium dose, □-low dose, ●-placebo.

The study was only continued for 12 months, so it is not possible to predict whether the initial rate of increase in bone mineral content would continue over many years. However, several studies have indicated an ap-

parent increase in bone mineral content over several years on estrogen treatment (75,94, Fig. 18). The study depicted in Fig. 18 is a 10 year prospective double blind study (75) with placebo controls involving 84 pairs of randomly chosen postmenopausal patients. Half the patients received conjugated estrogens (Premarin 2.5 mg daily for 21 days) and cyclic progesterone (Provera 10 mg daily last 7 days of cycle), while the other half received placebo. Bone density measurements were made by photon absorptiometry at the mid-point of the third metacarpal and the "linear absorption coefficient" was calculated. This measurement is proportional to bone mass; a positive change therefore indicates an increase in bone mass, and a negative change a decrease in bone mass. The study shows that prevention of bone loss by an estrogen/progestogen combination may persist for up to 10 years and suggests that an earlier initiation of therapy may actually increase bone mass. Any increase in bone mass would probably occur within the first 6 mos to 3 years of treatment, because after that bone formation decreases as well as bone resorption (10).

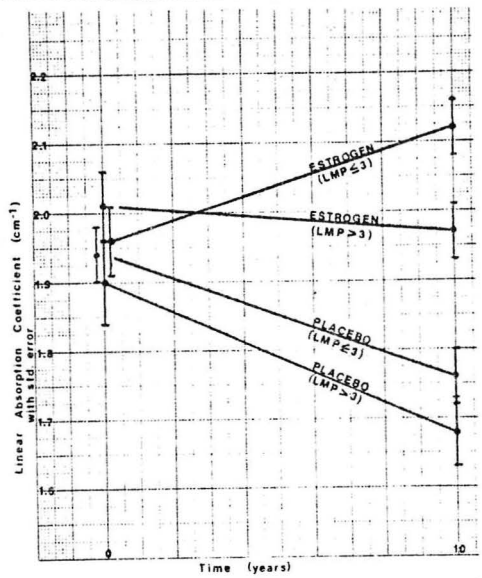


Fig 18. Densitometric linear absorption coefficients in treated patients with LMP ≤ 3 years, treated patients with LMP > 3 years, control patients with LMP ≤ 3 years, and control patients with LMP > 3 years.

Also of interest in the above study,there were 7 fractures in the control group, but none in the treated group. Several additional studies have now confirmed that estrogen therapy prevents the occurrence of hip, radial and vertebral fractures (95-97). Epidemiologic evidence suggests that estrogen may prevent osteoporotic fracture even in the elderly, but only if the estrogen treatment is continued (96). Estrogen plus calcium re-

placement may lower the vertebral fracture rate to 25% of that in untreated osteoporotic women (97). Thus, the evidence that estrogen may have a prophylactic value in the prevention of age-realted bone loss and fractures is solid.

It is believed that any estrogen preparation of equal potency would be equally effective in preventing bone loss of osteoporosis. The minimum effective dose of estrogen for protection against postmenopausal bone loss is .625 mg of conjugated equine estrogens (Premarin). This is the minimal dose which was found to stabilize bone density and lower fasting urinary Ca and urinary hydroxyproline in postmenopausal or oophorectomized women (98). A dose of ethinyl estradiol of 20 mcg/day is believed to be comparable. A single study found that higher doses of Premarin, up to .6 mg daily, was needed to prevent spinal bone loss in women during the first year following oophorectomy (72).

SOME PRACTICAL QUESTIONS CONCERNING ESTROGEN REPLACEMENT THERAPY.

1. Who should receive estrogen treatment?

Women with premature estrogen deficiency clearly require estrogen treatment (72,82). The question of whether all women should receive prophylactic estrogen therapy at the time of menopause is more difficult. In one prospective study in which radial bone density was measured, about 30% of the women with natural menopause did not lose bone for as long as 11 years (79). Among postmenopausal women, there is a group who may not need replacement estrogen for prevention of bone loss. Furthermore, although as many as 25% of women over age 60 will develop osteoporotic fractures, this is far from the entire population (76). If estrogen treatment carries a significant risk it would seem unwise to subject the entire population of postmenopausal women to long term estrogen therapy.

2. What are the side effects of estrogen treatment in postmenopausal women?

The side effects of estrogen therapy were reviewed by an NIH Consensus Development Conference in 1979 (99) and in detail by Dr. Jean Wilson during his July 1980 Medical Grand Rounds (100). Minor symptoms which are noted in 5-15% of women include breast tenderness, edema, abdominal bloating and weight gain. These minor discomforts may be offset by a reduction in hot flashes and improvement in urogenital epithelium. There is no convincing evidence that estrogens in customary doses increase the risk of thromboembolic phenomena, stroke or heart disease in women who have undergone natural menopause. Recent studies have suggested a protective effect of estrogen against arteriosclerotic heart disease (relative risk 0.43) (101,102). This beneficial effect occurs despite the induction of hypertension in a small percentage of patients. Hypertension is due to enhanced hepatic production of renin substrate, and is usually reversable when the hormone is discontinued (103). Estrogen therapy also influences hepatic lipid metabolism and the following alterations of circulating lipids are seen: ↓LDL, ↓VLDL, ↓cholesterol, ↑HDL, ↑TG, which may partially account for the beneficial effect on heart disease (104,105,106). However, an increased cholesterol saturation of the bile creates a 2.5 fold greater risk for symptomatic gallbladder disease (99). Most recent case control studies have not found any link between estrogen use and the development of

breast cancer (106,107). The major concern in the use of estrogens in postmenopausal women has related to the increased incidence of low-grade, non-invasive endometrial carcinoma. The expected incidence of this tumor is 1-2 per 1000 woman-years (108), and a 3-8 fold relative risk occurs after 10 years of estrogen treatment (109). Because these tumors are so well-differentiated and discovered early, this complication of estrogen use has had no signifciant effect on mortality (100). More recently, data has accumulated suggesting that addition of progestogens to the cyclic estrogen regimen may be protective against developing endometrial carcinoma (106,107,108). The progestogen (e.g. medroxyprogesterone acetate-Provera 10 mg) is given the last 7-10 days of the 3 week estrogen cycle. By decreasing the estradiol receptors in the uterine endometrium, progestogens convert the "proliferative" endometrium to the "secretory" phase and withdrawal bleeding ensues. The following table shows the results of a large prospective 5 year study (1975-1979) in postmenopausal women on various hormonal therapies, conducted at Wilford Hall USAF Medical Center (107). Women on a combined estrogen-progestogen regimen actually had a lower incidence of endometrial cancer than untreated women. Further studies need to be done on estrogen-progestogen combinations to determine whether they will alter the risk for cerebrovascular and cardiovascular occlusive disease (110).

Table 8 *Incidence of endometrial cancer in postmenopausal women: 1975 – 1979.*

	Therapy group	Patient-years of observation	Patients with cancer	Incidence (per 100,000)
I	Estrogen-progestogen users	7,063	5	70.8*
II	Estrogen users	2,302	10	434.4***
III	Estrogen vaginal cream users	1,318	1	75.9
IV	Progestogens or androgen users	761	0	–
V	Untreated women	2,477	6	242.2
	Total	13,921	22	158.0

* $p<0.05$ (difference between groups I and V). *** $p<0.001$ (difference between groups I and II).

Although the discomforts and risks of estrogen therapy may be relatively small, since only a small proportion of postmenopausal women require estrogen to prevent significant bone disease, this treatment should probably not be given to all postmenopausal women. The slender, white woman who is a smoker and who begins the menopause with a reduced bone mass may be worth selecting for treatment, and the risk-benefit ratio would also be worthwhile in women who have had hysterectomy (109). The risks should be fully explained to the patients who choose estrogen therapy and careful gynecologic follow-up is mandatory. Black patients who tend to have higher bone mass, and patients with PTH-deficient hypoparathyroidism rarely develop osteoporosis, and such patients would not be suitable candidates for prophyllactic estrogen therapy. It is hoped that in the near future, sensitive techniques such as computed tomography (72) or dual photon absorptiometry (80) of the spine will be able to tell us by 2 measurements 12 months apart which postmenopausal patients should be treated with estrogen (111). These techniques may even allow us to titrate the individual patient on the proper dose of replacement estrogen.

3. If part of the action of estrogen is to increase intestinal Ca absorption and restore Ca balance, how about the use of oral Ca supplements or vitamin D as "the cautious man's estrogen"? Administration of Ca supplements by mouth has also been shown to retard bone loss in postmenopausal women (76), although not as effectively as estrogen administration. Ca administration may also decrease fracture incidence, by 50% in one study (97). These events are perhaps not surprising considering that absorbed Ca would be expected to suppress PTH and stimulate calcitonin. To achieve Ca balance in postmenopausal women not receiving estrogen, a total Ca intake of about 1500 mg/day is necessary, whereas if estrogen is being administered, only about 1000 mg/day is necessary (112). The average American diet, exclusive of dairy products, provides about 500 mg of Ca daily. The contribution of a glass of milk is about 200 mg and that of a slice of cheese is about 100 mg. Thus, women taking estrogen should also drink at least two tall glasses of skimmed milk daily or should take a calcium supplement. A calcium intake of 1500 mg/day is unlikely to be achieved by dietary means, so calcium supplements are indicated for women who cannot take estrogen. The cheapest and most convenient Ca supplement is calcium carbonate which can be bought over the counter in tablets containing 250 to 500 mg of elemental Ca. Although the beneficial effect of Ca therapy is not quite as strong as with estrogen therapy, it may be more safely recommended as a preventive measure.

The use of vitamin D substances in postmenopausal osteoporosis would seem logical because of evidence for vitamin D deficiency and low Ca absorption (41,42,65,66). The treatment should increase intestinal Ca absorption, suppress PTH secretion and inhibit bone resorption. Certain vitamin D metabolites (25-OHD) and 24,25-$(OH)_2D$) may stimulate bone formation by affecting osteoblast function. The treatment with vitamin D or 1,25-$(OH)_2D$ in osteoporosis has been shown to lower serum PTH and bone resorption (113,114). However, as was the case with estrogen or Ca treatment, bone formation declined following vitamin D treatment, probably because of the coupling response to the decline in bone resorption (113). During 1,25-$(OH)_2D$ therapy, no improvement in Ca balance occurred despite stimulation of Ca absorption, because of a rise in urinary Ca (114). Moreover, 1,25-$(OH)_2D$ treatment was ineffective in preventing the decline in bone density of appendicular bone occurring in patients with postmenopausal osteoporosis (115). In long-term trials, addition of vitamin D to any of the therapeutic regimens failed to reduce the fracture rate, and was associated with substantial hypercalcemia, hypercalciuria or both (97). Riggs et al. concluded that vitamin D should not be used as a component of therapy. The problem with vitamin D metabolites is that in addition to raising intestinal Ca absorption, they directly stimulate bone resorption. This same property of 1,25-$(OH)_2D$ may be used to advantage as a short-term "stimulator" of bone remodeling units, as part of the "coherence therapy" protocol recently described by Dr. Pak (116). Used in this way, it is hoped the net result will be enhanced bone formation.

4. If estrogen has not been started immediately post-oophorectomy or postmenopause, is it useful many years later? Even many years after the onset of estrogen deficiency, estrogen will still inhibit further bone resorption (79) and reduce the risk of fractures (96). However, it is not likely to restore much of the bone that has been lost.

5. Once begun, how long should estrogen therapy be continued? Women who commit themselves to a course of estrogen therapy should be encouraged not to interrupt it. Cessation of estrogen therapy is followed by a rapid loss of bone mass that may negate the therapeutic benefit within a 2-4 year period (91).

6. Does estrogen treatment "merely" retard bone loss or does it produce an actual increase in bone mass? Some studies show that bone mass can actually increase transiently with estrogen treatment (73,75,78). Usually, this effect does not proceed beyong the first 6 months or so because of the coupling of bone formation with bone resorption (10). The best hope for actual replacement of lost bone rest with protocols employing fluoride (97) or coherence therapy (116). Uncertainties persist about the quality of "fluoridic" bone (117). Moreover, even replacement of bone will not correct deformities that have resulted from fracture. Once patients requiring estrogen replacement to prevent bone loss can be identified in the perimenopausal period, prophylactic treatment with estrogen would seem the ideal management (79,111).

EFFECTIVENESS OF ESTROGEN AS THERAPY FOR POSTMENOPAUSAL WOMEN WITH PRIMARY HYPERPARATHYROIDISM (PHPT).

Parathyroid surgery is the therapy of choice in patients with PHPT. However, there exists a subset of patients who are suboptimal, unwilling, or failed surgical candidates and who represent potential candidates for medical therapy. PHPT occurs most commonly in postmenopausal women, who are particularly sensitive to the effects of PTH on bone. Since estrogen may oppose PTH-mediated bone resorption, and moreover may stimulate production of 1,25-$(OH)_2D$ and intestinal Ca absorption, the negative Ca balance in these patients may be reversed. If a more positive Ca balance were to persist during estrogen therapy, bone density and histomorphometry should show improvement.

About a decade ago, it occurred to Gallagher and other members of the Leeds group that treatment with estrogens might be beneficial to postmenopausal women with PHPT (118,119). When estrogen was given to these patients, decreases were observed in serum and urinary Ca and P, urinary hydroxyproline, fasting urinary Ca, and bone resorption rate (as calculated by ^{47}Ca kinetic studies). By crude balance studies, no consistent effect of estrogen on net intestinal Ca absorption could be determined, although Ca balance improved in the majority of patients. These earlier studies brought to light the potential benefit of estrogen therapy in selected hyperparathyroid patients--both in protecting bone and reducing the hypercalciuric risk for stones.

We have begun to re-examine this issue using current laboratory techniques permitting assessment of the effect of estrogen on the PTH 1,25-$(OH)_2D$ axis, fractional intestinal ^{47}Ca absorption, serial bone density by single and dual photon absorptiometry, and bone histomorphometry. Six postmenopausal women with PHPT, ages 45 to 75 years were treated with ethinyl estradiol 50 mcg daily. Four of these women had previous hysterectomy. Evaluations were performed on constant metabolic diet during a control period and at 1-3 months of treatment (Table 9).

Table 9. Effect of short-term estrogen treatment in PHPT (N=6)

	Normal	Baseline	Estrogen
Serum Ca, mg/dl	8.5-10.2	10.4±0.3	10.0±0.4*
Serum P, mg/dl	2.5-4.5	2.8±0.2	2.4±0.2***
Tmp/GFR, mg/dl	2.5-4.2	2.6±0.2	1.9±0.3***
PTH, µl-eq/ml	10-30	32±3	31±5
25-(OH)-D, ng/ml	7-42	16±5	16±5
1,25-$(OH)_2$D, pg/ml	20-50	52±23	68±24***
Intes ^{47}Ca Abs, fraction	.37-.61	.58±.20	.69±.14**
24-hr urine Ca, mg/day	50-250	240±65	143±88***
2-hr fast urine Ca, mg/mg Cr	<.11	.17±.09	.07±.03*
24-hr urine OHP, mg/day	13-30	30±10	19±7***
Estimated Ca balance, mg/day	50±25	-8±78	+132±77***

Data expressed as mean ± SD. Significance by paired t test: *p<.05, **p<.01, ***p<.005.

As noted earlier by Gallagher's group, estrogen treatment caused decreases in serum Ca and P, fasting and 24-hr urine Ca and urinary hydroxyproline. Although we were unable to detect any change in serum PTH, there was a significant increase in serum 1,25-$(OH)_2$D and fractional intestinal ^{47}Ca absorption. The rise in serum 1,25-$(OH)_2$D may relate to the reduction in serum P, to a direct effect of estrogen on the renal 1α-hydroxylase, or to an augmentation of renal PTH-receptors by the estrogen (38). Ca balance was estimated by subtracting the mean 24-hr urine Ca excretion from the daily absorbed Ca (fractional Ca absorption multiplied by 400 mg Ca intake). Because of the increase in absorbed Ca and the decrease in 24-hr urine Ca excretion on estrogen treatment, estimated Ca balance improved from a mean value of -8 to +132 mg/day. In one patient who was reevaluated after 14 months of estrogen therapy, the inhibition of bone resorption and augmentation of intestinal Ca absorption persisted, such that estimated Ca balance remained +132 mg/day. It is to be noted that "actual" Ca balance would be 50-100 mg less than the estimated values because of endogenous fecal Ca losses.

Sequential measurements of bone density by ^{125}I photon absorptiometry in the distal radius (Norland-Cameron) were performed in the patient who had remained in positive Ca balance over 14 months of estrogen therapy (Fig. 19).

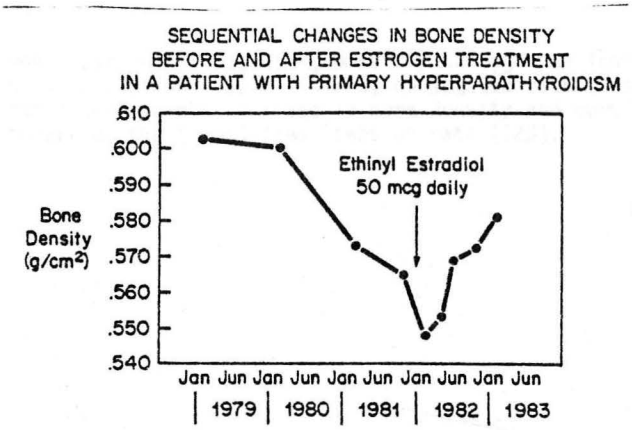


Fig. 19.

In the 3 years preceding estrogen therapy, bone density had progressively decreased by 10% to .547 g/cm^2, a value more than 2 SD below the normal value of .750 g/cm^2 for the age, sex and race matched control population. During 14 months of estrogen therapy, bone density progressively increased by 6.2%, an improvement comparable to that seen following parathyroidectomy over the same time course (120). It is uncertain how long this improvement in bone density would continue, but the situation may be different from that of postmenopausal osteoporosis (10) because of the continued stimulus to formation of new bone remodeling units.

Histomorphometric examination of undecalcified trans-iliac bone biopsy specimens from the above patient was performed in the laboratory of Dr. Kenneth Glass (UTHSCD), using the technique of Meunier (121). Biopsy material was obtained at baseline and after 14 months of estrogen treatment. Consistent with previous reports, trabecular bone volume was normal, but the relative trabecular resorption surfaces were increased at 6.4% (normal 3.6±1.1 SD%) (121). Following estrogen therapy, resorption surfaces had decreased to 4.3%, but the striking change was an increase in relative trabecular osteoid surfaces from 20.7 to 48.5% (normal 12.2±7.5 SD%). The latter change may represent a direct stimulus by estrogen of osteoblastic collagen synthesis (30,31), and is consistent with the sequential changes observed in bone density.

EFFECT OF ESTROGEN ON IMMOBILIZATION OSTEOPOROSIS.

Support for the parathyroid playing a permissive role in the bone resorption of immobilization comes from the work of Burkhart and Jowsey (122). They showed that immobilization of the hind limbs of dogs with plaster casts was associated with readily demonstrable demineralization within 6 to 12 weeks. This bone loss could be prevented by performing parathyroidectomy before immobilization. Since only the immobilized bone was demineralized, they postulated that a local factor produced by disuse may sensitize that particular bone to normal circulating levels of PTH. Orimo et al. reasoned that estrogen might diminish the severity of im-

mobilization osteoporosis, possibly through the inhibition of PTH-induced bone resorption (123). Indeed, conjugated estrogens significantly protected against the decrease in bone density and cortical thickness of the femurs of the immobilized limbs of rats (123).

References

1. Orimo H, Fujita T, Yoshikawa M. Increased sensitivity of bone to parathyroid hormone in ovariectomized rats. Endocrinology 90:760, 1972.
2. Houssain M, Smith DA, Nordin BEC. Parathyroid activity and postmenopausal osteoporosis. Lancet 1:809, 1970.
3. Pak CYC, Stewart A, Kaplan R, Bone H, Notz C, Browne R. Photon absorptiometric analysis of bone density in primary hyperparathyroidism. Lancet 2:7, 1975.
4. Reifenstein EC Jr, Albright F. Metabolic effects of steroid hormone in osteoporosis. J Clin Invest 26:24, 1947.
5. Wallach S, Henneman PH, Prolonged estrogen therapy in postmenopausal women. JAMA 171:1637, 1959.
6. Canniggia A and Gennari C. Sites and modes of action of an estrogen-gestagen combination on calcium and phosphate metabolism in postmenopausal osteoporosis. Clin Orthop 85:187, 1972.
7. Aitken JM, Hart DM, Smith DA. The effect of long-term mestranol administration on calcium and phosphorus homeostasis in oophorectomized women. Clin Sci 41:233, 1971.
8. Young MM, Jasani C, Smith DA, Nordin BEC. Some effects of ethinyl estradiol on calcium and phosphorus metabolism in osteoporosis. Clin Sci 34:411, 1968.
9. Riggs BL, Jowsey J, Kelly PJ, Jones JD, Maher FT. Effect of sex hormones on bone in primary osteoporosis. J Clin Invest 48:1065, 1969.
10. Riggs BL, Jowsey J, Goldsmith RS, Kelly PJ, Hoffman DL, Arnaud CD. Short- and long-term effects of estrogen and synthetic anabolic hormone in postmenopausal osteoporosis. J Clin Invest 51:1659, 1972.
11. Atkins D, Zanelli JM, Peacock M, Nordin BEC. The effect of estrogens on the response of bone to parathyroid hormone in vitro. J Endocrinol 54:107, 1972.
12. Stern PH. Inhibition by steroids of parathyroid hormone-induced ^{45}Ca release from embrynonic rat bone in vitro. J Pharmacol Exp Ther 168:211, 1969.
13. Caputo CB, Meadows D, Raisz L. Failure of estrogen and androgen to inhibit bone resorption in tissue culture. Endocrinol 98:1065, 1976.
14. Chen TL, Feldman D. Distrinction between alpha-fetoprotein and intracellular estrogen receptors: evidence against the presence of estradiol receptors in rat bone. Endocrinology 102:236, 1978.
15. Raisz LG, Kream BE. Regulation of bone formation. N Engl J Med 309:83, 1983.
16. Deftos, LJ. Osteoporosis. pp. 197-198. In: Judd HL, moderator. Estrogen replacement therapy: indications and complications. Ann Int Med 98:195, 1983.
17. McDermott MT, Kidd GS, Blue P, Ghaed V, Hofeldt FD. Reduced bone mineral content in totally thyroidectomized patients: possible effect of calcitonin deficiency. J Clin Endocrinol Metab 56:936, 1983.
18. Pechet MM, Bobadilla E, Carroll EL, Hesse RH. Regulation of bone resorption and formation. Am J Med 43:696, 1967.

19. Heath H III, Sizemore GW. Plasma calcitonin in normal man: differences between men and women. J Clin Invest 60:1135, 1977.
20. Deftos LJ, Weisman MH, Williams GW, Karpf DB, Frumar AM, Davidson BJ, Parthemore JG, Judd HL. Influence of age and sex on plasma calcitonin in human beings. N Engl J Med 302:1351, 1980.
21. Milhaud G, Benezeck-Lefevre M, Moukhtar MS. Deficiency of calcitonin in age-related osteoporosis. Biomedicine 29:272, 1978.
22. Taggart HM, Ivey JL, Sisom K, Chestnut CH III, Baylink DJ, Huber MB, Roos BA. Deficient calcitonin response to calcium stimulation in postmenopausal osteoporosis Lancet 1:475, 1982.
23. Chestnut CH III, Baylink KJ, Sisom K, Nelp WB, Roos BA. Basal plasma immunoreactive calcitonin in postmenopausal osteoporosis. Metabolism 29:559, 1980.
24. Stevenson JC, Hillyard CJ, Abeyasekera G. Calcitonin and the calcium regulating hormones in postmenopausal women: effect of estrogens. Lancet 1:693, 1981.
25. Morimoto S, Tsuji M, Okada Y, Onishi T, Kumahara Y. The effect of estrogens on human calcitonin secretion after calcium infusion in elderly female subjects. Clin Endocrinol (Oxf) 13:135, 1980.
26. Wallach S, Cohn SH, Atkins HL, Ellis KJ, Kohberger R, Aloia JF, Zanzi I. Effect of calcitonin on skeletal mass in osteoporosis. Current Ther Res 22:556, 1977.
27. Deftos LJ, First BP. Calcitonin as a drug. Ann Int Med 95:192, 1981.
28. Austin LA, Heath H III. Calcitonin physiology and pathophysiology. N Engl J Med 304:268, 1981.
29. Canalis E, Raisz LG. Effect of sex steroids on bone collagen synthesis in vitro. Calcif Tiss Res 25:105, 1978.
30. Henneman DH. Effect of estrdiol 17-β on collagen biosynthesis, degradation and re-utilization in vivo. Biochem Biophys Res Comm 44:326, 1971.
31. Simmons DJ. Collagen formation and endochondral ossification in estrogen-treated mice. P.S.E.B.M. 121:1165, 1966.
32. Ivey JL, Baylink, DJ. Postmenopausal osteoporosis: proposed roles of defective coupling and estrogen deficiency. Metab Bone Dis Relat Res 3:3, 1981.
33. Lauffenburger R, Olah AJ, Dambacher MA, Guncaga J, Lentner C, Hass HG: Bone remodeling and calcium metabolism: A correlated histomorphometric, calcium kinetic, and biochemical study in patients with osteoporosis and Paget's disease. Metabolism 26:589, 1977.
34. Kenny AD. Vitamin D metabolism: physiological regulation in egg-laying Japanese quail. Am J Physiol 230:1609, 1976.
35. Tanaka Y, Castillo L, DeLuca HF. Control of renal vitamin D hydroxylases in birds by sex hormones. Proc Natl Acad Sci USA 73:2401, 1976.
36. Castillo L, Tanaka Y, DeLuca HF, Sunde ML. The stimulation of 25-hydroxyvitamin D_3-1α-hydroxylase by estrogen. Arch Biochem Biophys 179:211, 1977.
37. Pike WJ, Spanos E, Colston KW, MacIntyre I, Haussler MR. Influence of estrogen on renal vitamin D hydroxylases and serum 1α,25-$(OH)_2D_3$ in chicks. Am J Physiol 235:E338, 1978.

38. Forte LR, Thomas ML. Estrogen regulates the number of functional receptors for parathyroid hormone in plasma membranes of chicken kidney. 5th Annual Scientific Meeting of Am Soc Bone and Min Research (Abstract) 1983.
39. Kumar R, Cohen WR, Epstein FH. Vitamin D and calcium hormones in pregancy. N. Eng. Med. 302:1143, 1980.
40. Gray TK, Lowe W, Lester GE, Vitamin D and pregnancy: The maternal-fetal metabolism of vitamin D. Endocrine Reviews 2:264, 1981.
41. Gallagher JC, Riggs BL, Eisman J, Hamstra A, Arnaud SB, DeLuca HF. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. J Clin Invest 64:729, 1979.
42. Gallagher JC, Riggs BL, DeLuca HF. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. J Clin Endocrinol Metab 51:1355, 1980.
43. Moses AM, Notman DD. Secondary hyperparathryoidism caused by oral contraceptive therapy. Arch Int Med 142:138, 1982.
44. Marcus R, Madvig P, Crim M, Kosek J, Pont A. Management of primary hyperparathyroidism with conjugated estrogens in postmenopausal women. 5th Annual Scientific Meeting of Am Soc Bone and Min Res (Abstract A36), 1983.
45. Miller RA, Russell JE, Birge SJ. Quantitation of the receptor proteins for 1,25-$(OH)_2D$ in the intestine of the aging rat. 5th Annual Scientific Meeting of Am Soc Bone and Min Res (Abstract A64) 1983.
46. Wilson JD. Use of estrogens after the menopause. Southwestern Medical School Grand Rounds. July 10, 1980.
47. Pak CYC. Metabolic Bone Disease with Special Emphasis on Osteoporosis. Southwestern Medical School Grand Rounds. January 3, 1980.
48. McDonald RC, Grodin JM, Siiteri PK. The utilization of plasma androstenedione for estrone production in women. Progress in Endocrinology. Proc Third Int Cong Endo, p 770, 1968.
49. Siiteri PK, MacDonald PC. Role of extraglandular estrogen in human endocrinology. In: Handbook of Physiology-Endocrinology II, Part 1. R.O. Greep and E.B. Astwood (eds). Am Physiol Soc (Washington, D.C.) p 615, 1973.
50. Riggs BL, Ryan RJ, Wahner HW, Jiang NS, Mattox VR. Serum concentrations of estrogen, testosterone and gonadotropins in osteoporotic and nonosteoporotic postmenopausal women. J Clin Endocrinol Metab 36:1097, 1973.
51. Lindsay R, Coutts JRT, Hart DM. The effect of endogenous estrogen on plasma and urinary calcium and phosphate in oophorectomized women. Clin Endocrinol (Oxford) 6:87, 1977.
52. Marshall DH, Crilly RG, Nordin BEC. Plasma androstenedione and estrone levels in normal and osteoporotic postmenopausal women. Brit Med J 2:1177, 1977.
53. Avioli LV. Postmenopausal osteoporosis: prevention versus cure. Federal Proc 40:2418, 1981.
54. Adlin EV, Korenman SG. Endocrine aspects of aging. Ann Int Med 92:429, 1980.
55. Davidson BJ, Ross RK, Paganini-Hill A, Hammond GD, Siiteri PK, Judd HL. Total and free estrogens and androgens in postmenopausal women with hip fractures. J Clin Endocrinol Metab 54:115, 1982.

56. Riggs BL. Osteoporosis--A disease of impaired homeostatic regulation Mineral Electrolyte Metab 5:265, 1981.
57. Meunier PJ, Courpron P, Edouard C, Alexandre C, Bressot C, Lips P, Boyce BF. Bone histomorphometry in osteoporotic states, In: Osteoporosis II, U.S. Barzel (ed) Grune and Stratton, p 27, 1979.
58. Riggs BL, Arnaud CD, Jowsey J, Goldsmith RS, Kelly PJ. Parathyroid function in primary osteoporosis. J Clin Invest 52:181, 1973.
59. Slovik DM, Adams JS, Neer RM, Holick MF, Potts JT Jr. Deficient production of 1,25-dihydroxyvitamin D in elderly osteoporotic patients. N Engl J Med 305:372, 1981.
60. Wiske PS, Epstein S, Bell NH, Queener SF, Edmondson J, Johnston CC Jr. Increases in immunoreactive parathyroid hormone with age. N Engl j Med 300:1419, 1979.
61. Gallagher JC, Riggs BL, Jerpbak CM, Arnaud CD. The effect of age on serum immunoreactive parathyroid hormone in normal and osteoporotic women. J Lab Clin Med 95:373, 1980.
62. Riggs BL, Hamstra A, DeLuca HF. Assessment of 25-hydroxyvitamin D-1-hydroxylase reserve in postmenopausal osteoporosis. J Clin Endocrinol Metab 53:833, 1981.
63. Sorensen OH, Lumholtz B, Birger L et al. Acute effects of parathyroid hormone on vitamin D metabolism in patients with the bone loss of aging. J Clin Endocrinol Metab 54:1258, 1982.
64. Horst RW, DeLuca HF, Jorgenson N. The effect of age on calcium absorption and accumulation of 1,25-$(OH)_2D_3$ in intestinal mucosa of rats. Metab Bone Dis Rel Res 1:29, 1978.
65. Lawoyin S, Zerwekh JE, Glass K, Pak CYC. Ability of 25-hydroxyvitamin D_3 therapy to augment serum 1,25- and 24,25-dihydroxyvitamin D in postmenopausal osteoporosis. J Clin Endocrinol Metab 50:593, 1980.
66. Zerwekh JE, Sakhaee K, Glass K, Pak CYC. Long term 25-hydroxyvitamin D_3 therapy in postmenopausal osteoporosis: demonstration of responsive and nonresponsive subgroups. J Clin Endocrinol Metab 56:410, 1983.
67. Breslau NA, Clarke M: unpublished observations.
68. Gallagher JC, Nordin BEC. Effects of oestrogen and progestogen therapy on calcium metabolism in postmenopausal women. Front Horm Res 3:150, 1975.
69. Heaney RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. J Lab Clin Med 92:953, 1978.
70. Cohn SH, Vaswani A, Zanzi I, et al. Effect of aging on bone mass in adult women. Am J Physiol 230:143, 1976.
71. Smith DM, Khairi MRA, Norton J et al. Age and activity effects on rate of bone mineral loss. J Clin Invest 58:716, 1976.
72. Genant HK, Cann CE, Ettinger B, Gordan GS. Quantitative computed tomography of vertebral spongiosa: a sensitive method for detecting early bone loss after oophorectomy. Ann Int Med 97:699, 1982.
73. Lindsay R, Hart DM, Aitken JM et al. Long-term prevention of postmenopausal osteoporosis by oestrogen: evidence for an increased bone mass after delayed onset of estrogen treatment. Lancet 1:1038, 1976.

74. Lindsay R, Hart DM, Forrest C, Baird C. Prevention of spinal osteoporosis in oophorectomized women. Lancet 2:1151, 1980.
75. Nachtigall LE, Nachtigall RH, Nachtigall RD, Beckman EM. Estrogen replacement therapy I: a 10-year prospective study in the relationship to osteoporosis. Obstet Gynecol 53:277, 1979.
76. Recker RR, Saville PD, Heaney RP. Effect of estrogens and calcium carbonate on bone loss in postmenopausal women. Ann Int Med 87:649, 1977.
77. Marshall DH, Horsman A, Nordin BEC. The prevention and management of postmenopausal osteoporosis. Acta Obstet Gynecol Scand (Suppl) 65:49, 1977.
78. Christiansen C, Christensen MS, Larsen NE, Transbol I. Pathophysiologic mechanisms of estrogen effect on bone metabolism. Dose-response relationships in early postmenopausal women. J Clin Endocrinol Metab 55:1124, 1982.
79. Meema S, Bunker ML, Meema HE. Preventive effect of estrogen on postmenopausal bone loss. A follow-up study. Arch Int Med 135:1436, 1975.
80. Krolner B, Pors Nielsen S. Bone mineral content of the lumbar spine in normal and osteoporotic women: cross-sectional and longitudinal studies. Clin Sci 62:329, 1982.
81. Riggs BL, Wahner HW, Dunn WL et al. Differential changes in bone mineral density of the appendicular and axial skeleton with aging. J Clin Invest 67:328, 1981.
82. Johannson BW, Kaij L, Kullander S et al. On some late effects of bilateral oophorectomy in the age range 15-30 years. Acta Obstet Gynecol Scand 54:449, 1975.
83. Klibanski A, Neer RM, Beitins IZ et al. Decreased bone density in hyperprolactinemic women. N Engl J med 303:1511, 1980.
84. Schlechte JA, Sherman B, Martin R. Bone density in amenorrheic women with and without hyperprolactinemia. J Clin Endocrinol Metab 56:1120, 1983.
85. Daughaday W, McKeel D. Clinicopathologic Conference: Pituitary mass and osteopenia in a middle-aged woman. Am J Med 72:259, 1982.
86. Daniell HW. Osteoporosis of the slender smoker. Arch Int Med 136:298, 1976.
87. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 304:313, 1981.
88. Schindler AE, Ebert A, Friedrich E. Conversion of androstenedione to estrone by human fat tissue. J Clin Endocrinol Metab 35:627, 1972.
89. Goldin BR, Adlercreutz H, Gorbach SL et al. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. N Engl J Med 307:1542, 1982.
90. Aitken JM, Hart DM, Lindsay R. Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. Brit Med J 3:515, 1973.
91. Lindsay R, Maclean A, Kraszewski A et al. Bone response to termination of oestrogen treatment. Lancet 1:1325, 1978.
92. Horsman A, Gallagher JC, Simpson M, Nordin BEC. Prospective trial of oestrogen and calcium in postmenopausal women. Brit Med J 2:789, 1977.
93. Nordin BEC, Horsman A, Crilly RG, Marshall DH, Simpson M. Treatment of spinal osteoporosis in postmenopausal women. Brit Med J :451, 1980.

94. Christiansen C, Christensen MS, Transbol I. Bone mass in post-menopausal women after withdrawal of oestrogen/gestagen replacement therapy. Lancet 1:459, 1981.
95. Hutchinson TA, Polansky SM, Feinstein AR. Post-menopausal oestrogens protect against fractures of hip and distal radius. A case control study. Lancet:705, 1979.
96. Weiss NS, Ure CL, Ballard JH, Williams AR, Daling JR. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 303:1195, 1980.
97. Riggs BL, Seeman E, Hodgson SF, Taves DR, O'Fallon WM. Effect of the fluoride/calcium regimen on vertebral fracture occurrence in post-menopausal osteoporosis. Comparison with conventional therapy. N Engl J Med 306:446, 1982.
98. Lindsay R, Fogelman I, Hart DM. Minimum effective dose of estrogen for protection against postmenopausal bone loss. Calcif Tissue Int 34 (Suppl):S33, 1982 (Abstract).
99. Ryan KJ et al. Estrogen ues and postmenopausal women: A National Institutes of Health Consensus Development Conference. Ann Int Med 91:921, 1979.
100. Wilson JD. Use of estrogens after the menopause. Southwestern Medical Grand Rounds. July 10, 1980.
101. Henderson BE. Arteriosclerotic heart disease, pp 198-199. In: Judd HL, moderator. UCLA Conference on estrogen replacement therapy: indications and complications. Ann Int Med 98:195, 1983.
102. Ross RK, Paganini-Hill A, Mack TM, Arthur M, Henderson BE. Menopausal oestrogen therapy and protection from death from ischaemic heart disease. Lancet 1:858, 1981.
103. Judd HL, Hypertension, p 201. In: Judd HL, moderator. UCLA Conference on estrogen replacement therapy: indications and complications. Ann Int Med 98:195, 1983.
104. Judd HL. Lipids, p 202. In: Judd HL, moderator. UCLA Conference on estrogen replacement therapy: indications and complications. Ann Int Med 98:195, 1983.
105. Wahl P et al. Effect of estrogen/progestin potency on lipid/lipoprotein cholesterol. N Engl J Med 308:862, 1983.
106. Council on Scientific Affairs, American Medical Association. Estrogen replacement in the menopause. JAMA 249:359, 1983.
107. Gambrell RD Jr. Role of hormones in the etiology and prevention of endometrial and breast cancer. Acta Obstet Gynecol Scand Suppl 106:37, 1982.
108. Greenblatt RB, Gambrell RD Jr, Stoddard LD. The protective role of progesterone in the prevention of endometrial cancer. Path Res Pract 174:297, 1982.
109. Weinstein MC. Estrogen use in postmenopausal women--costs, risks and benefits. N Engl J Med 303:308, 1980.
110. MacDonald PC. Estrogen plus progestin in postmenopausal women. N Engl J Med 305:1644, 1981.
111. Heath H III. Progress against osteoporosis. Ann Int Med 98:1011, 1983.
112. Heaney RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. J Lab Clin Med 92:953, 1978.

113. Riggs BL, Jowsey J, Kelly PJ, Hoffman DL, Arnaud CD. Effects of oral therapy with calcium and vitamin D in primary osteoporosis. J Clin Endocrinol Metab 42:1139, 1976.
114. Davies M, Mawer EB, Adams PH. Vitamin D metabolism and the response to 1,25-$(OH)_2D$ in osteoporosis. J Clin Endocrinol Metab 45:199, 1977.
115. Christiansen C, Christensen MS, Rodbro P, Hagen C, Transbol I. Effect of 1,25-dihydroxyvitamin D in itself or combined with hormone treatment in preventing postmenopausal osteoporosis. Europ J Clin Invest 11:305, 1981.
116. Pak CYC. Management of postmenopausal osteoporosis. Southwestern Medical School Grand Rounds. October 14, 1982.
117. Riggins RS, Rucker RC, Chan MM, Zeman F, Beljan JR. The effect of fluoride supplementation on the strength of osteopenic bone. Clin Orthop 114:352, 1976.
118. Gallagher JC, Nordin BEC. Treatment with estrogens of primary hyperparathyroidism in postmenopausal women. Lancet:503, 1972.
119. Gallagher JC, Wilkinson R. The effect of ethinyl estradiol on calcium and phosphorus metabolism of postmenopausal women with primary hyperparathyroidism. Clin Sci Mol Med 45:785, 1973.
120. Leppla DC, Northcutt C, Snyder W, Pak CYC. Sequential changes in bone density before and after parathyroidectomy in primary hyperparathyroidism. Invest Radiol 17:604, 1982.
121. Meunier PJ, Bressot C, Edouard C. Dynamics of bone remodeling in primary hyperparathyroidism: histomorphometric data. In: Endocrinology of Calcium Metabolism (Eds: Copp DH, Talmage RV) Excerpta Medica, Amsterdam, p 415, 1978.
122. Burkhart JM, Jowsey J. Parathyroid and thyroid hormones in the development of immobilization osteoporosis. Endocrinology 81:1053, 1967.
123. Orimo H, Fujita T, Yoshikawa M et al. Effect of estrogen on immobilization osteoporosis in rat. Endocrinology 88:102, 1971.