bination therapy with an inhibitor of osteolysis such as calcitonin. It may also prove more effective to interrupt the course of treatment with regular rest periods.

We thank Mrs C. Tait, Mr J. R. Green, Mr G. P. Gibbs, and Mr R. Blows for skilled technical assistance. The Armour Pharmaceutical Company allowed us to use their facilities for sterile ampouling of the hP-T-H- 1–34 and Miss W. Davis of the hospital pharmacy prepared the ampouled diluent. J. R. is a M.R.C. clinical research fellow.

Requests for reprints should be addressed to J. A. P.

REFERENCES

1. Parsons, J. A. *in* Biochemistry and Physiology of Bone (edited by G. H. Bourne; vol. IV. New York (in the press).
2. Bauer, W., Aub, J. C., Albright, F. *J. exp. Med.* 1929, **49**, 145.
3. Selye, H. *Endocrinology*, 1932, **16**, 547.
4. Jaffe, H. L. *Archs Path.* 1933, **16**, 63.
5. Pugsley, L. I., Selye, H. *J. Physiol.* 1933, **79**, 113.
6. Shelling, D. H., Asher, D. E., Jackson, D. A. *Bull. Johns Hopkins Hosp.* 1933, **53**, 348.
7. Burrows, R. B. *Am. J. Anat.* 1938, **62**, 237.
8. Kalu, D. N., Pennock, J., Doyle, F. H., Foster, G. V. *Lancet*, 1970, i, 1363.
9. Walker, D. G. *Endocrinology*, 1971, **89**, 1389.
10. Hodgkinson, A. *Clin. Sci.* 1963, **25**, 231.
11. Lloyd, H. M. *Medicine, Baltimore*, 1968, **47**, 53.
12. O'Riordan, J. L. H., Watson, L., Woodhead, J. S. *Clin. Endocr.* 1972, **1**, 149.
13. Parsons, J. A., Reit, B. *Nature*, 1974, **250**, 254.
14. Niall, H. D., Sauer, R., Jacobs, J. W., Keutmann, H. T., Segre, G. V., O'Riordan, J. L. H., Aurbach, G. D., Potts, J. T. Jr. *Proc. natn. Acad. Sci. U.S.A.* 1974, **71**, 384.
15. Tregear, G. W., Rietschoten, J. van, Greene, E., Keutmann, H. T., Niall, H. D., Reit, B., Parsons, J. A., Potts, J. T. Jr. *Endocrinology*, 1973, **93**, 1349.
16. Reeve, J., Hesp, R., Veall, N. *Br. med. J.* 1974, iii, 310.
17. Marshall, J. H. *Int. atomic Energy Tech. Rep.* 1964, **32**, 21.
18. Burkinshaw, L., Marshall, D. H., Oxby, C. B., Spiers, F. W., Nordin, B. E. C., Young, M. M. *Nature*, 1969, **222**, 146.
19. Reeve, J., Wootton, R., Hesp, R. *Calcif. Tissue Res.* (in the press).
20. Seymour, G. C., Jackson, M. J. *Clin. Chem.* 1974, **20**, 544.
21. West, R. R., Reed, G. W. *Br. J. Radiol.* 1970, **43**, 886.
22. Rafferty, B., Zanelli, J. M., Parsons, J. A. Unpublished.
23. Segre, G. V., Habener, J. F., Powell, D., Tregear, G. W., Potts, J. T., Jr. *J. clin. Invest.* 1972, **51**, 3163.
24. Hunter, M. W., Greenwood, F. C. *Nature*, 1966, **194**, 495.
25. Albright, F., Reifenstein, E. C. The Parathyroid Glands and Metabolic Bone Disease. Baltimore, 1948.
26. Reeve, J., Tregear, G. W., Parsons, J. A. *Calcif. Tissue Res.* (in the press).
27. Marshall, J. H., Linjecki, J., Lloyd, E. L., Marotti, G., Mays, C. W., Rundo, J., Sissons, H. A., Snyder, W. S. *Hlth Phys.* 1973, **24**, 125.

LONG-TERM PREVENTION OF POSTMENOPAUSAL OSTEOPOROSIS BY ŒSTROGEN

EVIDENCE FOR AN INCREASED BONE MASS AFTER DELAYED ONSET OF ŒSTROGEN TREATMENT

R. LINDSAY D. M. HART
J. M. AITKEN E. B. MACDONALD
J. B. ANDERSON A. C. CLARKE

Department of Medicine, Western Infirmary, Glasgow G11 6NT

Summary Treatment of 63 oophorectomised women with œstrogen for five years prevented the reduction in bone mineral content observed in 57 women treated with a placebo preparation. When onset of therapy was delayed for three to six years there was a highly significant increase in bone mineral content mainly during the first three years of treatment. During the next two years there was no further increase in bone mineral, while the placebo-treated groups continued to lose bone at about 1% per annum. In association with the changes in bone mass, the expected biochemical effects of œstrogen therapy also persisted for at least three years, and were compatible with a prolonged increase in parathyroid activity but a reduction in bone turnover.

Introduction

UNTIL recently postmenopausal osteoporosis was not diagnosed until clinical or X-ray changes were obvious, and when bone loss was substantial. Photon absorption techniques have enabled follow-up of an at risk population and evaluation of the comparatively small changes occurring annually. Using this technique we found that œstrogen therapy had a short-term preventive effect on bone loss after oophorectomy.[1] The more long-term study of Meema[2] using measurements at the proximal radius suggested not only a preventive effect but also a gain in bone mineral content in their œstrogen-treated group. However, their study was based on only two measurements of each subject, these estimations being separated by at least four years. Varying œstrogen-containing preparations were used, the most common being conjugated equine œstrogens, and the dosage varied from patient to patient.

In an attempt to verify that œstrogen therapy produced a gain in bone mineral content we examined data from a five-year follow-up programme involving a controlled trial of mestranol therapy in oophorectomised women. Preliminary observations had suggested that a slight increase in bone mass might be evident in women in whom treatment was started three years after oophorectomy.[1]

Patients and Methods

Altogether 120 patients who were part of the original trial of œstrogen therapy[1] were followed five years. Of these patients 24 were followed from oophorectomy, 64 from three years after oophorectomy, and 32 from six years after operation. None had received œstrogen replacement before and all were given identical active or placebo tablets in a double-blind fashion. Tablets were supplied in boxes of 120. Further supplies were obtained by returning a coded postcard to the hospital pharmacy. By counting these postcards it was possible to obtain a rough estimate of the number of tablets consumed by each patient. Placebo and mestranol tablets were of identical appearance during each year of the trial. The mean daily dose of mestranol was 24·8 μg. For the first three years of therapy the patients were followed up every six months, and fasting blood and urine samples were obtained annually.

Biochemical measurements on serum and urine were carried out following standard techniques. Calcium was estimated by atomic absorption spectrophotometry.[3] Standard 'AutoAnalyzer' techniques were used to estimate creatinine (N 11), phosphorus (N 4), alanine and aspartate transaminases (N 44). Serum-alkaline-phosphatase was estimated by the method of King and Wooton.[4] Urinary hydroxyproline was measured by the method of Stegemann and Stalder.[5]

Every six months during the first three years and thereafter annually, photon absorption measurements were made at the mid-point of the third metacarpal of the right hand.[6] In 46 of the 64 patients who started attending three years after oophorectomy, photon absorption estimations were carried out, at similar intervals, at the mid-radius site, in addition to the mid-metacarpal.

Results

The initial bone mineral content in the three groups of women at their initial review are shown in table I. These results agree closely with other examples of cross-

TABLE I—INITIAL BONE MINERAL MEASUREMENTS (MEAN ±S.E.M.) BEFORE MESTRANOL THERAPY

	Time after oophorectomy		
	2 mo (A)	3 yr (B)	6 yr (C)
No. of patients	24	64	32
Age (mean±S.E.D.)	44·6±0·7	47·9±0·5	50·8±0·6
Initial metacarpal mineral content (mg/mm)	45·1±0·97	41·1±0·61	40·1±0·78

TABLE II—MEAN (±S.E.M.) ANNUAL CHANGES IN METACARPAL BONE MINERAL CONTENT DURING 5-YEAR FOLLOW-UP

	Follow up from:		
	2 mo (A)	3 yrs (B)	6 yrs (C)
Mestranol group (mg mm^{-1} yr^{-1})	−0·02±0·23(12)	0·57±0·09(34)	0·31±0·11(17)
Placebo group (mg mm^{-1} yr^{-1})	−1·22±0·11(12)	−0·30±0·10(30)	−0·44±0·13(15)
P	$<10^{-4}$	$<10^{-7}$	$<25^{-6}$

Number of patients is shown in parentheses.

sectional data using radiological methods,[7] morphometric measurements,[8] and photon absorption.[9 10] The results in the table imply that bone is lost most rapidly in the initial three-year period after oophorectomy, and relatively more slowly thereafter. At this stage the amount of bone lost is still equivalent to 8% per decade, a value similar to that obtained from a review of published reports.[11]

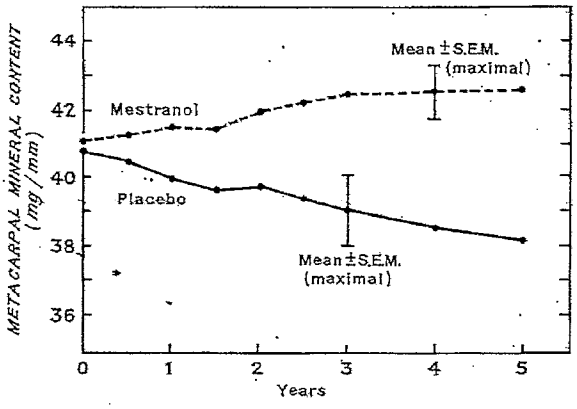
Changes in Bone Mineral Content during Therapy

The mean annual changes in bone mineral during the five-year follow-up are shown in table II. The placebo-treated patients followed from two months after operation (group A) lost bone at a mean rate of 1·22 mg mm^{-1} $year^{-1}$, which represents a fall in bone density of 2·7% per year. The difference between this value and that of 3·9% obtained in an earlier study[12] is almost entirely due to the longer follow-up period in our current study. The mean bone density in the mestranol-treated group fell by 0·02 mg mm^{-1} $year^{-1}$ or 0·4% per year; a result which was highly significantly different from the loss of bone experienced by the control group.

Those patients who were treated from three years after oophorectomy (group B) experienced a loss equivalent to 0·7% per annum, somewhat less than that achieved by group C who were not seen until six years after their operation (1·2% per annum). In groups B and C both mestranol-treated groups gained significant amounts of bone during the trial. The trial mean bone density of the mestranol-treated patients in group B was 42·6 S.E.M. mg/mm and for group C was 41·1±0·6 mg/mm, both values being significantly higher than the initial mean bone densities of each group (group B, P <0·0001; group P <0·004). Bone increase in group B occurred predominantly during the first three years of therapy, lessening somewhat during the final two years (see accompanying figure). Bone loss in the placebo-treated group occurred at a constant mean rate throughout the five years of observation.

Biochemical Changes

Studies of the biochemical changes in blood and urine are usually short-term ones.[1 13] In view of the evidence of a continuing action of œstrogen on bone we examined in detail the important biochemical parameters of mineral metabolism over a three-year period. All measurements were made yearly in fasting patients. The results, all obtained from group B, are shown in table III. Serum calcium, phosphate, and alkaline phosphatase fell significantly in the patients who received mestranol, and except for serum-calcium remained significantly below the values in the placebo-treated group throughout the next three years. After this period mean serum-



Mean metacarpal mineral content during 5-year follow-up of group observed from 3 years after bilateral oophorectomy (zero time).

Patients given a placebo lost bone mass continuously (with unexplained exception of the period between 1·5 and 2 yr) at an average rate of 0·7 mg/mm^{-1} yr^{-1}, mean fall becoming significant after 1·5 yr. Patients given mestranol gained bone mass for first 3 yr (P<0·001) showing very little increase thereafter. Treatment groups are significantly different (P<0·01) after only 1 year's treatment.

TABLE III—SERUM CALCIUM, PHOSPHATE, AND ALKALINE PHOSPHATASE (MEAN±S.E.M.) DURING A 3-YEAR PERIOD OF THERAPY WITH MESTRANOL (A) OR PLACEBO (P)

	No.	Therapy	Treatment yr 0	1	2	3
Serum-calcium (mmol/l)	30	P	2·46±0·02	2·45±0·02	2·44±0·02	2·43±0·02
	34	A	2·46±0·02	2·39±0·01	2·38±0·01	2·40±0·02
			N.S.	P<0·005	P<0·004	N.S.
Serum-phosphate (mmol/l)	30	P	1·11±0·02	1·14±0·02	1·12±0·02	1·13±0·02
	34	A	1·13±0·02	0·94±0·02	0·98±0·03	0·99±0·03
			N.S.	P<0·00001	P<0·00002	P<0·00001
Alkaline phosphatase (King Armstrong units)	30	P	7·69±0·39	7·96±0·42	8·78±0·53	9·18±0·34
	24	A	7·66±0·38	6·31±0·35	7·00±0·31	7·26±0·31
			N.S.	P=0·001	P=0·002	P<0·0005

TABLE IV—RENAL HANDLING OF CALCIUM, HYDROXYPROLINE, AND PHOSPHATE DURING 3 YEARS' THERAPY WITH MESTRANOL (A) AND PLACEBO (P)

—	No.	Therapy	Treatment yr 0	1	2	3
Calcium/Creatinine (mean±S.E.M.)	30 34	P A	0·113±0·009 0·116±0·008 N.S.	0·114±0·01 0·071±0·006 P<0·0001	0·126±0·01 0·082±0·01 P<0·0005	0·114±0·01 0·085±0·0 P<0·01
Hydroxyproline/Creatinine (mean±S.E.M.)	30 34	P A	0·026±0·003 0·022±0·002 N.S.	0·021±0·002 0·011±0·001 P<0·00005	0·020±0·001 0·013±0·001 P<0·0025	0·022±0·001 0·016±0·001 P<0·001
$TmPo_4$/G.F.R. (mean±S.E.M.)	30 34	P A	3·29±0·11 3·39±0·09 N.S.	3·33±0·10 2·76±0·08 P<0·00002	3·38±0·10 2·87±0·11 P<0·0005	3·37±0·09 2·89±0·10 P<0·005

calcium in the mestranol-treated group was not significantly less than that in the control group, although it was still significantly different ($P < 0.0025$ by paired t test) from the original concentration in the mestranol-treated group. This change in pattern was produced by an unexplained (and non-significant) fall in the mean serum-calcium in the control group.

Excretion of calcium and hydroxyproline, expressed as ratios per unit creatinine, was significantly reduced. This remained constant for the three years of observation, although the level of significance had fallen slightly by the end of the third year. The setting of phosphate reabsorption (tubular maximum for PO_4/glomerular filtration-rate) as expected was reduced by œstrogen therapy and remained highly significantly reduced during the three-year follow-up. The mean values of aspartate (S.G.O.T.) and alanine (S.G.P.T.) transaminases were unaltered by mestranol therapy.

Discussion

Œstrogen therapy had beneficial short-term effects in retarding bone loss after a natural or artificial menopause.[1 14 15] Memma et al.[2] demonstrated a possible rise in bone density during œstrogen replacement therapy, but their study had several faults. In particular, the œstrogen preparations and dosages were not standard and their most commonly prescribed single preparation contains several œstrogens. Not all patients who were prescribed œstrogen necessarily took the tablets regularly or for the complete period between measurements. Follow-up ranged from 4 to 10 years. Each patient was measured only twice and the intervals between natural or artificial menopause, the first bone mineral estimation, and the onset of œstrogen therapy, were not stated clearly.

Our trial procedure was conducted in a double-blind fashion and our patients consisted of three homogeneous groups of women followed for exactly five years, in whom bone density was measured at six-monthly intervals. We demonstrated that bone loss after oophorectomy in this age-group is pronounced, in accord with others.[1 2] It is likely that the rate of bone loss is greatest immediately after loss of ovarian function (mean 2·7% per year), diminishing after three or more years to a steady mean loss of about 0·7% per annum.

Therapy with mestranol prevented this bone loss in all three groups of patients. However, the response of patients whose therapy was delayed for three or six years after oophorectomy indicated an important increase in bone density which seemed to be maintained for the five-year period of follow-up. Since the metacarpal mineral content correlates well with that of the radius, femur, and spine,[16] it is reasonable to assume that the changes described here are representative of skeletal behaviour as a whole.

The short-term biochemical effects of œstrogen therapy are relatively well known.[13 17] Our present study indicates that the hypophosphatæmic, hyperphosphaturic, and hypocalciuric actions of œstrogen persist for at least three years after initiation of therapy. This is true also of the reduction in serum-alkaline-phosphatase and urinary hydroxyproline. Although serum-calcium at the end of three years was not significantly different in mestranol and placebo treated patients, the initial significant fall was still present when the paired t test was used to compare the initial and final values for serum-calcium in the mestranol group ($P < 0.0025$).

With the exception of the fall in serum-calcium which may be at least partially dilutional,[18] the reduction in serum-phosphate and increase in urinary phosphate and calcium are indicative of increased parathyroid activity. Riggs et al.[19] demonstrated increased immunoreactive parathyroid hormone in the plasma of patients treated with œstrogens and a rise in urinary cyclic-A.M.P. has also been observed.[20] Low doses of a fragment of human parathyroid hormone had an anabolic effect on the skeleton in postmenopausal osteoporosis.[21] However, serum-alkaline-phosphatase and urinary hydroxyproline also fall in patients treated with œstrogens, and this expression of the reduction in bone turnover cannot be directly attributed to increased parathyroid activity.

Prolonged administration of low-dose mestranol was not associated with overt clinical evidence of thromboembolic disease in our patients. The effects of such long-term therapy on clotting function, and fat and carbohydrate metabolism must be determined. We believe that œstrogen treatment is effective in preventing postmenopausal osteoporosis as measured by a reduction in bone mineral content. However, whether œstrogen given at this stage will prevent the clinical complications of osteoporosis, such as fractures of long bones and crush fractures of the spine, remains to be seen.

Requests for reprints should be addressed to R. L.

REFERENCES

1. Aitken, J. M., Hart, D. M., Lindsay, R. *Br. med. J.* 1973, iii, 515.
2. Meema, S., Bunker, M. L., Meema, N. E. *Archs intern. med.* 1973, 135, 1436.
3. Trudeau, D. L., Freier, E. F. *Clin. Chem.* 1967, 13, 101.
4. King, E. J., Wooton, L. D. P. Microanalysis in Medical Biochemistry; p. 85. London, 1956.
5. Stegemann, H., Stalder, K. *Clinica chim. Acta*, 1967, 18, 267.
6. Shimmins, J., Smith, D. A., Aitken, J. M., Anderson, J. B., Gillespie, F. C. *Clin. Radiol.* 1972, 23, 47.
7. Smith, D. A., Anderson, J. B., Shimmins, J., Speirs, C. F., Barnett, E. *ibid.* 1969, 20, 23.
8. van Gerven, D. P. *J. Gerontol.* 1973, 28, 40.

References continued at foot of next column