PAPERS AND ORIGINALS

Plasma androstenedione and oestrone levels in normal and osteoporotic postmenopausal women

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Summary

Plasma androstenedione and oestrone concentrations were measured in 72 postmenopausal women. The women included some who had undergone oophorectomy, some with osteoporosis, and normal controls; they were matched for years since menopause. Both hormone concentrations were significantly reduced in the women with osteoporosis. The women who had undergone oophorectomy had hormone concentrations intermediate between the normal and osteoporotic values. Oestrogen deficiency secondary to low androstenedione levels is a major risk factor in postmenopausal osteoporosis, and may be caused by failure of ovarian stromal androgen secretion or some abnormality in the pituitary-adrenal axis.

Introduction

We have shown that the vaginal smear maturation values in postmenopausal women with osteoporosis are significantly lower than those in normal postmenopausal women¹ and suggested that this implies a more severe degree of "oestrogen deficiency" in the osteoporotic patients. We now report significantly reduced plasma oestrone and androstenedione concentrations in postmenopausal osteoporotic women compared with values in normal women matched for years since menopause.

Patients and methods

Four groups of postmenopausal women were matched for years since menopause. Each group comprised 18 women. Those in groups

1 and 2 presented with menopausal symptoms, the former having intact ovaries and the latter having undergone oophorectomy. Bone radiographs were normal in these patients. The mean ages of the women in groups 1 and 2 ($\pm$ SD) were 55.3 ± 6.3 years (range 42-69) and 52.5 ± 8.3 (range 39-67) years respectively. Women in groups 3 and 4 presented with backache, the former without spinal osteoporosis and the latter with multiple vertebral crush fractures. Three of the patients in each of groups 3 and 4 had also undergone oophorectomy. Women in group 3 had a mean age of 56.8 ± 5.6 (range 52-74) years, and those in group 4 were aged 58.1 ± 5.2 (range 52-68) years. The years since menopause ranged from 2 to 20 (mean 9.2 years).

Blood and urine samples were obtained from all patients at about 9.0 am, after an overnight fast. Plasma androstenedione and oestrone assays were measured by radioimmunoassay using antisera supplied by Miles Laboratories. Plasma (1 ml) was extracted with sodium-dried ether for the oestrone assay and 0.1 ml for the androstenedione assay. For the oestrone assay the extract was evaporated to dryness, and to it was added antiserum and 0.045 pmol (12 pg) [3 H₄]-oestrone in potassium phosphate buffer (pH 7.4). The solution was vortexed and incubated overnight at 4°C. On the following day dextran-coated charcoal was added, the tubes were centrifuged at 2000 rpm for 10 minutes, and the supernatant was decanted and collected for 10 minutes. For the androstenedione assay the procedure was similar but 0.22 pmol (63 pg) of [3 H₄]-androstenedione was used. The oestrone antibody was found not to interact with androstenedione, and vice versa. The intra-assay coefficient of variation of plasma androstenedione was 2% and that of oestrone 7%. The corresponding interassay errors were 10% and 20%. All measurements were performed in duplicate.

Results

The plasma androstenedione values are shown in fig 1 together with the means. The highest mean values were in the normal women (groups 1 and 3) and the lowest in the osteoporotic women (group 4), the differences between groups 1 and 3 and group 4 being highly significant. The mean value in the women who had had oophorectomies (group 2) was intermediate between these extremes (see table).

The corresponding oestrone values are also shown in fig 1. The pattern was very similar. The highest mean values were in groups 1 and 3 and the lowest in group 4. The mean value in group 2 was again intermediate between these extremes.

There was a correlation between the androstenedione and oestrone levels in the whole series of 72 patients (fig 2), the values being scattered around the curve representing the relation between these two variables (to be reported elsewhere). Most of the osteoporotic patients

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FIG 1—Plasma androstenedione and oestrone concentrations in four groups of postmenopausal women. $\circ$ = Women with intact ovaries. $\blacktriangle$ = Women who had undergone oophorectomy.

Conversion: SI to traditional units—Androstenedione: 1 nmol/l $\approx$ 28.8 ng/100 ml. Oestrone: 1 pmol/l $\approx$ 0.027 ng/100 ml.

Mean values ($\pm$ SE) of plasma oestrone and androstenedione in the four groups

Group	Plasma androstenedione (nmol/l)	Plasma oestrone (pmol/l)
1	3.46 $\pm$ 0.21	143 $\pm$ 9
2	2.87 $\pm$ 0.24	114 $\pm$ 6
3	3.54 $\pm$ 0.35	127 $\pm$ 9
4	2.18 $\pm$ 0.17	105 $\pm$ 7
Significance of differences (P values)		
1 v 2	NS	<0.02
1 v 3	NS	NS
1 v 4	<0.001	<0.005
2 v 3	NS	NS
2 v 4	<0.025	NS
3 v 4	<0.005	<0.05

Conversion: SI to traditional values—Androstenedione: 1 nmol/l $\approx$ 28.8 ng/100 ml. Oestrone: 1 pmol/l $\approx$ 0.027 ng/100 ml.

FIG 2—Relation between plasma androstenedione and oestrone concentrations in 72 postmenopausal women. $\circ$ = Women with normal bones. $\bullet$ = Women with osteoporosis.

were clustered around the lower part of this line, which suggested that their low plasma oestrone levels resulted from their low plasma androstenedione levels.²

Discussion

Although menopausal women as a group lose bone at a mean rate of about 0.5% per year, this mean value conceals a wide variation in the rate of bone loss, and we have reported elsewhere that women with osteoporosis lose bone faster than other postmenopausal women.³ One factor in this accelerated bone loss is probably malabsorption of calcium,¹ which may be attributable to a low plasma level of 1,25-dihydroxycholecalciferol.⁴⁻⁶ The other factor appears to be oestrogen deficiency, previously suspected on the indirect evidence of reduced vaginal smear maturation values^{1,5} and now confirmed by the direct measurement of plasma oestrone. Since oestrone is the main postmenopausal oestrogen, the observation that plasma oestrone levels are significantly reduced in osteoporotic patients points to a true oestrogen deficiency state. Since postmenopausal oestrone is derived largely from androstenedione by peripheral conversion,⁷ the cause might be a low conversion rate or low plasma level of androstenedione. We have not, however, found a reduced conversion rate in patients with fractures,⁸ and our present data show that the low plasma oestrone values in osteoporosis are associated with (and probably the result of) correspondingly low plasma androstenedione values. Our findings also confirm previous reports of low urinary androgen concentrations in postmenopausal osteoporosis.^{9,10}

The cause of the low plasma androstenedione levels is not clear, but since postmenopausal women obtain androstenedione from the adrenal glands and from the ovarian stroma, it must be assumed that the deficiency originates in one or both of these sites. It may therefore be relevant to note that the mean plasma androstenedione concentration of the women who had undergone oophorectomy fell between the mean values of the osteoporotic women and the normal women. This presumably reflected the loss in the former of the normal secretion of androstenedione by the postmenopausal ovary.^{11,12} The osteoporotic group (all but three of whom had intact ovaries) might therefore have been suffering from a comparable lack of ovarian androgen due to functional failure of the ovarian stroma. If this is the whole explanation of our data in osteoporosis, however, it seems surprising that the mean hormone levels in the osteoporotic patients were even lower than those in the group of women who had undergone oophorectomy. Moreover, the proportion of women who had had an oophorectomy (three out of 18) was no higher in group 4 (osteoporotic) than in group 3 (normal), as might have been expected had deficiency of ovarian androgens been a major factor in the genesis of osteoporosis. It is true that Albright,¹³ who also found that 15% of his postmenopausal osteoporotic patients had undergone oophorectomy, postulated a significant association between oophorectomy and osteoporosis, but our data do not really confirm this. We do not know, of course, the proportion of women who have had an oophorectomy in the postmenopausal population as a whole—it may be less than 15%. Nevertheless, if ovarian stromal failure does predispose to osteoporosis we would have expected to find more women who had had an oophorectomy in group 4 than in group 3.

An alternative explanation is that the low plasma androstenedione levels in postmenopausal osteoporosis are due to reduced adrenal secretion. This may in turn be due to adrenal or pituitary factors and requires further investigation.

Although we cannot exclude the possibility that androstenedione (or the testosterone derived from it) is a bone-regulating hormone in its own right, we are assuming at present that its importance derives from its role as the main source of postmenopausal oestrogen. Our present data are therefore compatible with the concept that oestrogen deficiency is one of the two main risk factors in the genesis of postmenopausal osteoporosis, the other being malabsorption of calcium.^{1,14} It is

therefore not surprising that the most effective treatment, judged by balance studies, appears to be the combination of 1 α -hydroxycholecalciferol (or 1,25-dihydroxycholecalciferol) and oestrogens.^{15 16}

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Prolactin concentrations in ovulatory but infertile women: treatment with bromocriptine

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Summary

We measured basal plasma prolactin concentrations (in samples obtained during the early follicular phase) in 25 normal (control) women and in a similar group of 40 patients with a long-standing history of infertility. The infertile patients were all ovulating regularly, and had been unsuccessfully treated with clomiphene and in some cases dydrogesterone and human menopausal gonadotrophin. Although none of the patients had plasma prolactin concentrations > 1000 μ U/ml, 47.5% of the estimations were greater than 1 standard deviation (SD) above the mean established for our control group. This difference was highly significant ($P < 0.001$). Treatment with various bromocriptine regimens effectively reduced prolactin concentrations to below normal in all cases, and 16 pregnancies followed—13 during bromocriptine treatment and three in the first post-treatment cycle. The cumulative conception rate was 63.4% after 10 months' treatment.

Introduction

Plasma prolactin concentrations are consistently raised in patients with Chiari-Frommel syndrome¹ and in some amenorrhoeic women with or without galactorrhoea.²⁻⁶ Treatment with bromocriptine (2-bromo- α -ergocryptine) has effectively reduced circulating prolactin concentrations, and induced ovulation (and conception) in most.¹⁻⁸ Bromocriptine may also restore cyclical menstruation in euprolactinaemic amenorrhoea,⁹ and improve luteal function in normally men-

struating patients who nevertheless have hyperprolactinaemia and short luteal phases.^{10 11} We undertook the present study to determine whether an appreciable number of regularly ovulating women with long-standing infertility also have raised plasma prolactin concentrations, and to determine the therapeutic effectiveness of treatment with bromocriptine for this particular type of infertility.

Patients and methods

Initially 40 patients with long-standing infertility (range 2-10 years) were selected for assessment of basal prolactin concentrations and treatment with bromocriptine. Although 13 (32.5%) of the women had had a previous pregnancy this had ended in an abortion in 10 (25%) and a full-term intrauterine death in one patient. Only two patients had given birth to a living child. These women had all been fully investigated and were found to be ovulating regularly, with cycles of normal length (25-35 days). No reason for their infertility was shown by routine investigations (which included seminal analysis, hysterosalpingogram in all patients, and laparoscopy in 20), but we felt that these patients were similar to women with defective luteal phases.^{12 13} Preliminary findings showed that none of these women had a short luteal phase—that is, a luteal phase lasting less than 10 days (criteria of Sherman and Korenman¹⁴)—and that they had ovulated in the cycles investigated. We considered ovulation to have occurred if a surge of luteinising hormone preceded a rise of plasma progesterone in all cases. Blood samples for prolactin measurement were obtained on two occasions (between 0800 and 1000 hours) within the first five days of each patient's basal cycle. Similar samples were obtained at the same time in the cycle from a group of 25 normal (control) women—that is, fertile women with no known gynaecological or medical abnormality or relevant drug history. Plasma was separated within 45 minutes and stored at -20°C until assayed for prolactin.

Hormone assay—Plasma prolactin was measured by radioimmunoassay,¹⁵ using the reagents provided by the National Pituitary Agency (antisera and hormone for labelling), and the standard distributed by the Medical Research Council Division of Biological Standards (Research Standard A71/222), which was used to calibrate our own laboratory standard (a pool of serum from lactating women). All results are expressed in μ U/ml in terms of the MRC 71/222 preparation. (Approximately 40 μ U/ml of this are equivalent to 1 ng/ml NIH VLS1). All the prolactin estimations were performed in two assays to eliminate possible interassay variation.

Treatment regimen—The patients were randomly allocated to one of four groups. Bromocriptine (Parlodel, Sandoz) was given throughout

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