

# Effects on climacteric symptoms, bone and lipoprotein metabolism of hormone replacement therapy delivered by estradiol-releasing intravaginal rings: a pilot study

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## ABSTRACT

**Objective** To assess the efficacy and tolerability of intravaginal rings (IVRs) delivering estradiol.

**Design** This was a dose-escalating, continuous-dosing, pilot study.

**Methods** Sixteen women post surgical menopause were recruited at a hospital-based menopause clinic. Over 20 weeks, each patient had IVR devices releasing 50, 75, 100, 150 and 200 µg/day of estradiol inserted consecutively at 4-weekly intervals.

**Main outcome measures** Climacteric symptoms were assessed, and levels of serum estradiol, lipoproteins and biochemical indices of bone turnover were estimated prior to insertion of the first IVR and at each monthly visit, when the IVR was changed to one of a higher dose. The susceptibility of low-density lipoprotein (LDL) to oxidation was assessed at 0, 12 and 20 weeks.

**Results** Twelve women completed the study. The rings were well tolerated and serum estradiol levels increased in parallel with each increasing dose. Vasomotor and psychological symptoms and loss of libido were reduced by 76% ( $p < 0.001$ ), 44% ( $p < 0.001$ ) and 44% ( $p < 0.05$ ), respectively, by the end of the study. There were no significant changes in levels of serum lipoproteins, although the ratio of LDL cholesterol to high-density lipoprotein cholesterol decreased by 7.2% ( $p = 0.01$ ) after 20 weeks. The susceptibility of LDL to oxidation did not change. Urinary excretion of both calcium and deoxypyridinoline cross-links decreased significantly ( $p < 0.001$ ), indicating a reduction in bone resorption.

**Conclusions** The rings were effective in controlling climacteric symptoms and had beneficial effects on bone metabolism, but no significant effects on lipoprotein levels or the susceptibility of LDL to oxidation.

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## INTRODUCTION

Estrogen replacement therapy is an established treatment for the relief of climacteric symptoms. Long-term therapy protects against postmenopausal osteoporosis<sup>1</sup>, and evidence from numerous observational studies suggests that it reduces the risk of cardiovascular disease<sup>2,3</sup>. However, there is apparent dichotomy between observational studies and randomized clinical trials. Recently, the Women's Health Initiative (WHI) primary prevention trial was stopped early, owing to increased risk of invasive breast cancer and coronary heart disease (CHD) in the group of women treated with estrogen plus progestin<sup>4</sup>. A parallel WHI study of unopposed oral estrogen in hysterectomized women is ongoing, since the balance of risks and benefits in that component remains uncertain.

There are several routes of administration available for estrogen replacement: oral, transdermal, subcutaneous, nasal and vaginal. Estrogen given orally is subjected to first-pass metabolism in the liver and intestine. Non-oral therapy perhaps reflects more closely endogenous hormone activity, and is often well tolerated in women who experience gastrointestinal side-effects with oral therapy<sup>5</sup>.

However, there are some disadvantages with non-oral delivery systems. The main disadvantages of transdermal therapy are the possibility of localized irritation and the need to replace the patch frequently. This can lead to fluctuating plasma estrogen levels and problems with concordance. Subcutaneous implants require a minor surgical procedure for insertion, with the possibility of wound infection, and are difficult to remove should a problem occur; also, occasionally the patient experiences tachyphylaxis<sup>6</sup>. Intravaginal delivery overcomes many of these disadvantages, and several studies have indicated that plasma estradiol levels in the range associated with effective estrogen replacement therapy can be achieved by means of intravaginal rings (IVRs)<sup>7-11</sup>. A ring device that releases very low-dose 17 $\beta$ -estradiol, and is currently available as topical treatment for vaginal atrophy<sup>12,13</sup>, has demonstrated that intravaginal delivery of estrogen is both safe and acceptable.

Intravaginal ring devices containing 17 $\beta$ -estradiol-3-acetate have recently been developed to deliver estradiol for a minimum of 3 months, in predetermined daily doses ranging from those typically released from transdermal patches to the higher doses achieved by subcutaneous implants<sup>14</sup>. On contact with body fluids, 17 $\beta$ -

estradiol-3-acetate is hydrolyzed immediately to estradiol, the active moiety. An IVR that releases the equivalent of 50  $\mu$ g/day estradiol has recently become available in the UK for the relief of vasomotor and urogenital symptoms (Menoring<sup>®</sup>; Galen Ltd, Northern Ireland, UK).

A pilot dose-escalating study was initiated to establish whether the plasma levels of estradiol obtained from the rings were adequate for the relief of climacteric symptoms in a group of postmenopausal women. As a secondary objective, biochemical indices of bone turnover and lipoprotein cardiovascular risk markers, including susceptibility of low-density lipoprotein (LDL) to oxidative modification, were also monitored during this investigation of the efficacy, safety and tolerability of intravaginal ring devices.

## PATIENTS AND METHODS

Sixteen women, mean ( $\pm$  standard deviation (SD)) age 47  $\pm$  8 years, were recruited from patients attending menopause clinics at Stobhill Hospital, Glasgow, for the treatment of climacteric symptoms. All had undergone a hysterectomy and bilateral oophorectomy for benign gynecological disease at least 2 months prior to recruitment. None had received any previous hormonal therapy or was taking any drug known to affect lipid or bone metabolism. There was no contraindication to estrogen treatment in any case. The protocol was approved by the hospital ethical committee and informed consent was obtained from all participants.

The study period was 20 weeks, during which IVR devices manufactured by Galen Ltd, UK, releasing 50, 75, 100, 150 and 200  $\mu$ g/day of estradiol as its 3-acetate ester<sup>14</sup>, were inserted intravaginally, consecutively at 4-week intervals. There was no run-in or wash-out period between insertion of rings, as this would have been unacceptable to the patients.

Climacteric symptoms were assessed using the Greene menopausal symptoms scale<sup>15</sup> on two occasions prior to insertion of the first IVR and at each 4-weekly visit, when the ring was changed to one of a higher dose.

Fasting venous blood and urine samples were obtained for quantification of lipoproteins, biochemical indices of bone turnover and serum estradiol, at the same time-points. The susceptibility of LDL to oxidation was estimated at 0, 12 and 20 weeks.

The Greene climacteric scale questionnaire<sup>15</sup> is a widely used tool to provide a rapid but

comprehensive and valid measure of climacteric symptomatology. It is composed of a checklist that yields three main independent symptom measures: psychological (P), somatic (S) and vasomotor (V) symptoms. At every visit, each subject rated on a four-point scale (not at all = 0, a little = 1, quite a bit = 2, extremely = 3) the extent to which she was bothered by each of 20 symptoms contributing to these three factors. The calculation of scale scores is the sum of symptoms for each symptom category. The maximum possible scores (worst-case scenario) are P = 33, S = 21 and V = 6. The P scale can be subdivided to give measures of anxiety (A, maximum score = 18) and depression (D, maximum score = 15).

The scale can also be used to identify menopausal women who are severely and possibly clinically anxious and/or depressed. The recommended cut-off points are: clinically anxious = anxiety score of 10 or more; clinically depressed = depression score of 10 or more.

Loss of sexual libido score (Sx) was also assessed (maximum score = 3).

Serum was separated and stored at 4°C for a maximum of 5 days before lipoprotein fractionation and estimation of lipids. Aliquots of serum were also stored at -20°C for later batch measurement of estradiol levels. Further aliquots of heparinized plasma were separated within 30 min of being collected and stored at -80°C for later batch estimation of oxidation of LDL, and aliquots of urine were stored at -20°C for batch analysis of deoxypyridinoline.

Serum estradiol levels were measured by <sup>125</sup>I radioimmunoassay using a commercial kit (Immunodiagnostic Systems Ltd, Tyne and Wear, UK). Samples for all visits from each patient were analyzed in the same batch (within-batch coefficient of variation (CV) < 5% at all levels).

Serum cholesterol and triglyceride levels were estimated enzymatically. Cholesterol levels were measured in lipoprotein fractions isolated following ultracentrifugation, as described previously<sup>16</sup>.

The susceptibility to oxidation of LDL, isolated from heparinized plasma by single-step rapid ultracentrifugation, was assessed by measuring the lag time to copper-mediated oxidation, as described by McDowell and colleagues<sup>17</sup>. For each patient, samples taken at baseline and at 12 and 20 weeks were analyzed in the same batch (within-batch CV 1%).

Serum calcium (adjusted for albumin), phosphate, alkaline phosphatase and urine calcium/creatinine and hydroxyproline/creatinine were estimated by standard methods. Urinary levels of

deoxypyridinoline cross-links were estimated by an enzyme-linked immunosorbent assay method using a commercial kit (Metra Biosystems, Mountain View, CA, USA). For each patient, samples from all visits were analyzed in the same batch (within-batch CV < 8%, range 11–175 nmol/l).

All statistical analyses were carried out using the StatView statistical package (SAS Institute Inc., Cary, NC, USA). Results from the two pre-treatment visits were averaged to give a single baseline value for each parameter. Since the sample size was small, non-parametric analysis was used to compare within-group differences. Parameters during treatment were compared with pretreatment levels using the Friedman test, and individual time-point differences identified using Bonferroni-corrected Wilcoxon signed ranks tests.

## RESULTS

Four women withdrew from the study within the first 4-week period for the following reasons: one incurred a respiratory infection and expelled the ring on two occasions as a result of coughing, one withdrew owing to dysuria, the third owing to migraine headaches and rectal bleeding, which was later found to be secondary to hemorrhoids, and the fourth because of abdominal pain and bilateral lower limb pain. Results from these patients were not included in the final analyses.

The mean age of the remaining 12 women was 44.5 years (range 32–57). Six of the group were smokers and six were non-smokers. Smoking habits remained the same throughout the study. Baseline and end-of-study demographic details are given in Table 1. Two of the women were more than 25% above their ideal weight at baseline. Weight was increased in three women by 4 kg, 7 kg and 10 kg, respectively, while a more moderate increase (up to 2 kg) in weight occurred in five women. In two cases, there was a weight reduction, and no weight change in the remainder. There was no significant change in systolic blood pressure, but diastolic blood pressure was significantly reduced.

Two patients were hospitalized during the course of the study. One, with a history of ischemic heart disease, was admitted for 2 days with an attack of angina during the final 4 weeks, and the other was admitted for the elective removal of an ingrowing toenail.

A number of adverse events, probably estrogen-related, were reported during the course of the study. Four women experienced breast

tenderness, and another developed a lump in her right breast, with the 200- $\mu$ g ring. A mammogram reported as normal, and the lump disappeared after 2 weeks.

One patient experienced nausea, and another reported dysuria, with the 50- $\mu$ g ring.

Other adverse events that were possibly estrogen-related, but not associated with a specific dose of IVR, included headache ( $n = 5$ ), abdominal pain ( $n = 3$ ), lower back pain ( $n = 2$ ), fluid retention ( $n = 2$ ) and, as noted above, weight gain.

The occurrence of vaginal infection ( $n = 3$ ) and vaginal discharge ( $n = 5$ ) were possibly related to use of the IVR.

Adverse events which were unlikely to be related to estrogen included facial acne ( $n = 1$ ), urinary tract infection ( $n = 1$ ), chest tightness ( $n = 1$ ), respiratory infection ( $n = 3$ ), dizziness ( $n = 1$ ), sinusitis ( $n = 1$ ), joint pain ( $n = 1$ ) and diarrhea ( $n = 1$ ). Palpitations and mild right-ankle edema were experienced by one subject with the 50- $\mu$ g IVR only.

Estradiol levels before and during treatment are listed in Table 2. The results clearly demonstrate serum estradiol levels increasing in parallel with each increasing dose of estradiol-3-acetate delivered by IVR.

Results of the Greene scale scores are summarized in Table 3. At the start of this study, apart

from one patient who was free of somatic symptoms, the women had symptoms in all symptom categories. Five patients recorded anxiety and/or depression scores greater than 10. The IVR devices releasing 75  $\mu$ g/day and greater significantly reduced vasomotor and psychological symptom scores, while the device releasing the lowest concentration of estradiol had no significant effect on any symptom category. By the end of the study, a significant reduction of 38% and 48% in anxiety and depressive symptom scores, respectively, resulted in an overall 44% decrease in psychological symptoms. There were no significant changes in somatic symptom scores. Vasomotor symptoms were significantly reduced by 76% and loss of sexual libido by 44%. One patient alone, who recorded severe psychological, vasomotor and sexually related symptoms at baseline, was totally free of all symptoms at the end of the study.

Serum lipoprotein levels before and during treatment are given in Table 4. Although lipoprotein levels did not change significantly, by the end of the study the ratio of LDL cholesterol to high-density lipoprotein (HDL) cholesterol was significantly reduced by 7.2% ( $p = 0.006$ ), and an average reduction of 8.5% in levels of LDL cholesterol approached significance ( $p = 0.079$ ).

**Table 1** Characteristics of 12 postmenopausal women before and after treatment with estradiol released from dose-escalating intravaginal ring devices containing 17 $\beta$ -estradiol-3-acetate. Values are expressed as median (interquartile range)

Variable	Baseline	20 weeks
Weight (kg)	70 (63.3–73.5)	73 (63.3–76.3)*
Body mass index (kg/m <sup>2</sup> )	25.6 (23.1–27.3)	26.6 (24.1–27.8)*
Systolic BP (mmHg)	130 (117.5–130)	123 (105–135)
Diastolic BP (mmHg)	77.5 (70–93)	73 (64.5–90)*

\* $p < 0.05$ , significance of difference from baseline; BP, blood pressure

**Table 2** Serum estradiol levels in 12 postmenopausal women before and during treatment with estradiol released from dose-escalating intravaginal ring (IVR) devices containing 17 $\beta$ -estradiol-3-acetate

Dose of estradiol released from IVR ( $\mu$ g/day)	Attendance at clinic (week)	Ring in situ (weeks)	Serum estradiol (pmol/l)	
			Mean $\pm$ SD	Range
Nil (pretreatment)	Baseline	Baseline	45.1 $\pm$ 13.7	25–69
50	4	0–4	138.1 $\pm$ 32.8	92–205
75	8	4–8	166.3 $\pm$ 43.9	103–241
100	12	8–12	224.6 $\pm$ 91.9	142–479
150	16	12–16	348.2 $\pm$ 162.2	198–740
200	20	16–20	464.1 $\pm$ 127.1	240–646

**Table 3** Greene climacteric scale scores in 12 postmenopausal women before and during treatment with estradiol released from dose-escalating intravaginal ring (IVR) devices. Values are expressed as median (interquartile range)

<i>Dose of estradiol released from IVR (µg/day)</i>	<i>Attendance at clinic (week)</i>	<i>Anxiety symptoms score (A)</i>	<i>Depression symptoms score (D)</i>	<i>Psychological symptoms score (P)</i>	<i>Somatic symptoms score (S)</i>	<i>Vasomotor symptoms score (V)</i>	<i>Loss of libido score (Sx)</i>
Nil (pretreatment)	Baseline	8.5 (4.5–10)	9.0 (6.5–10)	17.0 (13–22)	4.0 (2–7)	4.5 (4–6)	2.0 (2–2.5)
50 (weeks 0–4)	4	6.5 (4–9.5)	7.5 (4.5–10)	13.5 (10.5–17)	2.0 (2–6)	4.0 (2–5)	2.0 (1–2)
75 (weeks 4–8)	8	5.5 (2–8)	4.5 (2.5–8)**	10.5 (4.5–16.5)**	3.0 (2–5.5)	2.5 (1–4)**	2.0 (1.5–2)
100 (weeks 8–12)	12	4.0 (1–6)**	3.0 (1.5–7)**	7.0 (2.5–12.5)**	4.5 (1.5–6)	2.0 (0–4.5)*	1.5 (1–2)
150 (weeks 12–16)	16	3.5 (2–6)*	2.5 (2–5)**	6.0 (3.5–10)**	3.0 (0–5)	0.5 (0–1.5)**	1.0 (0–1.5)*
200 (weeks 16–20)	20	4.0 (2–7)**	2.5 (2.5–6.5)**	8.5 (3–13.5)**	2.0 (0.5–5)	1.0 (0–1.5)**	1.0 (0.5–2)**
<i>p Value</i> <sup>†</sup>		0.0001	0.0003	0.0001	0.26	< 0.0001	0.019

\* $p < 0.05$ , \*\* $p < 0.01$ , significance of difference from baseline; <sup>†</sup>significance of changes during treatment (Friedman test)

**Table 4** Serum lipid and lipoprotein levels in 12 postmenopausal women before and during treatment with estradiol released from dose-escalating intravaginal ring (IVR) devices. Values are expressed as median (interquartile range)

<i>Dose of estradiol released from IVR (µg/day)</i>	<i>Attendance at clinic (week)</i>	<i>Triglycerides (mmol/l)</i>	<i>Cholesterol (mmol/l)</i>	<i>VLDL cholesterol (mmol/l)</i>	<i>LDL cholesterol (mmol/l)</i>	<i>HDL cholesterol (mmol/l)</i>	<i>LDL/HDL ratio (mmol/mmol)</i>
Nil (pretreatment)	Baseline	1.2 (1–2.05)	5.75 (4.55–6.9)	0.5 (0.4–0.85)	3.7 (2.55–4.6)	1.45 (1.35–1.65)	2.62 (1.83–3.29)
50 (weeks 0–4)	4	1.3 (0.85–1.85)	6.0 (4.5–6.8)	0.5 (0.4–0.7)	3.7 (2.5–4.65)	1.6 (1.25–1.75)	2.29 (1.71–3.21)
75 (weeks 4–8)	8	1.0 (0.7–1.55)	5.65 (4.6–6.2)	0.45 (0.2–0.7)	3.6 (2.6–4.65)	1.55 (1.25–1.65)	2.32 (1.73–3.16)
100 (weeks 8–12)	12	1.1 (0.8–1.7)	5.6 (4.65–6.75)	0.4 (0.25–0.75)	3.4 (2.75–4.65)	1.6 (1.4–1.75)	2.37 (1.66–3.17)
150 (weeks 12–16)	16	1.15 (0.95–1.85)	5.3 (4.35–6.15)	0.45 (0.3–0.8)	3.15 (2.4–4.1)	1.55 (1.35–1.7)	2.41 (1.62–2.66)
200 (weeks 16–20)	20	1.15 (0.95–1.7)	5.25 (4.45–6.05)	0.45 (0.3–0.6)	3.3 (2.5–4.05)	1.5 (1.3–1.7)	2.09 (1.79–2.92)**
<i>p Value</i> <sup>†</sup>		0.197	0.23	0.22	0.079	0.17	0.056

\*\* $p < 0.01$ , significance of difference from baseline; <sup>†</sup>significance of changes during treatment (Friedman test); VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein

**Table 5** Biochemical indices of bone metabolism in 12 postmenopausal women before and during treatment with estradiol released from dose-escalating intravaginal ring (IVR) devices. Values are expressed as median (interquartile range)

<i>Dose of estradiol released from IVR (µg/day)</i>	<i>Attendance at clinic (week)</i>	<i>Serum calcium (corrected for albumin) (mmol/l)</i>	<i>Serum phosphate (mmol/l)</i>	<i>Serum alkaline phosphatase (U/l)</i>	<i>Urine calcium/Creat ratio (mmol/mmol)</i>	<i>Urine OHPR/Creat ratio (mmol/mmol)</i>	<i>Urine DPD/Creat ratio (µmol/mol)</i>
Nil (pretreatment)	Baseline	2.38 (2.36–2.46)	1.24 (1.17–1.30)	150 (134–189.5)	0.33 (0.245–0.495)	0.023 (0.017–0.030)	7.1 (5.9–8.4)
50 (weeks 0–4)	4	2.42 (2.30–2.47)	1.22 (1.11–1.26)	159 (137–200)	0.23 (0.13–0.395)**	0.020 (0.018–0.026)	7.3 (5.7–7.7)
75 (weeks 4–8)	8	2.38 (2.30–2.44)	1.13 (1.04–1.19)**	167 (145–215)	0.21 (0.12–0.30)**	0.021 (0.020–0.028)	6.1 (4.9–7.3)*
100 (weeks 8–12)	12	2.32 (2.29–2.38)	1.12 (1.07–1.20)**	173 (152–189)	0.25 (0.14–0.39)*	0.019 (0.017–0.025)	5.85 (4.8–6.3)*
150 (weeks 12–16)	16	2.33 (2.24–2.38)**	1.05 (0.97–1.10)**	159 (138–195)	0.21 (0.14–0.32)**	0.018 (0.014–0.022)	5.5 (4.5–6.7)**
200 (weeks 16–20)	20	2.36 (2.27–2.38)**	1.08 (1.01–1.21)**	151 (134–187)	0.14 (0.08–0.30)**	0.016 (0.014–0.022)	5.45 (4.2–5.8)**
<i>p Value</i> <sup>†</sup>		0.0037	< 0.0001	0.044	0.0004	0.11	< 0.0001

\* $p < 0.05$ , \*\* $p < 0.01$ , significance of difference from baseline; <sup>†</sup>significance of changes during treatment (Friedman test); OHPR, hydroxyproline; Creat, creatinine; DPD, deoxypyridinoline

There was no change in the susceptibility of LDL to oxidation. The median (interquartile range) lag times at baseline and at 12 and 20 weeks were 61.4 (55.9–63.6) min, 60.8 (57.1–63.5) min and 60.5 (56.1–63.9) min, respectively.

At the start of the study, the median level of urinary deoxyypyridinoline cross-links was above the premenopausal reference range, but by 8 weeks had decreased into the premenopausal range (2.5–6.5  $\mu\text{mol/mol}$  creatinine), and continued to fall thereafter (Table 5). The urine hydroxyproline/creatinine ratio was not significantly altered, although the trend appeared to be downwards. The other indices of bone turnover were reduced, apart from alkaline phosphatase, which tended upwards over the first 12 weeks and subsequently returned to baseline levels.

After their participation in the study, all patients ( $n = 16$ ) completed an acceptability questionnaire. When asked, 'Which term best describes your experience with the ring?' (very good, good, fair, bad, very bad), the majority of subjects rated the ring as very good ( $n = 10$ ) or good ( $n = 3$ ). In answer to a question asking the women to rank their preference with regard to forms of hormone replacement therapy (HRT) delivery (estrogen implants, tablets, patches, gel, vaginal rings or vaginal creams), 75% of the patients opted for vaginal rings as their first choice.

## DISCUSSION

The IVR devices used in this study were well tolerated by the women, the majority of whom experienced no discomfort or awareness of the ring once it was inserted. Serum estradiol levels increased in parallel with each increasing dose of estradiol-3-acetate delivered by the IVR. Interindividual differences of estradiol levels at each dose were relatively large at the extremes, probably reflecting differences in absorption or metabolism. In a previous study, an estradiol-3-acetate-containing IVR delivering 100  $\mu\text{g/day}$  estradiol resulted in a mean plasma level of 300 pmol/l estradiol<sup>14</sup>, a concentration approximately 30% greater than in the present study. The difference in the mean levels of estradiol attained in the two studies is likely to be due to methodological differences in the estradiol assays. However, serum levels of estradiol in our study were comparable to those found by Nash and co-workers<sup>11,18</sup> in studies to examine the efficacy of 17 $\beta$ -estradiol-containing IVR devices.

At the start of the present study, all the women were highly symptomatic, including a minority who recorded high psychological Greene scale scores, probably indicating severe or clinical anxiety or depression unlikely to be alleviated by HRT alone. Both psychological and vasomotor symptoms improved over the course of the study, becoming statistically significant after 8 weeks. The results are in agreement with those of Nash and colleagues<sup>18</sup>, who found highly significant reductions in vasomotor symptoms and significant improvements in mood in a group of postmenopausal women treated with IVR devices releasing either 80  $\mu\text{g/day}$  or 160  $\mu\text{g/day}$  estradiol.

It is generally recognized that elimination of sweats and hot flushes will often improve depression and increase the sense of physical well-being. We realise that no account has been taken of a possible placebo effect in response to extra care and attention received during the course of the study. However, in a double-blind placebo-controlled study, in which quality of life was assessed with a series of standard questionnaires, Wiklund and associates<sup>19</sup> reported that self-rated psychological and vasomotor symptoms were reduced by 49% and 78%, respectively, for those on transdermal estrogen therapy (almost identical to the reductions obtained in our study), compared with 16% and 18%, respectively, in the placebo group. Similarly, the magnitude of the changes observed in the present study is in keeping with that found by Derman and colleagues<sup>20</sup> as assessed using the Greene climacteric scale. Significant reductions in psychological, somatic and vasomotor symptom scores were achieved in patients treated with a standard estrogen-progestin preparation, but in the placebo group these measures remained virtually unchanged<sup>20</sup>. The results from the above studies lead us to believe that any placebo effect in the present study is likely to be minimal.

In our study, some of the changes in serum lipid levels failed to achieve statistical significance, which may be a result of the small sample size. However, the pattern and magnitude of the changes are consistent with those previously observed with three IVR variants<sup>11</sup>, and other non-oral methods of estradiol delivery<sup>21–23</sup>. These circumvent first-pass intestinal and hepatic metabolism, and have a much less marked effect on lipoproteins than oral therapy. It may be important that non-oral estrogen, as opposed to oral, does not increase triglyceride levels, as it has been proposed that triglycerides are a strong

predictor of coronary heart disease in women over 50 years of age<sup>24</sup>.

Recently, similar changes in lipids were reported in a study of ultra-low doses of estradiol released from IVR devices intended for local treatment of vaginal atrophy, and in which circulating levels of estradiol remained within the normal postmenopausal range<sup>25</sup>. These results were surprising, but as the women were aged above 60 years, the authors tentatively suggested that the effects may be a result of increased estrogen receptor sensitivity after a long period of estrogen deprivation<sup>25</sup>.

It has been suggested that the susceptibility of LDL to oxidative modification, believed to play a key role in the pathogenesis of atherosclerosis<sup>26,27</sup>, is an independent risk factor for coronary atherosclerosis. There appear to be no previous reports of the effect of estradiol delivered by IVR devices on the susceptibility of LDL to oxidation. To date, *in vivo* studies involving non-oral routes of estrogen administration have produced conflicting results<sup>28-31</sup>. In our study, estradiol delivered by IVR devices had no effect on the susceptibility of LDL to oxidation.

With regard to the effect of estradiol on the biochemical indices of bone metabolism, the changes in urinary excretion of both calcium and deoxypyridinoline cross-links indicate a reduction in bone resorption during the study period. The absence of a significant effect on urinary hydroxyproline levels probably represents a lack of sensitivity and specificity of this marker. The pattern of change in markers is similar to those previously reported after 3 months' therapy with transdermal estradiol<sup>32</sup> and oral HRT<sup>33</sup>.

It is accepted that long-term therapy with estrogen, regardless of the route of administration, retards the rate of postmenopausal bone loss<sup>34</sup>. Earlier studies established minimum daily doses of estrogen sufficient to prevent postmenopausal bone loss as 0.625 mg conjugated equine estrogens<sup>35</sup>, or 2 mg estradiol<sup>36</sup>, given orally, or 50 µg transdermal estradiol<sup>37</sup>. Recent studies have suggested that these doses may be halved without loss of protection<sup>38-40</sup>.

The effect of estrogen on bone appears to be dependent on the circulating level attained. However, active metabolites which are not recognized in assays for estradiol in the case of oral therapy,

and differences in the estradiol assays themselves, cause difficulty in establishing a consensus estradiol level for adequate protection. For example, the average serum estradiol level attained after transdermal treatment with 50 µg/day estradiol has been variously reported as 90 pmol/l<sup>21</sup>, 213 pmol/l<sup>37</sup> or 122 pmol/l<sup>41</sup>. In the last study it was demonstrated that maximum therapeutic benefit for bone was obtained at a serum estradiol level of 150 pmol/l. In the present study, the 75-µg/day IVR device, which resulted in an average serum estradiol concentration of 166.3 pmol/l, was the lowest dose that significantly reduced levels of the markers of bone resorption. Interestingly, it has been suggested that ultra-low doses of estradiol (7.5 µg/day) delivered by IVR may potentially prevent bone loss in elderly women above age 60 years, despite there being no significant effect on serum estradiol levels<sup>42</sup>. Whether these latter results would apply in the early postmenopausal period is debatable.

In conclusion, the rings were well tolerated by the patients and were effective in controlling vasomotor symptoms and improving levels of anxiety and depression. They had beneficial effects on bone metabolism, indicating a protective effect on the postmenopausal skeleton. There was no evidence of an antioxidant effect on LDL, nor any significant changes in lipoprotein risk markers for coronary heart disease. The 75-µg/day IVR device appears to be the lowest effective dose to provide relief from climacteric symptoms and offer protection from bone loss. However, it must be borne in mind that each ring was *in situ* for only 4 weeks, as opposed to the anticipated treatment regimen of 12 weeks. The 50-µg ring may provide adequate symptom control and bone protection for some patients in the longer term.

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