The effect of hormone replacement therapy and route of administration on selected cardiovascular risk factors in post-menopausal women

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Background. There is increasing use of hormone replacement therapy (HRT) by post-menopausal women. Observational epidemiological studies have shown reductions in cardiovascular risk factors in HRT users in the USA, but no randomized controlled trials of HRT have been carried out in the primary practice setting. Previous studies of cardiovascular risk factors have shown a variety of responses according to type of progestagen and oral or topical administration. None has examined the effect of route using an identical progestagen.

Objectives. Our aim was to establish differences, if any, in alteration in cardiovascular risk factors with HRT in post-menopausal women according to route of administration of HRT, oral, transdermal and implant, using first oestrogen alone then oestrogen plus norethisterone, or testosterone for implant.

Methods. Subjects were recruited by letter of invitation to women aged 50–65 years from lists in general practices local to the Charing Cross Hospital Lipid Clinic in West London. Their menopausal status was confirmed and they were randomized to one of three treatment groups or acted as controls. They attended for three visits; at baseline, HRT was initiated as oestrogen alone, oral or transdermal. At the 3-month visit, HRT with the progestagen, norethisterone, was given cyclically, continuously or transdermally until the final visit at 6 months. A separate group of women from the menopause clinic at Chelsea and Westminster Hospital were studied on oestrogen implant then on implanted oestrogen and testosterone. The outcome measures studied were the separate effects of the four regimes as compared with controls on lipoproteins, glucose, insulin, fibrinogen, factor VII and E-selectin, together with weight, waist:hip ratio and blood pressure.

Results. The continuous combined oestrogen–progestagen therapy had similar effects on cardiovascular risk factors as oestrogen with cyclical progestagen. All regimes lowered low-density lipoprotein cholesterol, the oral route being more potent than the parenteral; the effect of transdermal HRT was similar to the implant. Lp(a) was reduced only with the oral route. Reductions in factor VII and E-selectin were observed in both the oral and transdermal routes. There was no increase in body mass index, waist:hip ratio, blood pressure or glucose and insulin levels with any of the HRT regimes used. Systolic blood pressure was reduced with the transdermal route.

Conclusions. This study supports the evidence that oestrogen–progestagen HRT, both oral and transdermal, although attenuating some of the benefit of oestrogen alone on fibrinogen and high-density lipoprotein, significantly reduces cardiovascular risk factors, which should diminish post-menopausal risk of coronary disease.

Keywords. Cardiovascular risk factors, hormone replacement therapy, lipoproteins, post-menopausal women.

Introduction

Pre-menopausal women have a low risk of coronary heart disease (CHD) compared with their male counterparts, and this gender gap continues to be maintained until a women is in her sixties.[1,2] The observation that premature menopause or oopherectomy lowers the age at which women begin to develop CHD by a decade suggested that gonadal steroids may offer protection against CHD.[3] Consistent with this are a number of observational studies that post-menopausal hormone replacement therapy (HRT) lowers CHD risk.[4–7]

Historically, the problem of CHD in women has been understudied. The increasing longevity of women in the UK in the last century has made the nature of the clinical problem more apparent. In the last decade, we have seen confirmation from clinical trials (4S, CARE, LIPID) that the major reduction in cardiovascular risk which can be achieved with lipid-lowering therapy in men is also applicable to women, but even in these studies the numbers of women involved were small.[8–10] Data from the only randomized controlled trial of the use of HRT on cardiovascular risk in secondary prevention, the Heart Estrogen Replacement Study (HERS), was neutral, reporting an increase in cardiovascular mortality within the first 12 months, and a reduction thereafter.[11] Primary prevention studies with large numbers of women in the Women's Health Initiative (WHI) and Women's International Study of long Duration Oestrogen in the Menopause (WISDOM) are due to report within the next few years.[12]

Most prescribing of HRT is primarily for relief of menopausal symptoms, so convenience of use is paramount. Consequently, a bewildering variety of regimes and preparations administered by oral, transdermal, intrauterine or implant routes exist. Prescribing HRT for its effect on cardiovascular risk factors therefore plays a secondary role. The original epidemiological studies from the USA were of HRT users on conjugated equine oestrogen (premarin) which in common with oral 17-β oestradiol lowers total and low-density lipoprotein (LDL) cholesterol and increases high-density lipoprotein (HDL) and triglycerides. Transdermal oestrogen has modest effects on lipids compared with oral oestrogen.[13] Here we report on the results of a study designed to compare different routes and regimes of administration of HRT on cardiovascular risk factors in urban post-menopausal women in order to assess the particular benefit of one or other regime. It also examines the effect of an androgen (testosterone) on these risk factors.

Methods

Subjects
Women aged 50–65 years from primary care practices were invited to attend a cardiovascular risk factor and lipid clinic at Charing Cross Hospital. General practices near the hospital who were enthusiastic about the study provided names of women patients from their practice lists within the age range. A letter describing the study with an invitation to participate was sent to 4000 women. Advertisements were also placed in a national newspaper, and a few women joined the study as a result. Altogether, 400 volunteered and, of these, 260 women attended for consultation and were recruited into the study. One hundred started as controls, and 83 of these completed the study. One hundred and sixty started in the actively treated group, 78 of whom completed the study. A separate group of 34 women who had undergone a hysterectomy were treated with HRT as implant in the menopause clinic at the Chelsea and Westminster Hospital.

Endocrinologically menopausal women aged 50–65 years, off HRT therapy for at least 6 months, consented to the study which had local ethics committee approval. Menopause was defined biochemically on the basis of an elevated follicle-stimulating hormone (FSH) $\geqslant 30$ IU/l and serum oestradiol <100 pmol/l. Women were excluded on the basis of other endocrine disorders, abnormal liver or renal function or the use of lipid-lowering therapy or other medication known to impact on lipoprotein metabolism.

Study design
This was an open parallel group study and subjects were allocated randomly to one of three treatment groups or as controls. The treatment regimes were two oral groups, with cyclical or continuous progestagen, and one transdermal regime with cyclical progestagen. The group of women who had had a hysterectomy received HRT by implant. Each regime lasted 6 months; for the first 3 months, oestradiol unopposed was given by each route; for the second 3 months, norethisterone was added as the progestagen, orally or transdermally as appropriate. At the time of the study, norethisterone was the only progestagen available for both routes of administration; the choice was made to ensure that the metabolic effects of the progestagen were comparable across all three regimes.

The implant group were treated with oestradiol alone then with oestradiol and testosterone.

Study medication
The oral cyclical preparation consisted of a 28 day cycle of 17-β oestradiol 2 mg for 16 days followed by 17-β oestradiol 2 mg and norethisterone 1 mg for 12 days (Climagest®). Three cycles of unopposed oestradiol 2 mg was taken for 12 weeks followed by three cycles of treatment when the progestagen was taken in the latter half of the cycle. Treatment was taken continuously for 24 weeks in total. The oral continuous preparation consisted of a 28-day cycle of 17-β oestradiol 2 mg and norethisterone 700 µg daily (Climesse®). Three cycles of
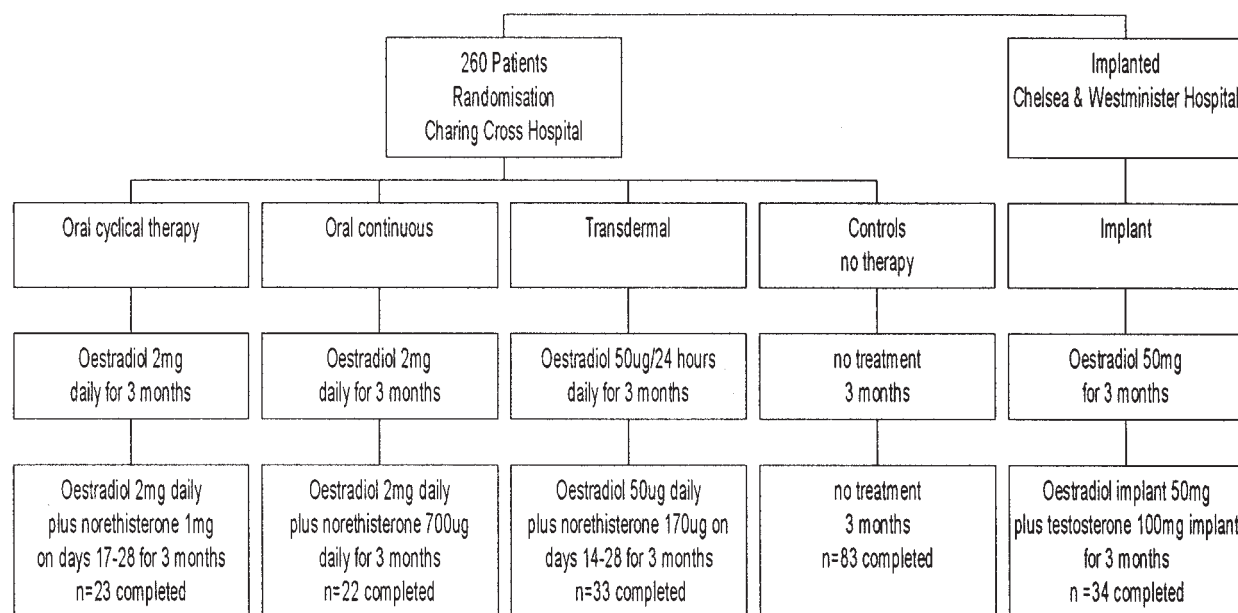
FIGURE 1   *Diagram to show the design of the study*

treatment with oestradiol 2 mg only was taken continuously for 12 weeks followed by a further three cycles with oestradiol 2 mg and norethisterone 700 μg daily continuously for 12 weeks.

The transdermal preparation was in the form of a patch releasing 17-β oestradiol 50 μg/24 h transdermally for 14 days followed by a different patch releasing both 17-β oestradiol 50 μg and norethisterone 170 μg/24 h for 14 days (Evorel Sequi®). The first three cycles of treatment were with 17-β oestradiol alone for 12 weeks followed by three further cycles when the progestagen was added in the second half of each cycle for 12 weeks.

The implant preparation consisted of a single 50 mg oestradiol pellet implanted under local anaesthetic subcutaneously. After 3 months, a further 50 mg oestradiol pellet together with a single 100 mg testosterone pellet were implanted under local anaesthetic.

Study visits
Subjects were seen three times during the study. At the first visit, they underwent consultation, examination, randomization and allocation to a study group and blood was taken for baseline biochemical measurements. Oestradiol was dispensed and, after the results of the baseline tests were available, a phone call was made so that the appropriate women started their medication. (This was to reduce the number of visits patients had to make to the hospital.) A contact phone number was made available to every patient for the duration of the study to respond to any anxieties. The second visit was in the 12th week following the start of medication, when the progestagen was added to their treatment and all the initial measurements were repeated. The third and final visit was in the 24th week when the final measurements

were obtained. All subjects included in the analysis therefore acted as their own controls. Data on those who were unsuitable on laboratory findings, for example who were not menopausal or with endocrine abnormalities, or who did not complete the three study visits, were not included in the analysis.

Anthropometric measurements
At all study visits, subjects had their weight recorded and waist (maximal abdominal girth between the subcostal margin and anterior superior iliac spine) and hip (measured at the greater trochanter) measurements made. The waist:hip ratio was derived from the waist (cm)/hip(cm). Body mass index (BMI) was calculated from the weight (kg)/height (m)^2, systolic and diastolic blood pressure were measured (mmHg) from the right arm and recorded as the mean of two separate readings with the subjects sitting relaxed for 30 minutes.

Biochemical measurements
Biochemical measures were taken at all study visits between 8 a.m. and 10 a.m. after an overnight fast from midnight. Serum liver, electrolytes and creatinine, and thyroid function tests were performed at all visits to exclude hepatic, renal and thyroid dysfunction. Blood samples were taken for fasting plasma glucose, insulin, cholesterol, HDL cholesterol and triglycerides, Lipoprotein (a) [Lp(a)], FSH, luteinizing hormone (LH), oestradiol, factor VII, fibrinogen, plasmin antiplasmin, thrombin anti-thrombin III, von Willebrand factor and E-selectin. LDL was calculated from the Friedewald equation.

Samples for glucose, insulin and sex hormones were stored at –70° C immediately following collection and

analysed at the end of the study. Samples for coagulation factors were taken into citrated tubes, immediately placed into ice and centrifuged (within 30 minutes) at 4°C for 15 minutes at 2000 *g*. Plasma was then separated and stored at –70° C until assay.

Serum insulin was measured using an enzyme-linked immunosorbent assay (ELISA) one-step sandwich assay that shows 40% cross-reactivity with pro-insulin. The intra-assay coefficient of variation (c.v.) was 5.2%. Glucose was collected into fluoride oxalate tubes and assayed using an automated glucose oxidase method; the c.v. for this technique is 2.3%. Serum cholesterol, HDL and triglycerides were measured using an enzyme-based automated assay on an Olympus AU600 (Olympus Optical Co. Ltd, UK); the c.v.s were 1.5, 4, and 2%, respectively. Apolipoprotein A1 (Apo-A1) and apolipoprotein B (Apo-B) were measured on a Beckman array nephelometer with Beckman antisera, c.v. 4%. Lp(a) was determined using an ELISA method (Biopool®, Sweden), c.v. 6%. FSH, LH and oestradiol were determined by standard ELISA assays on an automated analyser (ES700; Roche Diagnostics Ltd, Lewes UK), the c.v.s being 2.7, 2.4 and 5.1%, respectively.

The Department of Medicine at the University of Dundee undertook the following measurements. Fibrinogen and factor VII were measured using an automated clotting assay (Instrumentation Laboratory). Von Willebrand factor antigen was measured by an 'in-house' ELISA with Dako reagents. Plasmin anti-plasmin and thrombin anti-thrombin were both measured using ELISAs (Behring) as were E-selectin levels (R&D Systems UK).

Statistics

All values except triglycerides and Lp(a) are expressed as the mean ± standard deviation. Triglycerides and Lp(a) are expressed as the median and the range. Randomization between the three groups was compared using a Kruskal–Wallis test to ascertain adequacy of randomization. Paired data were analysed using a Wilcoxon signed rank test to determine the effect of treatment. *P*-values <0.05 were regarded as significant. Data was analysed using the statistical program SPSS for Windows.

Results

Parameters of interest were measured at entry. Results are summarized in the tables and expressed as mean ± SD, or median and range for triglycerides and Lp(a).

There were more smokers (22%) in the group who started HRT, compared with controls (11%), although this difference was not significant. The two groups were otherwise similarly matched in terms of age, BMI, waist: hip ratio, blood pressure and measured cardiovascular risk factors. Baseline blood pressure was by chance higher in those women on the oral cyclical regime; it did not change on treatment. We carried out a detailed examination of the effects of the continuous as compared with cyclical progestagen in the oral treatment arms of the study. We found no significant differences in measured cardiovascular risk factors between these two oral HRT regimes. Percentage changes in lipoproteins were therefore analysed for both oral groups together.
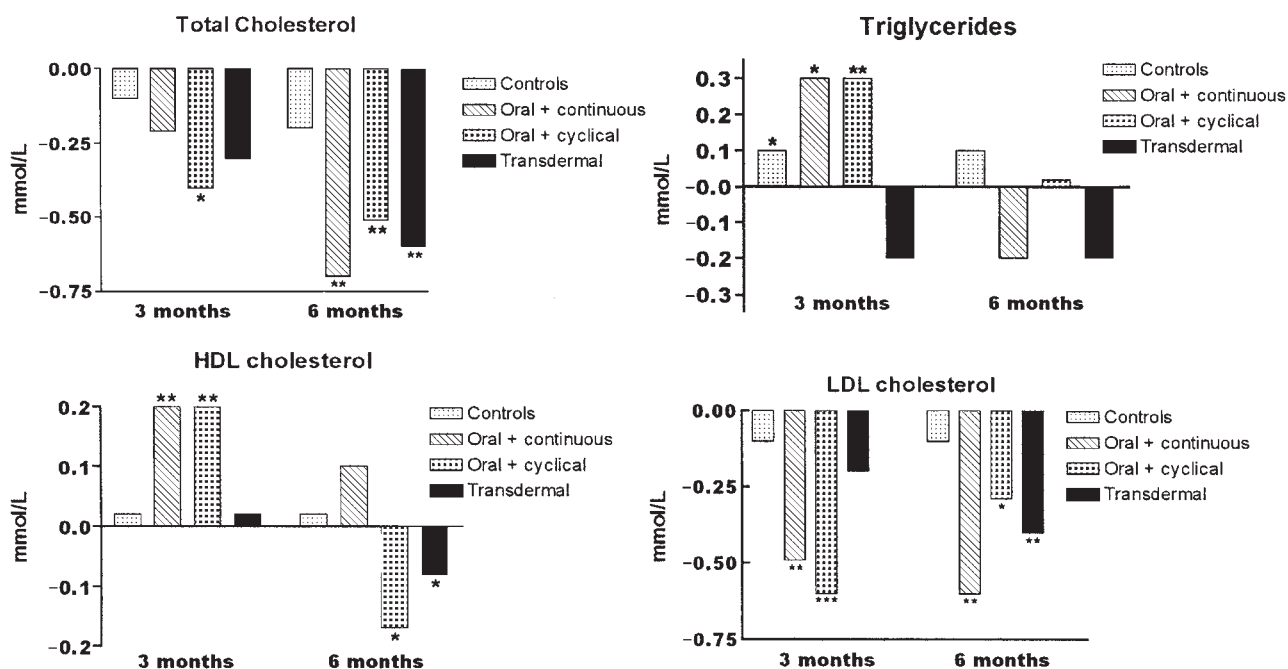

FIGURE 2   *Changes in lipoproteins (mmol/l) as compared with baseline values at 3 months (oestrogen alone) and 6 months (oestrogen plus progestagen)*
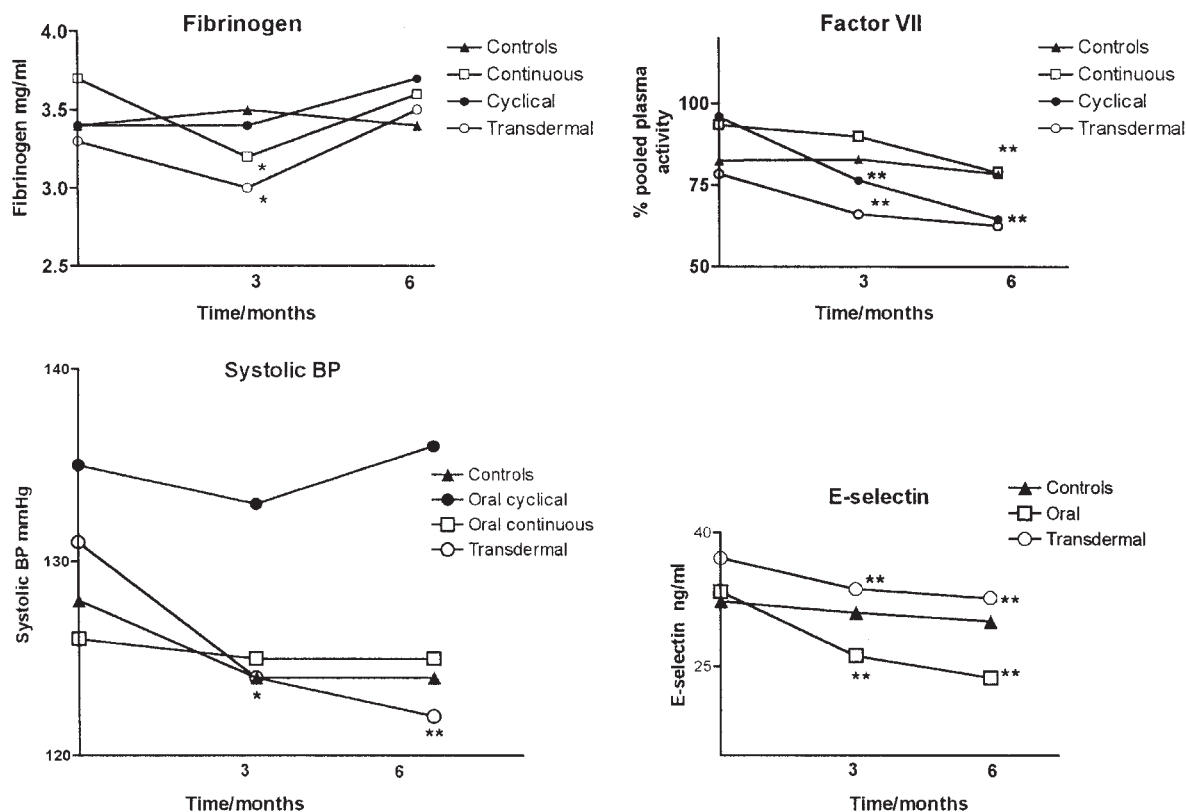
FIGURE 3   *Changes in fibrinogen, factor VII, systolic blood pressure and E-selectin at 3 months (oestrogen alone) and 6 months (oestrogen plus progestagen)*

The women in the separate group implanted with oestrogen were younger than the other treatment groups.

Oestrogen treatment
Cholesterol, LDL and Apo-B were significantly reduced in all the active treatment groups over the first 3 months of the study. The greatest reduction was 20% in the group with oral oestrogen (Fig. 4). Lp(a) levels were also reduced by 50% with oral oestrogen. HDL cholesterol, Apo-A1 and serum triglycerides were increased only with oral oestrogen. There were no significant changes in

triglycerides, HDL, Apo-A1 or Lp(a) in the parenteral (transdermal and implanted) groups after 3 months of oestrogen. Fibrinogen decreased by 10% in the oral oestrogen-treated group. Factor VII and E-selectin decreased significantly after 3 months of treatment with both oral and transdermal oestrogen.

Oestrogen and progestagen treatment
Following 3 months of therapy with oestrogen and progestagen, total cholesterol, LDL and Apo-B levels were reduced further in all active treatment groups,
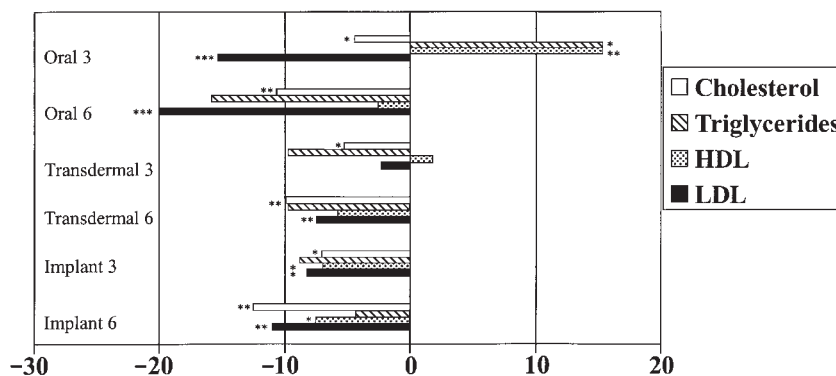

FIGURE 4   *Percentage change from baseline in lipoproteins for three HRT regimes: 3 months oestrogen alone, 6 months oestrogen plus norethisterone with implant 3 months oestrogen, and 6 months oestrogen plus testosterone*

TABLE 1 *Baseline characteristics of patients randomized to control or HRT groups*

	Controls: $n = 83$	HRT (oral and transdermal): $n = 78$
Smokers	11%	22%
Systolic blood pressure	128 ± 17	131 ± 20
Diastolic blood pressure	79 ± 8	79 ± 9
Age	58.7 ± 5.2	58.2 ± 6.7
BMI	25.7 ± 4.9	26.5 ± 5.5
Waist:hip ratio	0.78 ± 0.05	0.79 ± 0.06
Mean cholesterol	6.2 ± 1.1	6.3 ± 1.3
Triglycerides	1.0 (0.2–1.8)	1.2 (0.4–2.7)
HDL	1.7 ± 0.5	1.6 ± 0.5
LDL	4.0 ± 1.1	4.1 ± 1.2
Lp(a)	12 (3–94)	18 (0.13–82)
Apo-A1	1.6 ± 0.26	1.58 ± 0.21
Apo-B	1.10 ± 0.30	1.19 ± 0.34
Glucose	5 ± 0.5	5 ± 0.8
Insulin	9.1 ± 5.1	10.6 ± 4.2
FSH	81 ± 35	80 ± 32
Oestradiol	51 ± 30.5	52 ± 24.6
Fibrinogen mg/ml	3.6 ± 1.5	3.4 ± 0.9
Factor VII	135 ± 30	139 ± 40

$P < 0.01$ (Table 1). HDL cholesterol and Apo-A1 were reduced as compared with the oestrogen phase but were similar to the levels at baseline. There were no significant changes in triglycerides as compared with baseline values in any of the treatment groups.

Lp(a) levels continued to be reduced as compared with baseline in the oral group. There was no change in Lp(a) with the addition of implanted androgen or transdermal progestagen. The reduction in fibrinogen with oestrogen alone was reversed, as in the oestrogen and progestagen phase fibrinogen reverted back to baseline values. Factor VII and E-selectin levels continued to decline with the addition of progestagen in both the oral and transdermally treated groups. There was no change in any other of the haemostatic variables measured; levels of plasmin anti-plasmin, thrombin anti-thrombin III and von Willebrands factor were unaltered and remained within the normal range.

Systolic blood pressure was reduced in women on transdermal HRT.

Oestrogen and androgen
Implanted oestrogen achieved serum oestradiol levels twice the magnitude of the transdermal route. There was a 10% reduction in cholesterol, LDL and Apo-B which

was maintained with the addition of the implanted androgen testosterone. There was a small reduction in HDL over the study period with no specific effect of testosterone. However, there was a non-significant reduction in triglyceride, comparable with transdermal therapy. Lp(a) levels were unchanged.

Discussion

General
The difficulties associated with this study were 2-fold. The first was in the recruitment of patients directly from primary care practices; we achieved a response rate of only 1 in 10. The second was in the maintenance of women on their allotted HRT regime as 45% of the group starting HRT did not complete the 6-month study. The main reasons were anxiety over reports of the increased risk of venous thrombosis at that time and media mis-information about breast cancer risk. In addition, some women had unacceptably heavy bleeds during the second phase of the study.

These figures, while disappointing, are not unusual. The MRC pilot study had a similar loss of patients over the period of a year as did the Estradiol Clotting Factor Study Group.[14,15] Our results serve to highlight the difficulties both for medical research, and for the successful treatment of women. Such studies emphasize the problems inherent in completion of the large-scale randomized controlled trials of HRT at the primary intervention level, which are being carried out at present. For these to be successful, women are required to adhere to their treatment arm, either placebo or HRT, for at least 5 years.

Unopposed oestrogen
The effects of unopposed oestrogen on total cholesterol, HDL cholesterol, LDL cholesterol, serum triglycerides, Apo-A1 and Apo-B levels are consistent with those described in other reports.[16] Oestrogen lowers total plasma cholesterol, through reduction in LDL fractions by regulating lipoprotein binding and clearance. The increased binding of LDL to the hepatocyte is due to a specific up-regulation of the expression of the Apo-B/E receptor in the liver.[17] Increased LDL receptor expression leads to increased clearance of LDL and thus a reduction in intermediate density lipoproteins and LDL levels in the circulation.[18] The reduction of 10% in LDL is less in women treated with transdermal and implanted oestrogen even though implant achieved serum levels of oestrogen similar to those with the oral route in which LDL was lowered by 20%, indicating the importance of the hepatic first-pass hormonal effect. Plasma triglyceride concentrations are increased only with oral oestrogen, due to increased hepatic secretion of triglyceride-rich particles, an effect not seen with the parenteral route of oestrogen administration.[19] HDL cholesterol levels increase only with oral oestrogen, with increased synthesis

TABLE 2 Baseline values and responses to HRT for selected cardiovascular risk factors

	Controls: n = 83			Oral (continuous + cyclical): n = 45			Transdermal: n = 33		
	Baseline	3 months	6 months	Baseline	3 months	6 months	Baseline	3 months	6 months
BMI	25.7 ± 4.9	25.4 ± 5.1	25.7 ± 4.9	25.7 ± 4.0	25.4 ± 3.5	25.9 ± 3.7	26.8 ± 6.5	27.0 ± 6.6	27.5 ± 6.8
Oestradiol (pmol/l)	51 ± 31	64 ± 77	50 ± 31	52 ± 25	325 ± 183**	286 ± 165**	45 ± 17	146 ± 121*	149 ± 99*
Total cholesterol (mmol/l)	6.2 ± 1.1	6.1 ± 1.1	6.0 ± 1.0	6.38 ± 1.37	6.1 ± 1.4*	5.7 ± 1.3 **	6.44 ± 1.4	6.1 ± 1.2 *	5.8 ± 1.0**
HDL cholesterol (mmol/l)	1.73 ± 0.47	1.74 ± 0.45	1.74 ± 0.41	1.56 ± 0.47	1.8 ± 0.44**	1.52 ± 0.41	1.72 ± 0.46	1.75 ± 0.48	1.62 ± 0.45*
LDL cholesterol (mmol/l)	3.99 ± 1.2	3.9 ± 1.0	3.8 ± 1.1	4.25 ± 1.3	3.6 ± 1.4***	3.4 ± 1.2***	3.89 ± 0.96	3.8 ± 1.1	3.6 ± 1.0**
Triglyceride (mmol/l)	0.9 (0.32–5.0)	0.99 (0.33–4.2)	0.98 (0.35–3.3)	1.2 (0.44–2.7)	1.42 (0.67–3.19)*	1.01 (0.49–2.53)	1.23 (0.28–5.4)	1.11 (0.44–5.2)	1.11 (0.57–4.5)
Lp(a) (mg/dl)	12.4 (0–94)	11.7 (0–108)	12.7 (0–97)	25 (0.13–82)	15.5 (2–84)*	12 (0.2–3)*	11 (1.5–86)	9.7 (0.33–56)	11 (0.16–82)
Apo-A1 (g/l)	1.61 ± 0.26	1.66 ± 0.24	1.65 ± 0.22	1.51 ± 0.23	1.78 ± 0.4**	1.53 ± 0.24	1.65 ± 0.22	1.65 ± 0.24	1.61 ± 0.22
Apo-B (g/l)	1.1 ± 0.29	1.07 ± 0.31	1.06 ± 0.27	1.21 ± 0.36	1.13 ± 0.41*	1.10 ± 0.37*	1.2 ± 0.40	1.10 ± 0.37*	1.1 ± 0.29*
E-selectin (ng/ml)	46.7 ± 18.5	46.7 ± 18.6	44.7 ± 20.6	44.2 ± 22.5	37.8 ± 14.6***	32.8 ± 14.6***	51.3 ± 23	46.7 ± 20.6**	44.7 ± 20.6**
Factor VII %	135 ± 30	136 ± 30	123 ± 34	145 ± 50	125 ± 40*	113 ± 45**	131 ± 26	114 ± 18**	108 ± 17**
Fibrinogen (g/l)	3.4 ± 0.9	3.5 ± 0.8	3.4 ± 0.7	3.4 ± 0.7	3.2 ± 0.7*	3.6 ± 0.7	3.3 ± 0.7	3.0 ± 0.5	3.5 ± 0.7
Fasting glucose (mmol/l)	5.0 ± 0.51	4.8 ± 0.58	4.8 ± 0.6	5.0 ± 0.7	4.8 ± 0.7	4.8 ± 0.6	4.8 ± 0.6	5.0 ± 0.6	4.9 ± 0.6
Fasting insulin (IU/l)	9.1 ± 5.1	9.4 ± 6.0	9.3 ± 5.3	10.3 ± 4.2	9.7 ± 4.7	9.5 ± 5.3	10.3 ± 6.1	11.2 ± 6.6	9.6 ± 5.8

Data presented as the mean and standard deviation; triglyceride and Lp(a) shown as median and range.
*P < 0.05; **P < 0.01; ***P < 0.001.

TABLE 3 *Implanted group: baseline values and following treatment*

n = 34	Baseline	3 months oestrogen 50 mg	6 months oestrogen 50 mg + testosterone 100 mg
Age	51.7 ± 3.8 years		
BMI	25.64 ± 3.96	25.78 ± 4.02	26.32 ± 4.52
Testosterone (nmol/l)	1.15 ± 0.37	1.11 ± 0.37	6.44 ± 2.63**
Oestradiol (pmol/l)	53.94 ± 24.14	343.62 ± 263.55**	475.06 ± 219.91**
Cholesterol (mmol/l)	6.71 ± 0.96	6.24 ± 0.82**	5.87 ± 1.18**
Triglycerides(mmol/l)	1.14 (0.49–4.06)	1.04 (0.43–4.01)	1.091 (0.46–3.30)
HDL (mmol/l)	1.73 ± 0.53	1.61 ± 0.37*	1.60 ± 0.46*
LDL (mmol/l)	4.38 ± 0.97	4.02 ± 0.9*	3.9 ± 0.77**
Lp(a) (mg/dl)	8.9 (2–103)	10.1 (2–107)	10.0 (2–81.3)
Apo-A1(g/l)	1.59 ± 0.39	1.54 ± 0.28	1.52 ± 0.25
Apo-B (g/l)	1.42 ± 0.36	1.30 ± 0.32*	1.19 ± 0.27**

*$P < 0.05$; **$P < 0.01$.

of its major apoprotein Apo-A1 and reduction of hepatic lipase activity.[20]

A further effect of oral HRT is to reduce the concentration of Lp(a). Lp(a) consists of an LDL particle containing Apo-B linked to a second protein apolipoprotein (a), similar in structure to plasminogen.[21] High plasma concentrations of Lp(a) are associated with an increased risk of premature coronary atherosclerosis as well as other arterial disease.[22] Lp(a) competes with plasminogen for the plasminogen activator receptor and also competes with plasmin binding to fibrin. Endothelial fibrinolytic activity is thus impaired by Lp(a), inhibiting lysis of clots.[23] High concentrations of Lp(a) predict the presence of angiographic coronary disease in women.[24] Oestrogen-mediated decreases in Lp(a) levels may contribute to its antiatherogenic effects. Recent analysis of the Lp(a) data from the HERS showed that women with high Lp(a) levels had a higher number of coronary events and that these were reduced in women in whom Lp(a) was lowered in the HRT treatment arm of the study.[25] This is the first report of the association of Lp(a) lowering with a reduction of coronary events. Our study showed a decrease in Lp(a) with oral but not with parenteral HRT. Thus the first-pass effect appears to be important, particularly as the high concentrations of oestradiol obtained with implant did not lower Lp(a) levels.

Oestrogen reverses post-menopausal increases in fibrinogen, plasminogen activator inhibitor and factor VII.[26] In our study, we observed a decrease in fibrinogen and factor VII with oestrogen alone. The reduction in fibrinogen was reversed with the addition of the progestagen, in contrast to the effect on factor VII which was potentiated. We have also shown that the concentration of the pro-atherogenic adhesion molecule E-selectin was reduced by oestrogen without and with a progestagen, restoring concentrations to pre-menopausal levels.[27] These potentially beneficial changes were seen with both routes of HRT administration.

Oestrogen and progestagen

A vexing question when considering post-menopausal HRT in women with an intact uterus is the role of a progestagen. Progestagens are a necessary requirement in such women to prevent oestrogen-induced endometrial hyperplasia and malignancy. An adverse androgenic effect on HDL cholesterol levels has been seen with the sequential administration of certain progestagens.[28] However, analysis of epidemiological data from the large Nurses Health Study and of data from Sweden suggests that the addition of a progestagen does not attenuate the cardioprotective effects of oestrogen.[29] Unfortunately, there is no confirmatory evidence from randomized controlled trials on this issue. In our study, the addition of a progestagen, whether cyclical or continuous, had no effect on the LDL-lowering action of oestrogen. It did, however, prevent the oestrogen-induced rise in HDL, Apo-A1 and triglyceride, the last being a beneficial effect. *A priori* we would have expected progestagen taken continuously throughout the cycle to lower HDL to a greater extent than progestagen taken cyclically, but this was not the case in our study. A possible explanation is the achievement at hepatocyte level of a steady state of the antagonistic effects of these hormones, whereas with cyclical therapy there are fluctuations in the hormonal environment. By adding this particular progestagen, norethisterone, the increase in HDL levels is lost. Data from the Framingham study of cardiovascular risk factors in women showed risk to be inversely related to HDL, and the initial evaluation of the protective effect of oral oestrogen rested largely on the increases in HDL. However, recently the effect

of reduction in LDL levels has been well demonstrated in randomized controlled trials of statins in which women were included for both primary and secondary prevention.[8–10,30] It is likely that the reduction in LDL achieved with HRT is equally important. This is illustrated further by the Scottish Cancer Trials Breast group in which the selective oestrogen receptor modulator (SERM) tamoxifen reduced LDL without increasing HDL and in which cardiovascular events were reduced.[31] The effect of the SERM raloxifene is the subject of a large international secondary prevention study which is in progress and fully recruited.

In terms of the direct effect on the arterial wall, animal studies have shown that the addition of selected progestagens and testosterone to oestrogen replacement therapy did not oppose the inhibitory effects of oestrogen replacement on the development of atherosclerosis.[32] Specificity of the progestagen appears important, as this was not the case for the progestagen, medroxyprogesterone acetate.[33] Progestagens may, however, attenuate some of the beneficial effect of oestrogen on vasomotion. Studies of different progestagens on endothelial function and vasomotion would allow for choice of an appropriate progestagen in women at increased cardiovascular risk.

Androgens and oestrogens
This study is one of the few to examine the effect of post-menopausal implanted oestrogen and androgen therapy. Androgen replacement after the menopause improves libido, strength and well-being in some women.[34] In this study, the addition of parenteral testosterone in women who were well oestrogenized did not impact adversely on the insulin to glucose ratio or lipoproteins. The 10% reduction in LDL achieved with the oestrogen implant was maintained with the addition of implanted testosterone.

Summary
- The effect of the continuous combined oestrogen–progestagen therapy was not significantly different from oestrogen with cyclical progestagen. In women who are post-menopausal, the continuous combined no-bleed regime is more acceptable than the withdrawal bleed regime. This will encourage adherence to therapy.
- All regimes lowered LDL. The oral route was more potent in lowering LDL cholesterol than the transdermal route, which was equivalent to the LDL reduction found with implant. Lp(a) was reduced with the oral route.
- The addition of the androgen implant did not alter the effect of implanted oestrogen on lipoprotein levels.
- None of the routes of administration had deleterious effects on the haemostatic factors measured.

Reduction in factor VII and E-selectin was observed with both oral and transdermal routes.
- There was no increase in body mass index, waist:hip ratio, blood pressure or glucose and insulin levels with any of the HRT regimes used in this study. Systolic blood pressure was reduced with the transdermal route.

Conclusions
Within the lifetime of a women resident in the western world, the risk of death from cardiovascular disease greatly exceeds all other single causes. The benefit from modification of cardiovascular risk and protection from osteoporosis depends on long-term use of HRT, so that any convenient method of delivery should be encouraged to promote adherence to therapy. We were surprised to find no apparent metabolic disadvantage with the use of progestagen continuously as compared with cyclically, which is encouraging, as long-term use of HRT is predicated on a lack of withdrawal bleed. This can be achieved with continuous combined oestrogen and progestagen regimes.

Oral HRT had a greater effect in reducing cholesterol and LDL, and might be the route of choice for women at increased risk from lipoprotein abnormalities. The reduction in LDL of 20% is comparable with the LDL lowering achieved in two randomized controlled trials of statins in which cardiovascular events in women were reduced in both primary and secondary prevention.[9,30] In our study, the HRT prescribed (oestradiol and norethisterone) reduced LDL without a concomitant increase in triglyceride. This contrasts with the changes in lipoproteins in the HERS in which premarin combined with medroxyprogesterone acetate decreased LDL but increased triglyceride.[11] There was no reduction in cardiovascular events in the HRT arm of this randomized control trial of secondary prevention. A further beneficial effect of oral HRT can be to increase HDL, an effect not obtained with the particular progestagen we studied, norethisterone. Ideally, HRT should include a progestagen which does not reduce HDL or increase triglyceride (such compounds currently are becoming more widely available).[35] In terms of cardiovascular risk factors, the oral route might be preferable for women with raised LDL; however, a reduction in systolic blood pressure was obtained with transdermal but not with oral HRT. This might influence choice of route for particular women. Post-menopausal hormone replacement using the modern yardstick of prospective randomized trials has yet to show a reduction in cardiovascular mortality, despite compelling evidence from observational studies. This study supports the evidence that oestrogen–progestagen HRT, both oral and transdermal, although it attenuates some benefits of oestrogen alone on fibrinogen and HDL, overall demonstrated significant reductions in cardiovascular risk factors which should diminish post-menopausal risk

of coronary disease. However, we still need to prove that the number of coronary events will be reduced in women on HRT.

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