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Metabolic, inflammatory and haemostatic effects of a low-dose continuous combined HRT in women with type 2 diabetes: potentially safer with respect to vascular risk?

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Summary

BACKGROUND Conventional hormone replacement therapy (HRT) containing conjugated equine oestrogen (CEE) and medroxyprogesterone acetate (MPA) increases triglyceride, C-reactive protein (CRP) and coagulation Factor VII concentrations, potentially explaining their increased coronary heart disease (CHD) and stroke risk.

OBJECTIVE To assess the metabolic effects of a continuous combined HRT containing 1 mg oestradiol and 0·5 mg norethisterone or matching placebo.

DESIGN Double-blind, randomized placebo-controlled trial.

PATIENTS Fifty women with type 2 diabetes.

MEASUREMENTS Classical and novel risk factors for vascular disease.

RESULTS Triglyceride concentration was not altered ($P = 0·31$, change in active arm relative to placebo) and low-density lipoprotein (LDL) cholesterol concentration declined 13% ($P = 0·018$). IL-6 concentration (mean difference –1·42 pg/ml, 95% CI: –2·55 to –0·29 IU/dl, $P = 0·015$), Factor VII (–32 IU/dl, –43 to –21 IU/l, $P < 0·001$) and tissue plasminogen activator antigen (by 13%, $P = 0·005$) concentrations fell, but CRP was not significantly altered ($P = 0·62$). Fasting glucose ($P = 0·026$) also declined significantly, but there are no significant effects on HBA1c, Factor IX or APC resistance.

CONCLUSIONS HRT containing 1 mg oestradiol and 0·5 mg norethisterone may avoid the adverse metabolic effects potentially implicated in the elevated CHD and stroke risk induced by conventional higher dose HRT. This type of preparation may therefore be more suitable than conventional HRT for women at elevated CHD risk such as those with type 2 diabetes. Large randomized controlled trials of such low dose preparations, powered for cardiovascular end points, are now needed.

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Until the publication of the Heart and Oestrogen/Progestogen Replacement Study (HERS; Hulley *et al*., 1998), and more recently of the Women's Health Initiative (WHI) study (Rossouw *et al*., 2002), many women and their physicians were convinced of the cardio-protective effects of hormone replacement therapy (HRT). In HERS, women with established CHD were randomized to 0·625 mg/day conjugated equine oestrogen (CEE) plus 2·5 mg/day medroxy-progesterone acetate (MPA) or matching placebo. The HRT group experienced an elevation in coronary heart disease (CHD) risk in the first year of use and no overall difference in events over 4 years (Hulley *et al*., 1998). The WHI used the same preparation in a primary prevention setting and also reported an increased risk of CHD and of stroke in the active arm compared to placebo (Rossouw *et al*., 2002), and more recently a potential deleterious effect on cognitive function (Rapp *et al*., 2003). Therefore, despite a beneficial effect of this preparation on low-density lipoprotein (LDL) and HDL cholesterol concentrations, other effects may be unfavourable; the search for these is receiving intense scrutiny. Most current attention has focussed on possible triglyceride-raising, pro-coagulant and pro-inflammatory effects of CEE and 2 mg oestradiol containing HRTs (Kroon *et al*., 1994; Petitti, 1998; van Baal *et al*., 1999b; Cushman *et al*., 1999; Ridker *et al*., 1999; Lowe *et al*., 2001b) but data on preparations containing low dose oestradiol combined to norethisterone, particularly from randomised placebo-controlled trials, are sparse.

Women with type 2 diabetes have a markedly elevated baseline risk for CHD. A recent report from a prospective observational study (Lokkegaard *et al*., 2003) suggested that HRT use leads to

a significantly increased risk of death from all causes and ischaemic heart disease among women with diabetes. Current users of HRT with diabetes had a near 10-fold increased risk of myocardial infarction [9·2, 95% confidence interval (CI), 2·0–41·4] compared with never users with diabetes. By contrast, Ferrara *et al*. (2003) noted that among diabetic women who did not have a recent myocardial infarction, current HRT use was associated with a significant 16% lower risk of acute myocardial infarction. Thus HRT effects on CHD risk in diabetes are controversial and randomized trials are required.

HRT preparations are not homogeneous with respect to metabolic effects: metabolic actions are profoundly altered according to route of delivery, dose and chemical nature of the combined oestrogenic and progestogenic preparations (Knopp & Zhu, 1997). Data from trials with differing designs suggest that lower doses of oestradiol (1 mg) or transdermal preparations may have fewer deleterious and (perhaps) even beneficial effects on inflammatory and haemostatic pathways (van Baal *et al*., 1999a; Sattar *et al*., 1999; Lowe *et al*. *et al.*, 2001b; Perera *et al*., 2001; Vehkavaara *et al*., 2001). In addition, there is an increasing awareness that androgenic progestogens such as norethisterone may offer several advantages over MPA, particularly with respect to coagulation and inflammatory parameters (Sattar *et al*., 1999; Perera *et al*., 2001).

The aim of the present randomized double-blind placebo-controlled study therefore was to examine the metabolic effects of a novel continuous combined preparation containing 1 mg oestradiol and 0·5 mg norethisterone in women with type 2 diabetes. We comprehensively assessed key pathways, including lipids and glycaemic parameters, and haemostatic and inflammatory pathways, known to be influenced by hormonal regulation and relevant to CHD risk. The hypothesis was that this low-dose oestradiol preparation combined with norethisterone would continue to reduce LDL cholesterol, limit any triglyceride rise and would have fewer potentially adverse effects on key coagulation and inflammatory parameters than observed with conventional CEE/MPA-based preparations.

Methods

Subjects

From December 1998 to September 2000, 50 women with type 2 diabetes aged under 70 years of age were recruited from general diabetic clinics in Glasgow Hospitals. Women randomized were clinically and biochemically postmenopausal, i.e. at least 1 year since last menses and a FSH concentration of greater than 20 IU/l. Menopause could be either natural or surgically induced. A normal pelvic examination and mammogram within the year prior to inclusion in the trial was also required.

Exclusion criteria comprised: poor glycaemic control; severe hypertriglyceridaemia (> 10 mmol/l); moderate to severe hypertension (systolic > 160 mmHg, diastolic > 110 mmHg); renal impairement (serum creatinine greater than twice the upper limit of normal range); liver disease (serum transaminases and bilirubin greater than twice the upper limit of normal range); or established cardiovascular, cerebrovascular, or peripheral vascular disease. Subjects with either a personal history of – or first-degree relative with – breast cancer were excluded.

Table 1 Baseline characteristics of study groups

	Group	
	Active, $n = 19$	Placebo, $n = 22$
Age (years)	60·7 (5·5)	61·3 (4·8)
BMI (kg/m^2)	30·5 (6·5)	29·8 (5·61)
Waist circumference (cm)	93·9 (11·3)	93·7 (13·6)
Years postmenopausal	14·6 (8·5)	14·2 (6·3)
Smokers (yes/no)	6/19	5/22
Systolic blood pressure (mmHg)	152 (17)	151 (21)
Diastolic blood pressure (mmHg)	87 (8)	83 (9)
Medications		
Diet alone (*n*)	5	3
Oral hypoglycaemics (*n*)	10	11
Insulin (*n*)	4	9
Anti-hypertensives (*n*)	10	10
Lipid-lowering agents (*n*)	5	5

Mean (SD) reported.

Women eligible at screening were randomized to prescription of either active medication (1 mg oestradiol plus 0·5 mg norethisterone) or identical placebo daily for 6 months. Randomization was effected in blocks of four using computer-generated numbers. Compliance was assessed by returned medication packs at the final visit and by oestradiol and gonadotrophin concentration measures at the final visit. A requirement of oestradiol to rise by more than10 pmol/l and FSH to decline by more than 5 U/l was predefined.

The participating hospitals' local research ethical committees granted approval. All subjects gave written informed consent to a single investigator (JM). As far as possible, existing medications for glycaemic control, blood pressure or lipid lowering (detailed in Table 1) were not altered throughout the course of the study.

We determined that our sample size had 80% power to detect a 5% reduction in LDL cholesterol and 90% power to detect a 5% reduction in factor VII with $\alpha = 0{\cdot}05$.

Study visits

Women attended the Diabetes Centre, Glasgow Royal Infirmary, Glasgow at baseline and 6 months, having fasted for 10 h and avoided heavy exercise, alcohol and caffeine in the

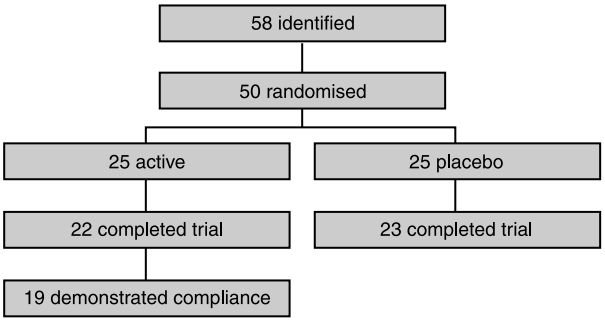

Fig. 1 Trial outcome flow chart.

preceding 24 h. Subjects rested prone for 15 min prior to blood pressure recordings being taken in triplicate (mean recorded). The women also had anthropometric measurements to include height (cm), weight (kg) and waist and hip circumferences (cm). From these, body mass index (BMI) was calculated as weight/(height)^2 in kg/m^2, as well as waist to hip ratio (WHR).

Laboratory methods

The reproductive hormones: oestradiol, LH, FSH, testosterone and SHBG were measured using semiautomated 'Immulite' technology (DPC, Los Angeles, CA, USA). Plasma total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol were determined by a modification of the standard Lipid Research Clinics Protocol. The intra-assay and interassay coefficients of variation (CVs) for lipid measures were both less than 3%. Fibrinogen, factor VII and factor IX, activated protein C (APC) ratio and tissue plasminogen activator (t-PA) antigen were measured in citrated plasma (0·11 M trisodium citrate; 9 : 1, v : v) as previously described (Woodward *et al*., 1997; Lowe *et al*., 1998, 2001a). The APC ratio measurement was an APTT-based test rather than a Factor V prediluted test. The intra-assay CVs for these haemostatic mediators were all less than 5%. C-peptide was measured using the DPC Immulite 2000 analyser with a CV of < 7%. Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit – Olympus AU5200, Olympus Optical Co Ltd, Tokyo, Japan).

CRP concentration was measured using an in-house sensitive double antibody sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (Sattar *et al*., 1999). The assay was linear up to 5 mg/l and logarithmic thereafter, and had a lower detection limit of 0·10 mg/l. The inter- and intra-assay coefficients of variation were less than 10% across the range of measured results. Sensitive IL-6 was measured by double antibody sandwich ELISA (R & D Systems, Minneapolis, MN, USA) with an intra-assay CV of 8%.

Statistical analysis

Mean differences in changes from baseline between the two treatment groups were compared using the unpaired *t*-test: the 95% CI for change in active group data relative to change in control group data are presented. Adjustment for baseline concentrations was made by linear regression. Baseline data are presented as mean and SD or median and interquartile range (IQR) for parameters exhibiting skewed distribution.

Results

Fifty-eight women were screened as potential recruits into the study. Of these, 50 were randomized (Fig. 1). The eight women not entered had unacceptably high HbA1c ($n = 3$), abnormal liver function tests ($n = 2$), abnormal mammogram ($n = 2$) or abnormal pelvic examination ($n = 1$). Five women did not complete the study due to either relocating or personal reasons. Thus, 45 women completed the study: of the 22 in the active group, 19 demonstrated adherence to study medications by predetermined criteria. Data from women falling outside these criteria were omitted from subsequent analyses. There were no serious adverse events. Breast tenderness and breakthrough bleeding were reported by three women on active treatment and by none on placebo.

Table 1 demonstrates the baseline characteristics of women completing the study. The two groups allocated to treatment were similar in age, BMI, blood pressure and years since menopause. All categories of diabetes therapy were represented and similar percentages were taking antihypertensive or lipid-lowering agents. The treatment groups also showed similar baseline hormonal and lipid concentrations (Tables 2 and 3).

A significant reduction in gonadotrophin concentrations and an elevation in oestradiol (all $P < 0·001$) and SHBG levels ($P = 0·042$) was observed in those women randomized to active therapy as a group (Table 2). Total testosterone was not altered significantly but free androgen index fell significantly ($P < 0·001$, data not shown).

Table 3 demonstrates lipid and glycaemic changes in the groups. Both total (10%) and LDL cholesterol (13%) concentrations were reduced significantly with active treatment ($P < 0·05$) but HDL cholesterol and triglyceride were not altered. Similarly, fasting C-peptide was reduced by 19% ($P < 0·01$ *vs.* change in placebo). Fasting glucose was also reduced ($P < 0·05$) in the active arm as was HbA1c but the latter change did not reach significance ($P > 0·10$).

Haemostatic and inflammatory variables are presented in Table 4. Significant reductions in Factor VII levels ($P < 0·001$), and t-PA antigen and IL-6 concentrations ($P < 0·02$) were observed without significant alteration in factor IX, APC resistance (APC ratio), fibrinogen or CRP concentrations.

Table 2 Sex hormone changes in active and placebo groups

	Active		Placebo			
	Baseline	Mean change	Baseline	Mean change	Difference for Δ active relative to Δ placebo (95% CI)	*P*
LH (IU/l)	35 (26–39)	−23·7	37 (24–44)	−0·4	−23 (−32–(−15))	< 0·001
FSH (IU/l)	53 (49–83)	−41·8	60 (48–81)	−2·5	−39 (−50–(−28))	< 0·001
Oestradiol (pmol/l)	63·3 (15·9)	165	65·4 (14·6)	2·0	163 (111–215)	< 0·001
Testosterone (nmol/l)	1·23 (0·63)	−0·12	1·19 (0·33)	0·0	−0·12 (−0·42–0·17)	0·410
SHBG (nmol/l)	33 (21–52)	15·7	37 (27–49)	1·4	14·3 (0·52–28·1)	0·042

Baseline data are given as mean (SD) or median (interquartile range).

Table 3 Lipids and insulin/glycaemia changes in active and placebo groups

	Active		Placebo			
	Baseline	Mean change	Baseline	Mean change	Difference for Δ active relative to Δ placebo (95% CI)	*P*
Lipids						
Cholesterol (mmol/l)	6·02 (1·07)	−0·62	5·68 (0·97)	−0·13	−0·49 (−0·05–(−0·90))	0·020
LDL-C (mmol/l)	4·14 (0·93)	−0·55	3·80 (1·00)	−0·10	−0·44 (−0·79–(−0·08))	0·018
HDL-C (mmol/l)	1·30 (0·32)	−0·07	1·36 (0·29)	−0·06	0·01 (−0·10–0·10)	0·830
Chol:HDL-C ratio	4·88 (1·58)	−0·28	4·46 (1·53)	0·20	−0·48 (−0·99–0·00)	0·050
Triglyceride (mmol/l)	1·75 (1·15–2·35)	−0·034	1·68 (1·25–2·21)	0·16	−0·19 (−0·58–0·19)	0·310
Glycaemia						
C-peptide (nmol/l)	0·97 (0·65–1·29)	−0·18	0·79 (0·49–1·51)	0·09	−0·27 (−0·44–(−0·09))	0·003
Glucose (mmol/l)	12·4 (4·2)	−1·74	11·3 (3·2)	0·42	−2·16 (−4·06–(−0·28))	0·026
HBA1c (%)	10·2 (1·8)	−0·37	10·2 (1·3)	0·22	−0·59 (−1·45–0·27)	0·170

Baseline data are given as mean (SD) or median (interquartile range).

Table 4 Haemostatic and inflammatory changes in active and placebo groups

	Active		Placebo			
	Baseline	Mean change	Baseline	Mean change	Difference for Δ active relative to Δ placebo (95% CI)	*P*
Haemostatic factors						
Factor VII (IU/dl)	160 (36)	−26·7	152 (31)	5·50	−32 (−43–(−21))	< 0·001
Factor IX (IU/dl)	163 (42)	7·00	155 (40)	3·60	3·7 (−9·9–16·3)	0·490
APC ratio	2·74 (0·6)	0·25	2·74 (0·5)	0·23	0·0 (−0·25–0·25)	0·990
tPA-antigen (ng/ml)	14·9 (5·6)	−2·01	12·7 (3·8)	0·97	−2·98 (−5·00–(−0·95))	0·005
Fibrinogen (g/l)	3·91 (0·68)	0·02	3·89 (0·92)	−0·12	0·14 (−0·19–0·47)	0·390
Inflammatory factors						
CRP (mg/l)	5·05 (4·46–8·53)	1·45	3·37 (1·76–8·10)	0·72	0·73 (−2·27–3·72)	0·620
IL-6 (pg/ml)	3·46 (2·48–4·89)	−0·32	3·55 (2·11–4·47)	1·10	−1·42 (−2·55–(−0·29))	0·015

Baseline data are given as mean (SD) or median (interquartile range).

Significance values were also checked with adjustment for baseline concentrations of all parameters measured. The results were in keeping with unadjusted values: specifically, reductions in cholesterol (adjusted $P = 0{\cdot}032$), Factor VII (adjusted $P < 0{\cdot}001$), tPA-antigen (adjusted $P = 0{\cdot}01$) and IL-6 (adjusted $P = 0{\cdot}045$) were similar and CRP remained similarly unchanged (adjusted $P = 0{\cdot}96$).

Finally, we determined the number of patients randomized to HRT or placebo that achieved 10% decline in LDL cholesterol

and Factor VII concentration using an intention to treat analysis. A 10% decline is either parameter in clinically relevant. Ten of the 25 in the HRT group had > 10% decline in LDL cholesterol compared to only four in the placebo group ($P = 0{\cdot}059$, Chi-square test). For Factor VII, 16 of the 25 in the HRT group had > 10% decline in Factor VII, whereas only one of the 25 in the placebo group did so ($P < 0{\cdot}0001$).

Discussion

Our study is one of the very few randomized double-blind placebo-controlled trials of HRT in diabetic women, albeit in an older group than those who would normally receive HRT. More importantly, it is the largest study to date to examine metabolic actions of a novel continuous combined preparation containing 1 mg oral oestradiol and 0·5 mg norethisterone in a high CHD-risk population. The key results were lowered plasma LDL cholesterol, Factor VII, t-PA antigen and IL-6 concentrations, and statistically similar triglyceride, Factor IX, APC resistance and CRP levels. This pattern of effects differs markedly from the profile produced by normal-dose HRT containing CEE and MPA used in HERS and WHI which increase triglyceride, Factor VII and promote a doubling in CRP concentrations (Hulley *et al*., 1998; Petitti, 1998). As a result, an HRT containing low-dose oestradiol and norethisterone may be more suitable for women who have an elevated risk of CHD (e.g. those with type 2 diabetes), who require HRT for menopausal symptom relief or bone protection. Formal clinical trials are required to test this suggestion.

The potentially better portfolio of metabolic effects of the HRT in this study may result either from the use of a low oestradiol dose or from the use of a more androgenic progestogen, norethisterone. More likely is that the balance of effects deriving from this HRT lean more towards androgenic rather than oestrogenic actions. This is an important point as conventional wisdom has dictated the use of nonandrogenic progestogens to minimize any HDL cholesterol reducing effect (Petitti, 1998). Clearly, this course of action needs re-evaluation as HDL cholesterol was unchanged in the present study.

With respect to the inflammation cascade, HRTs containing 2 mg oestradiol or CEE elevate CRP concentrations almost twofold (Ridker *et al*., 1999; Lowe *et al*., 2001b). In the observational arm of the (large) WHI, current HRT use was associated with higher CRP but, interestingly, similar IL-6 levels in those women not taking HRT (Pradhan *et al*., 2002). Moreover, transdermal delivery of oestradiol appears not to be associated with elevation of CRP (Sattar *et al*., 1999; Lowe *et al*., 2001b; Vehkavaara *et al*., 2001) which therefore may represent a 'first pass' effect on hepatic CRP synthesis (Lowe *et al*., 2001b). Because elevated CRP levels in men and women are independently linked to risk for CHD and stroke (Ridker, 2001), the HRT-induced CRP rise has been put forward as a potential pathway explaining the results of HERS and WHI (Kroon *et al*., 1994; Ridker *et al*., 1999). It should be acknowledged, however, that even though vascular literature suggests several mechanisms whereby CRP may be directly atherogenic (Ridker, 2001), it is not yet known whether the HRT-induced CRP rise leads directly or indirectly to a biologically adverse outcome.

Our data demonstrate a lack of significant rise in CRP in those assigned active therapy compared to the placebo group ($P = 0{\cdot}62$ crude, $P = 0{\cdot}96$ adjusted difference). Although power may be an issue here, studies in similar size to the present one, but employing CEE- or 2 mg oestradiol-based preparations, have demonstrated significant, near twofold elevations in CRP (van Baal *et al*., 1999b; Manning *et al*., 2002). A lack of CRP rise in the present study concurs with results from two other studies in nondiabetic women that used lower oestradiol doses (1 mg; van Baal *et al*., 1999a; Stork *et al*., 2002). Moreover, HRT combining oral norethisterone with transdermal oestradiol may lower CRP (Sattar *et al*., 1999); thus any tendency to an oestradiol-induced CRP elevation in the present study may have been attenuated by the oral norethisterone. The reduction in IL-6 concentration is of interest as androgens exhibit anti-inflammatory effects in several tissues (Gornstein *et al*., 1990). Because of the variability of circulating inflammatory markers and the wide confidence interval in the result reported in this and studies of similar size (van Baal *et al*., 1999a; Zanger *et al*., 2000), larger studies using low-dose HRT preparations are now urgently required to confirm our findings. In this respect, a potentially lower CHD risk with lower doses of HRT has recently been suggested by Ferrera *et al*. (2003) in their analysis of data from the Northern California Kaiser Permanente Diabetes Registry.

That triglyceride did not rise in this study is also relevant to CHD risk. Oral oestrogens, particularly CEE-based preparations, significantly increase circulating triglyceride concentrations by increasing hepatic synthesis of triglyceride-rich particles (Knopp *et al*., 1997). Increases in triglyceride concentration may enhance plaque instability by affecting platelet and endothelial function, as well as altering coagulation and vascular inflammation (Sattar *et al*., 1998; Dichtl *et al*., 1999). Triglyceride concentration is independently linked to CHD risk, particularly in women (Hokanson & Austin, 1996). Indeed, the HERS investigators speculated that the 10% rise in triglyceride concentration in their study may have contributed to the early increase in CHD events despite the positive changes in other lipid parameters (Hulley *et al*., 1998). In general, increase in triglyceride concentration is less pronounced with oral oestradiol-containing HRTs compared to CEE-based HRTs and absent with transdermal delivery (Knopp *et al*., 1997). Moreover, androgens decrease triglyceride concentration (Knopp *et al*., 1997; Perera *et al*., 2001), thereby opposing any tendency for an oestradiol-mediated increase.

Consistent with the reduction in Factor VII coagulation activity (by 17%) with active treatment in this study, similar reductions

have been reported with HRTs combining transdermal oestradiol with either oral MPA (10 mg; Kroon *et al*., 1997) or oral 1 mg norethisterone (Vehkavaara *et al*., 2001). By contrast, oral 2 mg oestradiol or 0·625 mg CEE alone increase Factor VII activity, whereas transdermal oestradiol has a negligible effect (Kroon *et al*., 1994; Lowe *et al*., 2001b; Vehkavaara *et al*., 2001). These data strongly suggest that oral progestogens reduce Factor VII coagulation activity. The reduction in Factor VII herein therefore indicates an overall hormonal balance favouring a dominant norethisterone action. Although elevated Factor VII coagulation activity has been associated with an increased risk of coronary thrombosis in one study of men (Meade *et al*., 1980), similar data in women are lacking. The relevance of HRT-mediated changes in Factor VII levels therefore requires clarification.

A further potential benefit noted in our study was a reduction in fasting glucose concentration. Whether this reflects an improvement in insulin action must be viewed with a degree of caution as we did not directly measure insulin action using clamp techniques, and many women were taking insulin therapy. Future studies should measures insulin action directly. Nevertheless, although HbA_{1c} was not significantly altered, an improvement in insulin sensitivity or reduced hepatic glucose production accords with findings of two previous randomized placebo-controlled trials in women with diabetes with unopposed oral 2 mg 17β-oestradiol alone (Anderson *et al*., 1997; Brussard *et al*., 1997). The recent report of a significant reduction in the incidence of diabetes (by 35%) in women with coronary disease assigned to active treatment in HERS study as compared to those given placebo (Kanaya *et al*., 2003) suggests that conventional HRT may lessen risk of diabetes. Whether low-dose HRT containing oestradiol and norethisterone has the same (or even larger) effect deserves further study.

The observed reduction in tPA-antigen (which largely measures circulating t-PA-PA1 complexes) is in keeping with a reduction in PAI-1, which was not directly measured. PAI-1 has also been shown to fall in oral HRT studies (Koh *et al*., 1997; Andersen *et al*., 1999; Lowe *et al*., 2001b). Alternatively, the fall in t-PA may reflect reduced endothelial disturbance (i.e. t-PA release). Interestingly, elevated tPA-Ag (but not PAI-1) levels independently predicted CHD event rate in a recent meta-analysis of prospective studies in general populations (Lowe *et al*., 2001a).

There are several notable strengths of this study. Firstly, it is one of the very few randomized double-blind placebo-controlled trials of HRT in diabetic women, a group at elevated baseline risk of CHD and at potentially elevated CHD risk with conventional HRT therapy (Lokkegaard *et al*., 2003). Secondly, the novel low-dose preparation was very well tolerated and the side-effect profile was excellent, helping maintain investigator blinding. Thirdly, we tracked adherence to study medication by measuring oestradiol and gonadotrophin concentrations. Finally, we assessed a number of key pathways for CHD risk simultaneously, an approach that facilitated a more comprehensive assessment of the overall balance of metabolic, haemostatic and inflammatory effects of the low-dose HRT used. A limitation of our study is the modest number of patients recruited. Nevertheless, statistically significant changes detected in several key parameters indicate sufficient power to detect meaningful changes in pathways of interest; moreover, the results have biological plausibility. Our data therefore provide a strong basis for future studies examining the clinical safety of low dose HRT containing oestradiol and norethisterone in preference to conventional HRT containing CEE and MPA, supporting an emerging consensus that lower doses may be the safer option for many women (Ferrara *et al*., 2003; Herrington, 2003).

In conclusion, our study shows that low-dose HRT containing 1 mg oestradiol and 0·5 mg norethisterone generates a vastly different portfolio of metabolic and haemostatic action compared to that observed with use of conventional higher dose HRT. Specifically, triglyceride, Factor IX, APC resistance and CRP levels were statistically similar, whereas IL-6 and Factor VII levels were significantly reduced and LDL cholesterol lowering was retained. On the basis of our data, we suggest that a preparation containing low-dose oestradiol combined with norethisterone may be more suitable for women who require HRT for menopausal symptom relief or bone protection but who are at higher risk of CHD, such as those with type 2 diabetes. However, before definitive recommendations are made, such novel formulations should be assessed in a large randomized controlled trial powered for cardiovascular endpoints.

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