

Different Effects of Oral and Transdermal Hormone Replacement Therapies on Factor IX, APC Resistance, t-PA, PAI and C-reactive Protein

A Cross-sectional Population Survey

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Keywords

Oestrogens, menopause, C-reactive protein, coagulation, fibrinolysis

Summary

The effects of hormone replacement therapy (HRT) on thrombosis risk, thrombotic variables, and the inflammatory marker C-reactive protein (CRP) may vary by route of administration (oral versus transdermal). We studied the relationships of 14 thrombotic variables (previously related to cardiovascular risk) and CRP to menopausal status and to use of HRT subtypes in a cross-sectional study of 975 women aged 40-59 years. Our study confirmed previously-reported associations between thrombotic variables and menopausal status. Oral HRT use was associated with increased plasma levels of Factor IX, activated protein C (APC) resistance, and CRP; and with decreased levels of tissue plasminogen activator (t-PA) antigen and plasminogen activator inhibitor (PAI) activity. Factor VII levels were higher in women taking unopposed oral oestrogen HRT. The foregoing associations were not observed in users of transdermal HRT; hence they may be consequences of the "first-pass" effect of oral oestrogens on hepatic protein synthesis. We conclude that different effects of oral and transdermal HRT on thrombotic and inflammatory variables may be relevant to their relative thrombotic risk; and suggest that this hypothesis should be tested in prospective, randomised studies.

Introduction

The risk of cardiovascular disease, especially ischaemic heart disease (IHD) increases after the female menopause. Observational studies suggest that hormone replacement therapy (HRT) decreases the risks of IHD and total mortality (1-3). Possible mechanisms for these associations include changes in blood lipids, blood pressure and insulin resistance; they also include changes in haematological variables related to thrombotic risk through their roles in blood coagulation, fibrinolysis or blood flow (rheology) (3, 4). Recently, case-control studies as well as clinical trials have shown that HRT increases the risk of venous thromboembolism 2-4 fold (5-10). One trial also suggested an early increase in risk of myocardial infarction in women with IHD who take HRT (10). The mechanisms for increased arterial and venous thrombotic risk are not defined, but may include acquired resistance to activated protein C (APC), which we have recently proposed as a common mechanism through which several genetic or acquired risk factors (including HRT) may promote venous thrombosis (11).

In recent years, transdermal HRT preparations are increasingly prescribed. Their potential advantages include acceptability; provision of relatively constant physiological levels of oestradiol while avoiding unnaturally high plasma levels of oestrogen and other metabolites; and avoiding the metabolic consequences of the high hormone concentrations in the hepatic portal vein following oral HRT, which include the hepatic synthesis of proteins involved in coagulation, fibrinolysis and the acute-phase response (12). Few comparisons of oral and transdermal HRT have been reported (3, 4, 12-14); however they suggest potentially important differences in their effects on coagulation and fibrinolysis.

The aim of the present study was to examine the relationships of 14 thrombotic variables which have previously been related to risk of IHD and/or venous thromboembolism (15) to menopausal status and to use of oral or transdermal HRT, in a cross-sectional study of women aged 40-59 years who participated in a family study of cardiorespiratory disease (Midspan Family Study) in the West of Scotland (16). Furthermore, we sought to examine the extent to which any such relationships might be explained by changes in blood lipids or lipoproteins (15, 17, 18) or by acute-phase reactants, as measured by serum levels of C-reactive protein (CRP) (19).

Subjects and Methods

The eligible population for the Midspan Family Study were offspring aged 30-59 years of parents who participated during middle-age in a general population study between 1972-1976, the Renfrew-Paisley (Midspan) Study (20). 3,202 such offspring were identified who lived within 45 min travel of the Renfrew-Paisley area, of whom 1,040 men and 1,298 women completed a questionnaire and examination: a response rate of 73% from the eligible population (16). The study was approved by the appropriate local research ethics committees, and all participants gave their written informed consent. Besides demographic and lifestyle information, for women, the questionnaire included questions on pregnancy, menopausal status, and use and type of HRT or oral contraceptives. On the day they attended for venepuncture participants were asked whether they had suffered from any infections in the previous seven days; there were specific questions about suffering from a sore throat, cold or influenza.

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There were 1056 women aged 40-59, of whom 965 had complete questionnaire data, underwent venepuncture, were not pregnant nor taking oral contraception, and were not on warfarin therapy. The sample also included 10 women aged 30-39 with an early menopause who met the same criteria. Examination included height and weight, for calculation of body mass index, BMI = weight (kg)/ height $(m)^2$.

A non-fasting venous blood sample was taken for measurement of plasma lipids (total cholesterol, triglyceride and HDL cholesterol; according to the Lipid Research Clinics Manual of Laboratory Operations) and CRP (ELISA, standardised using the International Reference Standard Lot 91/0619) in the Institute of Biochemistry, Glasgow Royal Infirmary. Thrombotic variables were measured in the University Department of Medicine, Glasgow Royal Infirmary. Plasma viscosity, microhaematocrit, whole blood viscosity (calculated from plasma viscosity and haematocrit) and white cell count were measured in fresh, dipotassium edetate (1.5 mg/ml) anticoagulated blood as previously described (21). Plasma fibrinogen, factor VII, factor VIII and factor IX (22); activated partial thromboplastin time (APTT) and activated protein C (APC) ratio (11); tissue plasminogen activator (t-PA) antigen, plasminogen activator inhibitor (PAI) activity, and fibrin D-dimer antigen (23); and von Willebrand factor antigen (24) were measured in citrated plasma (0.11 M trisodium citrate; 9 : 1 v : v) as previously described. Allowance was made for potential carriers of the factor V Leiden mutation by repeating statistical analyses, after excluding subjects who showed lack of correction of a low APC ratio (less than 2.15) when the assay was repeated in factor V deficient plasma.

Data were analysed using S-Plus for Windows v4.5. Each response variable was modelled using multiple linear regression to look for differences between women not on HRT (before or after menopause) and women on HRT (categorized primarily according to whether the route of administration was oral or transdermal, and secondarily whether the oestrogen was opposed or unopposed by progestogen). Each model also included a term for a linear age effect, regardless of whether or not it was statistically significant. Analyses were repeated after including a term for duration of HRT use. If necessary, response variables were transformed so that the models produced roughly normally distributed residuals. In most cases, the response was log transformed, except for white cell count which was given a square root transformation, and haematocrit, APC ratio, PAI and D-dimer which were left untransformed. Tests of significance and 95% confidence intervals were not adjusted for multiple comparisons; however, we adjusted for clustering of siblings within the same family by using generalised estimating equations.

Each logistic regression model provided age-adjusted effect estimates (with standard errors) of differences between subject groups. 95% confidence intervals were calculated by adding and subtracting 1.96 standard errors from each effect estimate. No adjustments were made for multiple testing. For untransformed variables these estimates and confidence intervals are directly interpretable as differences between group averages. For variables that were log transformed, the estimates and confidence intervals were back transformed by taking exponentials to give ratios between groups, and these were then converted into percentage differences, by subtracting 1 and multiplying by 100.

Results

Risk Factor Variables (Table 1)

After defined exclusions, analyses were performed on up to 522 premenopausal women (periods, no HRT); 222 post-menopausal women (no periods) not currently taking HRT; and 231 women currently taking HRT. Of the latter, 183 were taking oral HRT (58 taking unopposed oestrogen; 125 taking combined oestrogen and progestogen) and 48 were taking transdermal HRT (35 taking unopposed oestrogen, and 13 taking combined oestrogen and progestogen). As expected, there were small but statistically significant differences in age and smoking habit between premenopausal and postmenopausal women (Table 1); but there were no significant differences in age or smoking between women on oral HRT and those on transdermal HRT. However, users of unopposed oral HRT were younger than users of combined oral HRT (effect estimate – 2.65 years; 95% CI – 4.01, – 1.30; $p < 0.001$). As noted in the Methods section, all comparisons were age-adjusted.

There were no significant differences in body mass index between subject groups. There were significant postmenopausal increases in serum cholesterol, triglyceride and total cholesterol/HDL ratio. Overall, HRT use (oral or transdermal) was not associated with changes in triglyceride; however oral HRT appeared to partly reduce the postmenopausal increase in cholesterol, and also prevented the postmenopausal increase in total cholesterol/HDL ratio (Table 1). The only significant difference in serum lipids between oral HRT users and transdermal HRT users was a lower HDL cholesterol level in transdermal HRT

Table 1 Age, smoking habit, body mass index (BMI) and serum lipids in subject groups. IQR = inter-quartile range; a = significant difference relative to "Periods, No HRT" group; b = significant difference relative to "No Periods, No HRT" group; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Raw data with age-adjusted significance tests (except for age)

		Periods, No HRT	No Periods, No HRT	Oral HRT	Transdermal HRT	Oral vs Transdermal p-value
N Max		522	222	183	48	
Age (years)	Mean ±SE	44.9 ±0.14	50.9 ±0.33 a***	48.8 ±0.33 a*** b***	49.4 ±0.56 a*** b*	0.39
Current Smokers	N / Total (%)	116 / 522 (22.2)	68 / 222 a*** (30.6)	53/183 a** (29.0)	12 / 48 (25.0)	0.55
BMI (kg/m^2)	Median (IQR)	25.1 (22.3, 28.3)	25.5 (22.7, 28.9)	24.9 (23.1, 27.9)	25.6 (22.7, 28.3)	0.93
Cholesterol (mmol/l)	Median (IQR)	5.0 (4.4, 5.55)	5.6 a*** (5.05, 6.2)	5.25 a** b** (4.85, 5.85)	5.15 (4.74, 5.75)	0.98
Triglyceride (mmol/l)	Median (IQR)	1.05 (0.8, 1.4)	1.3 a*** (0.95, 1.9)	1.3 a*** (0.9, 1.85)	1.25 a* (0.89, 1.75)	0.80
HDL Cholesterol (mmol/l)	Median (IQR)	1.45 (1.25, 1.7)	1.45 (1.25, 1.7)	1.5 (1.3, 1.8)	1.4 (1.2, 1.55)	0.036
Total Cholesterol / HDL Ratio	Median (IQR)	3.41 (2.76, 4.09)	3.76 a** (3.15, 4.61)	3.36 b** (2.88, 4.27)	3.60 (3.00, 4.29)	0.17

users (effect estimate – 7.9%; 95% CI – 14.8, – 0.54; p = 0.036). Among oral HRT users, those using unopposed oestrogens had higher median levels of cholesterol (8.3%; 95% CI 2.8, 14.0; p = 0.0027), triglyceride (24.6%, 95% CI 7.7, 44.3; p = 0.0032) and HDL cholesterol (17.0%; 8.0, 26.8; p = 0.0001). There was no significant difference in total cholesterol/HDL ratio (-8.0%; 95% CI – 16.8, 1.6; p = 0.10). There were no significant differences in serum lipids comparing users of transdermal opposed oestrogen and users of transdermal unopposed oestrogen.

Coagulation Factors (Table 2)

Plasma fibrinogen was significantly higher in postmenopausal women compared to premenopausal women. HRT had no significant effect on plasma fibrinogen. There was no significant difference in fibrinogen levels between users of oral HRT and users of transdermal HRT, with or without adjustment for smoking habit as well as age (p = 0.74).

Factor VII and Factor IX levels were also significantly higher in post-menopausal compared to pre-menopausal women. Users of transdermal HRT had similar factor VII levels to premenopausal women. The effect of oral HRT on factor VII levels differed according to HRT type: higher median levels were seen in users of unopposed oral oestrogen (139 iu/dl; IQR 125,163) than in users of combined oral oestrogen, who also had similar levels to premenopausal women (114 iu/dl; IQR 101,132) (effect estimate + 26.0%; 95% CI 17.6, 34.9; p <0.0001). This effect was similar after adjustment for triglyceride and total cholesterol levels and body mass index (+21.7%; 14.3, 29.6, p <0.0001).

The effect of HRT on factor IX levels also differed by HRT type: higher levels compared to postmenopausal women not taking HRT were seen in users of oral HRT, while use of transdermal HRT was associated with significantly lower levels compared to use of oral HRT (effect estimate – 12.5%; 95% CI – 18.3, – 6.2; p = 0.0002), which were similar to those in premenopausal women.

Factor VIII and von Willebrand factor antigen did not vary significantly with menopause or HRT, apart from a slightly lower factor VIII level in users of transdermal HRT compared to post-menopausal women not using HRT (Table 2). Users of transdermal HRT also had a slightly lower level of factor VIII than users of oral HRT (effect estimate – 9.7%; 95% CI – 18.6, 0.2; p = 0.055).

APTT and APC ratio (Table 3)

A small increase in APTT was observed after the menopause, with a further increase in users of transdermal HRT. APTT was longer in users of transdermal HRT than in users of oral HRT (effect estimate 4.1%; 95% CI 1.2, 7.0; p = 0.0052). This effect was reduced on multi-

Table 2 Coagulation factors in subject groups. IQR = inter-quartile range; a = significant difference relative to "Periods, No HRT" group; b = significant difference relative to "No Periods, No HRT" group; * = p <0.05; ** = p <0.01; *** = p <0.001. Raw data with age-adjusted significance tests

		Periods, No HRT	No Periods, No HRT	Oral HRT	Transdermal HRT	Oral vs Transdermal p-value
N Max		517	218	181	48	
Fibrinogen (g/l)	Median (IQR)	3.09 (2.70, 3.55)	3.28 a* (2.84, 3.76)	3.13 (2.83, 3.54)	3.32 (2.66, 3.72)	0.74
Factor VII (iu/dl)	Median (IQR)	114 (99, 130)	128 a*** (111, 146)	124 a** (102, 144)	117 (104, 140)	0.61
Factor VIII (iu/dl)	Median (IQR)	132 (108, 160)	136 (115, 172)	136 (109, 165)	134 a* b* (95, 153)	0.055
vWF (iu/dl)	Median (IQR)	104 (84, 131)	114 (88, 146)	105 (84, 136)	100 (80, 130)	0.44
Factor IX (iu/dl)	Median (IQR)	117 (102, 140)	129 a** (113, 153)	136 a*** b* (118, 158)	119 b* (108, 139)	0.0002

Table 3 APTT, APC ratio and fibrinolytic variables in subject groups. IQR = inter-quartile range; a = significant difference relative to "Periods, No HRT" group; b = significant difference relative to "No Periods, No HRT" group; * = p <0.05; ** = p <0.01; *** = p <0.001. Raw data with age-adjusted significance tests

		Periods, No HRT	No Periods, No HRT	Oral HRT	Transdermal HRT	Oral vs Transdermal p-value
N Max		516	217	180	48	
APTT (s)	Median (IQR)	28.2 (26.7, 29.9)	28.4 a* (27.2, 29.9)	27.9 (26.7, 29.7)	29.3 a** (27.7, 31.0)	0.0052
APC Ratio	Median (IQR)	2.72 (2.51, 2.92)	2.75 (2.52, 2.92)	2.64 a* b** (2.41, 2.84)	2.80 (2.60, 2.93)	0.019
t-PA (ng/ml)	Median (IQR)	6.5 (5.0, 8.6)	8.0 a* (6.1, 10.6)	5.9 a*** b*** (4.3, 8.3)	7.1 (5.5, 9.2)	0.024
PAI (% pool)	Median (IQR)	81 (68, 98)	89 a* (72, 106)	74 a*** b*** (61, 87)	83 (67, 113)	0.0018
D-dimer (ng/ml)	Median (IQR)	15 (9, 22)	13 (7, 22)	14 (9, 23)	12 (7, 17)	0.74

variate analysis including factor VIII and factor IX levels (2.36%; –0.00, 4.84; p = 0.053).

APC ratio did not vary with menopause, but was significantly lower in the oral HRT group compared to pre-menopausal women and post-menopausal non-users of HRT. This effect was similar in users of unopposed oral HRT (2.59; IQR 2.33, 2.80) and combined oral HRT (2.64; IQR 2.45, 2.84); but was not seen in users of transdermal HRT. APC ratio was higher in users of transdermal HRT compared to users of oral HRT (effect estimate 0.11 (absolute change); 95% CI 0.02, 0.21; p = 0.019). This effect was attenuated only slightly in a multivariate analysis that included factor VIII and factor IX levels (effect estimate 0.09; -0.01, 0.18; p = 0.070), and in a repeat analysis excluding the 28 subjects who were potential carriers of the V Leiden mutation as defined in the Subjects and Methods section (0.08; – 0.01, 0.18; p = 0.073). The effect of oral HRT on APC ratio compared to post-menopausal non-users of HRT remained similar on multivariate analysis including these variables (-0.10 absolute units; 95% CI -0.16, -0.03, p = 0.0052), as did the lack of effect of transdermal HRT compared to post-menopausal non-users of HRT (effect estimate – 0.01 absolute units; –0.11, 0.19; p = 0.80).

Fibrinolytic Variables (Table 3)

t-PA antigen and PAI activity were higher in post-menopausal compared to pre-menopausal women; and were significantly lower in oral HRT users compared to both pre-menopausal women and post-menopausal non-users. This effect was greater in users of unopposed oral HRT (median t-PA 5.55 ng/ml; IQR 3.85, 6.9; PAI 69% pool; 57, 85) than in users of combined oral HRT (median t-PA 6.2 ng/ml; IQR 4.47, 8.62; PAI 75% pool; 63, 88); and was statistically significant for t-PA (effect estimate – 95% CI 14.1%; -25.2, – 1.3; p = 0.033) but not for PAI (p = 0.22). t-PA levels were significantly higher in users of transdermal HRT compared to users of oral HRT (effect estimate 17.4%; 95% CI 2.1, 35.0; p = 0.024), and this effect was similar after adjustment for triglyceride and total cholesterol levels and body mass index (18.4%; 3.0, 36.1; p = 0.018). PAI levels were also significantly higher in users of transdermal HRT compared to users of oral HRT (effect estimate 16.5% pool; 95% CI 6.1, 26.8; p = 0.0018), and again this effect was similar after adjustment for triglyceride and total cholesterol levels and body mass index (16.5% pool; 5.9, 27.1; p = 0.0023). t-PA and PAI levels in transdermal HRT users were similar to levels in non-users of HRT.

No significant effects of menopause or HRT use on D-dimer were observed.

C-reactive Protein, White Cell Count, Haematocrit and Viscosity (Table 4)

There were no significant associations of the menopause with CRP or white cell count. Oral HRT use was overall associated with significant increases in CRP. This effect was similar in users of unopposed oral HRT (median 2.40 ng/ml; IQR 0.95, 5.10) and in users of combined oral HRT (median 2.06 ng/ml; IQR 0.79, 4.44); but was not observed in users of transdermal HRT. CRP levels were significantly lower in users of transdermal HRT compared to users of oral HRT (effect estimate – 50.7%; 95% CI – 69.1, –21.2; p = 0.0031), and were similar after adjustment for smoking habit and history of recent infection (–50.3%; – 68.9, – 20.7; p = 0.0034).

Haematocrit, blood viscosity and plasma viscosity were each significantly higher in post-menopausal compared to pre-menopausal women. The post-menopausal increases in plasma and blood viscosity (but not haematocrit) were reduced by use of oral HRT; but not by transdermal HRT use. Haematocrit was higher in users of transdermal HRT compared to users of oral HRT (effect estimate 1.04 haematocrit %; 95% CI 0.03, 2.04; p = 0.044), and there was a similar but non significant trend, for blood viscosity (effect estimate 2.4%; – 0.10, 4.98; p = 0.061).

Duration of Use

Mean duration of use was 3.7 (SEM 0.2) years in the oral HRT group and 4.1 (SEM 0.5) years in the transdermal HRT group. No significant effects of duration of HRT use on the above results were observed (data not shown). Hence data are presented unadjusted for duration of use.

Discussion

We have shown that oral HRT use (but not transdermal HRT use) is associated with increased plasma levels of factor IX, APC resistance, and CRP; and with decreased levels of t-PA antigen and PAI activity.

Table 4 C-reactive protein, white cell count, viscosity and haematocrit in subject groups. IQR = inter-quartile range; a = significant difference relative to Periods, No HRT group; b = significant difference relative to No Periods, No HRT group; * = p <0.05; ** = p <0.01; *** = p <0.001. Raw data with age-adjusted significance tests

		Periods, No HRT	No Periods, No HRT	Oral HRT	Transdermal HRT	Oral vs Transdermal p-value
N Max		507	217	177	46	
C-reactive Protein (ng/ml)	Median (IQR)	0.68 (0.30, 1.52)	0.94 (0.43, 2.22)	2.29 a*** b*** (0.92, 4.89)	0.82 (0.41, 1.67)	0.0031
White Cell Count (10^9/l)	Median (IQR)	6.07 (5.00, 7.34)	5.94 (4.88, 7.24)	6.37 (4.99, 7.59)	6.30 (5.06, 7.68)	0.53
Plasma Viscosity (mPa.s)	Median (IQR)	1.22 (1.18, 1.26)	1.24 a** (1.20, 1.28)	1.22 b* (1.18, 1.27)	1.23 (1.19, 1.27)	0.88
Haematocrit (%)	Mean ±SE	38.94 ±0.14	40.14 ±0.21 a***	39.60 ±0.21 a*	40.65 ±0.47 a***	0.044
Blood Viscosity (mPa.s)	Median (IQR)	2.85 (2.71, 3.01)	2.95 a*** (2.79, 3.15)	2.91 a* b* (2.76, 3.07)	3.00 a** (2.85, 3.13)	0.061

The first three of these findings may be relevant to the increased risk of venous thromboembolism in users of oral HRT; which we have recently shown to be highest in women with high factor IXc, APC resistance, or low antithrombin activity (25). We suggest that the lack of effect of transdermal HRT on these variables be confirmed in further, larger studies (prospective as well as cross-sectional). If confirmed, such differences in effects may be one factor to consider when prescribing HRT to women with thrombophilias.

Effects of Menopause

We confirmed several potentially adverse effects of the female menopause (after age-adjustment) on thrombotic variables related to risk of IHD: increased blood viscosity, partly due to increases in haematocrit, and partly to increased plasma viscosity (21), which in turn reflected increases in fibrinogen and lipids (3, 4, 21, 22, 26). These rheological effects of the menopause were not associated with significant increases in markers of acute-phase reactions such as white cell count or C-reactive protein (19, 21). Increases in factors VII and IX; and increases in the fibrinolytic inhibitor PAI-1 (and in t-PA antigen, which probably reflects inactive PAI-1 – t-PA complexes) were also observed, confirming previous studies (3, 4, 13, 14, 22). No effect of menopause was observed on the factor VIII: von Willebrand factor complex, or on fibrin D-dimer.

Effects of HRT-Coagulation

Plasma fibrinogen levels were similar in users of oral HRT compared to premenopausal women in the present study, as in previous observational studies and randomised trials, which show a fibrinogen-lowering effect of oral HRT (3, 4, 14, 25, 27, 28). In contrast, users of transdermal HRT in the present study had similar levels of fibrinogen to post-menopausal non-users of HRT, consistent with previous reports (14). However the difference between users of oral HRT and users of transdermal HRT was not statistically significant, whether or not smoking was adjusted for as well as age.

Overall, factor VIIc levels were similar in users of oral HRT and in postmenopausal non-users of HRT. However, we observed that users of oral unopposed oestrogen had significantly higher levels, while users of combined oestrogen-progestogen HRT had significantly lower levels. These findings suggest (a) that unopposed oestrogen exerts a significant "first pass" effect on hepatic synthesis of factor VII (12); and (b) that progestogens significantly modify the effects of oestrogen in increasing factor VII. The latter effect has also been observed in another cross-sectional study (27) and in a randomized trial (28). Because of the important effects of cholesterol and triglyceride on factor VII antigen and activity (15, 17, 18), it has been suggested that differences between factor VII levels in users of different oral HRT preparations may partly reflect different effects of such preparations on blood lipids (29). However in the present study, adjustment for total cholesterol, triglycerides and body mass index did not explain these differences. We did not study the influence of factor VII genotypes on factor VII levels in the present study. We observed no significant effect of transdermal HRT use on factor VII levels compared to premenopausal women, consistent with previous reports (14).

We confirmed that use of oral HRT increases factor IX levels, compared to postmenopausal non-users of HRT (22). We observed no significant effect of transdermal HRT use on factor IX levels compared to premenopausal women not using HRT, consistent with a previous report (14). This difference in factor IX levels between users of oral and transdermal HRT appeared to contribute to the difference in APTT levels observed in the present study.

We observed little overall effect of HRT use on factor VIII or von Willebrand factor levels in the present study, consistent with most previous reports (3, 4, 14, 22, 27). However, we observed that users of transdermal HRT had a lower level of factor VIII than users of oral HRT, which while of borderline statistical significance appeared to contribute to the difference in APTT levels observed in the present study. We did not study the influence of blood group on factor VIII or von Willebrand factor levels.

We have confirmed that HRT use is associated with increased APC resistance, as shown by a lower APC ratio (11). In the present study, we have shown (a) that this association is similar in users of oral unopposed oestrogen HRT and in users of oral combined HRT; and (b) that this association was not present in users of transdermal HRT. These results were unaffected when we excluded subjects with a phenotype (lack of correction of low APC ratio when repeated in factor V deficient plasma) which is correlated with the factor V Leiden genotype. Our finding that 3% of women in the West of Scotland were potential carriers of V Leiden by this screening assay is very similar to our previous findings in this population using DNA analysis (11). Our observation of the lack of effect of transdermal HRT use on APC resistance has recently been confirmed in a prospective study, which indeed suggested a beneficial effect of transdermal HRT on APC resistance, possibly as a result of decreased factor VIII:c levels (30).

Effects of HRT-Fibrinolysis

We have confirmed previous reports (13, 14, 31) that oral HRT use, but not transdermal HRT use, is associated with lower levels of PAI activity and t-PA antigen compared to post-menopausal non-users of HRT. These effects of oral HRT may reflect a "first-pass" effect on hepatic PAI-1 synthesis (12). While both PAI-1 and t-PA antigen show significant associations with plasma total cholesterol and triglyceride levels, obesity, and insulin resistance (15, 17, 18), we observed no effect of adjustment for these lipid variables (and body mass index) on the effect of oral HRT on PAI activity or t-PA antigen in the present study. We did not observe any relationships of reduced PAI levels in oral HRT users to increased fibrin D-dimer levels, in contrast to a previous report (31). While reductions in PAI and t-PA antigen levels with oral HRT use have been postulated as potentially beneficial (13, 31), this hypothesis has not yet been tested in controlled trials of PAI-1 reduction.

Effects of HRT-CRP, White Cell Count, Haematocrit and Viscosity

We observed that oral HRT (but not transdermal HRT) significantly increases plasma C-reactive protein, a marker of the hepatic protein response to inflammation. This observation is consistent with two recent reports (32, 33) and may reflect oestrogenic effects on inflammatory monokines such as tumour necrosis factor and interleukin-6 (21, 34). It is possible that increases in acute-phase reactant proteins may be a cause of low APC ratios: ceruloplasmin may modulate APC resistance (35). The lack of effect of transdermal HRT on CRP may be relevant to risk of IHD, because CRP levels within the normal range are predictors of IHD in healthy men and women (36, 37). No effect of HRT use (oral or transdermal) on blood white cell count was observed, which suggests that the effect of oral oestrogens on CRP is due to an effect on hepatic synthesis of reactant plasma proteins, rather than a generalised inflammatory response.

Blood viscosity and its major determinants (plasma viscosity and haematocrit) are predictors of IHD and stroke in epidemiological studies (38). It has been suggested that the lower risk of IHD in premenopausal women may be partly due to their lower levels of these variables (21). We have confirmed two other recent reports from cross-sectional studies (21, 26) that oral HRT reduced the post-menopausal increases in plasma and blood viscosity. This effect was not observed in users of transdermal HRT in the present study.

Strengths and Limitations of Study

Strengths of this cross-sectional study, as with others (27), include the sampling of HRT users and non-users from the general population rather than secondary or tertiary care; the collection of a range of demographic, lifestyle, physiological and laboratory variables obtained using identical methods in HRT users and non-users; participants' lack of knowledge of the detailed scientific questions under investigation because of the broad base of the study; and blinding of laboratory staff to participants' HRT status. Limitations of this study include the lack of information about hormonal dose (because women were asked only to name the brand and duration of use of the HRT that they were taking), factor VII and factor V Leiden genotypes, and blood group. Multiple comparisons were made, hence p-values between 0.05 and 0.01 should be viewed with caution. We therefore suggest that further, larger studies be performed to confirm the lack of effect of transdermal HRT on risk markers for thrombosis, which may have implications for their consideration in women at increased risk of thrombosis.

Acknowledgements

We are grateful to the people of Renfrew and Paisley who took part in this study; Catherine Ferrell, Jane Goodfellow, Michere Beaumont and Helen Richards who contacted participants; Claire Bidwell, Iona MacTaggart, Evelyn Lapsley, Julie Hunter, Nicola McPherson and Sarah Morgan who checked questionnaires, performed venepuncture and made physiological measurements; and Karen McLoughlin for technical assistance. This study was funded by a NHS Research and Development Grant and the Wellcome Trust.

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Received December 24, 1999 Accepted after resubmission February 15, 2001