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Hormone replacement therapy and hypercoagulability. Results from the Prospective Danish Climacteric Study

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Objective To assess the influence of a variety of HRT regimens on the haemostatic balance using markers of fibrin turnover and inhibitors of coagulation.

Design An open randomised study allocating women to either a control group or five different HRT treatment groups.

Setting Gentofte Hospital, Hellerup, and Rigshospitalet, Copenhagen, Denmark.

Population One hundred and forty-nine postmenopausal women without previous venous thromboembolic disease.

Methods Prothrombin fragment 1+2 (F_{1+2}), fibrin degradation products, antithrombin, protein C, total protein S and activated protein C-normalised ratio were measured at baseline and after 6 and 12 months of HRT in six groups of healthy postmenopausal women: (A) no HRT (reference group), (B) continuous oestradiol valerate (E_2V) plus cyproterone acetate, (C) cyclic E_2V plus cyproterone acetate, (D) continuous combined oestrogen (E_2) plus norethindrone acetate, (E) E_2 combined with local delivery of levonorgestrel and (F) E_2V plus medroxyprogesterone.

Main outcome measures HRT-induced changes in the concentration of inhibitors of coagulation and markers of fibrin turnover during 12 months of treatment.

Results Significant decreases of antithrombin and protein S were found in all treatment groups, of protein C in Groups C, D, E and F and of activated protein C-normalised ratio in Groups E and F. Fibrin degradation products increased after three months of treatment, whereas F_{1+2} was persistently increased after three months in Group F. The cumulative response of antithrombin was significantly lower in Groups D, E and F than in the reference group. The cumulative response of protein S and activated protein C-normalised ratio was lower, whereas that of F_{1+2} was significantly higher in Group F than in the reference group.

Conclusion HRT reduces the inhibitory potential of coagulation significantly. The effect is related to the type of E_2/progestin combination administered, but seems to be oestrogen-derived as the most pronounced effect is found with only quarterly progestin intake. Such procoagulant activity of HRT may well translate into clinical manifestations in thrombosis-prone individuals.

INTRODUCTION

Hormone replacement therapy (HRT) is widely used among women in the industrialised part of the world. Various oestrogen/progestin regimens are available with systemic or local hormone delivery. Systemic oestrogen may be administered orally, transcutaneously, intranasally or as depots. Progestins can be administered via different routes (e.g. orally, transcutaneously or with local delivery in the uterine cavity). In addition, the composition of HRT varies according to the oestrogen component, the type of progestin used and the length and sequence of the oestrogen/progestin phases. In the HRT products currently marketed, the cycle length varies from 28 to 91 days, and several continuous combined regimens are available.

Epidemiological studies indicate that postmenopausal women using HRT have a reduced risk of coronary artery disease. Recent randomised primary and secondary prevention trials, however, did not report any benefit as previously reported[1,2]. Several studies have demonstrated a two- to fourfold increased risk for development of venous thromboembolism among women treated with HRT[1–6]. Most recently, the Women's Health Initiative study[7] reported a more than twofold increase in the risk for development of

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venous thromboembolism among HRT users compared with non-users of similar age.

A number of studies have demonstrated an HRT-induced decrease in the important inhibitory potential of coagulation (i.e. the concentration of antithrombin, protein C and total protein S)[8–14]. Additionally, a possible change towards increased resistance to activated protein C has been heavily debated[15–17]. Most of these studies have focussed on one or very few regimens of HRT. The findings indicate that HRT affects the haemostatic balance and induces a procoagulant state, but whether this shift is of clinical importance in healthy women is unclear.

We have undertaken a detailed study on women treated with various HRT formulations orally and intrauterine with varied length of the treatment cycles from 28 to 91 days. The study focussed on key risk markers for venous thromboembolism using standardised and internationally recommended assay procedures[18].

METHODS

The current study was part of the Collaborative Danish Climacteric Study. This study generates integrated data on a variety of key variables obtained from comparative investigations performed in healthy postmenopausal women during administration of different types of HRT. The study was conducted at the Copenhagen University Hospital (Rigshospitalet and Frederiksberg Hospital). The data comprise information from 12-month study periods in six groups of postmenopausal women with comparable clinical characteristics at baseline. Inclusion criteria were age between 40 and 60 years, FSH values $\geq$30 iu/L, at least five moderate hot flushes per week, intact uterus, no menstrual bleeding six months prior to recruitment or, in case of previous HRT, six weeks of washout period. All women were generally in good health. Exclusion criteria were drug intake due to any medical disorder, adiposity (BMI >30 kg/m^2), smoking $>$10 cigarettes/day, hypertension ($>$160/95 mmHg), alcohol intake $>$3 g/day, abnormal liver or thyroid function, diabetes, previous deep venous thrombosis or migraine. Recruitment and treatment allocations have previously been described[19]. Three distinct cohorts were established representing five different types of oestrogen/progestin combinations or no HRT (Fig. 1): (A) the reference group receiving no treatment ($n = 26$), (B) daily intake of 2 mg E_2V combined with 10 days of 1 mg cyproterone acetate (28/10 days) (Schering, Berlin, Germany) ($n = 25$), (C) cyclic intake of 2 mg E_2V plus 1 mg cyproterone acetate (21/10 days) (Schering) and placebo (7/28 days) ($n = 25$), (D) continuous combined intake of 2 mg E_2 plus 1 mg norethindrone acetate (Novo Nordic, Copenhagen, Denmark) ($n = 21$), (E) continuous oral intake of 2 mg of E_2 combined with local delivery of levonorgestrel (20 μg/24 h) (Leiras, Helsinki, Finland) ($n = 22$) and (F) a long cycle regimen with 2 mg oestradiol valerate (E_2V) (84/91 days) consisting of 20 mg medroxyprogesterone (14/91 days) and placebo (7/91 days) (Orion Pharma, Espoo, Finland) ($n = 30$). The accumulated study period for all cohorts covered 48 consecutive calendar months, and of the total study population, 149 subjects fulfilled the criteria of analysis per protocol (i.e. no protocol violation and participation during the whole study period). All women signed a written informed consent before entering the study. The study was conducted according to the guidelines of Good Clinical Practice in the European Community, which incorporates the principles of the Declaration of Helsinki II, and was approved by the Local Ethics Committee.

Venous blood samples were drawn after 10 min of standardised resting and after at least 12 h of fasting and non-smoking. The samples were collected with minimal stasis using evacuated tubes at baseline, after 6 and 12 months treatment or control. During sequential therapy (Groups B, C, and F), the samples were obtained during the last five days of the progestin phase. In Group F (long cycle), additional samples were obtained after 2 months of treatment with E_2V in cycles 1 and 4 (days 60 and 330) (Fig. 1). Citrate stabilised blood was collected using Hemogard 9NC tubes from Becton Dickinson (Plymouth, UK). All samples were centrifuged at $2000\times g$ for 20 min. Samples aimed for activated protein C resistance ratio, protein C and antithrombin were processed at room temperature. Samples aimed for protein S, fibrin degradation products and prothrombin fragment 1+2 (F_{1+2}) were supplemented with 10 μl 0.5 mmol/L D-Phe-Pro-Arg chloromethylketone HCl (PPACK, Calbiochem, Bad Soden, Germany) and collected on crushed ice to avoid *ex vivo* activation. These samples were centrifuged at 4°C. All samples were frozen and stored at −80°C. Before analysis, the samples were thawed on a water bath at 37°C.

Activated protein C-normalised ratio was determined with the Coatest APC Resistance kit from Chromogenix (Mölndal, Sweden). The activated protein C resistance was converted to normalised ratio by dividing the activated protein C resistance of the sample with that of normal pooled plasma collected from 25 healthy individuals not carrying the FVR506Q mutation. The assay was performed with the ACL 7000 from International Laboratories (Milan, Italy). This equipment was also used for determination of the activities of protein C and antithrombin employing the Coamatic Protein C and Coamatic Antithrombin kits (both from Chromogenix). The protein concentration of total protein S was determined with an ELISA employing antibodies from Dako (Glostrup, Denmark). The protein concentration of fibrin degradation products was determined with the Fibrinostika FbDP ELISA kit from Organon Teknika (Turnhout, Belgium), while the protein concentration of F_{1+2} was determined with the Enzygnost F_{1+2} micro-kit from Dade Behring (Marburg, Germany). The assay procedures were calibrated against

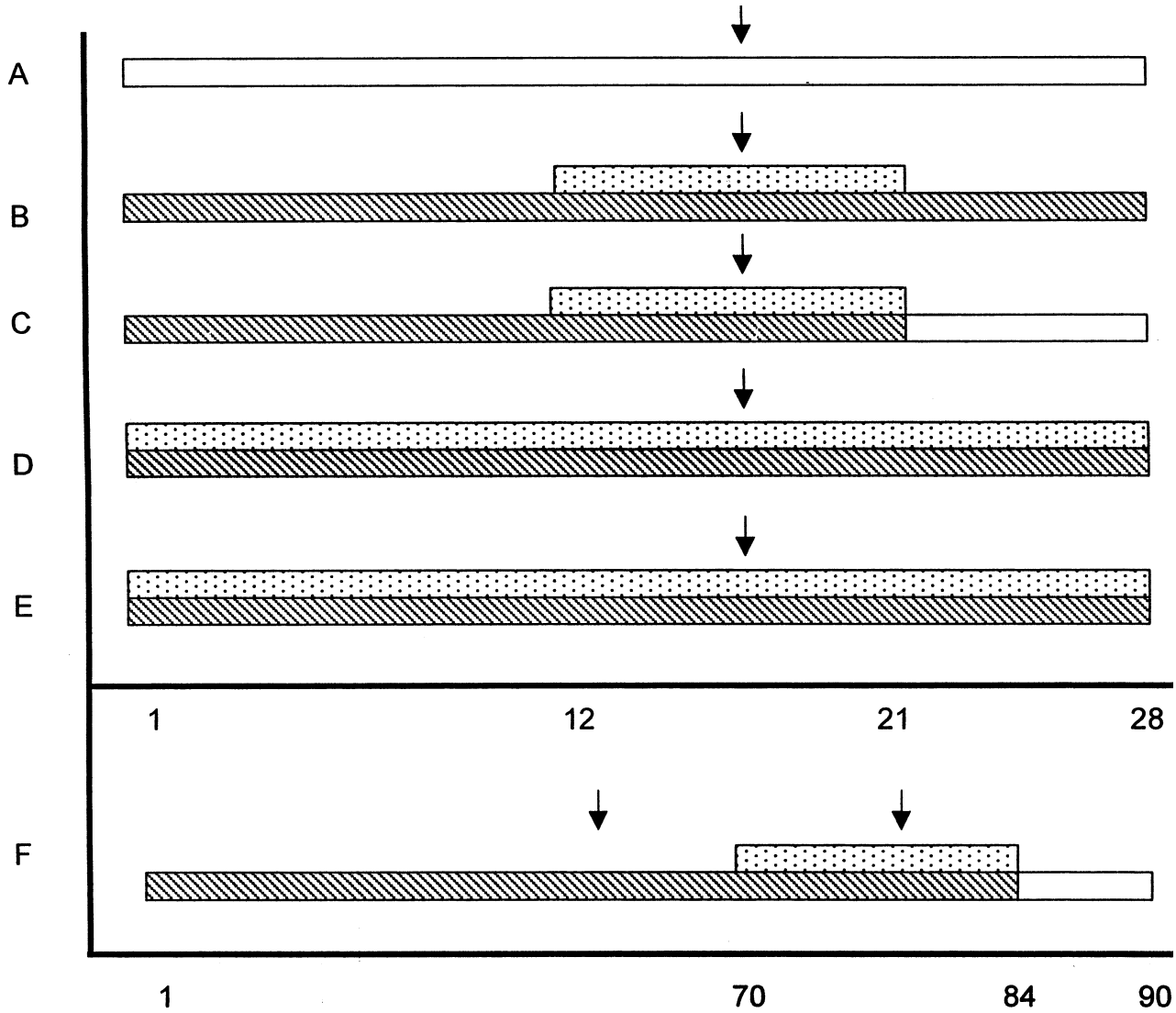

Fig. 1. The abscissas represent the days of oestrogen/progestin treatment, while the ordinate gives the various groups studied: (A) No treatment applied (reference group) ($n = 26$). (B) Continuous intake of 2 mg oestradiol valerate (E_2V) daily (day 1–28) plus 1 mg cyproterone acetate (day 12–21) ($n = 25$). (C) Cyclic intake of 2 mg E_2V (day 1–21) plus 1 mg cyproterone acetate (day 12–21) and placebo (day 22–28) ($n = 25$). (D) Continuous combined intake of 2 mg oestrogen (E_2) plus 1 mg norethindrone acetate ($n = 21$). (E) Continuous intake of 2 mg of E_2 combined with intrauterine delivery of levonorgestrel (20 fg/24 h) in the uterine cavity ($n = 22$). (F) Long cycle regimen with 2 mg E_2V (day 1–84) plus 20 mg medroxyprogesterone (day 71–84) and placebo (day 85–91) ($n = 30$). Open bars represent placebo or no treatment. Hatched bars represent periods of oestrogen treatment. Dotted bars represent periods of progestin treatment. The arrows represent time of blood collection.

WHO International Biological Standards and Reference materials when available. Antithrombin was calibrated against International Standard 93/768, protein C against International Standard 86/622, and protein S against International Standard 93/590, all provided by National Institute for Biological Standards and Controls (Potters Bar, UK). The assay procedures used for determination of F_{1+2} and fibrin degradation products were calibrated against standards provided by the manufacturers of the kits.

Non-parametric statistical methods were used due to non-Gaussian distribution of results. For each quantity, Kruskal–Wallis test was used to compare the results obtained in the groups at baseline. For each quantity, Friedman's repeated measures analysis of variance on ranks test was used to test possible within-group differences. If Friedman's test showed a significant change, Dunn's test was used to determine the period(s) significantly different from baseline. To test for possible differences between the treatment groups, a two-stage method that uses summary measures was used[20]. In the first stage, a summary of the response in an individual (i.e. the area under the curve for each quantity) was calculated. In the second stage, these cumulated responses were compared using Kruskal–Wallis test.

RESULTS

The baseline characteristics (age, BMI, blood pressure, FSH, oestradiol, smoking and previous use of HRT) of the five hormone groups and the reference group showed that the women in the reference group (median age 53 years) were slightly elder ($P < 0.05$) compared with the hormone allocated women (median age: Group B: 52 years, Group C: 51 years, Group D: 51 years, Group E: 52 years, and Group F: 52 years) and displayed higher FSH levels at

Table 1. Plasma concentrations of antithrombin, protein C, total protein S, activated protein C-normalised ratio, F_{1+2}, and fibrin degradation products during one year of different HRT or no HRT. See text for a detailed description of the treatment groups. Values are medians (25–75th centiles).

Variable	Months					AUC
	0	2	6	11	12	
Antithrombin (1)						
Group A	1.13 (1.08–1.21)		1.13 (1.08–1.21)		1.13 (1.02–1.19)	1.12 (1.05–1.19)
Group B	1.11 (1.07–1.16)		1.04*** (0.97–1.09)		1.04*** (0.98–1.12)	1.04 (0.99–1.12)
Group C	1.08 (1.03–1.17)		1.03*** (0.97–1.10)		1.06*** (0.97–1.10)	1.06 (0.99–1.10)
Group D	1.06 (1.02–1.12)		0.96*** (0.88–1.02)		0.91*** (0.89–0.96)	0.97[a] (0.93–1.03)
Group E	1.09 (0.99–1.18)		1.01*** (0.93–1.08)		0.98*** (0.94–1.05)	1.02[a] (0.97–1.12)
Group F	1.07 (0.98–1.14)	1.00*** (0.89–1.07)	1.04*** (0.96–1.13)	0.96*** (0.90–1.05)	1.03*** (0.91–1.12)	0.99[a] (0.90–1.05)
Protein C (iu/mL)						
Group A	117.0 (100.0–129.0)		112.5 (101.0–132.0)		115.0 (99.0–121.0)	111.4 (101.0–125.3)
Group B	113.0 (104.3–125.5)		108.0 (104.5–120.8)		119.0 (107.0–127.3)	110.8 (105.4–125.9)
Group C	112.0 (103.0–122.3)		108.0* (99.0–114.3)		112.0 (101.8–119.0)	109.0 (100.9–115.6)
Group D	109.0 (97.8–124.8)		98.0*** (90.7–111.0)		98.0*** (88.5–105.0)	99.0 (94.2–114.3)
Group E	120.0 (105.0–128.0)		108.0** (101.0–122.0)		103.0** (96.0–116.0)	109.1 (99.3–127.0)
Group F	116.0 (101.0–128.0)	110.0** (94.8–116.8)	111.5** (95.0–122.0)	115.0 (106.0–131.0)	115.0 (106.0–120.0)	110.3 (99.1–120.0)
Total protein S (iu/mL)						
Group A	99.7 (85.6–111.3)		97.2 (83.9–107.2)		95.9 (87.2–99.7)	97.8 (87.6–103.8)
Group B	98.0 (80.6–114.8)		86.4*** (68.7–90.3)		79.7*** (72.7–92.4)	86.8 (73.9–96.4)
Group C	94.7 (87.8–103.2)		81.4** (74.3–91.6)		85.6** (77.2–93.2)	84.1 (80.0–98.5)
Group D	102.2 (87.0–112.3)		87.2*** (77.5–94.1)		82.2*** (70.8–95.9)	92.8 (80.8–97.4)
Group E	104.7 (90.5–122.1)		86.0*** (72.3–89.7)		77.7*** (71.4–88.9)	87.8 (83.5–95.6)
Group F	95.5 (82.2–103.0)	78.1*** (73.1–95.5)	82.6*** (73.9–90.5)	73.9*** (68.1–81.4)	80.6*** (75.6–94.7)	82.6[b] (73.9–86.6)
Activated protein C resistance-normalised ratio (1)						
Group A	0.85 (0.81–0.92)		0.88 (0.79–1.00)		0.87 (0.75–0.91)	0.86 (0.81–1.00)
Group B	0.85 (0.81–0.98)		0.85 (0.77–0.92)		0.83 (0.75–0.93)	0.85 (0.80–0.91)
Group C	0.93 (0.81–1.08)		0.91 (0.80–1.00)		0.89 (0.80–1.05)	0.91 (0.80–1.01)
Group D	0.84 (0.74–0.92)		0.81 (0.75–0.88)		0.81 (0.74–0.91)	0.83 (0.74–0.87)
Group E	0.84 (0.69–0.93)		0.80 (0.61–0.87)		0.77* (0.68–0.89)	0.81 (0.65–0.88)
Group F	0.82 (0.74–0.92)	0.82 (0.70–0.87)	0.83 (0.75–0.87)	0.78* (0.67–0.84)	0.77 (0.68–0.85)	0.78[a] (0.71–0.83)
F_{1+2} (nmol/L)						
Group A	0.98 (0.79–1.40)		0.95 (0.84–1.18)		0.96 (0.79–1.38)	0.99 (0.82–1.37)
Group B	1.27 (0.96–1.48)		1.18 (0.96–1.41)		1.13 (0.88–1.41)	1.23 (1.00–1.53)
Group C	1.18 (0.87–1.55)		1.03 (0.82–1.36)		1.17 (0.93–1.44)	1.15 (0.92–1.52)
Group D	1.17 (1.01–1.49)		1.26 (1.06–1.54)		1.30 (0.93–1.46)	1.29 (1.06–1.46)
Group E	1.21 (0.84–1.67)		1.19 (1.04–1.85)		1.37 (1.01–1.85)	1.26 (1.02–1.91)
Group F	1.28 (0.97–1.44)	1.51*** (1.24–1.93)	1.41*** (1.06–1.91)	1.63*** (1.17–2.08)	1.44*** (1.12–1.79)	1.51[b] (1.22–1.79)
Fibrin degradation products (ng/mL)						
Group A	144 (115–200)		159 (130–195)		154 (121–202)	154 (132–191)
Group B	144 (118–227)		166 (138–227)		167 (127–232)	179 (150–247)
Group C	135 (119–225)		147 (112–202)		146 (124–217)	156 (118–205)
Group D	167 (140–220)		206 (145–304)		199 (152–384)	231 (159–317)
Group E	147 (122–181)		161 (127–285)		191 (128–242)	170 (137–230)
Group F	150 (135–248)	166 (131–299)	156 (113–307)	174 (131–252)	183* (140–297)	179 (135–242)

* $P < 0.05$ compared with baseline values (month 0) (Friedman's test).
** $P < 0.01$ compared with baseline values (month 0) (Friedman's test).
*** $P < 0.001$ compared with baseline values (month 0) (Friedman's test).
[a] $P < 0.001$ compared with AUC in Group A (reference group) (Kruskal–Wallis test).
[b] $P < 0.01$ compared with AUC in Group A (reference group) (Kruskal–Wallis test).

baseline. Otherwise, no significant differences between the groups in the baseline characteristics were observed[20]. Comparison of the baseline values among the six groups revealed no statistically significant differences in any of the haemostatic quantities.

Calculations on changes within groups are shown in Table 1. Friedman's repeated measures analysis of variance on ranks revealed a significant and persistent decrease in the concentration of antithrombin and protein S in all treatment groups. The activity of protein C was temporarily significantly reduced after 6 months of treatment in Group C, after 6 and 12 months of treatment in Groups D and E and after 2 and 6 months of treatment in Group F levelling off after 11 months. Activated protein C-normalised ratio was significantly reduced after 12 months of treatment in Group E and after 11 months during unopposed oestrogen in Group F, where also the concentration of fibrin degradation products was significantly increased after 12 months of treatment. In this group, the concentration of F_{1+2} showed a persistent and significant increase after three or more months. No significant differences were observed in the reference group over time.

Calculations on changes between groups are shown in Table 1 (right column). Kruskal–Wallis test showed that the cumulative response of antithrombin was significantly lower in Groups D, E and F than in the reference group ($P < 0.001$), while no difference was observed in protein C and fibrin degradation products. The cumulative response of protein S ($P < 0.01$) and activated protein C-normalised ratio ($P < 0.001$) was significantly lower, while that of F_{1+2} was significantly higher ($P < 0.01$) in Group F than in the reference group.

DISCUSSION

Previous studies dealing with the effect of HRT on venous thromboembolism have focussed on one or very few regimens of HRT, and the present study is the first to compare five different HRT regimens and a comparable reference group followed over an identical study period. We observed that all regimens of HRT persistently reduced the concentrations of antithrombin and protein S throughout the study period, while the concentration of protein C was significantly reduced in all groups except in Group B. Thus, HRT induces a profound reduction of three very important proteins involved in inhibition of coagulation, and this reduction may alone be responsible for the thrombosis-prone situation observed in women on HRT. Our results on antithrombin agree with several previous studies[8–14,21] and with a recent study by Gottsäter *et al.*[22] concluding that unopposed oestrogen had the most profound effect on antithrombin. In our study, unopposed oestrogen was used in Group F (blood samples obtained in cycle 1 [2 months] and cycle 4 [11 months]). We observed the lowest concentration of antithrombin in cycle 1. All samples obtained during the hormone treatment showed significantly lower concentrations of antithrombin compared with the concentration at baseline. Additionally, we observed a significant reduction of antithrombin in Groups B and D, and the subjects in these groups were all treated with oestrogen/progestin combined. This indicates that oestrogen might be responsible for the effect on antithrombin, and that this effect is not substantially attenuated by progestin. This may also be the case for total protein S because the lowest protein S concentration in Group F was observed in cycle 4 (11 months) representing unopposed treatment with oestrogen. Nevertheless, the effect of HRT on protein S is ambiguous. Protein S is present in plasma in two forms, as approximately 60% is bound to C4b-binding protein, while the remaining part circulates in free form. Apparently, HRT does not alter the concentration of free protein S in plasma[9,13,15,21,22], while the total protein S concentration is reduced[11,13,22]. However, HRT reduces the concentration of C4b-binding protein[23], and this can explain why HRT reduces the total concentration of protein S. Free protein S is an important co-factor in the activated protein C-induced degradation of factor Va, while bound protein S does not possess this function. However, it was recently demonstrated that C4b-binding protein-bound protein S retains its co-factor activity to activated protein C in the factor VIIIa inactivation[24]. Consequently, a reduction of the total protein S concentration may contribute to a procoagulant state. The observed HRT-induced reduction in protein C activity is in agreement with the results obtained by Høibraaten *et al.*[12,21], while others have reported that the concentration of protein C remains unchanged[9,10,14,22] or is increased during HRT administration[11]. A reduction in protein C seems to be associated with the use of orally administered oestrogen/progestin combined, as used in the studies by Høibraaten *et al.*[12,21] and in our study, while unaltered or increased concentration of protein C is observed in studies employing unopposed oestrogen[9,11,22] or transcutaneously administered HRT[10]. This apparent importance of unopposed oestrogen is also supported by the results we obtained on protein C in Group F. The concentrations of protein C in cycle 1 (2 months) and cycle 4 (11 months), representing unopposed oestrogen, were not significantly different from the baseline values. In contrast, all combined regimens studied resulted in a significant reduction of protein C. The only exception is Group B where a reduction reaching borderline statistical significance was observed ($P = 0.06$). The influence of HRT formulation on the protein C concentration deserves further attention.

Only few studies have dealt with the effect of HRT on the resistance to activated protein C[15–17,22], and the results are not conclusive. Our results underscore the uncertainty, because two out of five HRT regimens showed an increased resistance to activated protein C, while three were without effect. It should be noticed that both the increased activated

protein C-normalised ratio reported by Høibraaten *et al.*[17] and the decreased activated protein C-normalised ratio reported here correspond to an increased resistance to activated protein C. The apparent discrepancy is due to different assay procedures.

A reduction in the inhibitory potential of coagulation may result in an increased activation rate. We did observe a significant increase in F_{1+2}, a marker of thrombin activation, but exclusively in women treated with an oestrogen-dominated (long cycle) regimen of HRT (Group F). Other studies have demonstrated an HRT-induced increase in F_{1+2}[9,11,14,20,21] or no effect on F_{1+2}[12,16,25,26]. Two of these studies[12,21] have investigated women with previous episodes of venous thromboembolism, and such women might not be comparable with our groups of healthy women with respect to thrombin activation. We demonstrated that the effect of long cycle HRT on F_{1+2} was accompanied by a persistent reduction in the inhibitory potential of coagulation and an increase in the concentration of fibrin degradation products, but the latter exclusively after 12 months of treatment. This effect on fibrin turnover has been acknowledged in some studies[22,27,28], while other studies report unaltered concentrations of fibrin degradation products[12,14,25,26]. Changes in the cumulative response[20] of the haemostatic variables indicate the most persistent effect of the treatment on the haemostatic balance. We observed that also the cumulative response of the long cycle regimen used in Group F showed the most pronounced effect, with antithrombin, protein S and activated protein C-normalised ratio significantly lower, and F_{1+2} significantly higher, compared with the reference group. Thus, long cycle treatment with HRT induces a persistent reduction of the inhibitory potential of coagulation and a persistent increase in the activation of prothrombin.

Venous thromboembolism being a multifactorial disease entails several risk factors to precipitate, and both inherited risk factors such as factor V Leiden and the prothrombin G20210A mutation as well as acquired risk factors, such as obesity and smoking, are commonly present among women in the Western part of the world. Consequently, a number of women are potentially at risk for thrombosis.

The Women's Health Initiative study[7] and a very recent meta-analysis[29] have convincingly demonstrated that venous thromboembolism most prominently occurs during the very first period of HRT treatment, while venous thromboembolism is more rarely observed among women treated for a longer period. These epidemiological studies indicate that HRT can be the trigger precipitating thrombotic disease among women already at risk. Furthermore, women developing venous thromboembolism when treated with HRT have a higher frequency of inherited risk factors for thrombosis, as demonstrated in three recent case-control studies[30–32]. Herrington *et al.*[30] found that the factor V Leiden mutation was present in 16.7% of women having venous thromboembolism while only in 6.3% of the controls. Similar data were published by Rosendaal *et al.*[31], while Lowe *et al.*[32], in a re-analysis of the original Oxford case-control study[5], demonstrated that the presence of multiple risk factors increases the relative risk for venous thromboembolism among HRT users dramatically.

Taken together, these findings support that some women are in a thrombosis-prone condition prior to HRT treatment and that HRT might cause the thrombosis to precipitate. The present study suggests that the reduction of the inhibitory potential of coagulation induced by HRT potentially triggers the thrombotic event in such women.

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CORRECTION

Symphysiotomy: a lifesaving procedure. CB Wykes, TA Johnston, S Paterson-Brown & RB Johanson Vol 110 (2), 2003:219–221.

In the report of Case 2 of this paper, the last part of the first paragraph should have read ''Various manoeuvres, including McRobert's manoeuvre, supra-pubic pressure, internal rotation of both the anterior and posterior shoulder, attempted removal of the posterior arm, and rolling on to all fours and repeating the above manoeuvres, were all unsuccessful. General anaesthesia was then administered, and catheterization of the bladder was difficult because of the shoulder dystocia.''