0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(12):5723–5729

doi: 10.1210/jc.2003-030520

Venous Thrombosis and Changes of Hemostatic Variables during Cross-Sex Hormone Treatment in Transsexual People

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The incidence of venous thrombosis associated with estrogen treatment in male-to-female (M→F) transsexuals is considerably higher with administration of oral ethinyl estradiol (EE) than with transdermal (td) 17-β-estradiol (E_2).

To find an explanation for the different thrombotic risks of oral EE and td E_2 use, we compared the effects of treatment of M→F transsexuals with cyproterone acetate (CPA) only, and with CPA in combination with td E_2, oral EE, or oral E_2 on a number of hemostatic variables [activated protein C (APC) resistance and plasma levels of protein S, protein C, and prothombin], all of which are documented risk factors for venous thrombosis. APC resistance was determined by quantification of the effect of APC on the amount of thrombin generated during tissue factor-initiated coagulation; plasma levels of total and free protein S were determined by standard ELISA; and levels of prothrombin and protein C were determined with functional assays after complete activation of the zymogens with specific snake venom proteases.

CPA-only, td-E_2+CPA, or oral-E_2+CPA treatment produced rather small effects on hemostatic variables, whereas oral EE treatment resulted in a large increase in APC resistance from 1.2 ± 0.8 to 4.1 ± 1 ($P < 0.001$), a moderate increase in plasma protein C (9%; $P = 0.012$), and a large decrease in both total and free plasma protein S (30%; $P < 0.005$). The large differential effect of oral EE and oral E_2 indicates that the prothrombotic effect of EE is due to its molecular structure rather than to a first-pass liver effect (which they share). Moreover, these differences may explain why M→F transsexuals treated with oral EE are exposed to a higher thrombotic risk than transsexuals treated with td E_2. Testosterone administration to female-to-male transsexuals had an antithrombotic effect. (*J Clin Endocrinol Metab* 88: 5723–5729, 2003)

IN WOMEN, USE of estrogens and/or progestagens, by means of oral contraceptives (OC) or hormone replacement therapy, is associated with an increased risk of venous thrombosis (VT) (1–6). Epidemiological studies (6–8) and meta-analyses (8, 9) have shown that women using third-generation OC containing desogestrel are exposed to a higher thrombotic risk than women using second-generation OC that contain levonorgestrel. These reports initiated a large number of studies on the effects of OC on hemostatic variables (10–19). Particularly, the observation that women become activated protein C (APC) resistant during OC use and that third-generation OC users become more APC resistant than second-generation OC users (10, 12) attracted much attention (11, 13, 20) and was proposed to provide a biological explanation for the thrombotic risk and differences in risk associated with the use of various kinds of OC (21).

APC resistance is a major risk factor of VT, which in the majority of cases is due to the so-called factor V Leiden mutation (for a review, see Ref. 22). This mutation results in the replacement of an amino acid (Arg^{506}→Glu) at a major cleavage site for APC in factor Va. Heterozygous and homozygous carriers of factor V Leiden have a 6-fold (23) and a more than 50-fold increased risk of VT, respectively (24). Recently, it was shown that APC resistance in the absence of factor V Leiden (*e.g.* acquired APC resistance) is an independent risk factor of VT (25, 26).

An acquired APC-resistant phenotype due to hormone use is most easily demonstrated with the thrombin generation-based APC resistance test (27). The severity of APC resistance determined with this test correlates with reported risks of clinical VT (28), and analysis of the Leiden Thrombophilia Study showed that this test predicts for venous thrombotic risk (29). These observations support the proposition that acquired APC resistance may explain the increased thrombotic risk associated with OC use (10, 21).

Another less conspicuous group of users of (high-dose) estrogens are male-to-female (M→F) transsexuals. At present, there is no evidence that transsexuals differ in their biological functions from members of their biological sex (30). Cross-sex hormonal treatment of M→F transsexuals in our clinic consists of the administration of cyproterone acetate (CPA), an oral progestagen with strong antiandrogenic characteristics, combined with oral or transdermal (td) estrogens. This treatment regimen is accompanied by a significant number of thrombotic events, the incidence of which is significantly higher in M→F transsexuals using oral ethinyl estradiol (EE) than in transsexuals treated with td 17-β-estradiol (E_2) (both in combination with CPA) (31). At present, there is no good biological explanation for this observation.

Abbreviations: APC, Activated protein C; CPA, cyproterone acetate; E_2, 17-β-estradiol; EE, ethinyl estradiol; F→M, female to male; M→F, male to female; nAPCsr, normalized APC sensitivity ratio; OC, oral contraceptive; T, testosterone; td, transdermal(ly); VT, venous thrombosis.

In an attempt to gain more insight into the thrombotic risk associated with the type of estrogen treatment of transsexuals and to differentiate whether the molecular structure of estrogens (E_2 *vs.* EE) or the mode of administration (*i.e.* first-pass effects of estrogens through the liver contribute to different thrombotic risks), we have compared the effect of treatment of M→F transsexuals with CPA-only, td-E_2+CPA, oral-EE+CPA, and oral-E_2+CPA on a number of hemostatic variables. We investigated hemostatic variables (APC resistance and plasma levels of protein S, protein C, and prothombin), which are established venous thrombotic risk factors (22) and of which changes occurring during OC use in women have been implicated in the occurrence of VT (15, 17, 21, 29).

Because men in general are less resistant to APC than women (10, 32), we also monitored the effects of androgens on these hemostatic variables in female-to-male (F→M) transsexuals whose endocrine milieu changes from female to male after cross-sex hormone treatment with androgens.

Subjects and Methods

Patients

To study the effect of hormone treatment on hemostatic variables, we used plasma samples from an earlier study, which were collected before and after 4 months of hormone administration. The original study (33) included 40 M→F and 17 F→M transsexuals in whom the effects of oral and td administration of estrogens on tissue-type plasminogen activator levels were investigated. In this study (33), 30 M→F transsexuals were open label randomized to receive either oral EE (Lynoral; 50 μg, two times daily, one tablet; Organon, Oss, The Netherlands) (n = 15) or td E_2 (Estraderm TTS 100; two patches per week, with a daily delivery of 100 μg E_2; CIBA-Geigy, Basel, Switzerland) (n = 15), both combined with CPA (Androcur; 50 mg, two times daily, one tablet; Schering, Berlin, Germany). To investigate the effect of administration of CPA-only, 10 M→F transsexuals were studied before and after 4 months of administration of CPA (100 mg/d). Seventeen F→M transsexuals were treated with testosterone (T) esters (Sustanon; 250 mg, 2 wk im; Organon) receiving the standard treatment of our clinic. There was no evidence of hypertension, cardiovascular disease, VT, diabetes mellitus, or use of sex hormones in the volunteers included in this study. All F→M transsexuals had normal physiological menstrual cycles (28–31 d) before cross-gender sex hormone administration.

In the original study (33), plasma samples were collected from 57 subjects. However, in the present investigation, samples of seven individuals had to be excluded, because their plasma sample collection was incomplete. We were able to study 14 of 15 samples of both the group M→F transsexuals treated with oral-EE+CPA or with td-E_2+CPA. Of 10 M→F transsexuals treated with CPA, 8 subjects were studied, and 14 of 17 of the F→M transsexuals treated with androgen were included in the present investigation.

To differentiate whether the molecular structure of estrogens or the first-pass effects of estrogens through the liver influenced the hemostatic variables, plasma samples of an additional group of 20 M→F transsexuals who received orally administered E_2-valerate (Progynova; 2 mg, two times daily, one tablet; Schering) plus CPA (50 mg, two times daily, one tablet) (oral-E_2+CPA) were tested. The effects of oral-E_2+CPA were compared with those of oral-EE+CPA. At a certain point in time, oral EE was no longer available as a prescription drug, and to continue treatment with oral estrogens, subjects needed to be switched from treatment with oral-EE+CPA to oral-E_2+CPA. Sixteen subjects who had used oral EE for a mean duration of 1.4 yr (range, 0.6–2.4 yr) and who had switched to oral E_2 (mean duration, 0.5 yr; range, 0.4–1.6 yr) were included in the group of users of CPA+oral-E_2, whereas four subjects in this group had started their estrogen treatment with oral E_2 (mean duration, 0.6 yr; range, 0.4–1.2 yr) without having used oral EE before. In the 16 subjects, there was no wash-out period for EE; they continued estrogen treatment with oral E_2 immediately after cessation of oral EE. The switch from oral EE to oral E_2 was not motivated by medical complications. It is known that oral administration of 2 mg E_2-valerate yields approximately 50 μg E_2 to the circulation. The oral administration of 4 mg E_2-valerate is bioequivalent to the doses of 100 μg oral-EE or 100 μg td-E_2 administered in the other M→F groups. One molecule of E_2 is bioequivalent to one molecule of EE (34–36). In contrast to td administered, both orally administered EE and E_2-valerate share a first-pass effect through the liver. Time of blood sampling of these subjects is specified below.

Collection and handling of blood samples

With the exception of the samples collected during treatment with oral E_2 (see also above), blood samples were drawn before and 4 months after the start of cross-sex therapy. The sample of each subject obtained before treatment served as his or her own control. Blood was collected without a tourniquet into evacuated tubes (Diatube H; BD Biosciences, Mountain View, CA) containing 110 mM citrate, 15 mM theophylline, 3.7 mM adenosine, and 0.198 mM dipyridamole (pH 5.0). Blood samples were immediately placed on ice and centrifuged at 3500 × *g* for 30 min at 4 C to obtain platelet-poor plasma. Plasma was separated and snap-frozen within 1 h and stored at −70 C until analysis. For reference purposes, a normal pooled plasma was prepared from plasma of healthy volunteers not on medication, not using OC and nonpregnant (21 females and 44 males; mean age, 35 yr).

Laboratory methods

The chromogenic substrates D-Phe-(pipecolyl)-Arg-pNA (S2238) and L-pyro-Glu-Pro-Arg-pNA (S2366) were from Chromogenix (Mölndahl, Sweden) (supplied by Nodia, Amsterdam, Netherlands). Tissue factor (Dade Innovin) was from Behring (Marburg, Germany). Phospholipids were from Avanti Polar Lipids (Alabaster, AL). Small unilamellar phospholipid vesicles, composed of a mixture of 1,2-dioleoyl-sn-glycero-3-phosphoserine, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine, and 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (20 M/20 M/60 M), were prepared as described before (37). APC (Enzyme Research Laboratories, South Bend, IN), Protac (Pentapharm, Basel, Switzerland), and Ecarin (Pentapharm) were purchased from Kordia Laboratory Supplies (Leiden, The Netherlands). The concentrations of APC were determined according to Sala *et al.* (38). Ancrod was from the World Health Organization International Laboratory for Biological Standards (Hertfordshire, UK). Plasma samples used for determination of the normalized APC sensitivity ratios (nAPCsr) were defibrinated by addition of 1 U Ancrod per 0.5 ml plasma for 10 min at 37 C. The fibrin was then removed by winding onto a spatula. Plasma samples were kept on room temperature and were used within 3 h after thawing.

Determination of hemostatic variables

nAPCsr were determined as described before (12, 28) by quantification of the effect of APC on the total amount of thrombin generated during tissue factor-initiated coagulation (39).

Protein S total and free antigens were determined using the protein S antigen kits from Reaads Medical Products, Inc. (Westminster, CO). Protein C activity (Coamatic protein C activity kit; Chromogenix) was determined as described by the manufacturer by activation of protein C in diluted plasma with 0.05 U/ml Protac (final concentration) and quantification of APC with S2366. Prothrombin was determined after complete activation with ecarin, the partially purified prothrombin activator from the venom of *Echis carinatus* (Pentapharm) as described elsewhere (40). The plasma levels of coagulation factors (antigen or activity) were expressed as a percentage of that present in normal pooled plasma determined in the same experiment.

The occurrence of the factor V Leiden mutation was established by determination of the sensitivity of plasma factor Va for APC (37).

Determination of hormone levels

Standardized RIAs were used to measure serum levels of E_2 (Double Antibody; Sorin Biomedica, Saluggia, Italy) and T (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). Serum levels of LH and FSH were measured by immunometric luminescence assays (Amerlite; Am-

ersham, Buckinghamshire, UK). The lower limits of detection for LH, FSH, E_2, and T were 0.3 IU/liter, 0.5 IU/liter, 90 pmol/liter, and 1.0 nmol/liter, respectively. All laboratory measurements were carried out in blinded fashion with respect to gender and hormone treatment status.

Statistical analysis

Comparisons of variables between baseline and 4 months were performed by paired Student's *t* test. Comparison of baseline variables and variables after 4 months of hormone administration between the groups were performed by one-way ANOVA, with least significant difference as a multiple comparison procedure with an α set at 0.05. All statistical analyses were performed with the statistical software program SPSS 11.0 for Windows (SPSS, Chicago, IL). Descriptive data are reported as mean value ± SD. Values of $P < 0.05$ were considered statistically significant.

Results

Demographic characteristics and hormone levels in M→F transsexuals

Table 1 lists all values at baseline and after 4 months of treatment of M→F transsexuals with CPA-only, td-E_2+CPA, or oral-EE+CPA. Baseline values of demographic variables and hormone levels did not differ significantly between the three groups ($P_{baseline}$ in Table 1). The biological effects of oral-EE+CPA and td-E_2+CPA treatment were reflected by a significant decrease in levels of T, LH, and FSH, with both treatments showing comparable effects. td-E_2+CPA increased plasma E_2 levels up to values typical of the midfollicular phase in women (Table 1). Treatment of M→F transsexuals with oral-EE+CPA decreased the serum levels of E_2. This is due to the fact that EE suppresses endogenous E_2 production, and EE is not detected in conventional E_2 assays.

Levels of E_2 of M→F transsexuals treated with CPA-only were lowered, a phenomenon that is likely due to a suppressive effect of CPA on the production of T, the precursor of E_2. CPA-only was not able to fully suppress the pituitary release of LH and FSH.

Effects of hormone treatment of M→F transsexuals on hemostatic variables

None of the M→F transsexuals were positive for factor V Leiden. Nonetheless, the average baseline nAPCsr (mean ± SD, 1.3 ± 0.7) of plasma collected from the M→F transsexuals was somewhat higher than the nAPCsr of men without factor V Leiden, reported in an earlier study (nAPCsr, 1.0 ± 0.5) (27). This is most likely due to the lower citrate concentration present in the citrate theophylline adenosine dipyridamole anticoagulant (0.106 M) as opposed to the 0.130 M citrate anticoagulant present in the pooled plasma used for normalization of the nAPCsr (41). Because surreptitious estrogen use before inclusion of the study might also result in an increased APC resistance (see below), we carried out a control analysis in which the total M→F population was separated in two subgroups according the baseline nAPCsr values above or below the median value. Comparison of these two groups did not show any difference in values of LH, FSH, T, E_2, and prolactin (data not shown), which indicates that there was no evidence for estrogen use before inclusion in the study.

Compared with baseline, all treatment groups of M→F transsexuals showed an increase in nAPCsr (Table 1). CPA-

TABLE 1. Demographic variables, hormone levels, and hemostatic variables at baseline and after 4-month treatment of M→F transsexuals with CPA alone, CPA plus oral EE, or CPA plus td E_2

Variables	Oral CPA (n = 8)			td E_2 plus CPA (n = 14)			Oral EE plus CPA (n = 14)			$P_{baseline}$	$P_{4\ months}$
	Baseline	4 months	*P*	Baseline	4 months	*P*	Baseline	4 months	*P*		
Age (yr)	35 ± 8			30 ± 7			32 ± 6			0.36	
BMI (kg/m^2)	20.8 ± 1.8	21.5 ± 2.2	0.06	20.8 ± 2.7	21.7 ± 2.6	0.015	22.8 ± 2.8	23.3 ± 1.9	0.34	0.09	0.076
E_2 (pmol/liter)	82 ± 20	38 ± 9	0.002	82 ± 17	190 ± 133	0.013	97 ± 32	24 ± 5[a]	<0.001	0.23	<0.001
T (nmol/liter)	20.9 ± 5.8	8.1 ± 5.1	<0.001	21.2 ± 6.5	1.1 ± 0.22	<0.001	22.3 ± 6.0	1.0 ± 0.11	<0.001	0.85	0.93
LH (IU/liter)	2.6 ± 1.5	2.6 ± 1.4	0.85	2.55 ± 1.7	0.48 ± 0.47	<0.001	3.22 ± 1.7	0.30 ± 0.0	<0.001	0.51	0.50
FSH (IU/liter)	4.0 ± 3.0	2.6 ± 2.2	0.098	3.66 ± 3.95	0.51 ± 0.05	0.01	3.51 ± 2.1	0.50 ± 0.0	<0.001	0.94	0.97
nAPCsr	1.4 ± 0.6	1.8 ± 0.9	0.016	1.3 ± 0.6	2.0 ± 0.9	0.016	1.2 ± 0.8	4.1 ± 1.1	<0.001	0.798	<0.001[b]
Protein C antigen (%)	99 ± 14	103 ± 23	0.362	91 ± 23	88 ± 21	0.466	107 ± 28	117 ± 28	0.012	0.318	0.040[c]
Protein S total antigen (%)	115 ± 16	119 ± 14	0.400	108 ± 20	112 ± 29	0.368	115 ± 27	82 ± 16	0.002	0.693	0.001[b]
Protein S free antigen (%)	122 ± 26	131 ± 24	0.145	118 ± 20	121 ± 31	0.657	133 ± 30	91 ± 21	<0.001	0.299	0.003[b]
Prothrombin (%)	102 ± 8	107 ± 10	0.101	96 ± 13	105 ± 13	0.003	103 ± 18	112 ± 19	0.07	0.385	0.470

Values are mean ± SD. *P* value was determined by paired Student's *t* test. $P_{baseline}$ and $P_{4\ months}$ were determined by one-way ANOVA comparing baseline values and 4-month values from males treated with CPA-only, td-E_2 + CPA, and oral-EE + CPA. BMI, Body mass index.

[a] EE which suppresses endogenous E_2, is not detected in conventional E_2 assays.
P value of <0.05 was considered statistically significant.
[b] Significant difference between group with oral-EE + CPA *vs.* groups with CPA-only and td-E_2 + CPA.
[c] Significant difference between group with oral-EE + CPA and group with td-E_2 + CPA.

only showed a small but significant increase of the nAPCsr (1.4 ± 0.6 to 1.8 ± 0.9; $P = 0.016$), and a slightly larger increase of the nAPCsr was observed in M→F transsexuals receiving td-E_2+CPA (1.3 ± 0.6 to 2.0 ± 0.9; $P = 0.016$). However, in the latter treatment group, two individuals had a remarkably high nAPCsr after 4 months of hormone treatment. Using nonparametric tests, the increase in nAPCsr in the td-E_2+CPA group remained significant ($P = 0.002$; Wilcoxon signed rank test) but was not different ($P = 0.5$; Mann-Whitney test) from the increase observed after treatment with CPA-only. The group receiving oral-EE+CPA showed an increase in nAPCsr (1.2 ± 0.8 to 4.1 ± 1.1; $P < 0.000$) that was significantly higher than in the other two groups.

Treatment of M→F transsexuals with CPA-only or td-E_2+CPA did not influence the plasma levels of protein C or protein S (Table 1). Oral-EE+CPA treatment caused a significant increase of protein C, whereas the plasma levels of protein S were reduced. The reduction in protein S in the oral-EE+CPA-treated group was sufficient to achieve significance compared with the other two treatment regimens (Table 1).

Prothrombin levels showed a tendency to increase in all groups, a trend that became significant in the td-E_2+CPA-treated group (96 ± 13 to 105 ± 13%; $P = 0.003$).

Before and during treatment with estrogens and CPA of M→F transsexuals, no consistent pattern of correlations could be found between the absolute values or the observed changes of hemostatic variables and plasma levels of sex hormones (E_2, T) or LH or FSH. Age of M→F transsexuals did not correlate with hemostatic variables.

None of the subjects that participated in the present investigation developed clinical signs of VT during the 4-month study period.

Hemostatic variables in M→F transsexuals treated with oral-E_2+CPA

We studied an additional subset of 20 M→F transsexuals treated with oral-E_2+CPA to compare the effects of oral E_2 with those of oral EE. The intention was to explore whether first-pass liver effects of estrogens or molecular structure (EE *vs.* E_2) was relevant for their effects on hemostatic variables. The mean age of these subjects was 30 ± 8 yr, and the total duration of hormone treatment at the time of blood collection was 1.2 ± 0.6 yr (range, 0.4–2.4 yr). Values of hemostatic variables at baseline were not available for this group. Sixteen of these 20 individuals had started their cross-sex treatment with oral-EE+CPA for a mean duration of 1.4 yr (range, 0.6–2.4 yr) and, because oral-EE was no longer available as a prescription drug, had switched from oral-EE+CPA to oral-E_2+CPA for a mean duration of 0.5 yr (range, 0.4–1.6 yr). The remaining four had started their cross-sex treatment with oral-E_2+CPA for a mean duration of 0.6 yr (range, 0.4–1.2 yr). None of the 20 individuals included in this group developed clinical signs of VT.

There were no differences (Mann-Whitney test) between the 16 who had switched to oral-E_2+CPA and the four who had started with oral-E_2+CPA regarding age, duration of therapy with oral E_2, nAPCsr, and levels of protein C, protein S, and prothrombin.

The variables obtained in the group of 20 are shown in Table 2. The nAPCsr in the group treated with oral-E_2+CPA (1.4 ± 0.9) did not differ ($P = 0.375$) from the baseline values observed for all M→F transsexuals (1.3 ± 0.7, pooled average of all groups for which baseline values were available, *i.e.* CPA-only, td-E_2+CPA, and oral-EE+CPA; *cf.* Table 1). There were neither differences in the values of nAPCsr obtained in this group receiving treatment with oral-E_2+CPA when their values were compared with the results of treatment with td-E_2+CPA (nAPCsr, 2.0 ± 0.9; $P = 0.119$) or CPA-only (nAPCsr, 1.8 ± 0.9; $P = 0.419$). Comparison of the two E_2-treated groups with different modes of administration (oral E_2 *vs.* td E_2; see Tables 1 and 2) showed no differential effects on the plasma levels of protein C, protein S, and prothrombin.

The nAPCsr during treatment with oral-E_2+CPA (1.4 ± 0.9) was significantly lower ($P < 0.001$) than the nAPCsr obtained after treatment with oral-EE+CPA (4.1 ± 1.1). In addition to the large differences in nAPCsr observed between oral-E_2+CPA and oral-EE+CPA-treated groups, different effects were also seen in the plasma levels of protein S and prothrombin. Oral-EE+CPA treatment resulted in lower protein S levels than oral-E_2+CPA treatment (protein S, total, 82 ± 16 *vs.* 102 ± 16%, $P = 0.008$; protein S, free, 91 ± 21 *vs.* 106 ± 20%, $P = 0.092$), whereas higher prothrombin levels ($P = 0.002$) were reached in the group treated with oral-EE+CPA (112 ± 19) than in the group treated with oral-E_2+CPA (96 ± 11%).

Characteristics, hormone levels, and hemostatic variables in F→M transsexuals

Baseline characteristics and the effect of testosterone treatment on hormone levels and hemostatic variables in F→M transsexuals are summarized in Table 3. Four-month cross-sex hormone administration with androgens decreased the nAPCsr significantly (2.0 ± 0.8 to 1.3 ± 0.7; $P < 0.001$) in F→M transsexuals. All F→M transsexuals, except one carrier of factor V Leiden, showed a reduction of nAPCsr after treatment with T. The nAPCsr of this carrier of factor V Leiden was 2.18 at baseline and 2.24 after administration of T during 4 months. In one F→M transsexual, we observed high levels of nAPCsr of 3.25 at baseline and 2.47 at 4 months

TABLE 2. Demographic variables, hormone levels, and hemostatic variables after 4-month treatment of M→F transsexuals with CPA plus oral E_2

Variables	Oral E_2 plus CPA (n = 20)
Age (yr)	30 ± 8
BMI (kg/m^2)	22.7 ± 2.5
E_2 (pmol/liter)	154 ± 84
T (nmol/liter)	2.3 ± 4.4
LH (IU/liter)	1.0 ± 1.4
FSH (IU/liter)	0.7 ± 0.7
nAPCsr	1.4 ± 0.9
Protein C antigen (%)	102 ± 26
Protein S total antigen (%)	102 ± 16
Protein S free antigen (%)	106 ± 20
Prothrombin (%)	96 ± 11

Values are mean ± SD. BMI, Body mass index.

TABLE 3. Demographic variables, hormone levels, and hemostatic variables at baseline and after 4-month treatment of F→M transsexuals with T esters

Variables	T esters (n = 14)		
	Baseline	4 months	*P*
Age (yr)	26 ± 6		
BMI (kg/m^2)	23.4 ± 4.0	24.7 ± 3.8	0.003
Smoking (n)	8		
E_2 (pmol/liter)	178 ± 76	126 ± 40	0.024
T (nmol/liter)	2.1 ± 0.8	33.0 ± 9.7	<0.001
LH (U/liter)	6.0 ± 3.6	2.5 ± 2.1	0.009
FSH (U/liter)	4.3 ± 1.1	2.9 ± 1.1	0.008
nAPCsr	2.0 ± 0.8	1.3 ± 0.7	<0.001
Protein C antigen (%)	110 ± 18	100 ± 15	0.070
Protein S total antigen (%)	105 ± 22	118 ± 19	0.037
Protein S free antigen (%)	83 ± 20	97 ± 15	0.012
Prothrombin (%)	106 ± 18	107 ± 16	0.826

Values are mean ± SD. *P* value was determined by paired Student's *t* test. *P* value of <0.05 was considered statistically significant. BMI, Body mass index.

of androgen treatment, which appeared to be associated with a low factor V (*i.e.* 55% of normal). Plasma protein S levels increased during T treatment (protein S, total, 105 ± 22 *vs.* 118 ± 19%, $P = 0.037$; protein S, free, 83 ± 20 *vs.* 97 ± 15%, $P = 0.012$), whereas levels of protein C and prothrombin remained unchanged (Table 3).

As a control for use of OC by F→M transsexuals before inclusion in the study, they were separated in two subgroups according to baseline values of nAPCsr above or below the median value. Because comparison of these two groups did not show differences in values of LH, FSH, T, E_2, and prolactin (data not shown), there was no indication of previous use of OC.

T administration to F→M transsexuals led to steep increase of levels of T and a modest, although significant, decrease in plasma E_2 levels. Percent changes from baseline in levels of T and E_2 after androgen administration to biological women showed a strong trend to correlate inversely with changes from baseline in nAPCsr: $r = -0.48$, $P = 0.070$; and $r = -0.53$, $P = 0.053$, respectively. Percent changes of the other variables of coagulation did not correlate with proportional changes of hormonal variables (E_2, T, LH, or FSH). As opposed to percent changes of hemostatic variables, the absolute values before and during T treatment did not correlate with levels of hormone levels determined in F→M transsexuals.

Gender differences in nAPCsr

Baseline (mean ± SD) nAPCsr differed significantly between biological males and females: pooled average, 1.3 ± 0.7 *vs.* 2.0 ± 0.8; $P = 0.003$ (*cf.* Tables 1 and 3).

Figure 1 summarizes the changes in hemostatic variables (expressed as percentage of the values determined at baseline) as a result of hormone treatment of the M→F and F→M transsexuals. In general, the effects of hormone treatment on hemostatic parameters in M→F transsexuals became more pronounced in the order of CPA-only (*open bars*), td-E_2+CPA (*shaded bars*), and oral-EE+CPA (*solid bars*) treatment. Testosterone treatment of F→M transsexuals (*hatched bars*)

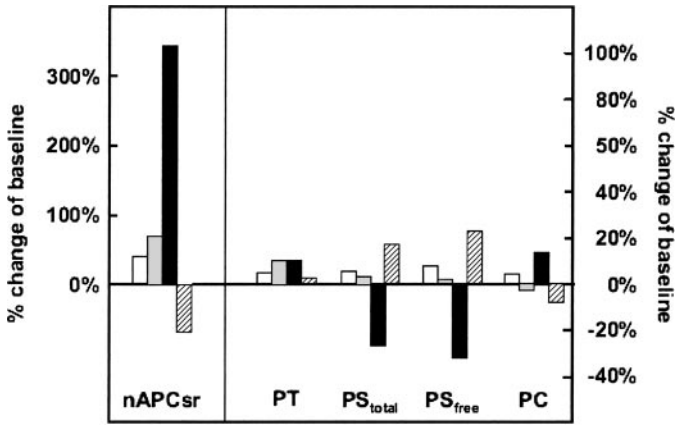

FIG. 1. Changes in hemostatic variables during cross-sex hormonal treatment. Summarized are the changes in hemostatic variables (expressed as percentage of the value determined at baseline) obtained during 4-month cross-sex hormonal treatment of M→F transsexuals with CPA-only (*open bars*), td-E_2+CPA (*shaded bars*), and oral-EE+CPA (*solid bars*), and of F→M transsexuals with T esters (*hatched bars*). Data are from Tables 1 and 3. PT, Prothrombin; PS, protein S; PC, protein C.

caused changes in hemostatic variables that were opposite to those observed in M→F transsexuals treated with oral EE.

It is interesting to note (*cf.* Tables 1–3) that the mean nAPCsr during oral-E_2 or td-E_2+CPA treatment of M→F transsexuals did not differ from the nAPCsr of F→M transsexuals at baseline, and, similarly, that the mean nAPCsr of F→M transsexuals after treatment with T was the same as the mean baseline nAPCsr of M→F transsexuals. This indicates that the normal gender differences in circulating T and E_2 are main determinants of the gender differences in the nAPCsr.

Discussion

An earlier, retrospective analysis of our group (31) indicated that cross-sex hormonal treatment of M→F transsexuals with oral-EE+CPA was associated with a 20-fold increase in the occurrence of VT compared with treatment with td-E_2+CPA. The clinical impression that subjects over 40 yr of age had a higher risk of VT than those younger than 40 had led in 1989 to the decision to recommend td-E_2 for treatment of M→F transsexuals over 40 yr of age. This clinical observation was reason to investigate the effects of oral-EE and td-E_2 combined with CPA and of CPA-only on a number of hemostatic variables known to be associated with increased risk for VT: APC resistance and plasma levels of protein S, protein C, and prothrombin (22).

Treatment of M→F transsexuals with CPA-only or td-E_2+CPA had a small but significant effect on the hemostatic variables determined in this study. However, much larger changes of hemostatic variables were observed in the group of transsexuals treated with oral-EE+CPA. On a quantitative basis, changes (*i.e.* an increase in APC resistance and levels of protein C and prothrombin and a decrease in protein S) in M→F transsexuals using oral EE were more pronounced than those reported in women using OC (15, 16, 18, 19, 42). This is likely due to the much higher dose of EE used by M→F transsexuals (100 μg/d EE) compared with the common dose in OC (30 μg/d EE) suggesting that the changes

in nAPCsr show a dose relationship with the amount of EE administered. The most impressive finding is the increase of nAPCsr in M→F transsexuals after 4 months of treatment with oral-EE+CPA. The nAPCsr in this group reached values comparable with values of heterozygous and homozygous factor V Leiden individuals (10, 12).

The findings of this study have clinical relevance, because it has been shown that nAPCsr as determined with the test used in this study predict for venous thrombotic risk (29), and, furthermore, that the magnitude of risk correlates with the magnitude of APC resistance determined (28, 29). Thus, the increase in nAPCsr observed during oral-EE+CPA administration substantially contributes to the increased thrombotic risk associated with this treatment. The observation that the changes of hemostatic variables did not correlate with changes of hormone levels (E_2, T, LH, and FSH) is indicative for a specific effect of EE rather than an effect of shifts in plasma levels of sex steroids associated with use of EE.

To investigate whether the profound differences between the effects of treatment with oral-EE+CPA and td-E_2+CPA on hemostatic variables were due to first-pass effects of estrogens through the liver or to the different molecular structures of EE and E_2, a group of 20 M→F transsexuals receiving oral-E_2+CPA was added at a later stage of the study. For this group, baseline hemostatic variables were not available. The mean values of nAPCsr after treatment with oral-E_2+CPA (daily doses of 4 mg E_2-valerate plus 100 mg CPA) were not different from baseline values of biological males and considerably lower than the nAPCsr obtained after treatment with oral-EE+CPA. This indicates that the large effect of oral-EE on the nAPCsr has to be attributed to its molecular structure rather than to a first-pass liver effect of pharmacological doses of estrogens.

The use of oral-EE+CPA in M→F transsexuals also affected levels of protein C, protein S, and prothrombin. In the majority of individuals treated with oral-EE+CPA, the plasma levels of these proteins remained within the normal range. It cannot be ruled out, however, that the changes in protein S and prothrombin may contribute to the increased risk of VT, particularly, because relatively small changes of circulating coagulation factors can be associated with an increased risk of VT (42–44). Furthermore, it should be emphasized that, in a number of individual subjects, changes in circulating coagulation factors are larger than the mean. Thus, it is possible that, in an individual with low protein S and/or high prothrombin at baseline, a small change in coagulation factor level may further disturb the hemostatic balance and increase the thrombotic risk (45).

Treatment with CPA-only caused mild APC resistance. The group treated with CPA-only is very small (n = 8), but it should be emphasized that each subject in this group showed an increase in nAPCsr. The antiandrogenic properties of CPA itself could have elicited some degree of APC resistance, because we observed an opposite effect, *i.e.* a decrease of the nAPCsr during androgen administration to biological females. This indicates that not only estrogens but also progestagens may contribute to the development of VT. However, thus far, there are no reports indicating an association between VT and the use of high doses of CPA.

Treatment of M→F transsexuals with td-E_2+CPA or oral-E_2+CPA induced the same (mild) APC resistance as observed with CPA-only treatment (*cf.* Table 1 and Fig. 1). The marginal effects of oral or td E_2 administration are in agreement with earlier reports that show that hormone replacement therapy with td E_2 (100 μg daily) alone does not influence the nAPCsr (46), and that increasing E_2 levels during ovarian hyperstimulation with FSH have a limited effect on the nAPCsr (47, 48).

In conclusion, we have shown that treatment of M→F transsexuals with sex steroid hormones (CPA combined with E_2 or EE) affects the hemostatic balance with a very pronounced difference in the effects of oral EE compared with the effects of both td E_2 or oral E_2. Oral EE induces a clinically relevant prothrombotic state. Treatment with T of F→M transsexuals has a mild antithrombotic effect. Our findings demonstrate that the gender difference in APC resistance largely results from the differences in circulating sex hormone levels. Men, who have lower levels of E_2 than premenopausal women, are less resistant to APC compared with women not using OC (10, 32). This is corroborated by our observations in F→M transsexuals whose APC resistance decreased significantly by im administration of T. Values of nAPCsr of biological females decreased to male values after administration of androgens, whereas after administration of estrogens, nAPCsr values of biological males increased to those of females.

Acknowledgments

Received March 26, 2003. Accepted August 21, 2003.

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References

1. **Jordan WM** 1961 Pulmonary embolism. Lancet 2:1146–1147
2. **Sartwell PE, Masi AT, Arthes FG, Greene GR, Smith HE** 1969 Thromboembolism and oral contraceptives: an epidemiologic case-control study. Am J Epidemiol 90:365–380
3. **Stadel BV** 1981 Oral contraceptives and cardiovascular disease (first of two parts). N Engl J Med 305:612–618
4. **Gerstman BB, Piper JM, Tomita DK, Ferguson WJ, Stadel BV, Lundin FE** 1991 Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. Am J Epidemiol 133:32–37
5. **Daly E, Vessey MP, Hawkins MM, Carson JL, Gough P, Marsh S** 1996 Risk of venous thromboembolism in users of hormone replacement therapy. Lancet 348:977–980
6. **Jick H, Jick SS, Gurewich V, Myers MW, Vasilakis C** 1995 Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet 346:1589–1593
7. 1995 Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet 346:1582–1588
8. **Bloemenkamp KW, Rosendaal FR, Helmerhorst FM, Buller HR, Vandenbroucke JP** 1995 Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen. Lancet 346:1593–1596
9. **Jick H, Kaye JA, Vasilakis-Scaramozza C, Jick SS** 2000 Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: cohort and case-control analysis. BMJ 321:1190–1195
10. **Rosing J, Tans G, Nicolaes GA, Thomassen MC, van Oerle R, van der Ploeg PM, Heijnen P, Hamulyak K, Hemker HC** 1997 Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women

using second- and third-generation oral contraceptives. Br J Haematol 97:233–238
11. **Winkler UH** 1998 Effects on hemostatic variables of desogestrel- and gestodene-containing oral contraceptives in comparison with levonorgestrel-containing oral contraceptives: a review. Am J Obstet Gynecol 179(3 Pt 2):51–61
12. **Rosing J, Middeldorp S, Curvers J, Christella M, Thomassen LG, Nicolaes GA, Meijers JC, Bouma BN, Buller HR, Prins MH, Tans G** 1999 Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. Lancet 354:2036–2040
13. **Kluft C** 2000 Effects on haemostasis variables by second and third generation combined oral contraceptives: a review of directly comparative studies. Curr Med Chem 7:585–591
14. **Meijers JC, Middeldorp S, Tekelenburg W, van den Ende AE, Tans G, Prins MH, Rosing J, Buller HR, Bouma BN** 2000 Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives. Thromb Haemost 84:9–14
15. **Middeldorp S, Meijers JC, van den Ende AE, van Enk A, Bouma BN, Tans G, Rosing J, Prins MH, Buller HR** 2000 Effects on coagulation of levonorgestrel- and desogestrel-containing low dose oral contraceptives: a cross-over study. Thromb Haemost 84:4–8
16. **Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH, Bouma BN, Buller HR, Rosing J** 2000 A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost 84:15–21
17. **Mackie IJ, Piegsa K, Furs SA, Johnson J, Bounds W, Machin SJ, Guillebaud J** 2001 Protein S levels are lower in women receiving desogestrel-containing combined oral contraceptives (COCs) than in women receiving levonorgestrel-containing COCs at steady state and on cross-over. Br J Haematol 113:898–904
18. **Kemmeren JM, Algra A, Meijers JC, Bouma BN, Grobbee DE** 2002 Effect of second- and third-generation oral contraceptives on fibrinolysis in the absence or presence of the factor V Leiden mutation. Blood Coagul Fibrinolysis 13: 373–381
19. **Kemmeren JM, Algra A, Meijers JC, Bouma BN, Grobbee DE** 2002 Effects of second and third generation oral contraceptives and their respective progestagens on the coagulation system in the absence or presence of the factor V Leiden mutation. Thromb Haemost 87:199–205
20. **Vandenbroucke JP, Helmerhorst FM, Bloemenkamp KWM, Rosendaal FR** 1997 Third-generation oral contraceptive and deep venous thrombosis: from epidemiologic controversy to new insight in coagulation. Am J Obstet Gynecol 177:887–891
21. **Vandenbroucke JP, Rosendaal FR** 1997 End of the line for "third-generation-pill" controversy? Lancet 349:1113–1114
22. **Rosendaal FR** 1999 Risk factors for venous thrombotic disease. Thromb Haemost 82:610–619
23. **Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM** 1993 Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 342:1503–1506
24. **Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH** 1995 High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 85:1504–1508
25. **de Visser MC, Rosendaal FR, Bertina RM** 1999 A reduced sensitivity for activated protein C in the absence of factor V Leiden increases the risk of venous thrombosis. Blood 93:1271–1276
26. **Rodeghiero F, Tosetto A** 1999 Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. Ann Intern Med 130:643–650
27. **Curvers J, Thomassen MC, Nicolaes GA, Van Oerle R, Hamulyak K, Hemker HC, Tans G, Rosing J** 1999 Acquired APC resistance and oral contraceptives: differences between two functional tests. Br J Haematol 105:88–94
28. **Curvers J, Thomassen MC, Rimmer J, Hamulyak K, van der Meer J, Tans G, Preston FE, Rosing J** 2002 Effects of hereditary and acquired risk factors of venous thrombosis on a thrombin generation-based APC resistance test. Thromb Haemost 88:5–11
29. **Tans G, van Hylckama Vlieg A, Thomassen MC, Curvers J, Bertina RM, Rosing J, Rosendaal FR** 2003 Activated protein C resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. Br J Haematol 122:465–470
30. **Gooren L** 1990 The endocrinology of transsexualism: a review and commentary. Psychoneuroendocrinology 15:3–14
31. **van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ** 1997 Mortality and morbidity in transsexual subjects treated with cross-sex hormones. Clin Endocrinol (Oxf) 47:337–342
32. **Henkens CM, Bom VJ, Seinen AJ, van der Meer J** 1995 Sensitivity to activated protein C; influence of oral contraceptives and sex. Thromb Haemost 73:402–404.
33. **Giltay EJ, Gooren LJ, Emeis JJ, Kooistra T, Stehouwer CD** 2000 Oral, but not transdermal, administration of estrogens lowers tissue-type plasminogen activator levels in humans without affecting endothelial synthesis. Arterioscler Thromb Vasc Biol 20:1396–1403
34. **Mashchak CA, Lobo RA, Dozono-Takano R, Eggena P, Nakamura RM, Brenner PF, Mishell Jr DR** 1982 Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol 144:511–518
35. **Hyder SM, Chiappetta C, Stancel GM** 1999 Synthetic estrogen 17α-ethinyl estradiol induces pattern of uterine gene expression similar to endogenous estrogen 17β-estradiol. J Pharmacol Exp Ther 290:740–747
36. **Prifti S, Mall P, Strowitzki T, Rabe T** 2001 Synthetic estrogens-mediated activation of JNK intracellular signaling molecule. Gynecol Endocrinol 15: 135–141
37. **Nicolaes GA, Thomassen MC, van Oerle R, Hamulyak K, Hemker HC, Tans G, Rosing J** 1996 A prothrombinase-based assay for detection of resistance to activated protein C. Thromb Haemost 76:404–410
38. **Sala N, Owen WG, Collen D** 1984 A functional assay of protein C in human plasma. Blood 63:671–675
39. **Nicolaes GA, Thomassen MC, Tans G, Rosing J, Hemker HC** 1997 Effect of activated protein C on thrombin generation and on the thrombin potential in plasma of normal and APC-resistant individuals. Blood Coagul Fibrinolysis 8:28–38
40. **Rosing J, Tans G, Govers-Riemslag JW, Zwaal RF, Hemker HC** 1980 The role of phospholipids and factor Va in the prothrombinase complex. J Biol Chem 255:274–283
41. **Curvers J, Christella M, Thomassen LG, de Ronde H, Bertina RM, Rosendaal FR, Tans G, Rosing J** 2002 Effects of (pre-)analytical variables on activated protein C resistance determined via a thrombin generation-based assay. Thromb Haemost 87:483–492
42. **Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR** 2000 High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 342:696–701
43. **Poort SR, Rosendaal FR, Reitsma PH, Bertina RM** 1996 A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3698–3703
44. **van Hylckama Vlieg A, van der Linden IK, Bertina RM, Rosendaal FR** 2000 High levels of factor IX increase the risk of venous thrombosis. Blood 95: 3678–3682
45. **Tans G, Bouma BN, Buller HR, Rosing J** 2003 Changes of hemostatic variables during oral contraceptive use. Semin Vasc Med 3:61–68
46. **Lowe GD, Upton MN, Rumley A, McConnachie A, O'Reilly DS, Watt GC** 2001 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. Thromb Haemost 86:550–556
47. **Wramsby ML, Bokarewa MI, Blomback M, Bremme AK** 2000 Response to activated protein C during normal menstrual cycle and ovarian stimulation. Hum Reprod 15:795–797
48. **Curvers J, Nienhuis SJ, Nap AW, Hamulyak K, Evers JL, Rosing J** 2001 Activated protein C resistance during in vitro fertilization treatment. Eur J Obstet Gynecol Reprod Biol 95:222–224