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 м/20 м/60 м), 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	0.076
BMI (kg/m²)	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	<0.001
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*)
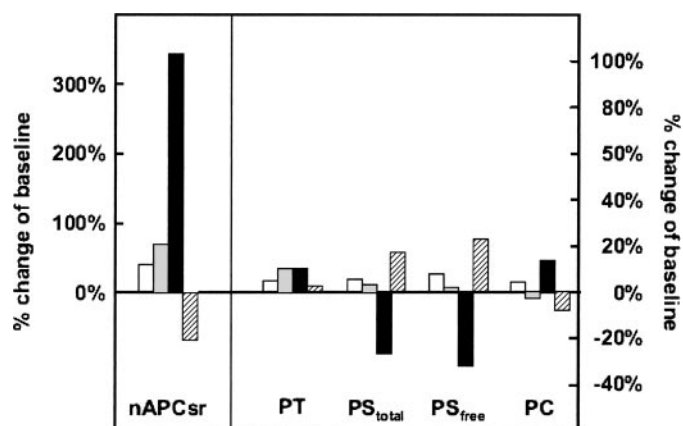

FIG. 1. Changes in hemostatic variables during cross-sex hormonal treatment. Summarized are the changes in hemostatic variables (expressed as percentage of the value determined at baseline) obtained during 4-month cross-sex hormonal treatment of M→F transsexuals with CPA-only (*open bars*), td-E_2+CPA (*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.

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