ORIGINAL RESEARCH—TRANSGENDER AND GENDER NONCONFORMANCE

Cross-Sex Hormone Therapy in Trans Persons Is Safe and Effective at Short-Time Follow-Up: Results from the European Network for the Investigation of Gender Incongruence

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ABSTRACT

Introduction. Data on the effects of cross-sex hormone therapy (CHT) are limited due to the low prevalence of gender dysphoria, small number of subjects treated at each center, lack of prospective studies, and wide variations in treatment modalities.

Aim. The aim of this study is to report the short-term effects of CHT on hormonal and clinical changes, side effects, and adverse events in trans men (female-to-male gender dysphoric persons) and trans women (male-to-female gender dysphoric persons).

Methods. This was a multicenter 1-year prospective study in 53 trans men and 53 trans women. Trans men received injections of testosterone undecanoate every 3 months. Trans women younger than 45 years received 50 mg cyproterone acetate (CA) and 4 mg estradiol valerate daily, whereas those older than 45 years received 50 mg CA daily together with 100 μg/24 hours transdermal 17-β estradiol.

Main Outcome Measures. Sex steroids, prolactin, liver enzymes, lipids, hematocrit, blood pressure, anthropometrics, Ferriman and Gallwey score, and global acne grading scale were measured. Side effects, adverse events, and desired clinical changes were examined.

Results. No deaths or severe adverse events were observed. Two trans men developed erythrocytosis, and two had transient elevation of the liver enzymes. Trans men reported an increase in sexual desire, voice instability, and clitoral pain (all $P \leq 0.01$). Testosterone therapy increased acne scores, facial and body hair, and prevalence of androgenetic alopecia. Waist–hip ratio, muscle mass, triglycerides, total cholesterol (C), and LDL-C increased, whereas total body fat mass and HDL-C decreased. Three trans women experienced transient elevation of liver enzymes. A significant increase in breast tenderness, hot flashes, emotionality, and low sex drive was observed (all $P \leq 0.02$). Fasting insulin, total body fat mass, and prolactin levels increased, and waist–hip ratio, lean mass, total C, and LDL-C decreased.

Conclusions. Current treatment modalities were effective and carried a low risk for side effects and adverse events at short-time follow-up. **Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher A, Toye K, Kaufman JM, and T'Sjoen G. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: Results from the European Network for the Investigation of Gender Incongruence. J Sex Med 2014;11:1999–2011.**

Key Words. ENIGI; Gender Dysphoria; Transsexualism; Hormone Treatment; Safety

Introduction

Trans persons undergo cross-sex hormone therapy (CHT) to induce the secondary sex characteristics of the desired sex while reducing those of the natal one [1]. The act of using cross-sex hormones is also an affirmation of gender identity in many trans persons. The choice of type and dosage of hormones have not yet been established as randomized controlled trials, and comparative studies are lacking [2], and so, a variety of hormone preparations are currently used [3]. Female-to-male gender dysphoric persons, referred to as trans men, are treated with testosterone (T) preparations to induce virilization, sometimes preceded with progestagens to suppress menstruation [1]. To promote feminization, trans women (male-to-female gender dysphoric persons) usually receive estrogens in combination with antiandrogen and/or gonadal axis suppression medication to lower T levels and/or action [1].

Many centers in Europe use cyproterone acetate (CA), a progestational agent with androgen receptor-blocking properties [4–6], whereas spironolactone, a diuretic with antiandrogen action, is mostly used in the United States [7,8]. Other centers also use gonadotropin-releasing hormone analogues [9,10], nonsteroidal androgen receptor blockers, or 5-alpha reductase inhibitors. In addition, type of formulation, hormone dosage, and route of administration (oral, transdermal, or intramuscular) may differ between centers. These wide variations in treatment modalities and the low prevalence of transsexuality, and therefore small number of subjects treated in each center, hamper our knowledge on the effects and side effects of CHT. Furthermore, hormonal therapies have changed considerably in the past years, and most current evidence is based on older treatment regimens. The use of long-acting T preparations has significantly increased in trans men, and the use of ethinyl estradiol (EE) has decreased in trans women due to the increased risk for cardiovascular disease [11].

Aims

The aim of this study was to investigate the physical and physiological effects, side effects, and adverse events of commonly used CHTs. We present the first multicenter prospective study in a well-described cohort of trans persons treated according to a standardized treatment protocol.

Methods

Study Population and Sex Hormone Therapy

This research is part of the European Network for the Investigation of Gender Incongruence (ENIGI), a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Hamburg, and Oslo) [12] created to study the diagnostics and treatment of gender dysphoria. We present current data from the Department of Endocrinology at the Ghent University Hospital and the University Hospital in Oslo. All patients diagnosed with gender dysphoria and referred to our departments between February 2010 and August 2012 were invited to participate in this prospective study (N = 152). We included only hormone-naive trans persons. After screening, by thorough medical history and determination of serum sex steroids, 44 individuals were excluded. A total of 53 trans men and 53 trans women participated in our study (Figure 1). Patients were followed every 3 months during the first treatment year.

Trans men received injections of 1,000 mg intramuscular T undecanoate (Nebido®, Bayer, Germany) at the start of the study, after 6 weeks, and every 12 weeks thereafter. Before T initiation, progestagens were sometimes taken to suppress the menstrual cycle. In case of nontolerance, injections with intramuscular T esters (T decanoate 100 mg, T isocaproate 60 mg, T fenylpropionate 60 mg, T propionate 30 mg/mL) (Sustanon 250®, MSD, Netherlands) every 2 weeks were prescribed.

All trans women younger than 45 years (N = 40) received 50 mg of CA (Androcur®, Bayer) in combination with 4 mg of estradiol valerate (EV) (Progynova®, Bayer) daily. Patients older than 45 years (N = 13) received 50 mg of CA daily in combination with 100 µg/24 hours transdermal 17-β estradiol (E2) patch (Dermestril®, Besins, Belgium). In case of nontolerance, 2 mg of transdermal 17-β E2 gel twice daily (Oestrogel®, Besins) or 4 mg EV per day was given. Based on the decision of the mental health professional and patient, some trans women who favored a slower procedure and/or needed an extra diagnostic evaluation received a dual-phase protocol (N = 16). In the first phase, sex-specific features were suppressed by administration of CA 50 mg daily for about 3 months, and estrogens were added to induce feminization in the second phase. This study complied with the recommendations of the Declaration of Helsinki and was approved by the ethical
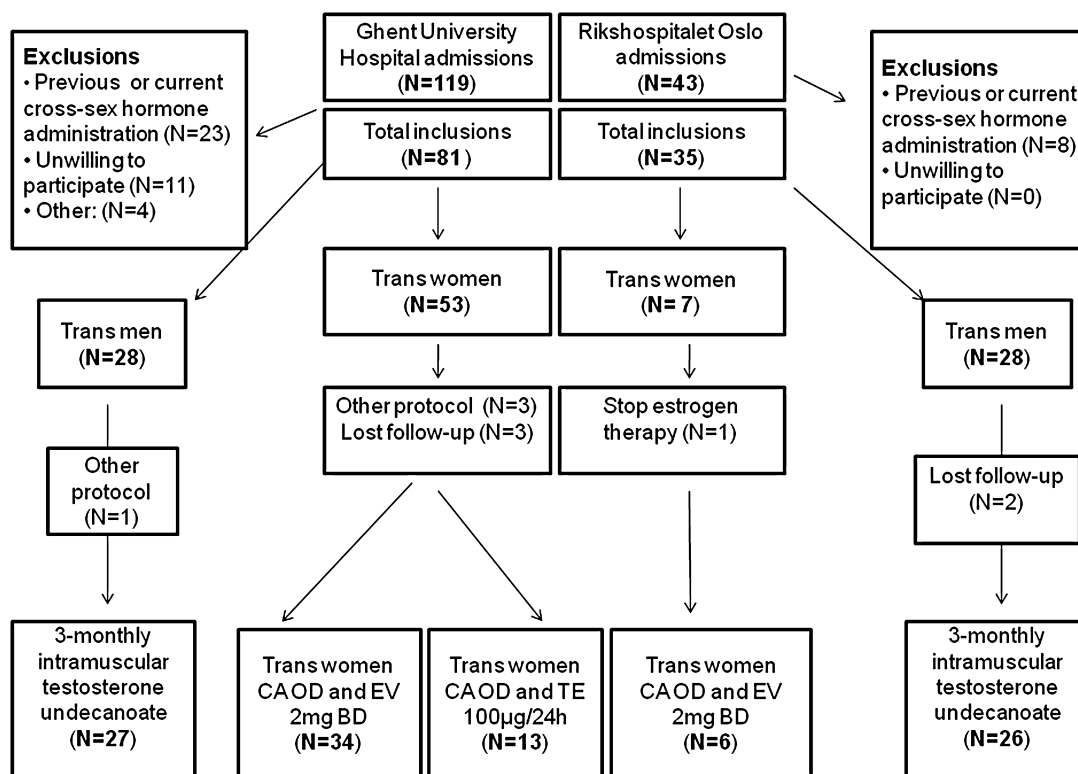
Figure 1 Subject enrollment

committee of the Ghent University Hospital and the University Hospital of Oslo. All participants gave written informed consent. Clinical trial number: NCT01072825.

Main Outcome Measures

Medical History and Examination
Descriptive data were collected from all individuals, including physical and psychiatric medical history, current and past medication use, familial medical history, and lifestyle factors such as smoking and alcohol consumption. Information was compared with data from medical files for accuracy and corrected if necessary.

Physical Parameters
Anthropometrics
Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymuch, UK). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. Waist circumference, defined as the smallest abdominal circumference, and hip circumfer-

ence, defined as the largest hip circumference, were determined to the nearest 0.1 cm.

Body Composition
Whole body lean mass and fat mass were measured using dual-energy X ray absorptiometry with Hologic Discovery (Hologic Inc., Bedford, MA, USA) in Belgium and with Lunar Prodigy Advance (GE, Madison, WI, USA) in Norway.

Acne
Clinical assessment of acne was performed every 3 months in each patient by the same endocrinologist with the Gradual Acne Grading Scale (GAGS) [13], a semiquantitative scoring system in which the total severity score is derived from the summary of six regional subscores. Each subscore was obtained by the score of the most heavily weighted lesion within each region (one for one or more comedones, two for one or more papules, three for one or more pustules, and four for one or more nodules) multiplied by the factor for each region (the factor for forehead and each cheek is 2, chin and nose is 1, and chest and upper back is 3).

Scores between 6 and 18, 20 and 30, and 31 and 36 were classified as mild, moderate, or severe acne, respectively.

Body Hair and Distribution

The effects of T therapy on hair growth and distribution of body hair in trans men was evaluated every 3 months using the modified Ferriman and Gallwey classification [14] in each patient by the same endocrinologist. This scale scores nine androgen-dependent areas on a five-point Likert scale (from 0 = no to 4 = very dense). A score for an androgen-dependent area of more than 8 indicated hirsutism. Androgenetic alopecia was assessed using the Norwood/Hamilton classification [15].

We were unable to evaluate the effects of CHT on hair growth and distribution of body hair in trans women because almost all of them underwent laser epilation during the course of the study.

Side Effects and Adverse Events

Clinical adverse events, including cardiovascular events, venous thrombosis and/or pulmonary embolism, osteoporotic fractures, abnormal liver function tests, hypertension, and death (including suicide), were recorded. We also evaluated whether hormone levels reached the target values for the desired gender. Development of erythrocytosis, hyperprolactinaemia, hypercholesterolemia, and hyperglycemia was also assessed.

We evaluated symptoms possibly related to hormonal status every 3 months, including hot flashes, night sweats, sleeping problems, fatigue, memory or cognition problems, mood swings, irritability, anxiety, low or high sexual desire, migraines, nail and gum problems, breast tenderness, joint pain, and muscle soreness using a four-point Likert scale (no, mild, moderate, or severe complaints).

Biochemical Determinations

Venous blood was obtained at baseline and at 12 months, and serum was stored at –80°C until hormones were analyzed in one batch. Blood samples for routine clinical parameters were drawn at the 3-, 6-, and 9-month time points.

Luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin, dehydroepiandrosterone sulfate (DHEAS), and prolactin were measured by electrochemiluminiscence immunoassay (ECLIA) (Modular, Roche Diagnostics, Mannheim, Germany). The interassay CVs were as follows: LH 2.19%, FSH 2.55%, SHBG 2.8%, prolactin

4.8%, DHEAS 4.8%, insulin 2.3%. E2, estrone (E1), DHEAS, androstenedione, cortisol, and T were determined using liquid chromatography tandem mass spectrometry (AB Sciex 5,500 triple-quadrupole mass spectrometer; AB Sciex, Toronto, Canada). The serum limit of quantification was 0.3 pg/mL for E2 and 0.5 pg/mL for E1, and the interassay CVs were 4% at 21 pg/mL for E2 and 7.6% at 25 pg/mL for E1 [16]. Serum limit of quantification was 1 ng/dL (35 pmol/L) for T, and the interassay CV was 6.5% at 3 ng/dL. Hemoglobin, hematocrit (Hct), glucose, creatinin, and the liver enzymes glutamic-oxaloacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), cholesterol (C), LDL-C, HDL-C, and triglycerides were measured using routine clinical chemistry methods.

Statistical Analysis

Descriptive statistics were expressed as means and standard deviations, or medians [first to third quartiles] for in case of a non-normal distributions. Statistical analyses of categorical variables were carried out using χ^2 and Fisher's exact tests as appropriate. Statistics of means in prospective data were carried out using the paired Student t-tests or Wilcoxon signed-rank tests when variables were not normally distributed. Between two groups, statistics of means was evaluated using independent Student t-tests and Mann–Whitney U-tests when variables were not normally distributed. Significance was set at $P < 0.05$ (two-tailed). Data were analyzed using SPSS software, v.21 (SPSS Inc., Chicago, IL, USA). For all analyses, missing values were excluded.

Results

General Characteristics

General characteristics of the study population are shown in Table 1. Trans women were significantly older than trans men at the time of presentation ($P < 0.001$). A significantly greater proportion of trans men were at the Oslo center, and the subjects were significantly older at the Ghent center.

Hormonal and Biochemical Changes in Trans Men

All trans men achieved T levels within the male reference range (321–1,005 ng/dL) during treatment (Table 2). Three trans men (5.7%) had T levels that exceeded the upper limit of 1,005 ng/dL (one at 3-month, one at 9-month, and one at 12-month time point of treatment). Hct levels

Table 1 Baseline characteristics of the study population

	Trans men		Trans women	
	Ghent (N = 27)	Oslo (N = 26)	Ghent (N = 47)	Oslo (N = 6)
Age (years)	27.3 ± 8.5	21.7 ± 5.1	31.7 ± 14.8	19.3 ± 2.4
Current smoker (%)	25.9	14.4	19.1	0
Former smoker (%)	33.3	30.8	38.3	0
Alcohol (U/week)	0 (0–0)	0 (0–0.75)	0 (0–7)	0 (0–0)
Height (cm)	163.4 ± 4.4	168.3 ± 6.0	178.4 ± 6.2	179.5 ± 3.8
Weight (kg)	65.7 ± 15.0	71.2 ± 15.9	76.1 ± 13.9	73.7 ± 18.5
BMI (kg/m^2)	24.5 ± 5.2	25.2 ± 5.5	23.9 ± 4.1	22.9 ± 5.7

Data are presented as mean ± standard deviation or median (first to third quartiles)
BMI = body mass index

gradually increased during T treatment. Two trans men developed erythrocytosis according to male reference ranges (Hct levels above 52%), one developed it after 9 months, and one after 12 months of treatment. T levels were within the male reference range in these men. Erythrocytosis was present in 20.1% of trans men according to female reference ranges (Hct levels above 48%). E2, E1, prolactin, and SHBG decreased significantly, whereas DHEAS, androstenedione, and cortisol levels were not influenced by T treatment. T treatment induced a less favorable lipid profile, as total C, LDL-C, and triglycerides increased, whereas HDL-C decreased (Table 2).

Hormonal and Biochemical Changes in Trans Women
All trans women achieved adequate gonadotropin and T suppression during antiandrogen and estrogen administration (Table 2). Two trans women (both on oral EV) who initially had an adequate T and gonadotropin suppression showed T levels within the normal male range at 12 months, possibly due to poor adherence to treatment. Gonadotropins, T, and androstenedione decreased during oral and transdermal estrogen therapy associated with CA, whereas E2 and E1 increased.

Trans women using oral EV, unlike those using transdermal estrogen, experienced a significantly increased SHBG, decreased DHEAS, and a trend toward increased cortisol levels. Prolactin levels significantly increased during both oral and transdermal estrogen treatments (Figure 2A) and during 12 weeks of CA treatment alone (Figure 2B).

CHT induced a more favorable lipid profile as total C and LDL-C decreased during both oral and transdermal estrogen treatments. HDL-C decreased during both forms of estrogen treatment, and triglycerides decreased during transdermal but not oral estrogen treatment. No significant differences were observed between trans women initially

treated with CA plus estrogens compared with those initially treated with CA alone (data not shown).

Physical Changes
Trans Men
Total body weight significantly increased due to an increase in total lean mass, whereas total fat mass decreased. An android pattern of fat distribution was observed as the waist–hip ratio increased during treatment ($P = 0.02$), mainly due to reduced hip circumference (Table 3). As expected, Ferriman and Gallwey score significantly increased ($P < 0.001$), with a wide between-subject variability ranging from 2 to 28.

Trans Women
In contrast to trans men, total body weight remained unchanged for trans women, although they experienced an increase in total body fat mass and a decrease in total body lean mass. A gynoid pattern of fat distribution was induced in trans women as the waist–hip ratio decreased during treatment (Table 3).

Antiandrogen with estrogen treatment resulted in a significant increase (average 3.3 cm) in breast circumference at the nipple, with a wide inter-individual range of increase. Trans women using oral estrogens experienced similar changes in physical measures as those using transdermal estrogens (Table 3). No significant differences were observed between trans women treated with CA plus estrogens from start compared with those initially treated with CA alone (data not shown), although the former tended to have a larger breast circumference ($P = 0.06$).

Side Effects and Adverse Events
Trans Men
We recorded no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or

Table 2 Hormonal and biochemical changes in trans persons

	Trans men (N = 53)		Trans women (N = 53)			
	Intramuscular T undecanoate (N = 53)	*P*	Oral estrogens (N = 40)	*P*	Transdermal estrogens (N = 13)	*P*
Testosterone (ng/dL)						
Baseline	30.2 (20.4–39.9)	<0.001	517.5 (419.2–631.9)	<0.001	567.0 (484.8–678.2)	0.001
12 months	595.8 (481.1–715.7)		10.7 (8.3–14.3)		14.0 (12.4–17.2)	
Estradiol (pg/mL)						
Baseline	50.3 (24.2–99.3)	0.001	19.1 (15.3–24.9)	<0.001	23.6 (18.3–29.3)	0.001
12 months	29.4 (21.7–34.7)		56.5 (42.3–70.8)		95.4 (58.5–127.6)	
Estrone (pg/mL)						
Baseline	51.7 (30.3–80.9)	0.01	29.8 (24.1–40.1)	<0.001	45.8 (33.8–50.3)	0.028
12 months	45.5 (32.9–53.8)		360.9 (253.3–485.3)		57.8 (47.3–90.7)	
SHBG (nmol/L)						
Baseline	51.6 (23.0–81.2)	<0.001	31.2 (24.0–40.1)	<0.001	50.3 (36.0–59.7)	0.33
12 months	25.1 (19.7–34.0)		41.7 (25.8–50.7)		48.1 (29.0–90.7)	
LH (U/L)						
Baseline	6.7 (4.2–9.9)	<0.001	4.8 (3.7–6.4)	<0.001	5.5 (3.5–6.8)	0.001
12 months	2.4 (0.7–5.8)		0.1 (0.1–0.1)		0.1 (0.1–0.1)	
FSH (U/L)						
Baseline	4.8 (3.4–7.3)	0.02	3.5 (2.8–5.3)	<0.001	5.4 (3.5–6.8)	0.001
12 months	4.2 (1.2–6.6)		0.2 (0.1–0.4)		0.2 (0.1–0.4)	
Prolactin (ng/mL)						
Baseline	13.7 (9.3–19.9)	<0.001	8.1 (6.3–11.3)	<0.001	6.6 (4.1–9.3)	0.001
12 months	9.6 (7.9–14.4)		20.6 (16.7–28.9)		26.2 (18.6–36.2)	
DHEAS (μg/dL)						
Baseline	254.0 (170.1–351.4)	0.97	289.9 (253.1–401.7)	<0.001	285.9 (220.4–404.5)	0.42
12 months	257.6 (174.1–355.7)		236.8 (192.9–348.5)		275.6 (171.9–410.3)	
Androstenedione (ng/dL)						
Baseline	113.9 (90.9150.5)	0.42	101.2 (77.0–121.3)	<0.001	117.4 (94.6–161.4)	0.001
12 months	113.8 (80.2–151.9)		56.2 (43.4–76.4)		69.6 (55.9–106.7)	
Cortisol (μg/dL)						
Baseline	13.3 (8.9–16.0)	0.42	15.2 (12.9–18.7)	0.06	15.4 (13.4–19.9)	0.28
12 months	11.8 (9.4–15.2)		15.7 (12.8–20.8)		15.0 (11.8–18.9)	
Hematocrit (%)						
Baseline	40.8 ± 2.9	<0.001	45.2 ± 2.5	0.003	45.5 ± 1.7	<0.001
12 months	45.8 ± 3.0		42.0 ± 5.7		42.0 ± 2.3	
Fasting glucose (mg/dL)*						
Baseline	0.80 ± 0.1	0.28	0.83 ± 0.1	0.78	0.92 ± 0.06	0.25
12 months	0.77 ± 0.1		0.84 ± 0.1		0.88 ± 0.1	
Fasting insulin (mU/L)*						
Baseline	9.1 (5.8–16.3)	0.02	7.2 (4.9–9.7)	0.019	7.3 (6.6–18.7)	0.1
12 months	7.5 (5.2–13.2)		9.3 (7.2–11.3)		12.0 (9.1–17.0)	
Creatinin (mg/dL)						
Baseline	0.74 ± 0.1	<0.001	0.9 ± 0.1	0.001	0.93 ± 0.1	0.011
12 months	0.84 ± 0.1		0.8 ± 0.1		0.85 ± 0.1	
AST (U/L)						
Baseline	20.0 (17–23)	0.01	24.1 ± 9.2	<0.001	26.8 ± 8.3	0.013
12 months	24 (18–29.5)		17.7 ± 3.6		19.6 ± 4.8	
ALT (U/L)						
Baseline	16.0 (11.5–20)	0.02	25.0 ± 17.4	0.01	29.9 ± 15.3	0.12
12 months	20 (15–25)		18.6 ± 9.5		21.0 ± 10.1	
Total C (mg/dL)						
Baseline	171.9 ± 28.1	0.04	171.5 ± 32.7	0.001	227.2 ± 35.6	0.004
12 months	178.2 ± 30.6		152.3 ± 28.3		181.3 ± 20.6	
LDL-C (mg/dL)						
Baseline	98.4 ± 26.3	0.006	99.4 ± 29.0	0.03	138.3 ± 24.8	0.001
12 months	116.1 ± 28.9		92.3 ± 28.9		106.3 ± 20.4	
HDL-C (mg/dL)						
Baseline	56.3 ± 12.7	<0.001	52.9 ± 13.5	<0.001	58.2 ± 15.2	0.26
12 months	47.8 ± 10.7		45.7 ± 9.2		54.8 ± 15.7	
Triglycerides (mg/dL)						
Baseline	69.0 (51.7–89.5)	<0.001	79.5 (54.7–108)	0.1	87.0 (68.0–176.5)	0.03
12 months	81.1 (65.3–124.6)		70.8 (50–133.1)		85.0 (70.0–110)	

*Based on subsample of trans women (N = 44) or trans men (N = 23)

Data are presented as mean ± standard deviation or median (first to third quartiles). *P* value results from paired *t*-test or Wilcoxon signed-rank test

ALT = glutamic-pyruvic transaminase; AST = glutamic-oxaloacetic transaminase; DHEAS = dehydroepiandrosterone sulfate; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol; FSH = follicle stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone binding globulin
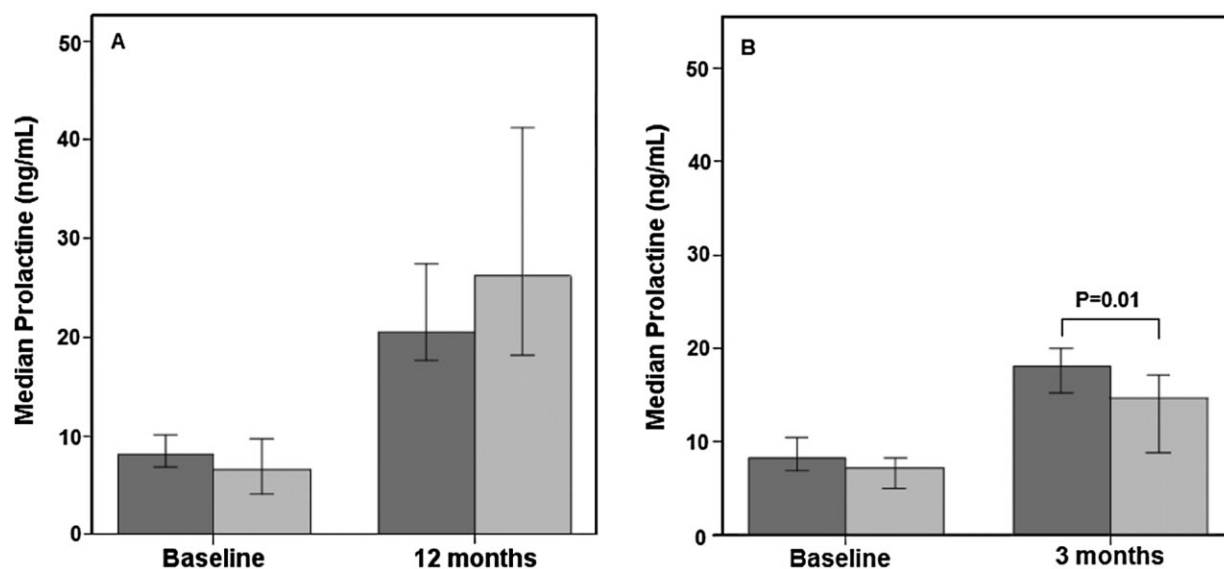
Figure 2 (A) Changes in prolactin levels during cyproterone acetate (CA) plus oral or transdermal estrogen treatment. Data are presented as median; error bars represent 95% confidence interval (CI). Dark grey square: CA plus oral estrogens; light grey square: CA plus transdermal estrogens. We observed a comparable increase in prolactin levels during both oral and transdermal estrogen treatment ($P < 0.001$ and $P = 0.002$, respectively). (B) Changes in prolactin levels during CA plus estrogens or CA alone. Data are presented as median; error bars represent 95% CI. Dark grey square: CA plus estrogens; light grey square: CA alone. We observed an increase in prolactin levels during CA + estrogen treatment ($P < 0.001$) and during CA treatment alone ($P = 0.009$). CA + estrogen treatment induced higher prolactin levels compared with CA treatment alone ($P = 0.01$).

pulmonary embolisms in trans men. Two were switched to short-acting intramuscular T esters after 9 and 12 months, respectively, of T undecanoate therapy mainly because of muscle and joint aches. Liver enzymes increased during T therapy, but only 1.9% of trans men had liver enzymes values exceeding twice the upper limit of normal according to female reference ranges. No subject had liver enzymes values exceeding twice the upper limit of normal according to male reference ranges.

Blood pressure increased slightly during treatment, but none of the subjects developed hypertension during our observation. Fasting insulin levels decreased ($P = 0.02$), and nobody developed type 2 diabetes. GAGS acne scores increased ($P < 0.001$), but the majority of trans men (94.3%) had mild acne lesions. The remaining 5.7% had moderate acne lesions after 12 months of therapy, and no individuals had severe or very severe acne lesions. Eighteen persons (34.0%) initiated topical or oral acne treatment.

Seventeen percent of trans men developed androgenetic alopecia. No indication of troublesome aggression, hostility, or sleep apnea was present. All trans men reported loss of vaginal bleeding during treatment. Spotting was reported

in about one-third of participants, generally limited to the first 6 months of treatment (Figure 3).

Trans Women

Similar to trans men, no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or pulmonary embolisms were observed in trans women. One trans woman had to stop estrogen treatment due to major depression and was excluded from our analyses. Serum prolactin levels exceeding twice the upper limit of normal were observed in 15.7% and 3.9% according to the male and female reference range, respectively. One trans woman experienced galactorea, which spontaneously stopped after several weeks. Transient elevation of the liver enzymes exceeding twice the upper limit of normal was observed in 5.7% and 1.9% according to the female and male reference ranges, respectively. One trans woman developed hypertension during the study observation (defined as a systolic blood pressure above 140 mm Hg or diastolic above 90 mm Hg at three different time points). Fasting insulin increased during antiandrogen and estrogen treatment ($P = 0.005$), but no subject met criteria for diagnosis of type 2 diabetes. Two trans women using transdermal estrogen patches (15.4%) were switched to other

Table 3 Physical changes in trans persons

	Trans men (N = 53)		Trans women (N = 53)			
	Intramuscular T undecanoate (N = 53)	P	Oral estrogens (N = 40)	P	Transdermal estrogens (N = 13)	P
Weight (kg)						
Baseline	68.4 ± 15.5	0.01	73.3 ± 13.8	0.10	82.1 ± 13.1	0.99
12 months	70.6 ± 13.2		74.6 ± 14.3		82.1 ± 11.6	
BMI (kg/m^2)						
Baseline	24.8 ± 5.3	0.01	23.1 ± 4.2	0.42	26.1 ± 3.5	0.91
12 months	25.6 ± 4.4		23.7 ± 4.4		26.1 ± 3.4	
Total body fat mass (kg)						
Baseline	22.9 ± 11.4	<0.001	15.4 ± 7.4	<0.001	16.6 ± 5.4	0.01
12 months	19.9 ± 9.7		20.0 ± 8.1		18.7 ± 4.6	
Total body lean mass (kg)						
Baseline	43.0 ± 6.6	<0.001	5.6 ± 7.5	<0.001	62.6 ± 9.3	0.02
12 months	48.3 ± 5.6		5.3 ± 8.0		59.7 ± 8.1	
Waist circumference (cm)						
Baseline	80.3 ± 13.6	0.74	81.2 ± 10.1	0.21	91.6 ± 11.1	0.50
12 months	80.1 ± 11.2		79.7 ± 10.5		90.9 ± 10.1	
Hip circumference (cm)						
Baseline	97.3 ± 10.5	0.02	94.2 ± 9.3	<0.001	96.9 ± 7.8	0.07
12 months	95.4 ± 9.2		98.1 ± 9.3		100.4 ± 7.1	
Waist–hip ratio						
Baseline	0.82 ± 0.09	0.03	0.9 ± 0.1	<0.001	0.94 ± 0.06	0.07
12 months	0.84 ± 0.08		0.8 ± 0.1		0.91 ± 0.07	
Breast circumference (cm)*						
Baseline	—	—	92.9 ± 10.0	0.03	98.2 ± 9.0	0.09
12 months			95.7 ± 11.2		101.2 ± 9.0	
Systolic blood pressure (mm Hg)						
Baseline	111.5 ± 12.6	0.05	125.1 ± 13.8	0.005	131.6 ± 15.8	0.47
12 months	115.6 ± 11.7		118.8 ± 13.9		128.8 ± 15.5	
Diastolic blood pressure (mm Hg)						
Baseline	70.2 ± 10.5	0.18	76.8 ± 10.8	0.32	76.7 ± 9.0	0.43
12 months	72.5 ± 9.2		75.7 ± 10.6		79.7 ± 9.3	
Ferriman and Gallwey score						
Baseline	0 (0–2)	<0.001	—	—	—	—
12 months	10 (6–16)					
Acne score						
Baseline	2 (0–5)	<0.001	2 (0–7)	<0.001	0 (0–0)	1.0
12 months	7.5 (2–11.8)		0 (0–0)		0 (0–0)	

*Based on subsample (n = 20)
Data are presented as mean ± standard deviation or median (first to third quartiles) in case of non-Gaussian distribution; *P* value results from paired *t*-test or Wilcoxon signed-rank test in case of non-Gaussian distribution
BMI = body mass index

therapies because of skin irritation after 3 and 9 months of treatment, respectively.

Treatment-Related Symptoms
Trans Men
Treatment-related symptoms were investigated every 3 months in a subsample of trans men (N = 25). The vast majority of the trans men reported an increase in sexual desire (Figure 3). T treatment resulted in variable levels of voice deepening and an increased voice instability ($P = 0.024$). About 20% of trans men reported clitoral pain, with a peak incidence observed at 6 months of treatment. Symptoms of emotionality decreased ($P = 0.01$).

We observed no changes in symptoms of night sweats, hot flashes, abdominal pain, anxiety, breast tenderness, irritability, palpitations, joint pain, muscle soreness, headache, mood swings, fatigue, concentration difficulties, memory, or sleep-related problems (data not shown).

Trans Women
We examined treatment-related symptoms every 3 months in a subsample of trans women (N = 30) treated with 50 mg of CA daily in combination with estrogens from the start. A significant increase in breast tenderness, emotionality, low sexual desire, and hot flashes was observed ($P < 0.001$, $P = 0.001$, $P < 0.001$, and $P = 0.02$, respectively) (Figure 3).

We found no changes in night sweats, abdominal pain, anxiety, irritability, palpitations, skin dryness, joint pain, muscle soreness, headache,
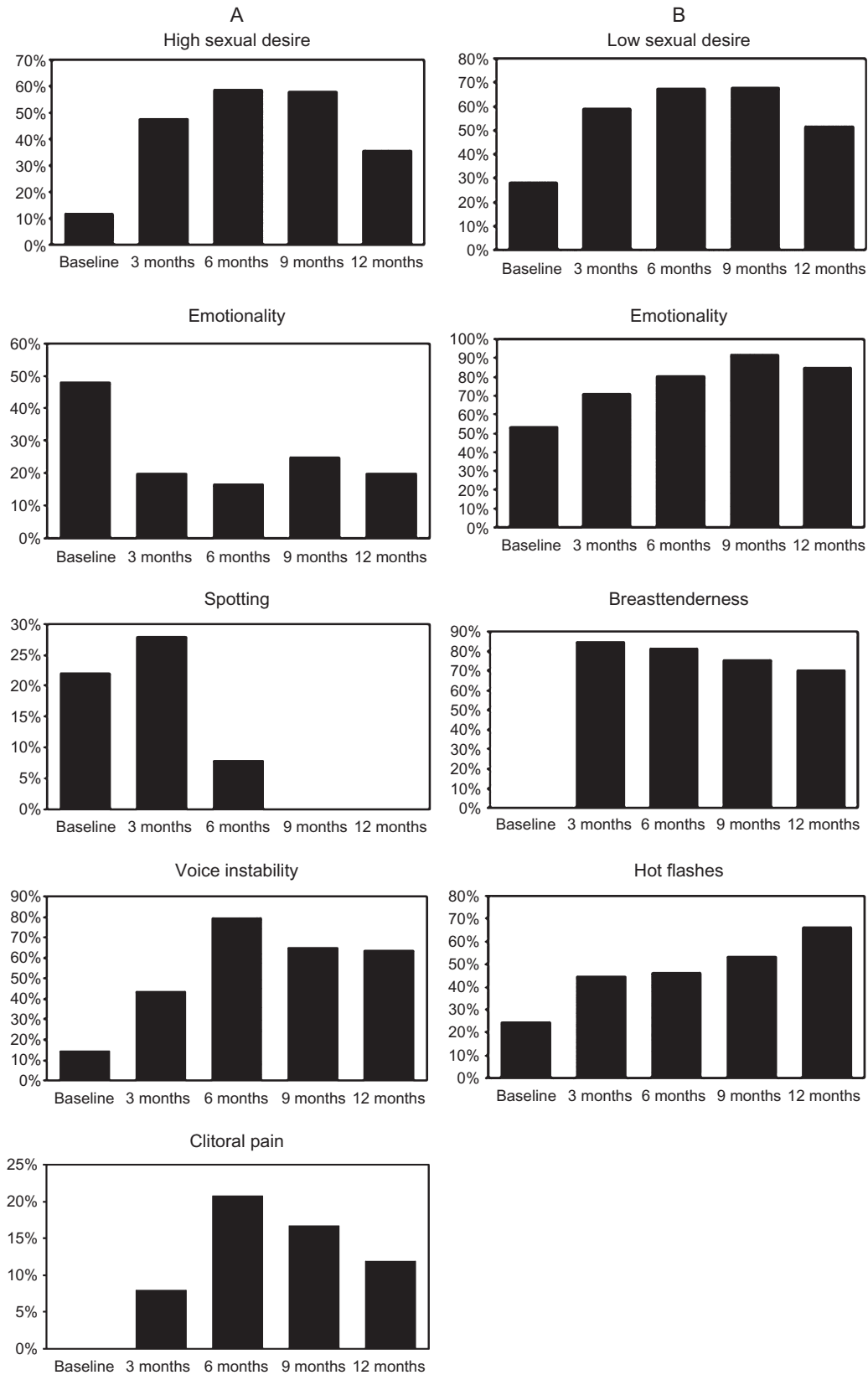
Figure 3 Prevalence of treatment-related symptoms in trans men (A) and trans women (B)

mood swings, fatigue, concentration difficulties, memory, or sleep-related problems (data not shown).

There were no significant differences in the presence of treatment-related symptoms at the 3-month time point between trans women treated with oral or transdermal estrogen. No significant differences were observed at the 3-month time point between trans women treated with CA plus estrogens from start compared with those initially treated with CA alone (data not shown), except for a higher prevalence of breast tenderness in those women treated with CA plus estrogens ($P = 0.001$).

Discussion

We presented the first multicenter prospective study describing the effects of the current CHT on hormonal and clinical changes, side effects, and adverse events in trans men and trans women. The main findings of our study were that our therapies (injections with T undecanoate every 3 months in trans men and 50 mg CA plus either 4 mg oral EV or 100 μg/24 hours transdermal 17-β E2 daily in trans women) were effective and safe. The majority of trans persons had desired levels of sex steroids according to the Endocrine Society Guidelines [1] and developed the secondary sex characteristics of the desired sex. Trans men experienced cessation of menses and developed a male body habitus with an android pattern of fat distribution, a deepening of the voice, an increase in lean mass, male pattern baldness, and both facial and body hair. Trans women experienced an increase in fat mass with a gynoid pattern of fat distribution and an increase in breast circumference.

None of the trans persons experienced severe adverse events such as cardiovascular events or death. These findings are in line with previous reports that demonstrated T treatment for trans men was effective and relatively safe in the short term [17–19]. The results in trans women confirm a recent study [10] showing a low risk for adverse events at short-time follow-up but contrast earlier reports that have indicated a high incidence of venous thrombosis and/or pulmonary embolism during the first year of CHT [17,19]. Current treatment modalities in trans women, including the avoidance of EE usage and using transdermal estrogens in older trans women, may therefore be less detrimental to the coagulation system. Indeed, Toorians and colleagues [20] described a

higher activated protein C resistance with oral EE compared with oral EV and transdermal 17-β E2 usage. In addition, Van Kesteren et al. [17] reported a decreased incidence of venous thrombosis with the use of transdermal estrogens in older trans women.

Similar to Mueller and colleagues [18], we observed a small but significant increase in systolic blood pressure during T therapy. However, considering the small increase (about 3.5%) we observed, it is likely that sample sizes in other studies [21,22] were too small to detect a statistically significant difference. Importantly, no subject developed a clinically significant blood pressure increase during our study observation. T treatment also increased liver enzymes in our study, similar to the study from Mueller and colleagues [18], although the clinical relevance of this finding remains to be determined.

Previous studies have not investigated systematically the treatment-related symptoms during T therapy in trans men. However, the majority of trans men reported an increase in voice instability, acne, and sexual desire. Most acne cases were mild, and none was severe or very severe according to the GAGS. Nevertheless, about a third of our patients underwent acne treatment, indicating that even mild and moderate acne lesions were clinically relevant.

One trans woman discontinued treatment because of depression. Indeed, it has been previously reported that CHT increases depression risk [19]. However, others show that CHT lowers anxiety and depression scores possibly due to improvements in mental health after initiation of cross-sex reassignment therapy [23]. Similar to others [17,19], we found a transient elevation of the liver enzymes during antiandrogen and estrogen treatment, although, generally, a decrease in liver enzymes is observed [24].

Concerning the risk for hyperprolactinemia during CHT in trans women, our findings substantiate most other studies showing an increase in prolactin levels during administration of CA combined with estrogen therapy [1,17,25–28] and CA alone [25,27]. However, our previous retrospective follow-up studies do not show a further increase in the long term [5,29]. Moreover, the clinical relevance of increased prolactin levels during CHT remains undetermined. Although a few case reports of prolactinomas have been reported following CHT, none was reported in large follow-up studies, perhaps suggesting a low risk associated with CHT.

Trans women also reported treatment-related symptoms. Although some of them, such as breast tenderness and low sexual desire, were well-known from our clinical practice, subjects unexpectedly reported a significant increase in hot flashes during antiandrogen and estrogen treatment. Because we did not use validated questionnaires or objective measurements to analyze these symptoms, further exploration and characterization are needed.

Investigation of cardiovascular risk factors during CHT is important as recent studies show that trans women have an increased cardiovascular morbidity [30] and mortality [11,31] compared with the general population. Similar to previously published studies using EE plus CA, we found a reduction in LDL-C and an increase in fat mass and fasting insulin during the first year of CHT in trans women [21,32]. Estrogen therapy increases removal of LDL apolipoprotein B-100 [33], but the pathophysiological mechanism of increased insulin resistance during antiandrogen and estrogen therapy is not fully understood. CHT induces important changes in body composition, with an increase in fat mass, which may affect glucose metabolism. Sex steroids may also exert direct effects, as acute T withdrawal in men decreases insulin sensitivity in the absence of any detectable changes in body composition [34]. Augmentation of E_2 to supraphysiological levels may also induce insulin resistance through liver hyperinsulinemia and reduced GLUT 4 expression within the muscles [35].

In line with Dittrich and colleagues [10], we observed no increase in body weight, triglycerides, or blood pressure in trans women. These findings differ from a previously published study using EE plus CA [21] and are likely to be related to differences in type of estrogen used. Different types of estrogen exert divergent metabolic effects. EE has a stronger hepatic impact due to its 17α-ethinyl group, which prevents the inactivation of the molecule and results in a slower metabolism [36,37]. Additionally, the higher estrogen dosage in the study by Elbers and colleagues [21] may also contribute to differences in these health outcomes.

HDL-C decreased in trans women (in both oral and transdermal group), which was somewhat unexpected under estrogen therapy. A potential explanation for these findings may be found in the progestagenic effects of CA, as progestogens decrease HDL-C concentrations [33]. Decreased HDL-C levels have been previously shown in trans women using transdermal estrogen therapy [38,39]. Because the transdermal route avoids the first pass effect of the liver, it may have different metabolic effects than oral estrogen therapy. Decreased triglycerides were also observed with transdermal but not oral estrogen therapy. In addition, trans women using oral EV showed significantly higher E1/E2 ratio than those using transdermal therapy. A lower E1/E2 ratio has been reported in postmenopausal women receiving transdermal hormone replacement therapy [40]. No other differences were observed between these two modes of estrogen treatment, suggesting both are equally effective.

T undecanoate treatment decreased fat mass but induced a less favorable lipid profile and an android pattern of fat distribution in trans men. Although these changes were also seen in studies using two or three weekly injections of intramuscular T esters [41,42], most of the evidence suggests that T treatment is relatively safe at short- and medium-term follow-up [17–19]. However, outcome studies in trans men are generally performed in much smaller sample sizes and at younger ages compared with trans women. Large, long-term (>20 years) follow-up studies are needed to investigate the cardiovascular safety of T therapy in trans men.

As previously described [12], the age and sex ratio differed significantly between our two centers. This age difference should be kept in mind in future outcome studies as older age may be associated with a worse cardiovascular outcome [43].

The strengths of the present study were its relatively large sample size compared with most other prospective studies; the use of widely prescribed (but scientifically not well documented) treatment modalities; our detailed description of adverse events, side effects, and treatment-related symptoms; and the use of a mass spectrometry-based methodology to measure serum sex steroid levels in trans persons. Although we did not use a validated questionnaire to measure treatment-related symptoms, we were the first to examine these symptoms systematically. Validation of such a questionnaire may be valuable for future studies as no standardized assessment of symptoms exists presently. Secondly, our treatment protocol was to administer oral EV to trans women younger than 45 years and transdermal estrogen therapy to those over 45 years. This age discrepancy may have influenced the difference in treatment response between the two groups. In addition, some trans women initially received CA alone without concomitant estrogen use. This may have influenced

our results, although no significant differences were observed between these two groups. Moreover, the lack of difference between the groups may indicate that most relevant changes occur during the first 9 months of CHT. Thirdly, we did not have a blinded clinician to determine the clinical effects of the treatment, which may possibly induce a bias. Finally, we described average differences associated with each treatment, but we observed large between-subject differences in clinical outcome measures, which may be due to differences in sex steroid metabolism or sensitivity.

Conclusion

We observed that our current treatment modalities in both trans men and women were effective and carried a low risk for side effects and adverse events at short-time follow-up.

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