

CLINICAL STUDY

The effects of sex-steroid administration on the pituitary–thyroid axis in transsexuals

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Abstract

Objective: Estrogen and androgen administration modulate the pituitary–thyroid axis through alterations in thyroid hormone-binding globulin (TBG) metabolism, but the effects of sex steroids on extrathyroidal thyroxine (T₄) to triiodothyronine (T₃) conversion in humans are unknown.

Design and methods: We studied 36 male-to-female and 14 female-to-male euthyroid transsexuals at baseline and after 4 months of hormonal treatment. Male-to-female transsexuals were treated with cyproterone acetate (CA) 100 mg/day alone ($n = 10$) or in combination with either oral ethinyl estradiol (or-EE) 100 µg/day ($n = 14$) or transdermal 17β-estradiol (td-E) 100 µg twice a week ($n = 12$). Female-to-male transsexuals were treated with i.m. testosterone 250 mg twice a week. A *t*-test was used to test for differences within groups and ANOVA with *post hoc* analysis to test for differences between the groups.

Results: Or-EE increased TBG ($100 \pm 12\%$, $P < 0.001$) and testosterone decreased TBG ($-14 \pm 4\%$, $P = 0.01$), but free T₄ did not change. Td-E and CA did not affect TBG concentrations. TSH was not different between groups at baseline or after treatment. CA decreased T₃/T₄ ratios ($-9 \pm 3\%$, $P = 0.04$), suggesting that T₄ to T₃ conversion was lower. Testosterone increased T₃/T₄ ratios ($30 \pm 9\%$, $P = 0.02$), which probably reflects higher T₄ to T₃ conversion.

Conclusion: Oral but not transdermal estradiol increases TBG, whereas testosterone lowers TBG. Testosterone increases T₃/T₄ ratios. Estradiol does not affect T₃/T₄ ratios, irrespective of the route of administration.

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Introduction

Administration of sex hormones can interfere substantially with the hypothalamic–pituitary–thyroid (HPT) axis. Oral estrogen administration increases thyroid-hormone-binding globulin (TBG) concentrations (1). This glycoprotein is produced in the liver and binds about two-thirds of serum thyroxine (T₄). The rise in TBG is paralleled by a T₄ increase to maintain a physiological concentration of free T₄. Therefore, the T₄ substitution dose in women with primary hypothyroidism, characterized by impaired endogenous T₄ production, must be increased when oral estrogens are administered (1). In contrast to oral administration, transdermal estrogen administration does not raise TBG concentrations (2). It is assumed that this discrepancy is caused by a first-pass effect due to high portal vein estrogen concentrations after oral administration. Opposite to estrogen, androgen administration in women decreases TBG concentrations and requires reduction of T₄ substitution in patients with primary hypothyroidism to avoid thyrotoxicosis (3).

Besides the effects on TBG concentrations, sex hormones also affect deiodinase activity. Peripheral conversion of inactive T₄ to biologically active triiodothyronine (T₃) is catalyzed by 5'-deiodinase activity and is the main source of circulating T₃. Two of the three deiodinase subtypes, type 1 (D1) and 2 (D2), have 5'-deiodinase capability. D1 is expressed in the liver of rodents and humans. D2 is expressed in brown adipose tissue of rodents and in muscle of humans. It was recently shown that muscle D2 activity is the major source of circulating T₃ in euthyroid humans (4).

In rats, hepatic activity of 5'-deiodinase was not altered by ovariectomy (5), but increased after a supraphysiological dose of 17β-estradiol (6). The latter effect was blunted by concurrent administration of progestins (6). In orchidectomized rats, hepatic D1 activity was reduced, but could be restored to normal by the substitution of testosterone (5,6). These observations suggest that physiological concentrations of testosterone stimulate D1 activity in male rats and might provide an explanation for higher D1 activity in the liver of normal male rats than in female rats (7).

The effects of androgens and estrogens on 5'-deiodinase activity in humans are not known. For evident reasons, 5'-deiodinase activity cannot be measured as easily in humans as in rodents, but serum T₃/T₄ ratios can be used as a marker for 5'-deiodinase activity, since the majority of circulating serum T₃ is produced by peripheral conversion of T₄ to T₃.

To explore the effects of androgens and estrogens on 5'-deiodinase activity, we studied transsexuals receiving standard cross-gender sex-hormone administration and measured the effects on the HPT axis, including T₃/T₄ ratios and TBG. The standard hormone administration regimens in male-to-female transsexuals include, among others, a regimen with single agent administration of cyproterone acetate (CA). CA is a progestin with anti-androgenic action by competitive binding to the testosterone receptor. The effects of CA administration on the HPT axis in humans have not been described before and will also be presented.

Materials and method

Materials

To study the effect of hormone treatment on the HPT axis, we used plasma samples from an earlier study, which were collected before and after 4 months of hormone administration. The original study was published elsewhere (8) and described the effects of oral and transdermal estrogen administration on tissue-type plasminogen activator levels in 40 male-to-female and 17 female-to-male transsexuals. In this study, 36 male-to-female transsexuals were treated with CA (Androcur, 100 mg/day, Schering, Berlin, Germany) and subsequently open-label randomized to receive oral ethinyl estradiol (EE) (Lynoral, 100 µg/day, Organon, Oss, the Netherlands; *n* = 14), transdermal 17β-estradiol (Estraderm TTS 100, 100 µg twice a week, CIBA-Geigy, Basel, Switzerland; *n* = 12) or no additional treatment (*n* = 10). Female-to-male transsexuals were treated with testosterone esters (Sustanon, 250 mg/2 week i.m., Organon, Oss, the Netherlands). The number of patients in the present study differs slightly from the original study, because the amount of available plasma in seven patients was insufficient to perform the complete evaluation of the pituitary–thyroid axis. All the female-to-male transsexuals had regular menstrual cycles (28–31 days) before cross-gender sex-hormone transformation.

All the subjects gave written informed consent and the study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Committee of the University Hospital Vrije Universiteit (VUMC).

Blood sampling and analysis

Each subject served as his or her own control. Blood samples for HPT axis hormones and TBG were

drawn before and after 4 months of cross-gender sex-hormone administration. In female-to-male transsexuals, blood was drawn at baseline between days 5 and 9 of the follicular phase of the menstrual cycle. During testosterone treatment, blood was drawn within 5–9 days after the most recent testosterone injection.

In-house RIAs were used to measure serum levels of 17β-estradiol and testosterone. Serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by immuno luminometric assays (ILMA).

For thyroid hormones and TSH, the following assays were used: T₄ (reference value 70–150 nmol/l, detection limit 5 nmol/l, intraassay coefficient of variance values (CV) 2–4%, interassay CV 3–6%), T₃ (reference value 1.3–2.9 nmol/l, detection limit 0.3 nmol/l, intraassay CV 3–4%, interassay CV 7–8%) and rT₃ (reference value 0.11–0.44 nmol/l, detection limit 0.03 nmol/l, intraassay CV 4–5%, interassay CV 5–9%) were measured by in-house RIA methods (9); free T₄ (FT₄) and TSH were measured by time-resolved fluoroimmunoassay (Delfia FT₄ and Delfia hTSH Ultra respectively, Wallac Oy, Turku, Finland; reference value 10–23 pmol/l and 0.4–4.0 mU/l, detection limit 2 pmol/l and 0.01 mU/l, intraassay CV 4–6 and 1–2%, interassay CV 5–8 and 3–4% respectively); TBG by a commercial RIA (Eiken Chemical Co., Tokyo, Japan; reference value 200–650 nmol/l, detection limit 30 nmol/l, intraassay CV 2–4%, interassay CV 4–6%).

Statistical analysis

Data are reported as mean ± s.e.m. ANOVA was used to test for differences between treatment groups. When appropriate a *post hoc* analysis was performed using Fisher's least significant difference test. Student's *t*-test for paired samples was used to test for differences within treatment groups, i.e. differences between values at baseline and after 4 months of treatment. A *P*-value of less than 0.05 was considered statistically significant. SPSS for windows 11.5 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis.

Results

At baseline, all subjects were eugonadal and euthyroid by clinical and laboratory criteria. Baseline characteristics are presented in Tables 1 and 2.

Pituitary–gonadal axis (Table 2)

Administration of oral EE or transdermal 17β-estradiol, in combination with CA, suppressed serum LH, FSH, and testosterone concentrations to similar levels. Oral administration of EE decreased serum 17β-estradiol concentrations, because EE suppresses endogenous 17β-estradiol and is not detected in conventional 17β-estradiol assays. Administration of CA alone did not reduce LH or FSH, but decreased serum levels of

Table 1 Patient characteristics.

	Male-to-female transsexuals			Female-to-male transsexuals
	Oral ethinyl estradiol plus CA (n=14)	Transdermal 17 β -estradiol plus CA (n=12)	Cyproterone acetate (n=10)	Testosterone (n=14)
Age (years)	32 \pm 2	30 \pm 2	34 \pm 3	28 \pm 2
BMI (kg/m ²)	23 \pm 1	21 \pm 1	22 \pm 1	23 \pm 1
Smoking (n)	8	6	4	8

Data are means \pm s.e.m. Cyproterone acetate, CA; body mass index, BMI.

17 β -estradiol and testosterone, although not as extensively as in combination with estrogens. Testosterone administration in female-to-male transsexuals increased plasma testosterone 16-fold and decreased 17 β -estradiol by suppression of LH and FSH.

Pituitary–thyroid axis and thyroid-binding globulin (Table 3)

As anticipated, TBG concentrations increased by 100% after oral, but not after transdermal estrogen administration. TBG concentrations decreased by 14% after testosterone administration and were not affected by CA. Changes in T₄ concentrations in response to treatment paralleled the changes observed with TBG. Plasma-free T₄ concentrations were not different between the treatment groups. Comparable to T₄, T₃ increased after oral, but not after transdermal estrogen administration. As a consequence, T₃/T₄ ratios were not different between patients treated with oral or transdermal estrogens. In contrast to estrogens, the administration of testosterone differently affected T₄ and T₃. T₄ decreased, while T₃ did not change, which resulted in a higher T₃/T₄ ratio after testosterone administration. In contrast to testosterone, CA

administration significantly decreased the T₃/T₄ ratio, but the magnitude of this decrease was not large enough to result in a difference compared to combined administration of estrogens and CA. TSH concentrations were not different at baseline or after treatment, irrespective of the type of treatment.

Discussion

Sex-hormone administration in cross-gender transformations provides a unique setting to evaluate the effects of sex hormones on the pituitary–thyroid axis and peripheral thyroxine metabolism in humans. As anticipated, oral estrogen administration increased TBG concentrations, whereas testosterone decreased TBG concentrations. Testosterone administration increased T₃/T₄ ratios, indicating increased 5'-deiodinase activity, whereas CA decreased T₃/T₄ ratios, suggesting a decreased activity of 5'-deiodinase. Oral or transdermal estrogen administration, combined with CA, had no effect on T₃/T₄ ratios.

Plasma T₃/T₄ ratios may be used as a marker of extrathyroidal T₄ to T₃ conversion, but several assumptions have to be met:

- (i) *Steady state is achieved:* sex-steroid administration alters TBG concentrations. As a result, under

Table 2 Gonadal and gonadotropic hormone concentrations at baseline and after 4 months of hormonal treatment in cross-gender transformations.

	Male-to-female transsexuals			Female-to-male transsexuals
	Oral ethinyl estradiol plus CA (n=14)	Transdermal 17 β -estradiol plus CA (n=12)	Cyproterone acetate (n=10)	Testosterone (n=14)
LH (IU/l)				
Baseline	3.2 \pm 0.4 ^a	2.6 \pm 0.5 ^a	2.4 \pm 0.4 ^a	5.7 \pm 1.0 ^b
4 months	0.3 \pm 0.0 ^a	0.5 \pm 0.1 ^a	2.4 \pm 0.4 ^b	2.4 \pm 0.6 ^b
FSH (IU/l)				
Baseline	3.5 \pm 0.5 ^a	3.9 \pm 1.2 ^a	3.7 \pm 0.9 ^a	4.3 \pm 0.3 ^a
4 months	0.5 \pm 0.0 ^a	0.5 \pm 0.0 ^a	2.4 \pm 0.6 ^b	2.9 \pm 0.3 ^b
17 β -estradiol (pmol/l)				
Baseline	97 \pm 9 ^a	83 \pm 5 ^a	83 \pm 6 ^a	193 \pm 26 ^b
4 months	24 \pm 1 ^{a†}	175 \pm 37 ^b	41 \pm 4 ^a	126 \pm 11 ^b
Testosterone (nmol/l)				
Baseline	22.3 \pm 1.6 ^a	22.0 \pm 1.8 ^a	19.6 \pm 2.0 ^a	1.9 \pm 0.2 ^b
4 months	1.0 \pm 0.0 ^a	1.1 \pm 0.1 ^a	8.8 \pm 1.9 ^b	32.9 \pm 2.6 ^c

Data are means \pm s.e.m. Means in a row with different superscript letters are significantly different, $P < 0.05$. †Ethinyl estradiol, which suppresses endogenous 17 β -estradiol, is not detected in conventional 17 β -estradiol assays. Cyproterone acetate, CA.

Table 3 Thyroid hormone and TBG concentrations at baseline and after 4 months of hormonal treatment in cross-gender transformations.

	Male-to-female transsexuals			Female-to-male transsexuals
	Oral ethinyl estradiol plus CA (n=14)	Transdermal 17 β -estradiol plus CA (n=12)	Cyproterone acetate (n=10)	Testosterone (n=14)
T₄ (nmol/l)				
Baseline	109 \pm 7	118 \pm 7	125 \pm 7	129 \pm 6
4 months	150 \pm 9*	135 \pm 8	122 \pm 6	94 \pm 4*
% change	42 \pm 10 ^a	17 \pm 7 ^b	-2 \pm 4 ^b	-26 \pm 3 ^c
T₃ (nmol/l)				
Baseline	1.71 \pm 0.11	1.78 \pm 0.14	2.08 \pm 0.13	1.83 \pm 0.11
4 months	2.32 \pm 0.15*	1.93 \pm 0.19	1.83 \pm 0.11*	1.66 \pm 0.07
% change	44 \pm 14 ^a	12 \pm 12 ^b	-11 \pm 2 ^b	-7 \pm 5 ^b
T₃/T₄ ratio				
Baseline	1.60 \pm 0.10	1.57 \pm 0.14	1.67 \pm 0.07	1.44 \pm 0.10
4 months	1.57 \pm 0.08	1.46 \pm 0.13	1.51 \pm 0.06	1.80 \pm 0.10*
% change	2 \pm 7 ^a	-3 \pm 8 ^a	-9 \pm 3 ^a	30 \pm 9 ^b
T₃/rT₃ ratio				
Baseline	8.38 \pm 0.75	7.81 \pm 0.43	6.69 \pm 0.58	5.49 \pm 1.05
4 months	10.10 \pm 0.59	8.63 \pm 0.75	6.61 \pm 0.47	5.22 \pm 1.02
% change	34 \pm 15 ^a	14 \pm 10 ^a	5 \pm 11 ^a	32 \pm 39 ^a
fT₄ (pmol/l)				
Baseline	15.8 \pm 0.5	16.4 \pm 0.7	15.8 \pm 0.6	16.3 \pm 0.4
4 months	14.2 \pm 0.5*	15.3 \pm 0.8	15.2 \pm 0.4	13.8 \pm 0.4*
% change	-9 \pm 4 ^a	-6 \pm 4 ^a	-3 \pm 3 ^a	-15 \pm 2 ^a
rT₃ (nmol/l)				
Baseline	0.22 \pm 0.02	0.23 \pm 0.02	0.34 \pm 0.05	0.40 \pm 0.04
4 months	0.24 \pm 0.02	0.23 \pm 0.02	0.29 \pm 0.02	0.38 \pm 0.03
% change	14 \pm 8 ^a	0 \pm 5 ^a	-9 \pm 7 ^a	13 \pm 22 ^a
TBG (nmol/l)				
Baseline	289 \pm 19	288 \pm 18	336 \pm 19	338 \pm 19
4 months	569 \pm 43*	345 \pm 31	337 \pm 22	285 \pm 15*
% change	100 \pm 12 ^a	23 \pm 14 ^b	0 \pm 2 ^{bc}	-14 \pm 4 ^c
TSH (mIU/l)				
Baseline	1.57 \pm 0.22	1.39 \pm 0.19	1.80 \pm 0.34	1.57 \pm 0.16
4 months	1.59 \pm 0.26	1.59 \pm 0.15	1.28 \pm 0.24	1.36 \pm 0.22
% change	9 \pm 12 ^a	43 \pm 27 ^a	-16 \pm 17 ^a	-3 \pm 16 ^a

Data are means \pm s.e.m. Baseline values between the treatment groups are not different with two exceptions: rT₃ is higher in the cyproterone and testosterone groups compared to the estrogen groups and T₃/rT₃ ratio is lower in the testosterone group compared to the estrogen groups, $P < 0.05$. Means in a row with different superscript letters are significantly different, $P < 0.05$. *Indicates a significant difference compared to the baseline value, $P < 0.05$. Cyproterone acetate, CA.

non-steady state conditions, the availability of free T₄ and T₃ for deiodination could fluctuate, resulting in different T₃/T₄ ratios independent of 5'-deiodinase activity. However, it seems safe to assume that steady state was achieved, since we studied the subjects after 8 weeks of sex-steroid administration, which is more than five times the half-life of T₄ and TBG (10).

- (ii) *The ratio of thyroidal T₃ to T₄ secretion is constant:* different plasma T₃/T₄ ratios could just reflect changed T₃/T₄ secretion ratios. Various conditions influence thyroidal T₃/T₄ secretion ratios. Iodine deficiency results in preferential excretion of T₃ and consequently lower T₃/T₄ secretion and plasma ratios. During transition from euthyroidism to hypothyroidism, T₄ decreases before T₃ and vice versa for hyperthyroidism. Neither of these conditions, iodine deficiency nor dysthyroidism, were present in our study subjects.
- (iii) *Deiodinase type 3 (D3) activity is constant:* recently, D3 tissue distribution in humans has been

characterized (11, 12), but the regulation of D3 activity in humans is largely unknown. Because of this lack of information on D3 regulation, there is currently no basis to support or reject the third assumption.

- (iv) *Plasma T₃ and T₄ concentrations reflect tissue concentrations:* disproportionate T₃/T₄ ratios in tissue and plasma would invalidate the plasma T₃/T₄ ratio as an indicator of peripheral T₄ to T₃ conversion, but it was recently shown that plasma T₃ and T₄ concentrations correlate closely to liver and muscle concentrations (13).

TBG binds approximately 75% of circulating T₄. The remainder is bound to transthyretin or albumin and only a very small fraction (0.1%) remains unbound. TBG has the highest affinity for T₄ of the T₄ binding proteins. The present study shows that oral estradiol administration increases TBG concentrations, whereas transdermal administration did not, confirming the previous observations (1). Two mechanisms are involved in the

estrogen stimulation of TBG concentrations, namely increased production and reduced clearance of TBG. In primates, high-dose estrogen administration stimulated TBG production and secretion by the liver (14). Estrogens also stimulated the formation of more heavily sialylated TBGs (15), which exhibit a slower clearance rate from plasma than less sialylated TBG (16). It appears that estrogen stimulation of TBG only occurs above a certain portal threshold, which explains why only oral administration of low-dose estrogens, with a relatively high portal concentration, and high systemic concentrations, as observed in pregnancy (17), increase TBG concentrations. As anticipated (3, 18), testosterone decreased TBG concentrations. Whether testosterone decreases TBG by reduced synthesis or enhanced clearance is not known. CA did not affect TBG concentrations. CA has three modes of action; it has progestinic, anti-androgenic and anti-gonadotropic effects. Previous studies do not support an effect of progestins on TBG (19). Although the anti-androgenic effects of CA could theoretically increase TBG concentrations, we did not observe such an effect.

In humans plasma T_3 comes from two, relatively independent sources, namely thyroid secretion and extrathyroidal conversion of T_4 by 5'-deiodinase with a relative contribution of 20 and 80% respectively (20). Three types of deiodinase exist, but only D1 and D2 have 5'-deiodinase capability. In euthyroid humans, the relative contributions of D1 and D2 to extrathyroidal T_3 production are approximately 34 and 66% (4). The effects of sex steroids on 5'-deiodinase activity have thus far only been studied in rats, but thyroid hormone metabolism in rats is markedly different from humans. In rats, thyroid secretion accounts for 40% of plasma T_3 and extrathyroidal 5'-deiodination of T_4 by D1 and D2, each for 30%. In rats, D2 is not expressed in muscle and contributes significantly less to plasma T_3 as compared to humans. Only a limited number of studies (summarized in the Introduction) have studied the effects of sex hormones in rats on D1 activity in the liver. Low-dose estrogens did not affect hepatic D1 activity, whereas testosterone increased D1 activity. Currently, there is no evidence to support an effect of androgens or estrogens on D2 activity, but the data are limited to D2 activity in the rat pituitary (6) and the mouse bone (21). Whether testosterone increased T_4 to T_3 conversion by effects on D1 or D2 remains speculative. CA decreased T_3/T_4 ratios suggesting decreased T_4 5'-deiodination. The effect of CA on T_3/T_4 ratios could be induced by the anti-androgenic effect, but also by the progestinic effect of CA. In rats progestin decreased hepatic and pituitary D1 activity (6). Unfortunately, we could only study the effects of estrogens with concurrent CA administration. Therefore, we cannot exclude that a potential effect of estrogens was blunted by CA.

In conclusion, oral but not transdermal estrogens increased TBG and testosterone decreased TBG. Estradiol combined with CA did not affect T_3/T_4 ratios, whereas testosterone increased T_3/T_4 ratios.

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