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Clinical Research Article

Changes in Serum Testosterone and Adrenal Androgen Levels in Transgender Women With and Without Gonadectomy

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Abstract

Background: Initiating feminizing gender-affirming hormone therapy (GAHT) in transgender women causes a steep decline in serum testosterone. It is unknown if testosterone concentrations change further and whether adrenal androgen levels change during feminizing GAHT and after gonadectomy. This limits clinical decision making in transgender women with symptoms attributed to GAHT or gonadectomy.

Methods: Transgender women (n = 275) initiating estradiol and cyproterone acetate (CPA) were included at baseline, and had follow-up visits after 3 months, 12 months, and 2 to 4 years. During follow-up, 49.5% of transgender women underwent a gonadectomy. Total testosterone (TT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and androstenedione (A4) were measured using liquid chromatography tandem mass spectrometry.

Results: After 3 months of GAHT, mean TT, calculated free testosterone (cFT), and A4 decreased by 18.4 nmol/L (95% CI, −19.4 to −17.4, $P < 0.001$ [ie, −97.1%]), 383 pmol/L (95% CI, −405 to −362, $P < 0.001$ [ie, −98.3%]), and 1.2 nmol/L (95% CI, −1.4 to −1.0, $P < 0.001$ [ie, −36.5%]), respectively, and remained stable thereafter. DHEA and DHEAS decreased by 7.4 nmol/L (95% CI, −9.7 to −5.1 [ie, −28.0%]) and 1.8 µmol/L (95% CI, −2.2 to −1.4 [ie, −20.1%]), respectively, after 1 year and did not change thereafter. After gonadectomy, CPA therapy is stopped, which induced no further change in TT, cFT, DHEA, DHEAS, and A4 compared with those who did not undergo gonadectomy.

Conclusions: Our findings confirm that after an initial drop, testosterone levels in transgender women remain stable. Adrenal androgens decrease in the first year of CPA and estrogen supplementation and remain unchanged after gonadectomy. Androgens did not change after gonadectomy and cessation of CPA. Correlates with clinical symptoms remain to be elucidated.

Key Words: transgender women, gender-affirming hormone therapy, liquid chromatographytandem mass spectrometry, androgens, testosterone, gonadectomy

Abbreviations: A4, androstenedione; BMI, body mass index; cFT, calculated free testosterone; CPA, cyproterone acetate; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol; ENIGI, European Network for the Investigation of Gender Incongruence; GAHT, gender-affirming hormone therapy; GnRHa, gonadotropin-releasing hormone analogue or antagonist; IQR, interquartile range; LC-MS/MS, liquid chromatography tandem mass spectrometry; LH, luteinizing hormone; LLOQ, lower limit of quantitation; SD, standard deviation; SHBG, sex hormone binding globulin; TT, total testosterone.

Transgender women experience an incongruence between their assigned male sex at birth and their female gender identity. To align their physical appearance and gender identity, transgender women may choose to start feminizing gender-affirming hormone therapy (GAHT) and to undergo gender-affirming surgery (1, 2). In transgender women, GAHT aims to induce female secondary sex characteristics through estrogen supplementation (3). In addition, anti-androgen therapy is often initiated in some centers by the administration of cyproterone acetate (CPA), which blocks the androgen receptor and

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suppresses luteinizing hormone (LH), thereby reducing testosterone concentrations and action (4). If, at a later stage, transgender women undergo a surgical removal of the testicles (gonadectomy) or the additional creation of a vagina (vaginoplasty), anti-androgens are stopped while estradiol therapy is continued. Transgender women who do not undergo gonadectomy may remain on long-term anti-androgen therapy and estradiol supplementation. Of note, gender-affirming treatment is customized and aims to achieve comfort with self and identity (1).

Feminizing GAHT and gonadectomy may induce several hormonal changes through different mechanisms. Upon the initiation of GAHT with estradiol and CPA, the serum concentration of estradiol (E2) rises whereas total testosterone (TT) drops, approximating the female physiologic sex hormone range (5–8). The supplementation of oral estradiol in particular is associated with an increase in sex hormone binding globulin (SHBG) (9), which may additionally reduce the concentration of free testosterone (10). Because CPA acts as an androgen receptor antagonist with progestogenic and glucocorticoid activity (11), some studies suggest it also induces changes in other androgen concentrations such as androstenedione (A4), dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS), but findings are inconsistent (12, 13). After gonadectomy, anti-androgens are stopped, and androgen profiles may change once more because androgen production also takes place in the adrenals and at peripheral sites (14). In our clinical experience, some transgender women report increased bodily hair growth after gonadectomy when CPA therapy is stopped. It remains unknown whether this may be a result of changes in adrenal androgen concentrations or the inhibition of the androgen receptor by CPA. Additionally, as low androgen concentrations as well as high CPA dosages may be associated with clinical symptoms such as reduced sexual desire, tiredness, depressed mood, and muscle weakness, understanding androgen dynamics in transgender women may benefit clinical decision making (7, 15, 16).

Until recently, the use of immunoassays limited our understanding of androgen concentrations in transgender women because this technique is known to be unreliable for measuring steroids in the low concentration range from a combined lack of sensitivity and accuracy (17–19). Measurement of androgens has gained accuracy by the use of liquid chromatography—tandem mass spectrometry (LC-MS/MS), which does not suffer from cross-reactivity and currently is the gold standard for measuring testosterone, its precursors, and metabolites (19). The use of LC-MS/MS allows for a better understanding of how androgen concentrations change after initiating GAHT and after gonadectomy. To our knowledge, no studies have used LC-MS/MS to assess androgen concentration changes in transgender women throughout hormonal and/or surgical gender-affirming care.

This study aims to describe changes in TT and the adrenal androgens DHEA, DHEAS, and A4 (measured by LC-MS/MS) in transgender women using anti-androgens and after gonadectomy in the context of continued estradiol supplementation. We hypothesized that testosterone levels remain low throughout feminizing GAHT and after gonadectomy, and that adrenal androgens decrease during CPA use and increase after gonadectomy when CPA is stopped.

Materials and Methods

Participants

This prospective cohort study was part of the European Network for the Investigation of Gender Incongruence (ENIGI). The protocol for the endocrine part of the ENIGI was published before (20) and its main finding have been summarized (21). Participants who initiated GAHT with CPA and E2, and who had received check-ups at regular follow-up visits at the Ghent University Hospital or Amsterdam University Medical Center (UMC) (location VUmc) were included. In this study, only transgender women whose serum testosterone concentrations were determined using LC-MS/MS were included. In Amsterdam, this technique has been used since October 2018 for routine testosterone measurements, whereas in Ghent LC-MS/MS was introduced in 2019. In Amsterdam, the inclusion period for participants started after the implementation of LC-MS/MS. In Ghent, the inclusion period was from 2010 to 2018. Because LC-MS/MS was introduced in Ghent only in 2019, measurements had only been carried out by LC-MS/MS for a fraction of the cohort during follow-up visits. Therefore, all Ghent samples at all visits were reanalyzed by LC-MS/MS in 2021.

Participants received GAHT according to the regular care protocol with estradiol (oral or transdermal) and anti-androgen therapy with CPA (Table 1). Participants who had undergone gonadectomy stopped CPA and continued estradiol therapy in unchanged dosage. Subjects using anti-androgen therapy other

Table 1. Baseline characteristics of the study population

	Transgender women
Center	
Amsterdam	162
Ghent	113
Age at baseline (median, IQR), y	25.0 (21.0-39.0)
BMI (median, IQR), kg/m^2	22.9 (20.7-26.2)[a]
Underwent gonadectomy	49.5%
Daily CPA dose at baseline	
10 mg	0.36%
25 mg	47.3%
50 mg	52.0%
100 mg	0.36%
Estradiol formulation and accumulated daily dose at baseline	
Tablet	
2 mg	0.73%
4 mg	73.8%
6 mg	0.36%
Patch	
50 µg/24 h	2.2%
100 µg/24 h	22.1%
Gel	
0.36 mg	0.36%
1.5 mg	0.73%
3 mg	0.73%

Abbreviations: BMI, body mass index; CPA, cyproterone acetate; IQR, interquartile range.
[a]Data available in 225 transgender women.

than CPA such as spironolactone or GnRH analogues (n = 18) were excluded.

Clinical Data

Data on age and body mass index (BMI) were collected at baseline. Dose and formulation of the GAHT at each visit and information on whether participants had undergone a gonadectomy was retrieved from the medical files.

Laboratory Analyses

Both in Ghent and Amsterdam, TT, A4, E2, and SHBG were measured in serum before the initiation of GAHT and at 12 months, and between 2 to 4 years after baseline. In Amsterdam, these hormones were also measured at 3 months after the start of GAHT. Only in Ghent, DHEA and DHEAS were additionally measured at baseline, 12 months, and 2 years. The number of hormonal analyses per time point is displayed in Table 2. In both centers, calculated free testosterone (cFT) was calculated using the Vermeulen formula (22). We present our data in SI units. Conversions factors are: TT (3.47 × ng/mL = nmol/L), A4 (3.49 × ng/mL = nmol/L), DHEA (3.47 × ng/mL = nmol/L), and DHEAS (2.71 × ng/mL = nmol/L).

In Amsterdam, measurements were performed at the Endocrine Laboratory of the Amsterdam UMC. TT, A4, and E2 were measured using in-house developed LC-MS/MS (23, 24). Inter-assay coefficient of variation (CV) for TT was 9.8% at 0.62 nmol/L and 6.6% at a level of 15 nmol/L (both N > 800) with a lower limit of quantitation (LLOQ) of 0.10 nmol/L. Inter-assay CV for A4 was 9.2% at 1.4 nmol/L and 7.7% at a level of 20 nmol/L (both N > 700) with an LLOQ of 0.10 nmol/L. Inter-assay CV for E2 was 10.6% at 23 pmol/L, 5.0% at a level of 171 pmol/L, and 4.2% at a level of 533 pmol/L (all N > 300) with an LLOQ of 20 pmol/L. An automated immunoassay (Architect, Abbott Diagnostics) was used to determine the SHBG concentration (CV < 6%).

In Ghent, measurements were performed at the endocrine laboratory of Ghent University Hospital. TT, A4, and DHEA were assessed using in-house developed LC/MS-MS. Inter-assay CV for TT was 4.5% at 0.88 nmol/L (N > 100) with an LLOQ of 0.035 nmol/L. For A4, LLOQ was 0.17 nmol/L with an inter-assay CV of 14.9% at 0.2 nmol/L (N > 7) and 10.0% at 1.6 nmol/L (N > 180). For DHEA, LLOQ was 0.35 nmol/L with an inter-assay CV of 11% (N = 8) and 7.7% at 6.3 nmol/L (N > 300). E2, SHBG, and DHEAS were measured by immunoassay (Roche Diagnostics). All CVs for the commercial immunoassays were ≤10%.

To study the comparability between the TT, A4, and SHBG results from Ghent and Amsterdam, we performed a method comparison on 40 randomly selected samples. All samples were measured at both sites and compared: TT (Ghent) = 1.00 × TT (Amsterdam) + 0.00 ($R^2 = 0.997$) for the whole concentration range (N = 40), also below 2.5 nmol/L (N = 30); A4 (Ghent) = 0.95 × A4(Amsterdam) + 0.11 ($R^2 = 0.989$) for the whole concentration range (N = 40); SHBG (Ghent) = 1.00 × SHBG(Amsterdam) + 1.00 ($R^2 = 0.969$) for the whole concentration range (N = 40). Method comparisons for TT, A4, and SHBG between the methods of Ghent and Amsterdam were evaluated using Passing & Bablok regression analysis and Pearson correlation coefficient using MedCalc Statistical Software version 18.5 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). In summary, TT, A4, and SHBG were comparable in both centers and no conversions of data were conducted. Detailed results of the method comparison can be found in the supplemental material (25).

Table 2. Hormone concentrations in transgender women with GAHT and after gonadectomy

	Baseline	3 months	12 months	2–4 years[a]	After gonadectomy
TT, nmol/L	18.6 (13.8–23.1)	0.40 (0.30–0.50)	0.40 (0.30–0.54)	0.40 (0.30–0.58)	0.48 (0.33–0.60)
	n = 185	n = 67	n = 165	n = 93	n = 95
cFT, pmol/L	378 (294–484)	7.1 (5.1–9.9)	5.9 (4.2–8.4)	6.6 (4.9–9.3)	5.7 (3.9–8.0)
	n = 173	n = 59	n = 154	n = 84	n = 86
SHBG, nmol/L	35.1 (25.6–51.8)	40.5 (27.0–53.0)	46.7 (34.0–66.0)	41.6 (31.0–57.2)	61.1 (46.0–99.7)
	n = 173	n = 60	n = 161	n = 86	n = 90
DHEA, nmol/L	23.7 (14.0–36.3)		15.6 (10.4–25.6)	18.0 (10.9–32.6)	15.9 (10.0–26.4)
	n = 112		n = 110	n = 51	n = 60
DHEAS, µmol/L	8.7 (5.9–10.5)		6.9 (4.6–8.6)	6.9 (4.8–10.0)	6.2 (4.1–8.7)
	n = 113		n = 110	n = 52	n = 59
A4, nmol/L	3.3 (2.5–4.3)	1.6 (1.1–2.1)	1.9 (1.4–2.8)	1.8 (1.3–2.9)	2.1 (1.4–3.0)
	n = 168	n = 66	n = 162	n = 88	n = 92
E2, pmol/L Ghent (immunoassay)[b]	94.0 (91.0–120.0)		255 (151–371)	245 (187–363)	232 (142–331)
	n = 97		n = 92	n = 36	n = 46
E2, pmol/L Amsterdam (LC-MS/MS)[b]	74.5 (57.0–93.0)	229 (170–392)	275 (151–375)	179.5 (112–289)	262 (195–451)
	n = 72	n = 67	n = 55	n = 42	n = 36

All values are reported as median (IQR).
Abbreviations: A4, androstenedione; cFT, calculated free testosterone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; E2, 17-beta estradiol; IQR, interquartile range; SHBG, sex hormone-binding globulin; TT, total testosterone.
[a]Only including transgender women who had not undergone gonadectomy and used cyproterone acetate.
[b]Estradiol concentrations were measured using immunoassay in Ghent and using liquid chromatography tandem mass spectrometry in Amsterdam; therefore, data on estradiol concentrations are not pooled and are presented separately per center.

With respect to estradiol, both centers used a different method (ie, LC-MS/MS vs immunoassay). Because the aim of reporting estradiol concentrations was merely to provide evidence that the transgender women (1) were compliant to estradiol supplementation and (2) had concentrations within the reference ranges of clinical guidelines on gender-affirming endocrine care, no estradiol method comparison was performed.

Statistical Analyses

Data were analyzed using IBM SPSS Statistics version 27. Data were verified for normal distribution using Shapiro-Wilk test. Non-normally distributed data were reported with median and interquartile range (IQR), whereas normally distributed data were reported with mean and standard deviation (SD). Outliers in hormone concentrations that may represent treatment noncompliance were defined for testosterone and estradiol when the concentration differed more than 3 SDs from the mean (low testosterone or high estradiol at baseline, and vice versa for all following time points). Because excluding these outliers (n = 5) did not alter results, they were not removed. Data were analyzed longitudinally. To evaluate changes in androgen concentrations over time in transgender women using CPA and after gonadectomy, a mixed-model analysis was conducted with androgen concentration at each visit as repeated measurement and a random intercept for center and participant. Of note, as demonstrated by the method comparison results for TT, A4, and SHBG and the lack of DHEA or DHEAS measurements in Amsterdam, there was no rationale to additionally include a covariate for the laboratory method used per center throughout analyses. Initially, age at baseline and BMI were added to the model as potential covariates. However, because we aimed to longitudinally assess changes clustered within individuals and centers, these covariates were not included in the final model as these did (1) not alter the results and (2) random intercepts for patient and center had already been added to the model. To assess whether hormone levels changed after gonadectomy compared with continuation of anti-androgens, baseline data were excluded from the mixed model to account for high variation between baseline and 3-month hormone levels. Here, age at baseline and BMI remained included as potential covariates because we aimed to identify differences in hormone levels that were a result of variations in treatment rather than group characteristics. Moreover, an interaction term for visit and gonadectomy status was added. To display reference ranges for cisgender men and women in the figures, reference ranges for people aged 18 to 40 years from the Endocrine Laboratory of the Ghent University Hospital were used for TT (ie, those by Travison et al (26)), cFT, SHBG, DHEA, DHEAS, and A4. These were highly similar with those used in clinical practice in Amsterdam. A P value less than 0.05 was considered significant; all tests were two-sided.

Ethics and Informed Consent

The ethics committee of the Amsterdam UMC (location VUmc) declared that this study is not subject to the Medical Research Involving Human Subjects Act (2014.322-A2016.465 and 2019.469). This study was approved by the ethical committee of the Ghent University Hospital (2009/622). All participants provided written informed consent.

Results

Baseline characteristics and dose and formulation of GAHT are shown in Table 1. In total, 275 transgender women were included, of whom 162 initiated GAHT in Amsterdam and 113 in Ghent. The majority of individuals participating in the study were young adults with a median age of 25.0 (21.0-39.0) years and a BMI of 22.9 (20.7-26.2) kg/m². The most commonly initiated GAHT regimen was oral estradiol valerate 2 mg twice daily (73.8%) and CPA 50 mg once daily (52.0%). Almost one-half of all transgender women underwent gonadectomy (49.5%), for which they are considered eligible after 1 year of GAHT with effective TT suppression. Mean time to endocrine evaluation between the initiation of GAHT and after gonadectomy was 24.5 ± 2.0 months. Transgender women who underwent gonadectomy were older than those who did not (32.0 [22.0-46.0] years vs 23.0 [21.0-32.0] years, $P < 0.001$). BMI did not differ between groups. The serum concentrations of TT, cFT, SHBG, DHEA, DHEAS, A4, and E2 at each visit are displayed in Table 2. For estradiol, concentrations are presented separately because of the use of immunoassay in Ghent and LC-MS/MS in Amsterdam.

Changes in Androgen Profiles Over Time

Three months after initiation of estradiol and anti-androgen therapy, serum TT levels decreased by 18.4 nmol/L (95% CI, −19.4 to −17.4, $P < 0.001$ [ie, −97.1%]) and cFT levels decreased by 383 pmol/L (95% CI, −405 to −362, $P < 0.001$ [ie, −98.3%]). Later on, both TT and cFT levels remained stable. SHBG increased between 3 months and 12 months (mean Δ = 8.4 nmol/L; 95% CI, 3.1-13.7; $P = 0.002$ [ie, + 19.0%]), and increased further between 12 months and 2 and 4 years (mean Δ = 10.0 nmol/L; 95% CI, 4.4-15.7; $P < 0.001$ [ie, + 19.0%]). DHEA and DHEAS decreased by 6.6 nmol/L (95% CI, −9.1 to −4.0, $P < 0.001$ [ie, −24.9%]) and 1.8 µmol/L (95% CI, −2.2 to −1.4; $P < 0.001$ [ie, −23.5%]), respectively, in the first year, but remained stable thereafter. After 3 months of GAHT, A4 had decreased by 1.2 nmol/L (95% CI, −1.4 to −1.0; $P < 0.001$ [ie, −36.5%]) and did not change at a later stage. Changes in concentrations of these hormones over the course of 2 to 4 years of GAHT are displayed in Fig. 1.

Changes in Androgen Levels in Transgender Women Using GAHT Before and After Gonadectomy

In the second year of GAHT (ie, between 12 months and 2 to 4 years), no changes in testosterone, adrenal androgens, or SHBG levels emerged when separately considering the group of women who did or did not undergo gonadectomy. Over the course of time, there was no differential change in androgen and SHBG concentrations between women who did and did not undergo a gonadectomy. Androgen concentrations did not differ between groups after 2 to 4 years, whereas SHBG levels were found to be higher in women who underwent a gonadectomy compared with those who continued CPA (mean Δ = 24.6 nmol/L; 95% CI, 13.1-36.1; $P <$ 0.001). For SHBG, adjusting for TT, E2, and mode of estradiol administration did not alter results.

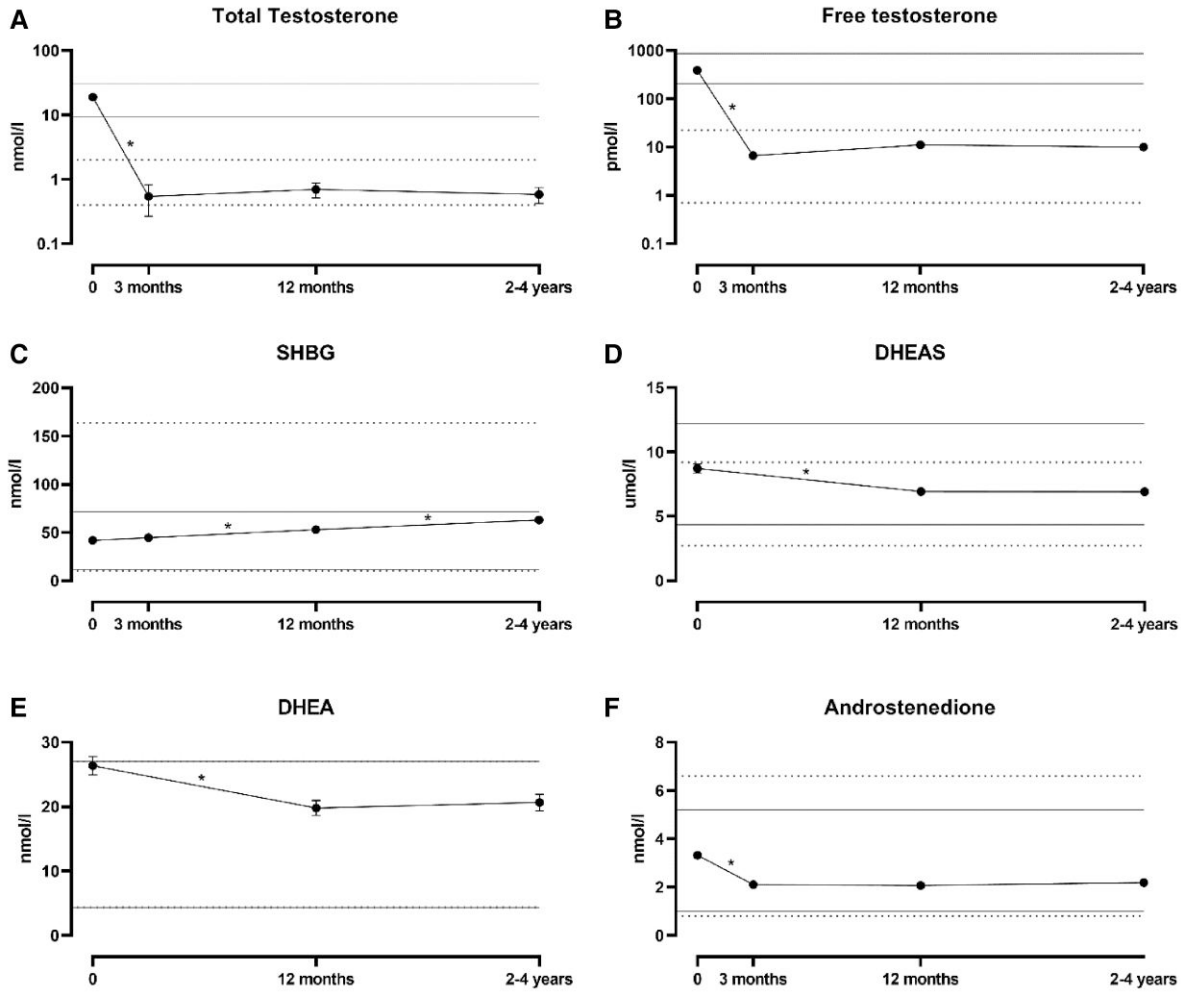

Figure 1. Androgen concentrations before the start of feminizing GAHT, at 3 months, 12 months, and after 2 to 4 years of feminizing GAHT regardless of gonadectomy status. Concentrations depicted as estimated mean with bars representing SEs obtained from mixed-model analyses. Asterisks indicate a significant change. Reference ranges for cisgender men (continuous line) and cisgender women (dotted line) are provided.

Discussion

This is the first study to report changes in androgen concentrations during the first years of GAHT and after gonadectomy in transgender women using LC-MS/MS. Upon initiation of GAHT with estradiol and CPA, TT and cFT dropped in the first 3 months and remained stable after gonadectomy. Adrenal androgens DHEA, DHEAS, and A4 decreased in the first year and remained stable thereafter, which was in line with our hypothesis. SHBG rose from 3 months on throughout the follow-up period of 2 to 4 years. After gonadectomy, TT and adrenal androgens did not change compared with transgender women who continued with antiandrogen therapy.

Overall, our findings are in line with other studies (12, 27). After initiation of GAHT, androgen concentrations decrease to levels comparable to cisgender women. The gradual decrease of DHEA, DHEAS, and A4 in the first year of antiandrogen therapy has been observed in other studies, both in populations of transgender women as well as in men who received anti-androgen therapy in the context of prostate cancer. Polderman et al reported a decrease of DHEA, DHEAS, and A4 after 4 months in transgender women treated with CPA and estradiol (12). Several studies in prostate cancer patients treated with gonadotropin-releasing hormone analogues or antagonists (GnRHa) or LH-releasing hormone agonist also reported a decrease in DHEA, DHEAS, and A4 upon initiation of anti-androgen therapy (27–29). A study in transgender girls receiving puberty suppression through a gonadotropin-releasing hormone analogue and feminizing hormone therapy through estradiol supplementation found no changes in A4 and DHEAS during the first year of therapy, which may be a result of the small sample size or the age at which GnRHa therapy was started (14.0 years ± 1.6 years) (13).

The mechanism underlying the decrease in DHEA, DHEAS, and A4 in the first year of CPA and estradiol therapy is unknown. Adrenal steroidogenesis may in part be regulated by ACTH, which in older studies was shown to be suppressed by high dosages of CPA (30–34). However, our finding that adrenal androgens do not change after ceasing CPA after gonadectomy suggests that ACTH-mediated adrenal steroidogenesis may not fully account for the adrenal androgen dynamics found in our study. Studies reporting a decrease in adrenal androgen during GnRHa/LH-releasing hormone agonist therapy in prostate cancer patients have suggested that LH receptors in the adrenal reticular cells regulate

adrenal androgen production, resulting in a decrease in adrenal androgens when LH is suppressed (28, 35). In line with this, a CPA-induced decrease in LH may explain the decreased adrenal androgen synthesis found in our study. Although we had hypothesized adrenal androgens to increase upon ceasing CPA after gonadectomy, the finding that adrenal androgen concentrations did not increase upon ceasing CPA after gonadectomy may suggest that dynamics in DHEA, DHEAS, and A4 are the result of the continued LH suppression through estradiol therapy. Multiple studies have demonstrated reduced concentrations of these androgens following estradiol administration (10, 36–38). More specifically, combined oral contraceptives are known to suppress both ovarian and adrenal androgen synthesis in cisgender women, resulting in decreased levels of androgen precursors DHEAS and A4, along with TT and free testosterone (39, 40). Although the exact mechanisms mediating the adrenal androgen decrease found in our study are unknown, LH suppression through CPA and estradiol may reduce LH receptor-mediated steroidogenesis in the adrenal cortex (35, 41).

Following cessation of CPA after gonadectomy, we found that TT and cFT levels remained stable. We did not replicate findings from an earlier study by van der Sluis et al in a mixed cohort of adult transgender women and cisgender men who underwent surgical or hormonal castration because of prostate cancer. They reported lower TT in subjects using an LH-releasing hormone agonist compared with after gonadectomy (14). This discrepancy may be explained by the smaller sample size (66 subjects), the use of different androgen-reducing medication, differential effects on SHBG, or the heterogeneity of the sample. Similar to this study, we found that serum DHEA, DHEAS, and A4 concentrations remained stable after gonadectomy. In addition, like Defreyne et al (42), we observed lower SHBG levels in transgender women with continued antiandrogens compared with those who underwent gonadectomy. This is in line with findings by Marcondes et al reporting a SHBG reduction in women with hirsutism receiving CPA albeit without coadministration of estradiol (43). In the past, a study with CPA at high doses in combination with transdermal estradiol gel was found to suppress SHBG, suggesting a progestogenic or androgenic effect of CPA on hepatic SHBG production (44).

Throughout the use of anti-androgens and after gonadectomy, transgender women in this study had TT concentrations that were in the lowest part of the reference range used for cisgender women. This is in line with the hypothesis presented earlier by Elaut et al, who reported that transgender women had testosterone levels comparable with those of postmenopausal women (45). However, the concentrations found in our study appear to be even lower than ranges reported in postmenopausal cisgender women by Eisenhofer et al (median, 0.75 nmol/L; minimum, 0.24 nmol/L; maximum, 2.75 nmol/L) (46). Although formal comparison was not carried out, TT reference ranges in cisgender women in other studies using LC-MS/MS suggest similar results where the transgender women in our study seemed to have lower TT concentrations (47–50). Considering the age-specific testosterone reference ranges measured by LC-MS/MS in cisgender women reported by Haring et al, our results seem lower than those reported in any of the age groups (50). Because very low levels of testosterone in cisgender women have been associated with clinical symptoms such as reduced sexual desire, tiredness, depressed mood, and muscle weakness, these low testosterone levels may explain similar symptoms in some transgender women as well (15, 16). Besides the desired changes induced by antiandrogen therapy, such as reduced body and facial hair growth or decreased muscle mass, these interventions may also induce these undesired clinical effects (1, 51). Because appropriately dosed testosterone supplementation in cisgender women has been shown to improve some of previously mentioned symptoms, the question remains whether this may also be a valuable treatment strategy in transgender women who experience these symptoms after gonadectomy (52). For women continuing anti-androgens, less strong testosterone suppression may alleviate symptoms although dose-response titration of anti-androgens may be necessary (7). However, our findings suggest that GAHT with anti-androgens is an adequate preparation for the hormonal situation after gender-affirming surgery with no further changes in serum androgen levels.

The findings of our study should be considered in the context of some limitations. Current GAHT regimens have been subject to change because of advancing scientific insights. CPA has been associated with side effects such as an increase in prolactin, changes in liver enzyme and lipid concentrations, increased prevalence of thrombosis, and the development of meningioma after long-term use of high-dose CPA (7, 53). Following these concerns, the standard CPA dose has successfully been reduced to 10 mg in recent years and is even gradually being replaced by GnRH analogues in Amsterdam (7). This was until recent not the case for Belgium as the choice of anti-androgen therapy depended on multiple factors including reimbursement policies. Repeating this study at the contemporary CPA dosage, or upon treatment with a GnRH analogue or spironolactone should be considered. However, as was recently shown by Kuijpers et al, 10 mg daily CPA results in equivalent TT suppression <2 nmol/L as higher dosages (ie, 25 mg, 50 mg) (7); hence, it is unlikely that androgen changes over time were conferred by CPA dosage changes throughout the current study period. Nonetheless, the possibility of clinical symptoms resulting from high dosages of CPA should not be overlooked. Unfortunately, for this cohort, information on clinical symptoms was not available. Moreover, because LH levels were not measured in this study, no conclusions can be drawn on whether estradiol supplementation fully suppresses the anticipated LH rise upon ceasing CPA. Therefore, findings of unchanged TT after gonadectomy should be interpreted with caution.

Despite these limitations, this study has several strengths. This is the first study to longitudinally evaluate androgen concentrations in transgender women using LC-MS/MS, which is, to date, acknowledged as the gold standard for accurate measurement of testosterone, its precursors, and metabolites. In addition, a cross-validation of analytes measured was conducted between centers. Other strengths are the prospective design and well-defined cohorts in which participants adhered to a strict treatment regimen.

Conclusion

In conclusion, using LC-MS/MS, this study confirms that TT and cFT levels decrease rapidly upon the initiation of feminizing GAHT with estradiol and CPA and remain stable at low concentrations before and after gonadectomy. Adrenal androgens DHEA, DHEAS, and A4 decreased in the first year and remained stable thereafter. After gonadectomy, when CPA

therapy was stopped while estradiol therapy was continued, no further changes occurred in TT, DHEA, DHEAS, and A4. Clinical symptoms that start after gonadectomy when CPA therapy is ceased are therefore unlikely to result from changes in testosterone or adrenal androgen concentrations.

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Data Availability

Data are available upon request.

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