4.1 Lower Serum Estradiol Levels in Assigned Female at Birth Transgender People with Initiation of Testosterone Therapy: Results from the European Network for the Investigation of Gender Incongruence

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ABSTRACT

Purpose: Concerns have been raised about undesired estrogenic effects in assigned female at birth (AFAB) transgender people on testosterone therapy. How serum estradiol levels change after initiation of testosterone therapy and if these levels should be monitored, remains unclear.

Methods: This prospective cohort study was part of the European Network for the Investigation of Gender Incongruence. Serum levels of sex steroids were assessed in 746 AFAB transgender people during a three-year follow-up period, starting at the initiation of hormone treatment.

Results: Estradiol levels decreased from median [P25-P75] 45.6 [24.0–102.2]pg/mL to 36.5 [25.0–46.2]pg/mL over three years (P<0.001); a change was already noticeable during the first three months (mean - 17.1 pg/mL, 95% confidence interval [CI] -23.8 to -10.6, P<0.001). Serum estradiol levels were lower in people without endogenous estradiol production from ovarian source (contraceptive users or post hystero-oophorectomy) at baseline and after three months, compared to people with endogenous estradiol production. Using long acting testosterone undecanoate injections resulted in a more prominent decrease in serum estradiol values over 12 months, compared to short acting mixed testosterone esters (P<0.001) or testosterone gel (P=0.001). Changes in serum estradiol were positively correlated to changes in luteinizing hormone (ρ = 0.107, P<0.001) and negatively correlated to changes in follicle-stimulating hormone levels (ρ= -0.167, P<0.001) and body mass index (ρ= -0.082, P<0.001).

Conclusion: Testosterone administration in AFAB transgender people resulted in decreasing serum estradiol levels. Our results suggest that testosterone therapy leads to central suppression of estradiol production, with partial restitution due to aromatization.

Introduction

Cisgender people are persons whose gender identity is congruent with their birth-assigned sex, whereas transgender people are persons whose gender identity differs from their birth-assigned sex. Transgender people may request gender-affirming hormone therapy (HT)(1), aimed at suppressing the secondary sex characteristics of the birth-assigned sex and inducing the secondary sex characteristics of the experienced gender. HT in assigned female at birth (AFAB) transgender people who desire masculinizing hormone therapy consists of testosterone, which can be administered intramuscularly, transdermally, or orally (1). If suppression of the menses is desired or menstrual bleeding does not cease, serum testosterone levels should be aimed at cisgender male reference ranges. Testosterone dose and administration frequency can be increased if subphysiological levels are measured.(2) Another option is the addition of progestogens (3).

Testosterone therapy in AFAB transgender people is aimed at achieving serum testosterone levels in the male reference ranges (1), although it is not known if serum estradiol levels should be evaluated and/or influenced. It is possible that initiating testosterone therapy may increase serum estradiol levels through aromatization of exogenous testosterone to estradiol (1), combined with a (not completely suppressed) endogenous production of estradiol in the ovaries and adrenal cortex. While one paper references concern that exogenous testosterone will lead to increases in estradiol levels (4), previous data either show decreased estradiol levels (5–10) or are insufficiently powered to demonstrate statistically significant change (11).

Aromatization of exogenous testosterone might result in increased estradiol levels. Therefore, concerns have been raised about undesired estrogenic effects after initiation of testosterone treatment in AFAB transgender people, including persistent menstrual bleedings, pelvic pain, and increased breast volume (9). Low estradiol on the other hand could lead to decreased bone density, vaginal dryness, decreased sexual desire, and menopausal symptoms in females (12).

The administration of testosterone therapy has been suggested as a risk factor for endometrial

cancer (13), also due to aromatization. However, no actual cases of endometrial cancer in transgender people have been reported, while three cases of ovarian cancer (14–16) and twenty-two cases of breast cancer (17,18,27–29,19–26) have been described, although there have been reports of hyperplasia of the ovarian cortex and stroma (30,31). Previous studies have also described higher estrogen receptor, modest increase of androgen receptor, and unchanged Ki67 expression in the endometrial cells of AFAB transgender people receiving testosterone therapy (31). Studies on oncological risk in AFAB transgender people after the initiation of testosterone therapy remain inconclusive and lack power (21,24,32–36). Therefore it is advised to adhere to the screening protocols for the general population, depending on the tissues present (1). The aim of this study was to evaluate changes in measured serum estradiol levels during testosterone treatment.

Methods

Cohort

The European Network for the Investigation of Gender Incongruence (ENIGI) study is a multicenter prospective cohort study conducted in four European treatment centers (Ghent, Oslo, Florence, and Amsterdam) (37). The study was reviewed and approved by the Ghent University Ethics Committee. For the present substudy, data from Ghent and Amsterdam were selected.

From February 2010 until July 2018, 1730 transgender persons were included in the Belgian–Dutch sample of the study, of whom 858 were AFAB. All participants were at least 17 years old and underwent a standardized diagnostic procedure to confirm the diagnosis of gender dysphoria before initiating treatment (37). Participants were included in the ENIGI endocrine protocol when they started HT. Every participant was treated in accordance with the World Professional Association for Transgender Health Standards of Care, Version 7 (38). Exclusion criteria were previous HT use and insufficient knowledge of the native languages (Dutch or French). At the start of the study, participants received oral and written information about the ENIGI endocrine protocol. A written informed consent was obtained according to the institution's ethics review board guidelines.

Short-term follow-up currently consists of a baseline visit and visits after 3, 6, 9, 12, and 36 months in Amsterdam and baseline, 3, 6, 9, 12, 18, 24, and 36 months in Ghent. A venous blood sample was obtained upon each visit, independent of the time to testosterone administration and at baseline randomly relative to the menstrual cycle. Serum levels of estradiol in the AFAB cohort were compared to serum levels of estradiol in 224 male controls. Baseline estradiol values were available in 746 AFAB people (86.9%). Baseline statistics of the study and control population can be found in table 1.

Gender-affirming hormone therapy

In Ghent, AFAB transgender people receive intramuscular long-acting testosterone undecanoate (Nebido® 1000 mg once every 12 weeks, n=160). In Amsterdam, they can choose between testosterone gel in a daily dose of 50 mg (n=316) or intramuscular administration, either as short-acting mixed testosterone esters (Sustanon® 250 mg every 2-3 weeks, n=175) (hereafter referred to as 'testosterone esters') or as testosterone undecanoate (Nebido® 1000 mg every 12 weeks, n=46) (missing: 49). Usually, suppression of menstrual bleeding occurs after initiation of testosterone therapy. A progestogen or a gonadotropin-releasing hormone analogue can be added to the treatment regimen if suppression of the menses is desired or menstrual bleeding does not cease. The main cycle suppressing agents used in AFAB transgender people are progestogens (e.g. oral lynestrenol 5mg once daily, injectable medroxyprogesterone acetate 150mg once every 3 months), which results in serum estradiol values comparable to levels observed in early follicular menstrual phase (39).

At baseline, 154 (20.6%) people were using contraceptive agents (either progestogens or combined oral contraceptives containing ethinyl estradiol) to suppress menses. Combined oral contraceptives were stopped or switched to progestogen only treatment. The use of contraceptives was logged at each visit, which allowed us to update the group of actual contraceptive users at each follow-up visit. Therefore, the numbers of people using contraceptives varies over time.

Laboratory analyses

In Ghent, competitive chemiluminescent immunoassays were run for estradiol (E170 Modular, Roche, Mannheim, Germany, Gen III, LOQ 25 pg/mL, interassay CV 3.2%), serum testosterone (E170 Modular, Roche, Mannheim, Germany, Gen II, LOQ 0.4 nmol/L, interassay CV 2.6%), luteinizing hormone (LH) (E170 Modular, Roche, Mannheim, Germany, Gen III, LOQ 0.1 mIU/mL, interassay CV 3.48%), and follicle stimulating hormone (FSH) (E170 Modular, Roche, Mannheim, Germany, Gen III, LOQ 0.1 mIU/mL, interassay CV 3.3%).

Before March 19, 2015, estradiol was measured using an E170 Modular (Gen II; Roche, Mannheim, Germany). For conversion of estradiol values measured before March 19, 2015, the formula Gen III = 6.687940 + 0.834495 * Gen II was used (E170 Modular, Roche, Mannheim, Germany).

In Amsterdam, estradiol was measured using a competitive immunoassay (Delfia, PerkinElmer, Turku, Finland, LOQ 20 pmol/L, interassay CV 10%-13%) until July 2014. After July 2014, estradiol was measured using a LC-MS/MS (VUmc, Amsterdam, the Netherlands, LOQ 20 pmol/L, interassay CV 7%). For conversion of the Delfia values, the formula LC-MS/MS = 1.60 * Delfia–29 was used. Testosterone was measured using a radioimmunoassay (RIA) (Coat-A-Count, Siemens, Los Angeles, CA, USA, LOQ 1nmol/L, interassay CV 7-20%) until January 2013. Thereafter, testosterone was measured using a competitive immunoassay (Architect, Abbott, Abbott Park, IL, USA, LOQ 0.1 nmol/L, interassay CV 6%-10%). The RIA values were converted to the competitive immunoassay values. For testosterone levels below 8 nmol/L, the formula Architect = 1.1 * RIA + 0.2 was used; for testosterone levels above 8 nmol/L, the formula Architect = 1.34 * RIA – 1.65 was used. LH and FSH were measured using chemiluminescent microparticle immunoassay (Architect System, Abbott), with an interassay CV of 4% and a LOQ of 2 U/L for LH and FSH.

Body composition

Body composition was measured in a subset of the study population using whole-body dual-energy X-ray absorptiometry (DXA) at the start and after one year of HT. In Amsterdam and Ghent, Hologic

Discovery A was used. Body fat, lean body mass, and total mass of the whole body were measured using DXA. All scans were analyzed using software, version 13.5.3. More information can be found in Klaver et al (40).

Data analysis

Data were analyzed using IBM SPSS 24.0 (IBM Corp., Armonk, NY, 2016). The analyses in this article focus on the 3-month (short term follow-up), 12-month (largest sample size), 24-month, and 36-month (longest follow-up) visits, as discussing all results on all study time points would result in excessive data. Data on other time points can be found in the tables.

Data were verified for normal distribution using the Shapiro–Wilk test. Normally distributed values are shown as mean ± standard deviation (SD), non-normally distributed values as median [percentile 25 – percentile 75]. Data were analyzed prospectively (individual mean changes, Δ, with 95% confidence intervals [CIs]) as well as cross-sectionally after 3, 12, 24, and 36 months and compared to baseline estradiol values in 224 male controls. Differences between groups were analyzed by unpaired Student's t-test (normally distributed data) and the Mann–Whitney U-test (non-normally distributed data).

To evaluate estradiol differences in time, a mixed model was applied to the log-transformed estradiol values, with visit (number of months) as fixed factor and with a random intercept for baseline serum estradiol levels. As serum estradiol levels were not distributed normally, data were log transformed for mixed models analyses. Type of testosterone, follow-up visit, use of contraceptives, previous hystero-oophorectomy, and previous hystero-oophorectomy*visit (interaction) were used as a factor and body mass index (BMI), serum testosterone, LH and FSH levels were used as covariates. Previous mastectomy, total body fat percentage, smoking status, number of packyear, recreational drugs, and alcohol habits were tested but not included as factors/covariates. Center was not included in the model, as mean estradiol levels did not differ between centers at any given visit. In addition, when used in the linear mixed models, SPSS Statistics labeled this category as ‘redundant’.

Prospective changes in serum estradiol, testosterone, LH and FSH levels were calculated as 'visit x – baseline'. Correlations were tested using Spearman's Rho for non-normally distributed values. As changes already occurred during the first three months of HT and use of contraceptives and/or undergoing hystero-oophorectomy may influence correlations between other biochemical parameters, subgroup analyses were performed for prospective changes in sex steroids in the group using contraceptives (assessed at each study visit) versus those who did not and groups who underwent hystero-oophorectomy (assessed at each study visit) versus those who did not. Bonferroni-Holm correction was applied to all P-values to limit the chance of type I error (41). Only significant P-values are shown in the results section.

Results

Baseline (endogenous) estradiol values were available in 746 AFAB people (86.9%) and compared to values of 224 male controls. Baseline characteristics of the study population, as well as characteristics of the male control population are shown in table 1.

Table 1. Baseline characteristics of the assigned female at birth transgender population and assigned male at birth control population in whom baseline serum estradiol values were available.

	AFAB transgender people (n = 746)	Male control group (N=224)
Age (years)	22.0 [19.0 – 26.0]	28.0 [21.0 – 42.3]
BMI (kg/m^2)	23.6 [21.3 – 28.8]	22.4 [20.4 – 26.0]
Total body fat percentage (%)	36.5 [31.5 – 40.3]	18.9 [14.6 – 22.3]
Total body fat mass (kg)	17.1 [14.6 – 22.0]	16.0 [12.5 – 20.4]
Serum estradiol (pg/mL)	45.6 [24.0 – 102.2]	27.1 [24.9 – 33.8])
Serum testosterone (nmol/L)	1.20 [0.90 – 1.50]	17.3 [12.7 – 21.8]
Serum LH (U/L)	4.20 [2.30 – 7.03]	5.0 [3.6 – 6.5]
Serum FSH (U/L)	5.20 [3.25 – 7.20]	3.5 [2.3 – 5.2]
On contraceptives (n, %)	154 (20.6%)	NA
Mastectomy (n, %)	11 (1.5%)	NA
Hysterectomy-oophorectomy (n, %)	3 (0.4%)	NA

For values that are not normally distributed, median values and IQR (interquartile range) [P25 and P75] are shown. BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; NA, not applicable.

Prospective analyses

Prospective changes in serum levels of sex steroids

Upon initiation of HT, serum testosterone levels increased from median [P25-P75] 1.2 [0.9 – 1.5] nmol/L to 19.0 [12.0 – 30.0] nmol/L over the first 3 months, with comparable levels after 36 months (P=0.851). Individual mean levels increased with Δ +23.4 nmol/L (95% CI 21.5 – 25.3, P<0.001).

Serum estradiol levels decreased from 45.6 [24.0 – 102.2] pg/mL at baseline to 42.8 [30.2 – 59.4] pg/mL after 3 months of HT (mean Δ - 17.1 pg/mL, 95% CI -23.8 – -10.6, P<0.001), remaining stable over the next year with a second decrease between 12 and 18 months (mean Δ -19.6 pg/mL, 95% CI -23.9 – -15.3, P<0.001). (figure 1)

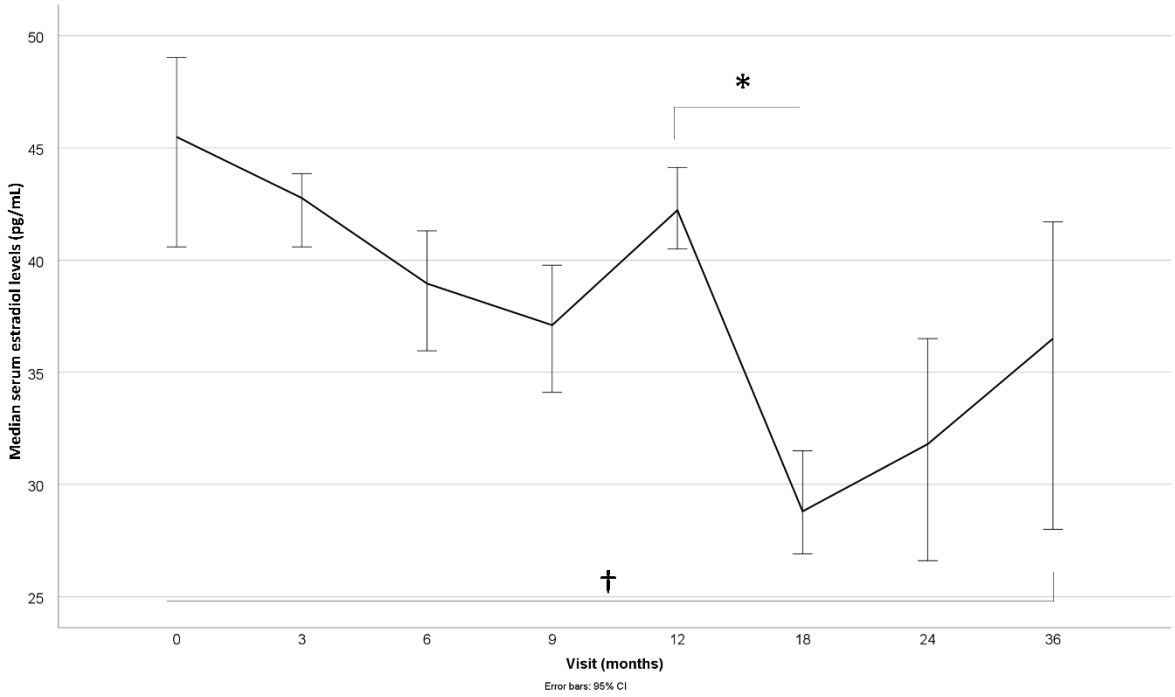


Figure 1: Prospective analysis of serum estradiol levels in AFAB transgender people.
Line graphs for the evolution of mean serum estradiol levels in AFAB transgender people on gender-affirming hormone therapy with standard error. * indicates a significant decrease in serum estradiol levels over the 12 to 18 months timeframe, † indicates a significant decrease over the entire study follow-up.

Factors associated with prospectively assessed changes in serum estradiol levels

Serum levels of sex steroids

Individual changes in serum estradiol over the entire follow-up period were positively correlated to changes in serum LH ($\rho = 0.107$, $P<0.001$) and negatively correlated to changes in serum FSH levels ($\rho = -0.167$, $P<0.001$). There was no correlation between changes in serum estradiol levels and changes in serum testosterone levels (P=NS). Correlations between individual changes in serum estradiol and serum testosterone, LH and FSH across time varied at different follow-up visits, as shown in table 2.

Table 2. Prospective (Δ) and cross-sectional correlations (Spearman's rho ρ) between serum estradiol levels and other levels of sex steroids at each visit. Bonferroni-Holm's correction was applied.

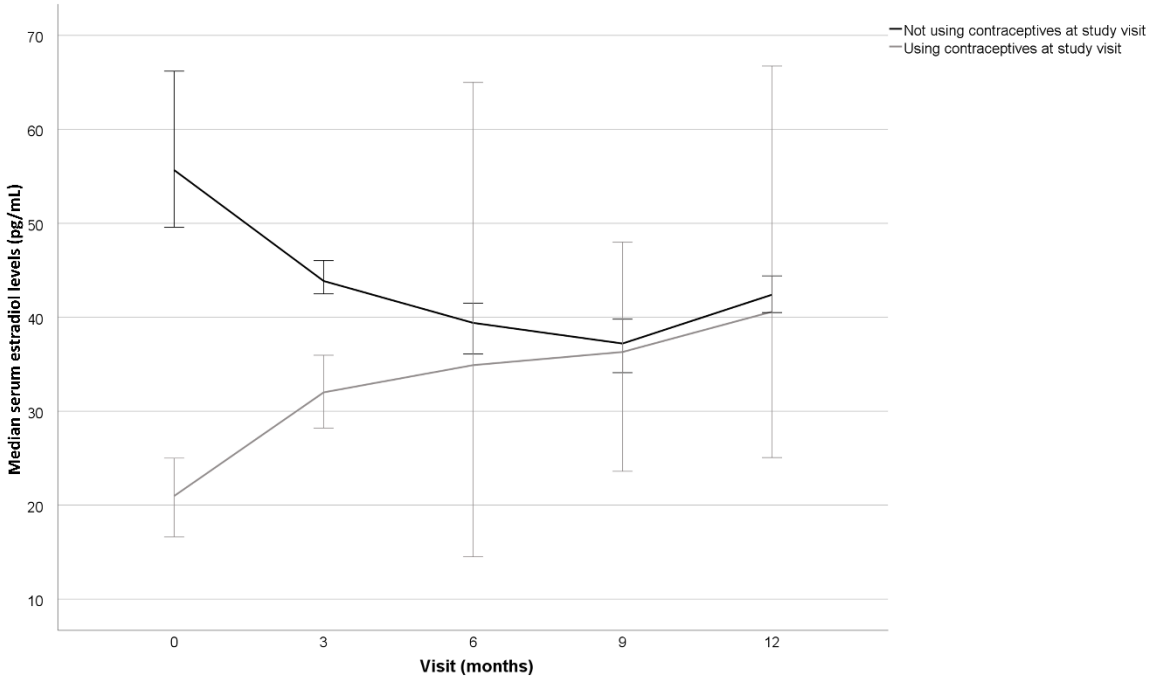
	Months							
	0	3	6	9	12	18	24	36
Correlations to prospective changes in serum estradiol								
Δ serum testosterone (nmol/L)	/	ρ = 0.085, P=0.045	ρ = 0.078, P=0.308	ρ = 0.036, P=0.669	ρ = 0.147, P=0.003*	ρ = 0.017, P=0.878	ρ = 0.081, P=0.462	ρ = 0.225, P=0.105
Δ serum LH (U/L)	/	ρ = 0.156, P<0.001*	ρ = 0.158, P=0.044	ρ = 0.130, P=0.138	ρ = 0.164, P=0.001*	ρ = 0.017, P=0.0878	ρ = -0.175, P=0.115	ρ = -0.086, P=0.542
Δ serum FSH (U/L)	/	ρ = -0.295, P=0.001*	ρ = -0.052, P=0.580	ρ = -0.136, P=0.173	ρ = -0.057, P=0.549	ρ = -0.243, P=0.027	ρ = -0.356, P=0.001*	ρ = -0.318, P=0.037
Correlations to cross-sectional levels of serum estradiol								
Serum testosterone (nmol/L)	ρ = 0.234, P<0.001*	ρ = 0.248, P<0.001*	ρ = 0.137, P=0.059	ρ = 0.189, P=0.017	ρ = 0.248, P<0.001*	ρ = 0.208, P=0.049	ρ = 0.441, P<0.001*	ρ = 0.78, P=0.004*
Serum LH (U/L)	ρ = 0.315, P<0.001*	ρ = 0.192, P<0.001*	ρ = 0.195, P=0.008*	ρ = 0.158, P=0.055	ρ = -0.219, P<0.001*	ρ = -0.317, P=0.002*	ρ = -0.458, P<0.001*	ρ = -0.409, P=0.002*
Serum FSH (U/L)	ρ = -0.101, P=0.206	ρ = -0.011, P=0.900	ρ = 0.061, P=0.502	ρ = 0.072, P=0.450	ρ = -0.093, P=0.313	ρ = -0.378, P<0.001*	ρ = -0.515, P<0.001*	ρ = -0.407, P=0.006*

Significant P-values are indicated with * (for the first significant value: adjusted P-values <0.017, the second: P<0.025, and the third: P<0.05).

Contraceptive use

As shown in figure 2, no prospective changes in serum estradiol levels over the first year of HT were observed in the AFAB group using contraceptives (P=NS), whereas the measured serum estradiol levels decreased over the first year of HT in people not using contraceptives (mean Δ -27.6 pg/mL, 95% CI -60.0 – -4.8, P<0.001). Using contraceptives at the time of assessment did not affect the prospective changes in serum estradiol levels (P=NS).

In AFAB transgender people not on contraceptives, the decrease in serum estradiol levels over the first 3 months was correlated to a decrease in serum LH levels ($\rho = 0.180$, P<0.001). In contraceptive users, changes in serum estradiol levels over the first 3 months were positively related to changes in serum testosterone levels ($\rho = 0.504$, P<0.001) and inversely related to changes in serum FSH levels ($\rho = -0.578$, P=0.001). In this group, there was no correlation between changes in serum estradiol and changes in serum LH (P=NS).

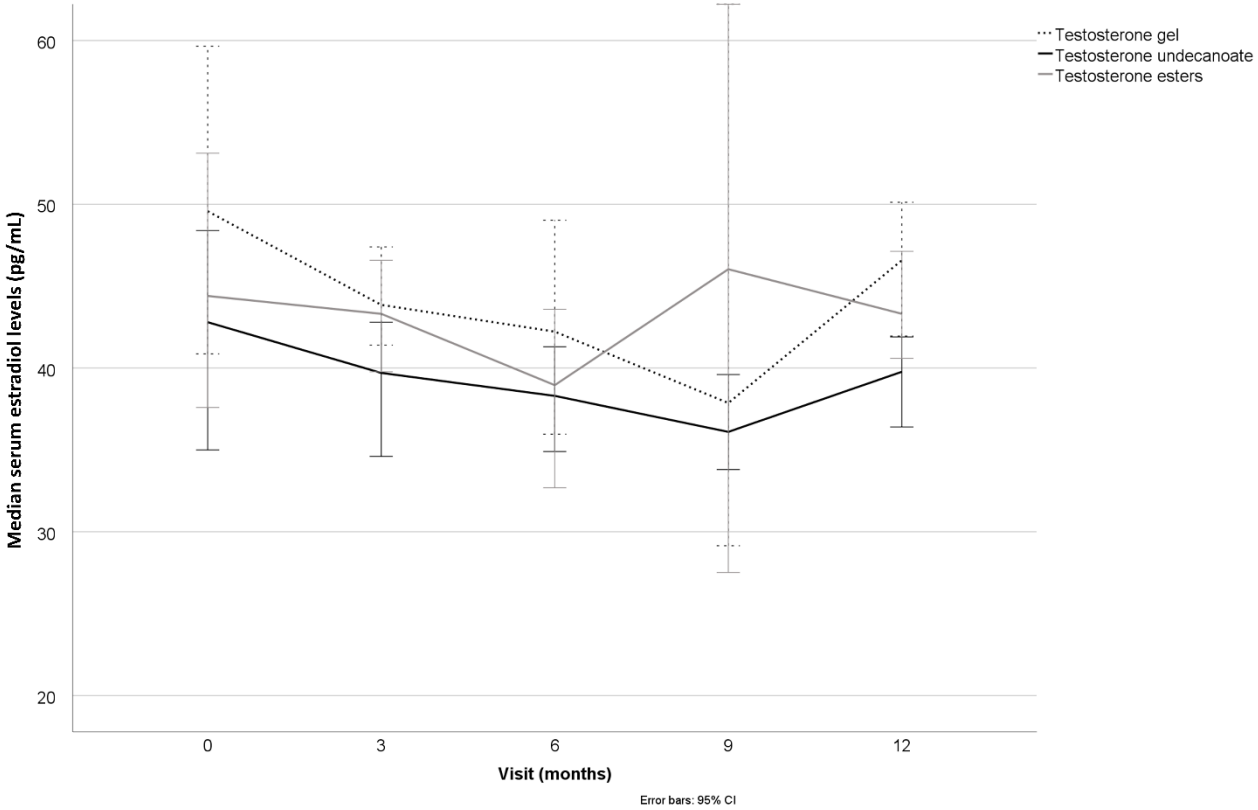


Visit (months)	0	3	6	9	12
Not using contraceptives at study visit	592/746 79.4%	561/651 86.2%	158/193 81.9%	136/159 85.5%	476/498 95.6%
Using contraceptives at study visit	154/746 20.6%	90/651 13.8%	35/193 18.1%	23/159 14.5%	22/498 4.4%

Figure 2: Prospective analysis of serum estradiol levels in contraceptive users versus non-contraceptive users. Line graphs for the evolution of mean serum estradiol levels with 95% confidence intervals. Tables underneath the graphs describe the number of persons included in each group at each given time point, shown as n/total valid and % of total valid.

Type of testosterone therapy

The use of testosterone undecanoate resulted in a more prominent decrease in serum estradiol values over 12 months (mean Δ - 32.61 pg/mL, 95% CI -44.70 – -20.52), compared to testosterone esters (mean Δ - 13.10 pg/mL, 95% CI -26.68 – 0.47, P<0.001) or testosterone gel (mean Δ - 6.91 pg/mL, 95% CI -23.15 – 9.32, P=0.001) (figure 3). These results remained significant in subgroups with versus without hystero-oophorectomy and subgroups with versus without contraceptive use. Type of testosterone therapy did not influence changes in se rum estradiol values over the first 3 months (P=NS).



Visit (months)	0	3	6	9	12
Testosterone gel	316/700 45.1%	274/646 42.4%	37/193 19.2%	26/158 16.5%	141/492 28.7%
Testosterone undecanoate	206/700 29.4%	193/646 29.9%	127/193 65.8%	122/158 77.2%	202/492 41.1%
Testosterone esters	178/700 25.4%	179/646 27.7%	29/193 15.0%	10/158 6.3%	149/492 30.3%

Figure 3: Prospective analysis of serum estradiol levels in AFAB transgender people, based on mode of testosterone administration. Line graphs for the evolution of mean serum estradiol levels in AFAB transgender people on different types of testosterone, with 95% confidence intervals. Tables underneath the graphs describe the number of persons included in each group at each given time point, shown as n/total valid and % of total valid.

Body composition

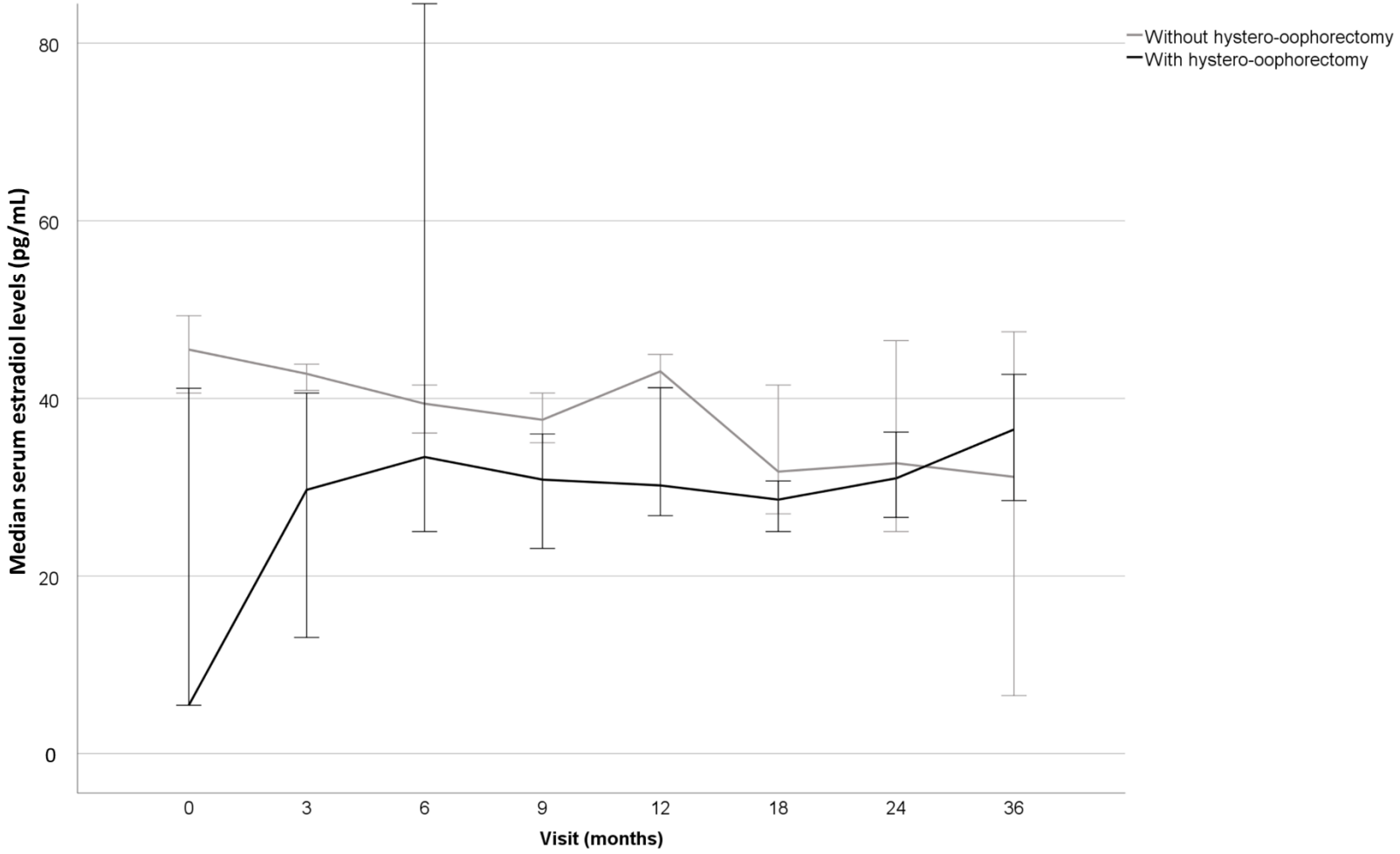
Mean BMI values increased over the first 3 months of HT (mean Δ + 0.980 kg/m², 95% CI 0.328 – 0.1632, P=0.003), remaining stable between 3 and 12 months (P=NS). During the second year of HT, a decrease in BMI values was observed (mean Δ - 1.498 kg/m², 95% CI -2.698 – -0.297, P=0.015), to BMI values comparable to baseline values (P=NS). Mean BMI values remained stable over the third year of HT (P=NS). Mean total body fat mass decreased over the first (mean Δ - 0.48 kg, 95% CI -0.88 – -0.07, P=0.038), second (mean Δ - 1.63 kg, 95% CI -2.77 – -0.47, P=0.008), and third year (mean Δ - 0.54 kg, 95% CI -1.46 – 0.38, P=0.048). Mean total body fat percentage also decreased over the first and second year of HT, with no further changes in body fat percentages over the third year of HT (mean Δ - 4.5%, 95% CI -6.1 – -3.0, P<0.001, mean Δ - 7.4%, 95% CI -10.2 – -4.3, P<0.001 and P=NS, respectively).

Prospective changes in serum estradiol levels were not correlated to prospective changes in total body fat mass (P=NS) or percentage (P=NS) over the entire follow-up period. Prospective changes in serum estradiol over the entire follow-up period were negatively correlated to changes in BMI (ρ = -0.082, P<0.001).

Surgery

As shown in figure 4, people who underwent hystero-oophorectomy during the first year of follow-up experienced an increase in serum estradiol levels over 12 months (mean Δ + 33.42 pg/mL, 95% CI 12.97 – 53.86), whereas those who did not undergo hystero-oophorectomy experienced a decrease in serum estradiol levels (mean Δ - 17.60 pg/mL, 95% CI -26.13 – -9.07, P<0.001).

Figure 4: Prospective analysis of median serum estradiol levels in AFAB transgender people with versus without hystero-oophorectomy. Line graphs for the evolution of median serum estradiol levels in AFAB transgender people on gender-affirming hormone therapy with substratification in groups who underwent hystero-oophorectomy versus those who did not (yet), with 95% confidence intervals. Tables underneath the graphs describe the number of persons included in each group at each given time point, shown as n/total valid and % of total valid.



Visit (months)	0	3	6	9	12	18	24	36
Did not undergo hystero-oophorectomy (yet)	743/746 99.6%	646/651 99.2%	186/193 96.4%	143/159 89.9%	453/498 91.0%	26/90 28.9%	21/89 23.6%	8/57 14.0%
Underwent hystero-oophorectomy	3/746 0.4%	5/651 0.8%	7/193 3.6%	16/159 10.1%	45/498 9.0%	64/90 71.1%	68/89 76.4%	49/57 86.0%

Cross-sectional analyses

Factors associated with levels of serum estradiol levels

Serum levels of sex steroids

Correlations between serum estradiol levels and serum testosterone, LH and FSH varied at different follow-up visits, as shown in table 2.

Contraceptive use

Over the entire study population, people using contraceptives had lower serum estradiol levels, compared to those not using contraceptives (25.9 pg/mL [14.5 – 40.6] versus 42.0 pg/mL [29.1 – 63.2], P<0.001). Cross-sectional analyses revealed lower serum estradiol levels in contraceptive users at baseline and after three months of HT, compared to non-contraceptive users at baseline (baseline: 20.7 pg/mL [9.5 – 37.1] versus 43.9 pg/mL [24.0 – 103.0], P<0.001, 3 months: 32.2 pg/mL [23.8 – 42.9] and 55.6 pg/mL [29.5 – 111.5], P=0.018) (other visits: P=NS).

Type of testosterone therapy

Cross-sectionally, the use of testosterone undecanoate resulted in lower serum estradiol levels in the entire study population (limited to one-year follow-up), compared to testosterone gel and testosterone esters (P<0.001). Lower serum estradiol levels were observed in testosterone undecanoate users after 12 months (37.8 pg/mL [29.7 – 49.0]), compared to gel (46.6 pg/mL [30.0 – 61.7]) and testosterone esters (43.3 pg/mL [31.9 – 63.5], P=0.005). These results remained significant in subgroups with versus without hystero-oophorectomy and subgroups with versus without contraceptive use.

Body composition

Over the entire study population, no correlation was observed between serum estradiol levels and total body fat mass (P=NS) or percentage (P=NS). Cross-sectional analyses revealed a positive correlation between serum estradiol levels and total body fat percentage after 12 months of HT (ρ = 0.233, P=0.002), but not after 36 months (P=NS) of HT. Body composition data was available in only 24 people after 36 months of HT.

Surgery

All data pooled and independent of duration of testosterone therapy, people on testosterone therapy who underwent hystero-oophorectomy had lower serum estradiol levels compared to those who did not undergo this procedure (yet) (34.11 pg/mL [32.31 – 35.90] versus 58.92 pg/mL [56.63 – 61.22], P<0.001). The serum estradiol levels were higher in people who did not undergo hystero-oophorectomy (yet) after 12 months, compared to those who did (43.0 pg/mL [31.9 – 57.1] versus 30.2 pg/mL [25.0 – 43.1], P<0.001). However, after 36 months of HT, serum estradiol levels were comparable in people with versus without hystero-oophorectomy (P=NS), based on markedly decreased sample sizes.

Comparison to serum estradiol levels in male controls

Compared to serum estradiol levels in the male control group (n=224), serum estradiol levels were higher in AFAB people at baseline and after 3, 12, and 36 months (27.1 pg/mL [24.9 – 33.8] versus baseline: 45.6 pg/mL [24.0 – 102.1], P<0.001, 3 months: 42.8 pg/mL [30.2 – 59.4], P<0.001, 12 months: 42.2 pg/mL [30.5 – 55.7], P<0.001, 36 months: 36.5 pg/mL [25.0 – 46.2], P=0.001).

However, AFAB people who underwent hystero-oophorectomy showed serum estradiol levels comparable to male levels during the first 1.5 years of HT (P=NS), and once again higher after two and three years of HT (31.8 pg/mL [25.0 – 43.9], P=0.006, and 36.5 pg/mL [25.0 – 46.2], P<0.001).

Discussion

Testosterone administration in AFAB transgender people was associated with lower serum estradiol levels compared to baseline, which already became apparent during the first three months of HT. Therefore, it is unlikely that testosterone therapy in AFAB people could be a risk factor for undesired estrogenic effects including persistent menstrual cycle, pelvic pain, and gynecomastia.

Earlier research in AFAB transgender people showed no risk for decreased bone mineral density (42) or decreased sexual desire (43) after initiation of HT, unlike hypogonadism in cisgender females. Moreover, our results display that serum estradiol levels after the start of HT in this population remain higher than those observed in a male control group and those described in post-menopausal females (44). This may be explained by enzymatic conversion of testosterone to estradiol by aromatase in adipose tissues, with higher aromatase activity in visceral fat of birth-assigned females, compared to males (45). In birth-assigned females, estrogens inhibit periosteal apposition and stimulate endosteal bone formation (46). Lower estrogen levels at menopause lead to accelerated bone loss (46). Therefore, aromatase inhibitors should not be used in the masculinizing hormone regimen due to potential of bone loss.

Subgroup analyses revealed a decrease in the measured serum estradiol levels in people with presumably remaining endogenous estradiol production: those without hystero-oophorectomy and those not using contraceptives. Serum estradiol levels increased in people with (partially) suppressed endogenous estradiol production (those with hystero-oophorectomy and those using contraceptives). In addition, AFAB transgender people who underwent hystero-oophorectomy showed serum estradiol levels comparable to male levels during the first one and a half years of HT, but higher levels after two and three years of HT. These results indicate that administering exogenous testosterone to AFAB people will lead to aromatization of the exogenously administered testosterone into estradiol. However, this does not explain the observed decrease in measured serum estradiol levels.

We show that testosterone administration results in a decrease of endogenous estradiol production. Although it is hard to disentangle the effects of decreasing estradiol values and exogenous testosterone administration on the gonadotropin axis, the observed serum estradiol levels were lower in people receiving testosterone undecanoate, compared to those receiving testosterone gel. Testosterone undecanoate is less susceptible to fluctuations in serum testosterone levels and may provide a more sustained gonadotropin and menstrual cycle suppression and may result in less aromatization to estradiol, compared to testosterone gel (47).

Serum estradiol levels were lower in people with hystero-oophorectomy or contraceptive use versus those without during the first year of HT. Nevertheless, after the first year of HT, we observed comparable serum estradiol levels in groups with versus without hystero-oophorectomy, and with versus without contraceptive use. Hystero-oophorectomy and contraceptive use resulted in lower serum estradiol levels. We hypothesize that testosterone therapy also leads to (partial) suppression of endogenous estradiol production over time. The comparable estradiol levels in groups with versus without hystero-oophorectomy and with versus without contraceptive use were probably due to a comparable degree of aromatization after the first year of HT.

However, when interpreting these changes, we should note that it is not possible to distinguish between endogenous estradiol and the contribution of exogenous estradiol that develops after initiation of testosterone therapy, through aromatization of testosterone, separately. We should also take into account that exogenous administration of testosterone can confound these findings, rendering interpretation of gonadotropins as a marker of endogenous estradiol production difficult in AFAB transgender people on testosterone therapy, as shown in the current results.

Although a decrease in serum estradiol values in AFAB people may hypothetically be explained by a decrease in body fat tissue, resulting in a decrease in aromatase activity, a correlation between prospective changes in estradiol and prospective changes in body fat mass or percentage was not demonstrated. These findings may be limited by the small number of people in whom DXA analysis

was available. However, a prospective decrease in serum estradiol levels was associated with a prospective increase in BMI, which may be explained by the prospective changes in body composition after initiation of testosterone therapy, including a decreasing body fat mass and percentage and increasing lean body mass percentage (40).

Limitations

Our study results may have been affected by several limitations. Of note, it is impossible to determine which proportion of the measured serum estradiol levels has an endogenous or exogenous (aromatization of exogenous testosterone) origin after initiating testosterone therapy. Therefore, baseline estradiol values (before the initiation of testosterone therapy) reflect the endogenous estradiol production, whereas values during follow-up are comprised of both aromatization of exogenous testosterone therapy as well as remaining endogenous production, which may be altered due to gonadotropin suppression induced by testosterone therapy.

Follow-up in Amsterdam only consisted of visits at baseline and after 3, 6, 9, 12, and 36 months, with limited data at the 36-month visit, leading to a decrease in sample size and power in the analyses of the 18th, 24th, and 36th months. As a further consequence of this, along with the fact that AFAB transgender people included in the ENIGI study in Ghent exclusively received testosterone undecanoate injections, we were not able to prospectively evaluate serum estradiol levels on other testosterone agents after more than one year of treatment. In addition, due to performing a data lock, the number of cases decreased after each follow-up visit, which leads to a decrease in power in the analyses of the 18th, 24th, and 36th months.

Blood samples were obtained at fixed time points during the follow-up period, independent of the time interval to the last administration and at baseline randomly relative to the menstrual cycle. This may have led to fluctuations in measured serum testosterone and estradiol levels. Unfortunately, type of contraceptives was not logged in Amsterdam, whereas 77% of those using contraceptives in Ghent (n=70) were using progestogens (21.1% oral progestogens, 11.4% injectable progestogens). In

addition, the results of the current study may not be applicable to other regions with different obesity rates.

Strengths

Despite these limitations, this study has a number of strengths. To our knowledge, this is the largest prospective study to date in which serum estradiol levels in AFAB transgender people receiving testosterone were evaluated. Our study cohorts are well defined and participants adhered to a strict treatment regimen. In addition, this is the first large study that directly compared the effects of testosterone gel, intramuscular testosterone esters, and intramuscular testosterone undecanoate on serum estradiol values.

Conclusion

Testosterone administration in AFAB transgender people resulted in lower serum estradiol levels compared to baseline, most likely due to suppression of endogenous estradiol production. This hypothesis was supported by the observed comparable serum estradiol levels in people with versus without hystero-oophorectomy or contraceptives after the first year of HT. Therefore, the need for estradiol lowering agents (e.g. aromatase inhibitors) seems unlikely. We do not assume that testosterone therapy in AFAB transgender people could be a risk factor for undesired estrogenic effects. We also conclude from previous research that the observed decrease in serum estradiol levels does not lead to adverse outcomes, unlike in hypogonadal females. However, serum estradiol levels after initiation of testosterone therapy in this population remained higher than those observed in the male control group.

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