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LOWER SERUM OESTRADIOL LEVELS IN ASSIGNED FEMALE AT BIRTH TRANSGENDER PEOPLE AFTER INITIATION OF TESTOSTERONE THERAPY

Xavier-Philippe AERS

Promotor: Prof. dr. Guy T'Sjoen

Masterproef voorgedragen in de master in de specialistische geneeskunde
Endocrinologie

UNIVERSITEIT GENT

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Lower serum oestradiol levels in assigned female at birth transgender people after initiation of testosterone therapy: results from the European Network for the Investigation of Gender Incongruence.

Abstract

Introduction

The role of serum oestradiol levels in assigned female at birth (AFAB) transgender people receiving testosterone therapy has not been elucidated and it is unknown whether serum oestradiol levels change after initiation of testosterone therapy, or if these levels should even be monitored. The effects of altered oestradiol levels in AFAB people receiving testosterone therapy on health outcomes have not been described.

Methods

This prospective cohort study was part of the European Network for the Investigation of Gender Incongruence (ENIGI). Serum oestradiol levels were prospectively assessed in 746 AFAB transgender people during a three-year follow-up period, starting at the initiation of hormone treatment. Data were analysed cross-sectionally and prospectively.

Results

Oestradiol levels decreased from 45.49 [24.00 – 102.15] pg/mL (baseline) to 42.77 [30.24 – 59.38] pg/mL (three months of hormone therapy (HT)) (mean - 17.13, 95% CI -23.82 – -10.56, P<0.001), remaining stable over the next year with a gradual increase between eighteen and thirty-six months (mean +7.47, 95% CI 2.16 – 12.77, P<0.001). Serum oestradiol levels were lower in contraceptive users at baseline and after three months, compared to non-contraceptive users (P<0.001). Compared to baseline serum oestradiol levels in the male control group, serum oestradiol levels were higher in AFAB people (P<0.001).

Prospective changes in serum oestradiol were positively correlated to prospective changes in serum LH ($\rho = 0.107$, $P < 0.001$) and negatively correlated to changes in serum FSH levels ($\rho = -0.167$, $P < 0.001$) and changes in BMI ($\rho = -0.082$, $P < 0.001$). There was no correlation between changes in serum oestradiol levels and serum total testosterone levels as well as total body fat percentage.

Conclusion

Testosterone administration in AFAB transgender people results in lower serum oestradiol levels. Therefore, there is no evidence that testosterone therapy in AFAB people could be a risk

factor for gynaecological cancers or the cause of undesired oestrogenic effects. However, the oestradiol levels after initiation of testosterone therapy in this population remain higher than those observed in the male control group. We conclude from previous research that the observed lower serum oestradiol levels do not lead to adverse outcomes.

Lower serum oestradiol levels in assigned female at birth transgender people after initiation of testosterone therapy: results from the European Network for the Investigation of Gender Incongruence.

Introduction

Transgender people are persons whose gender identity differs from their birth-assigned sex. When associated with distress or impairment in social, educational, or other important areas of functioning, gender dysphoria may occur. Transgender people may request gender affirming hormonal treatment to alleviate their gender dysphoria (1), aimed at suppressing the secondary sex characteristics of the birth-assigned sex and inducing the secondary sex characteristics of the experienced gender. Gender affirming hormone therapy in assigned female at birth (AFAB) transgender people who desire masculinizing hormonal therapy consists of testosterone, which can be administered intramuscularly, transdermally or orally (1).

Testosterone therapy in AFAB transgender people is aimed at achieving serum testosterone levels in the cisgender male reference ranges (1), although it is not known if serum oestradiol levels should be evaluated and/or influenced. It is possible that serum oestradiol levels increase after the initiation of testosterone therapy through aromatization of testosterone to oestradiol (1), although the effects of testosterone therapy on serum oestradiol levels in AFAB transgender people are inconclusive in the current literature, with some papers reporting no prospective change in serum oestradiol levels (2–4), whereas others report a significant decrease (5–8).

The administration of testosterone therapy has been suggested as a risk factor for endometrial cancer (9), through the aromatization of testosterone to oestradiol, which might result in increased serum oestradiol levels. However, no actual cases of endometrial cancer in transgender people have been reported, although three cases of ovarian cancer have been described (10–12). Studies on oncological risk in AFAB transgender people after the initiation of testosterone therapy remain inconclusive and lack power (13–19). Therefore it is advised to adhere to the screening protocols for the general population, according to birth-assigned sex, depending on the anatomical situation (1).

There are also concerns that testosterone treatment in AFAB transgender people may result in undesired oestrogenic effects including menstrual cycle, pelvic pain and gynaecomastia, due to aromatization of androgens (3). Low oestradiol on the other hand can lead to decreased bone density, vaginal dryness, decreased sexual desire and menopausal symptoms in females (20).

The aim of this study is to evaluate the evolution of measured serum oestradiol levels, to explore the underlying mechanism of changes in serum oestradiol levels (exogenous and/or endogenous contributions) and to determine need for measuring serum oestradiol levels in AFAB transgender people after initiation of testosterone therapy in a larger cohort

Methods

Cohort

The ENIGI study is a multicenter prospective cohort study conducted in four European treatment centers (Ghent, Oslo, Florence, and Amsterdam) (21). For the present substudy, data from Ghent and Amsterdam were selected due to logistical reasons. From February 2010 until July 2018, 1730 transgender persons have been included in the Belgian–Dutch sample of the European Network for the Investigation of Gender Incongruence (ENIGI) study, of which 858 assigned female at birth (AFAB) (figure 1). All patients were at least 16 years old (in the Belgian cohort) or 17 years old (in the Dutch cohort) and underwent a standardized diagnostic procedure to confirm the diagnosis of gender dysphoria before initiating treatment (21). Patients were included in the ENIGI endocrine protocol when they started gender-affirming hormonal treatment. Every patient was treated in accordance with the World Professional Association for Transgender Health Standards of Care, edition 7 (22). Exclusion criteria were previous use of gender-affirming hormones and insufficient knowledge of the native languages (Dutch or French). At the start of the study, patients received oral and written information about the ENIGI endocrine protocol. A written informed consent was obtained according to the institution's guidelines. Short-term follow-up currently consists of a baseline visit and visits after 3, 6, 9, 12 and 36 months in Amsterdam and at 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. Baseline oestradiol values were available in 746 AFAB people (86.9%). Serum oestradiol levels in AFAB transgender people were compared to serum oestradiol levels in a male control group (n=224, age 28.0 [21.0 – 42.0], range 16-69).

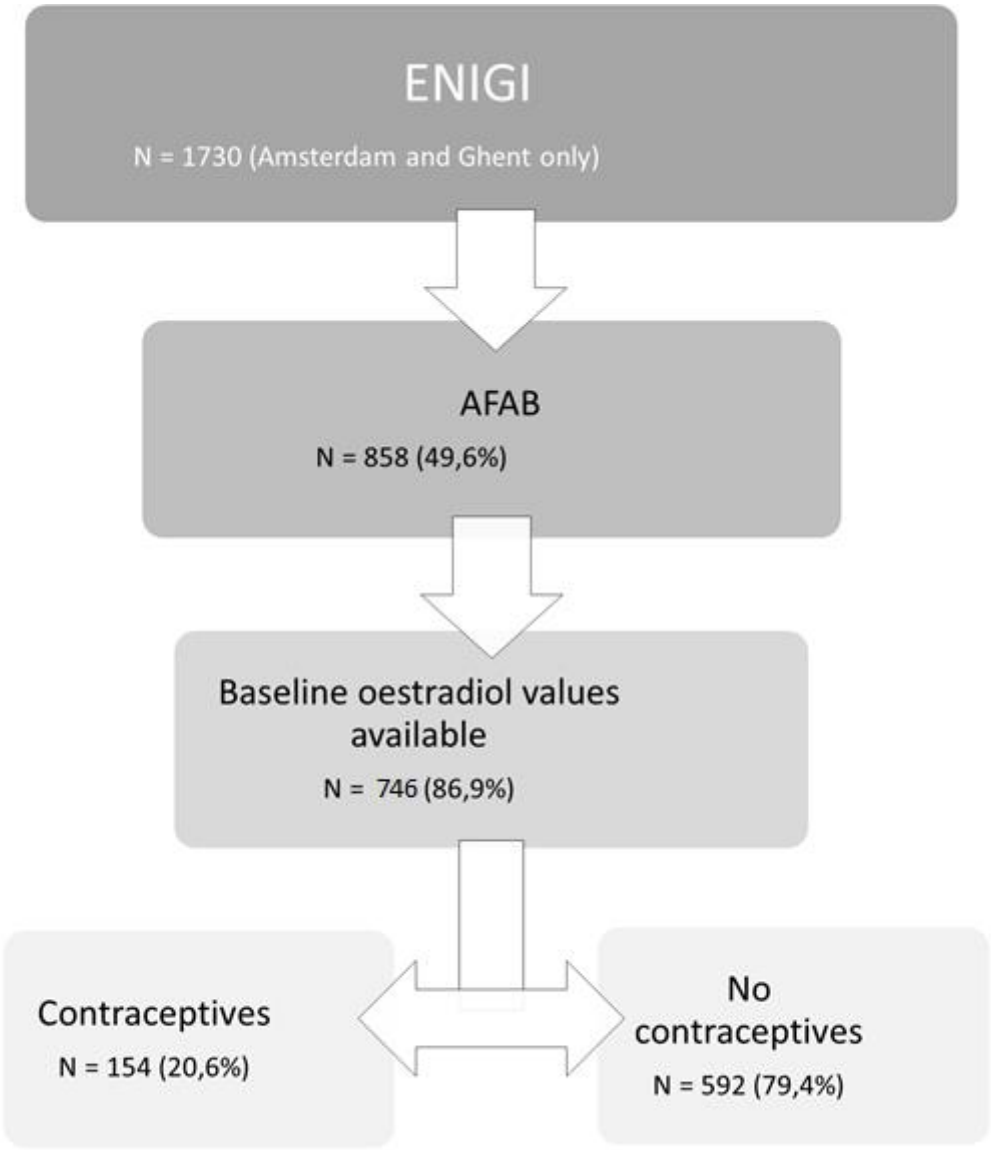


Figure 1: Flowchart for the inclusion of the assigned female at birth (AFAB) study population.

Gender affirming hormonal treatment

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 testosterone esters (Sustanon® 250 mg every 2 weeks, n=175) or as testosterone undecanoate (Nebido® 1000 mg every 12 weeks, n=46) (missing: 49). As follow-up only consisted of 12 months in Amsterdam, testosterone therapy after one year of follow-up only consisted of testosterone undecanoate. Usually, suppression of menstrual bleeding occurs after initiation of testosterone therapy. A progestational agent or a GnRH analogue can be added to

the treatment regimen if suppression of the menses is desired or menstrual bleeding does not cease.

At baseline, 154 (20.6%) people were using contraceptive agents to suppress menses.

Laboratory analyses

In Ghent, competitive chemiluminescent immunoassays were run for oestradiol (E170 Modular, Roche, Gen III, LOQ 25 pg/mL, interassay CV 3.2%), serum testosterone (E170 Modular, Roche, Gen II, LOQ 10 ng/dL (0.4 nmol/L), interassay CV 2.6%), LH (E170 Modular, Roche, Gen III, interassay CV 3.48%, LOQ 0.1 mIU/mL), and FSH (E170 Modular, Roche, Gen III, interassay CV 3.3%, LOQ 0.1 mIU/mL), and for SHBG, a sandwich-type chemiluminescent immunoassay was employed (E170 Modular, Roche, Gen III, interassay CV 4.06%, LOQ 0.35 mIU/mL).

Before March 19, 2015, oestradiol was measured using an E170 Modular (Gen II; Roche Diagnostics, 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 Diagnostics, Mannheim, Germany).

In Amsterdam, oestradiol was also measured using an E170 Modular (Gen II; Roche Diagnostics) until March 19, 2015. Thereafter, oestradiol was measured using an E170 Modular (Gen III; Roche Diagnostics); the same conversion formula as in Ghent was used to convert oestradiol values. Testosterone was measured using a radioimmunoassay (RIA) (Coat-A-Count; Siemens, Los Angeles, CA, USA) with an interassay CV of 7–20% and a LOQ of 1 nmol/L until January 2013. Thereafter, testosterone was measured using competitive immunoassay (Architect; Abbott, Abbott Park, IL, USA) with an interassay CV of 6–10% and a LOQ of 0.1 nmol/L. RIA values were converted to 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, FSH, and SHBG were measured using chemiluminescent microparticle immunoassay (Architect System; Abbott), with an interassay CV of 4% and a LOQ of 2 U/L for LH, FSH, and SHBG.

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 and specific regions as the arm region, leg region, trunk region, android region and gynoid region were measured using DXA. All scans were analyzed using software, version 13.5.3. More information can be found in Klaver et al. (23)

Data analysis

Data were analyzed using IBM SPSS 24.0 (SPSS, Chicago, IL, USA). 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 as well as cross-sectionally and compared to baseline oestradiol scores in 224 assigned male at birth transgender persons (AMAB). Prospective differences in serum oestradiol levels as well as prospective differences in the serum oestradiol to serum testosterone ratio were calculated. Differences between groups were analyzed by unpaired Student's t-test (normally distributed data) and the Mann–Whitney U-test (non-normally distributed data). As serum oestradiol levels were not distributed normally, data were log transformed. To evaluate oestradiol differences in time, a mixed model was applied to the logistic transformation of the outcome variable oestradiol, with visit (number of months) as fixed factor and with a random intercept for baseline serum oestradiol levels. 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, FSH and SHBG 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 oestradiol 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 oestradiol, testosterone, LH, FSH and SHBG 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 may influence correlations between other biochemical parameters, subgroup analyses were performed for prospective changes in sex steroids in the group using

contraceptives versus those who did not. Bonferroni-Holm correction was applied to all P-values to limit the chance of type II error (24).

Results

Cross-sectional analyses

Baseline oestradiol values were available in 746 AFAB people (86.9%). Baseline characteristics are shown in table one (table 1).

	AFAB transgender people (n = 746)
Age (years)	22.0 [19.0 – 26.0]
BMI (kg/m^2)	23.6 [21.3 – 28.8]
Total body fat (%)	36.5 [31.5 – 40.3]
Serum oestradiol (pg/mL)	45.5 [24.0 – 102.2]
Serum testosterone (nmol/L)	1.20 [0.90 – 1.50]
Serum SHBG	51.0 [32.5 – 72.8]
Serum LH (U/L)	4.20 [2.30 – 7.03]
Serum FSH (U/L)	5.20 [3.25 – 7.20]
On contraceptives (n yes, %)	154 (20.6%)
Mastectomy (n yes, %)	11 (1.5%)
Hysterectomy-oophorectomy (n yes, %)	3 (0.4%)

Table 1: Baseline characteristics of the AFAB population in whom baseline serum oestradiol values were available. For values that are not normally distributed, median values and IQR [P25 and P75] are shown. LH = luteinizing hormone, FSH = follicle stimulating hormone, SHBG = sex-hormone binding globulin.

Over the entire study population (all data pooled), people using contraceptives had lower serum oestradiol levels (25.9 [14.5 – 40.6] pg/mL), compared to those not using contraceptives (25.9 [14.5 – 40.6] pg/mL, P<0.001). Cross-sectional analyses revealed lower serum oestradiol levels (20.70 [9.47 – 37.06] pg/mL) in contraceptive users at baseline and after three months of HT

(32.21 [23.77 – 42.93] pg/mL) and (2.3 [1.5 – 22.0] pg/mL), compared to non-contraceptive users at baseline (43.0 [24.00 – 102,97] pg/mL, P<0.001) and after three months of HT (55.66 [29.45 – 111.51] pg/mL, P=0.018) (other visits: P-value ranges 0.080 – 0.628).

Cross-sectionally, the use of testosterone undecanoate resulted in lower serum oestradiol levels in the entire study population (limited to one year follow-up), compared to testosterone gel and testosterone esters (P<0.001). Lower serum oestradiol levels were observed in testosterone undecanoate users after 12 months (37.8 [29.7 – 49.0] pg/mL) compared to gel (46.6 [30.0 – 61.7] pg/mL) and testosterone esters (43.3 [31.9 – 63.5] pg/mL, P=0.005) months, but not at the other visits (P-values range 0.034 – 0.563, adjusted Bonferroni-Holm significance level P<0.0125).

All data pooled, people on testosterone therapy who underwent hystero-oophorectomy had lower serum oestradiol levels (34.11 [32.31 – 35.90] pg/mL) compared to those who did not undergo this procedure (yet) (58.92 [56.63 – 61.22] pg/mL, P < 0.001), independent of duration of testosterone therapy. The serum oestradiol levels were higher in people who did not undergo hystero-oophorectomy (yet) after 9 (30.9 [29.2 – 48.0] pg/mL) and 12 (43.0 [31.9 – 57.1] pg/mL) months, compared to those who underwent this procedure (30.9 [23.2 – 35.7] pg/mL with P = 0.007 and 30.2 [25.0 – 43.1] pg/mL with P<0.001, respectively).

Over the entire study population, no correlation was observed between serum oestradiol levels and total body fat percentage (P=0.700). Cross-sectional analyses revealed a positive correlation between serum oestradiol levels and total body fat percentage after 12 months of HT ($\rho = 0.233$, P = 0.002), but not after 24 (P = 0.733) or 36 months (P = 0.134) of HT. Body composition data was available in only 35 people after 24 months and 24 people after 36 months of HT.

Comparison to cisgender male serum oestradiol levels

Compared to serum oestradiol levels in males (27.1 [24.9 – 33.8] pg/mL), serum oestradiol levels were higher in AFAB people at baseline (45.5 [24.0 – 102.1] pg/mL, P<0.001) and after three (42.8 [30.2 – 59.4] pg/mL, P<0.001), six (39.0 [29.8 – 50.0] pg/mL, P<0.001), nine (37.1 [28.9 – 46.4] pg/mL, P<0.001), twelve (42.2 [30.5 – 55.7] pg/mL, P<0.001), twenty-four (31.8 [25.0 – 43.9] pg/mL, P<0.001) and thirty-six (36.5 [25.0 – 46.2] pg/mL, P=0.001) months. However, serum oestradiol levels were comparable to levels of males in AFAB people who underwent hystero-oophorectomy during the first 1.5 years of HT (P-values range 0.025 –

0.899, adjusted Bonferroni-Holm P-value <0.01), and once again higher after two (31.8 [25.0 – 43.9] pg/mL, P=0.006) and three (36.5 [25.0 – 46.2] pg/mL, P<0.001) years of HT.

Prospective analyses

Prospective changes in serum levels of sex steroids

Upon initiation of gender affirming hormone therapy, serum testosterone levels increased from 1.2 [0.9 – 1.5] nmol/L to 19.0 [12.0 – 30.0] nmol/L (mean +23.4, 95% CI 21.5 – 25.3, P < 0.001) over the first three months, with a small decrease over the next three months (mean -3.8, 95% CI -6.2 – -1.5, P < 0.001), to 18.5 [14.0 – 24.4] nmol/L after six months. The measured values remained stable over the next three months (P = 0.249), although they increased from 21.1 [14.2 – 26.1] nmol/L to 23.0 [16.0 – 33.2] nmol/L between nine and twelve months of follow-up (mean +7.8, 95% CI 5.0 – 10.6, P < 0.001), again decreasing over the next six months (mean -9.4, 95% CI -12.1 – -6.8, P < 0.001) to 18.3 [15.0 – 18.3] nmol/L after eighteen months, remaining stable thereafter (P-values range: 0.030 – 0.498).

As shown in figure 2, serum oestradiol levels decreased from 45.49 [24.00 – 102.15] pg/mL at baseline to 42.77 [30.24 – 59.38] pg/mL after three months of HT (mean - 17.13, 95% CI - 23.82 – -10.56, P<0.001), remaining stable over the next year with a gradual increase between eighteen and thirty-six months, from 28.80 [25.00 – 36.65] pg/mL to 36.50 [25.00 – 46.20] pg/mL (mean +7.47, 95% CI 2.16 – 12.77, P<0.001) (figure 2).

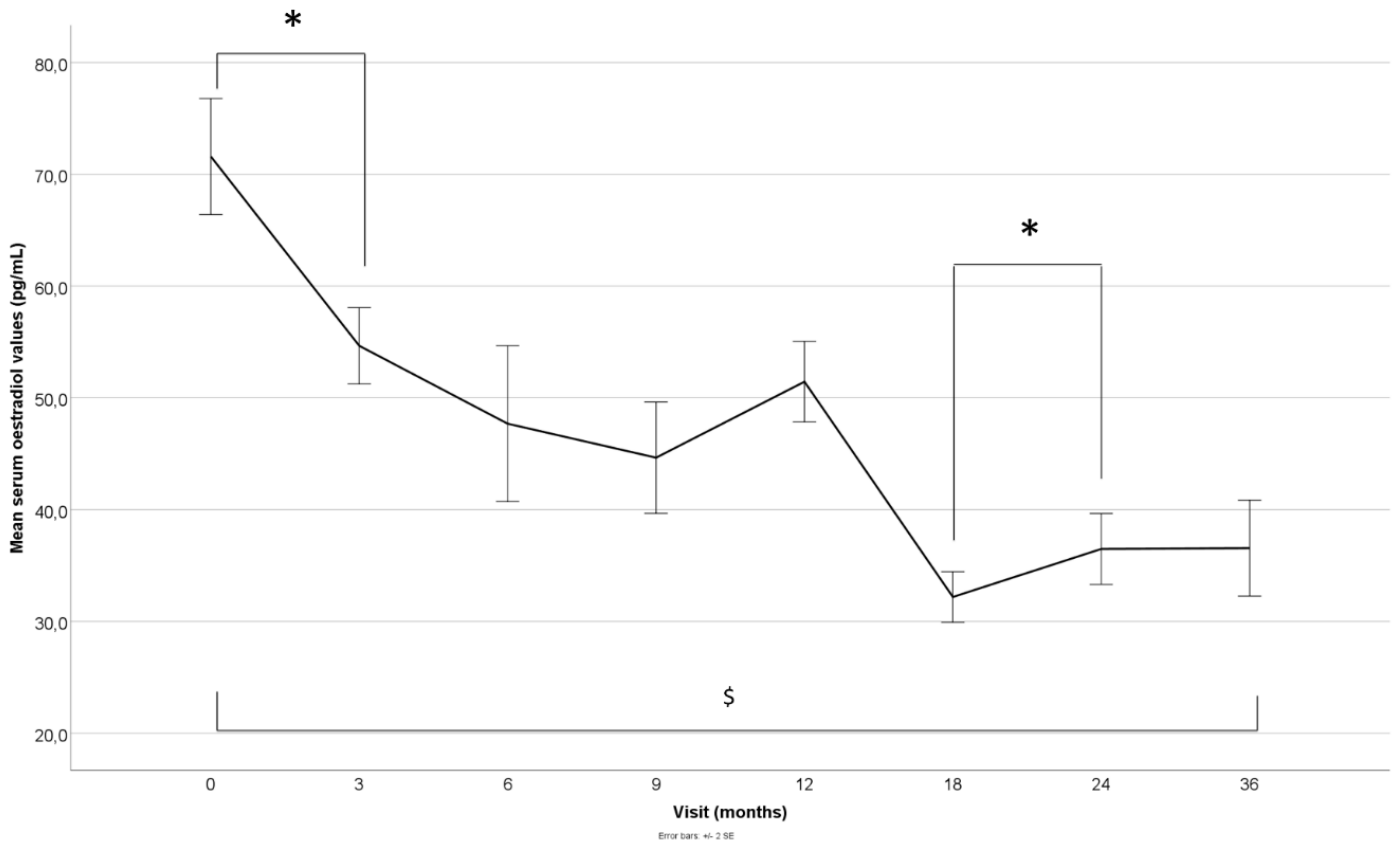


Figure 2: Prospective Analysis of serum oestradiol levels in AFAB Transgender People. Line graphs for the evolution of mean serum oestradiol levels in AFAB transgender people on gender affirming hormonal treatment with standard error. * indicates a significant decrease in serum oestradiol levels over the 0 to 3 month timeframe and 18 to 24 months timeframe, $ indicates a significant decrease over the entire study follow-up.

Factors associated with prospective changes in serum oestradiol levels

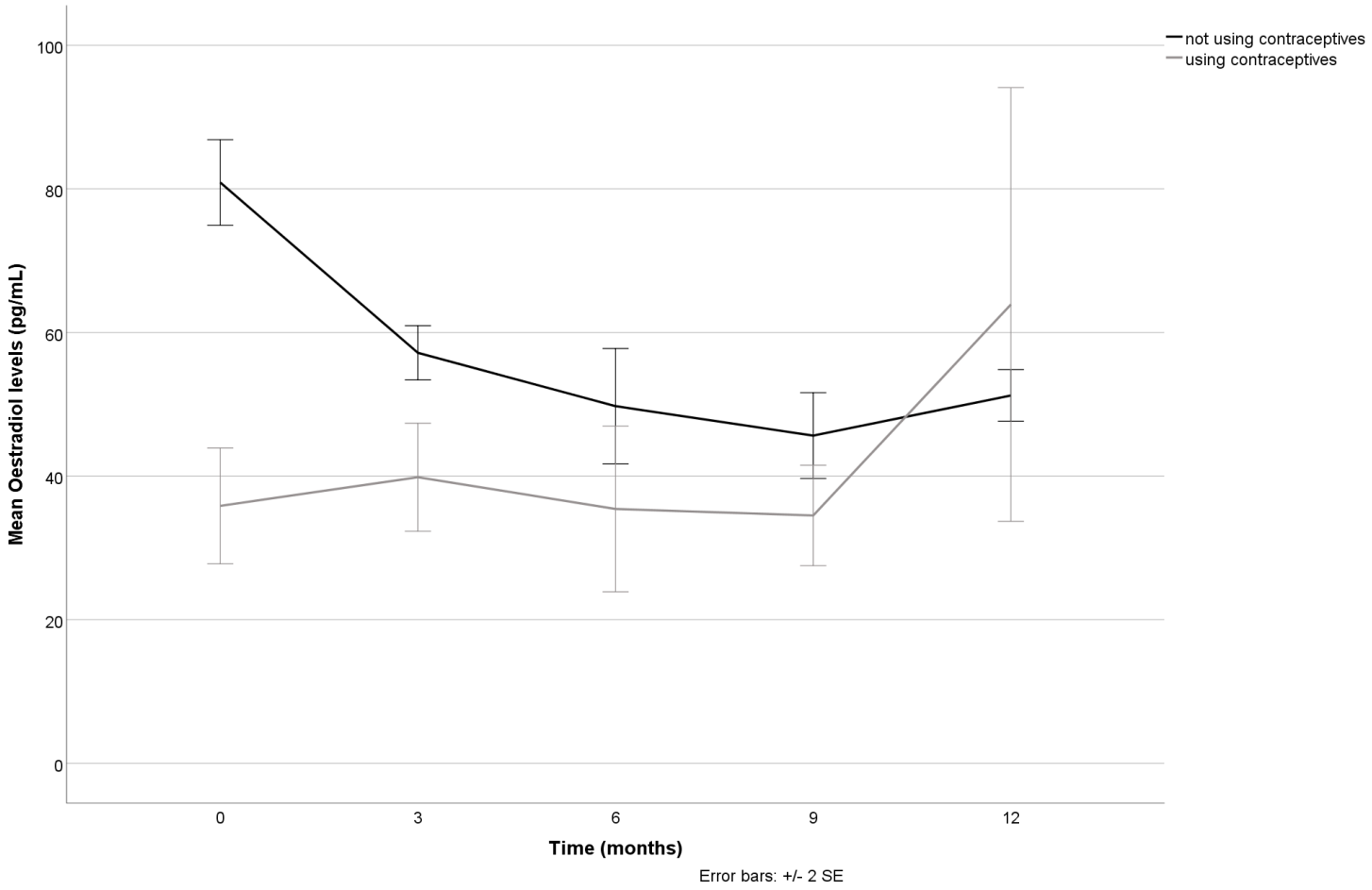
Serum levels of sex steroids

Prospective changes in serum oestradiol over the entire follow-up period were positively correlated to changes in serum LH ($\rho = 0.107$, $P < 0.001$) and SHBG ($\rho = 0.233$, $P < 0.001$) levels and negatively correlated to changes in serum FSH levels ($\rho = -0.167$, $P < 0.001$) over the entire follow-up period. There was no correlation between changes in serum oestradiol levels and changes in serum testosterone levels ($P = 0.973$).

Correlations between prospective changes in serum oestradiol and serum testosterone, LH, FSH and SHBG differed during different follow-up visits. Changes in serum oestradiol values were positively correlated to changes in LH ($\rho = 0.156$, $P < 0.001$) and inversely correlated to serum FSH ($\rho = -0.295$, $P = 0.001$) levels after three months. Changes in serum oestradiol levels were once again positively correlated to changes in serum LH ($\rho = 0.164$, $P = 0.001$), testosterone ($\rho = 0.147$, $P = 0.003$) and SHBG levels ($\rho = 0.222$, $P < 0.001$) after twelve months. After twenty-four months, changes in serum oestradiol levels were only (inversely) correlated to changes in serum FSH levels ($\rho = -0.356$, $P < 0.001$). After thirty-six months, changes in serum oestradiol levels were only correlated to serum SHBG levels ($\rho = 0.413$, $P = 0.004$).

Contraceptive use

The use of contraceptives did not impact the changes in serum oestradiol levels over the entire study population (all data pooled, $P = 0.257$), as well as each prospective time interval (P-values ranges for prospective changes over individual follow-up visits: 0.484 – 0.825) (figure 3).



Visit (months)	0	3	6	9	12
Not using contraceptives	656/746 87,9%	561/651 86,2%	158/193 81,9%	136/159 85,5%	476/498 95,6%
Using contraceptives	90/746 12,1%	90/651 13,8%	35/193 18,1%	23/159 14,5%	22/498 0,4%

Figure 3: Prospective analysis of serum oestradiol levels in contraceptive users versus non-contraceptive users. Line graphs for the evolution of mean serum oestradiol 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, % of total valid.

In AFAB people not taking contraceptives, a prospective decrease in serum oestradiol levels over the first three months was correlated to a prospective decrease in serum LH levels (ρ = 0.180, P < 0.001 and ρ = 0.140, P < 0.001). In contraceptive users, a prospective decrease in serum oestradiol levels over the first three months were positively related to changes in serum SHBG (ρ = 0.302 P<0.001 and ρ = 0.232, P=0.003, respectively) and inversely related to serum FSH levels (ρ = -0.578, P=0.001 and ρ = -0.372, P<0.001). Prospective changes in serum oestradiol levels over the first three months were also correlated to prospective changes in serum testosterone levels (ρ = 0.504, P < 0.001). In this group, there was no correlation between prospective changes in serum oestradiol and prospective changes in serum LH (P = 0.954).

Type of testosterone therapy

The use of testosterone undecanoate resulted in more decrease in serum oestradiol values over twelve months (mean -32.61 pg/mL, 95% CI -44.70 – -20.52), compared to testosterone esters (mean -13.10 pg/mL, 95% CI -26.68 – 0.47, P<0.001) or testosterone gel (mean -6.91 pg/mL, 95% CI -23.15 – 9.32, P=0.001) (figure 5b) Type of testosterone therapy did not influence prospective changes in serum oestradiol values over the first three to nine months (P-values range: 0.078 – 1.000).

Body composition

Figure 4b shows an increase in mean BMI values over the first three months of HT, from 25.35 ± 5.67 kg/m^2 at baseline to 26.32 ± 5.69 kg/m^2 after three months (mean + 0.980, 95% CI 0.328 – 0.1632, P = 0.003), remaining stable over the next nine months of HT (P-values range 0.339 – 0.512) (figure 4a). During the second year of HT, a decrease in BMI values was observed (mean -1.498, 95% CI -2.698 – -0.297, P = 0.015), to BMI values comparable to baseline (P = 0.277). Mean BMI values remained stable over the third year of HT (P = 0.511). As shown in figure 4a, mean total body fat percentage decreased over the first (mean -4.546, 95% CI -6.071 – -3.022, P<0.001) and second (mean -7.386, 95% CI -10.242 – -4.259, P<0.001) year of HT, with no further changes in body fat percentages over the third year of HT (P = 0.967) (figure 4).

Prospective changes in serum oestradiol levels were not correlated to prospective changes in total body fat percentage (P=0.688) over the entire follow-up period. Prospective changes in serum oestradiol over the entire follow-up period were negatively correlated to changes in BMI (ρ = -0.082, P < 0.001) over the entire follow-up period.

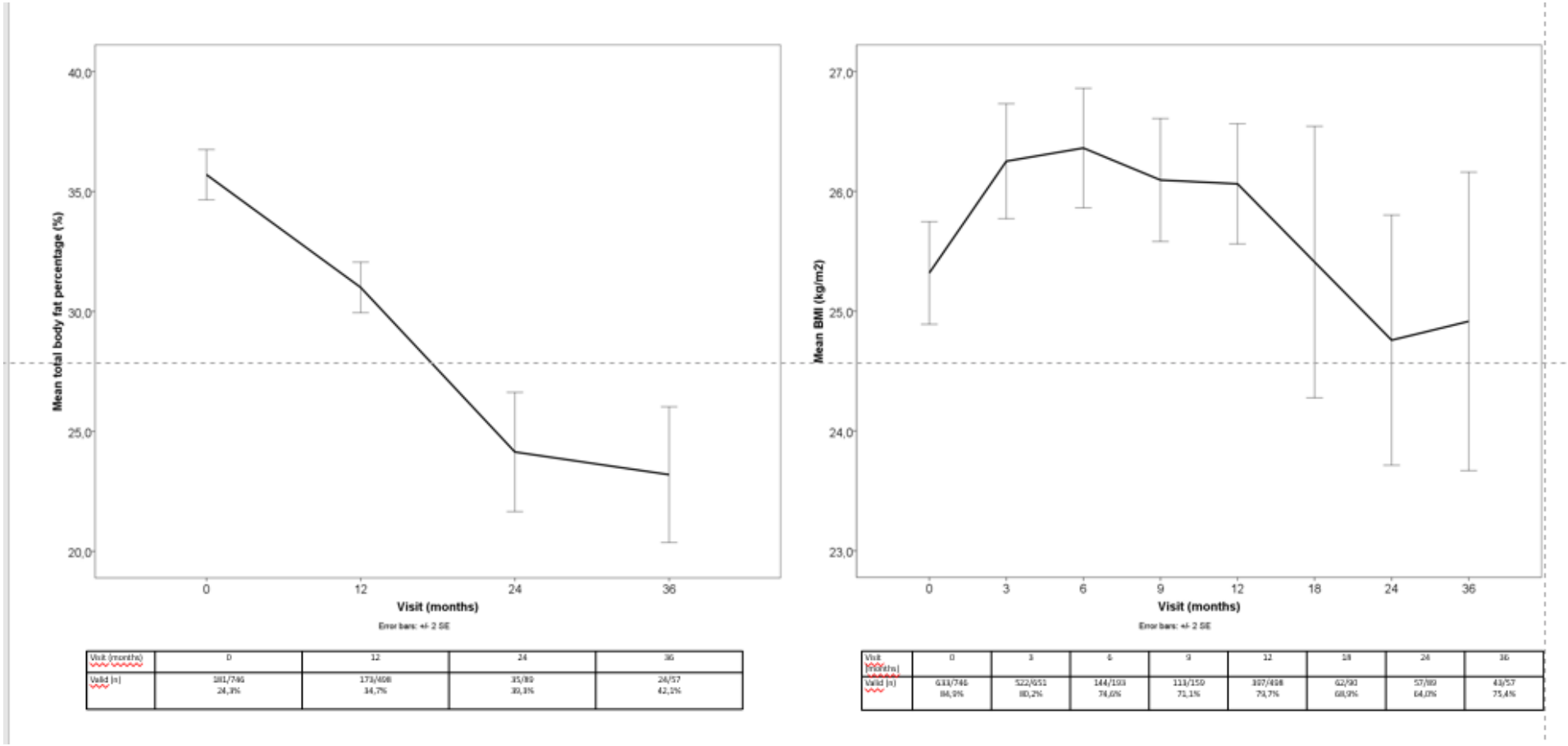
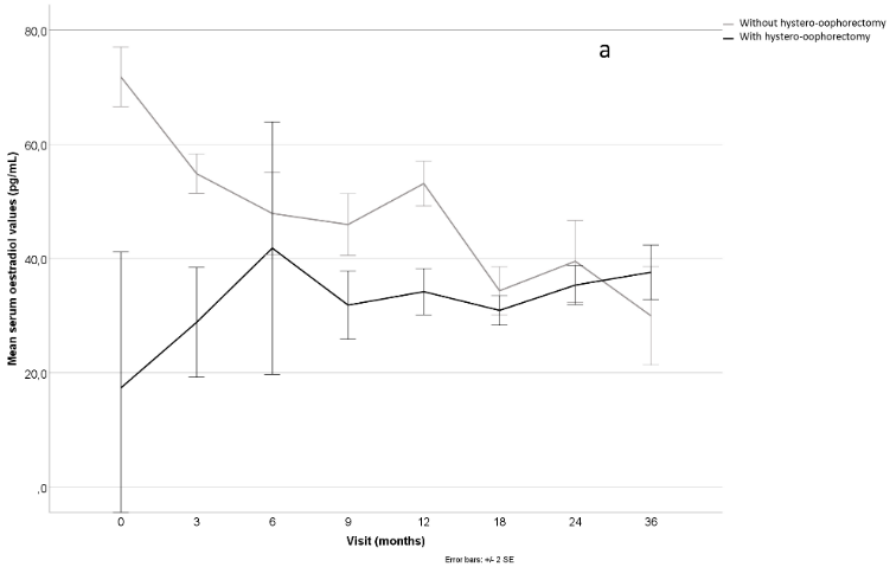


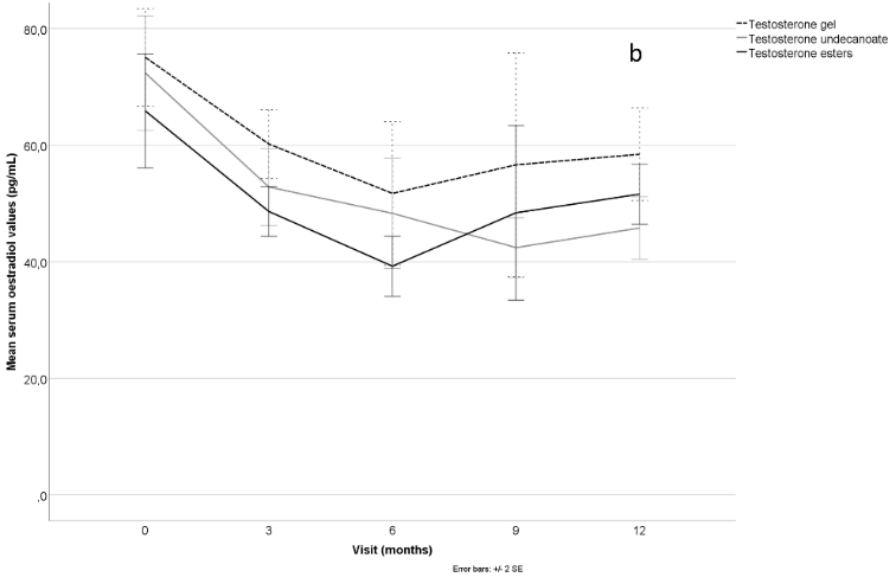
Figure 4: Prospective analysis body fat percentage (4a) and body mass index (BMI, 4b) AFAB transgender after initiation of testosterone therapy. Line graphs for the evolution of mean body fat percentage in AFAB transgender people on gender affirming hormonal treatment (4a) and line graphs for the evolution of mean BMI in AFAB transgender people on gender affirming hormonal treatment (4b), 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, % of total valid.

Surgery

People who underwent hystero-oophorectomy experienced a steep increase in serum oestradiol levels over nine (mean +38.20 pg/mL, 95% CI 9.50 – 38.20) and twelve months (mean +33.42 pg/mL, 95% CI 12.97 – 53.86), whereas those who did not undergo hystero-oophorectomy experienced a decrease in serum oestradiol levels (nine months: -26.87 pg/mL, 95% CI -41.03 – -12.72, $P = 0.016$ and 12 months: -17.60 pg/mL, 95% CI -26.13 – -9.07, $P < 0.001$) (figure 5a). However, undergoing hysterectomy did not influence prospective serum oestradiol levels over three ($P = 0.050$), six ($P = 0.424$), twenty-four ($P = 0.165$) and thirty-six ($P = 0.234$) months.



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%	68/57 76,4%	49/57 86,0%



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 5: Prospective analysis of serum oestradiol levels in AFAB transgender people with hystero-oophorectomy versus those without hystero-oopherectomy (5a) and prospective analysis of serum oestradiol levels in AFAB transgender people, based on mode of testosterone administration. (5b) Line graphs for the evolution of mean serum oestradiol levels in AFAB transgender people on gender affirming hormonal treatment with substratification in groups who underwent hystero-oophorectomy versus those who did not undergo hystero-oophorectomy (yet) (5a) and transgender people on different types of testosterone (5b), 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, % of total valid.

Discussion

Testosterone administration in AFAB transgender people is associated with lower serum oestradiol levels, which already becomes apparent during the first three months of gender affirming hormone therapy. Therefore, there is no evidence that testosterone therapy in AFAB people could be a risk factor for gynaecological cancers or the cause of undesired oestrogenic effects including menstrual cycle, pelvic pain and gynaecomastia.

The lower serum oestradiol levels don't seem to result in adverse outcomes related to hypogonadism in females, as decreased bone mineral density (25) or decreased sexual desire (26) have not been described in AFAB transgender people after initiation of testosterone therapy. Moreover, our results display that serum oestradiol levels after start of HT in this population remain higher than those observed in the male control group and those described in post-menopausal females (27). This may be explained by enzymatic conversion of testosterone to oestradiol by aromatase in adipose tissues, which is higher in visceral fat of birth-assigned females, compared to males (28). When interpreting these results, it is important to note that it's not possible to evaluate the distinction between the endogenous oestradiol that's present at baseline (part of which may or may not remain active after start of HT) and the oestradiol that develops after initiation of exogenous testosterone therapy (through aromatization of testosterone).

We have examined several potential mechanisms causing the lower serum oestradiol levels in AFAB people, including decreased aromatase action due to changes in body composition, more specifically a decrease in body fat percentage, and feedback inhibition of luteinizing and follicle stimulating hormone.

The current study describes an increase in BMI values over the first year of HT, returning to baseline thereafter, a decrease in total body fat percentage over the first two years of HT and a decrease in serum oestradiol values after initiation of testosterone therapy. Although a decrease in serum oestradiol values in birth assigned females may hypothetically be explained by a decrease in body fat tissue, resulting in a decrease in aromatase activity, the current study was not able to find a correlation between prospective changes in oestradiol and prospective changes in body fat percentage. These findings may be limited by the small number of people in whom DXA analysis was available. In addition, a prospective decrease in serum oestradiol 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, with a decreasing body fat percentage and increasing lean body mass percentage (29).

Subgroup analyses revealed a negative correlation between prospective changes in serum oestradiol levels and prospective changes in FSH, which may suggest an intact oestradiol – FSH feedback loop in AFAB people, without complete suppression of the gonadotrophic axis by testosterone. Contrary to our expectations however, we observed a positive correlation between prospective changes in serum LH and serum oestradiol. The reason for this is unclear. A possible explanation might be that the serum oestradiol values are lowered to levels insufficient for initiation of the mid-cyclic increase in serum LH levels and, subsequently, ovulation. However, we should also take into account that exogenous administration of testosterone can confound these findings, rendering interpretation of gonadotropins as a marker of endogenous oestradiol production useless in AFAB transgender people on testosterone therapy.

Lower serum oestradiol levels were observed in AFAB people using long-acting injectable testosterone undecanoate, compared to people using injectable testosterone esters or transdermal testosterone gel. We hypothesize that this is due to the pharmacological profile of testosterone undecanoate, which is less susceptible to fluctuations in serum testosterone levels and may provide a more sustained gonadotropin and menstrual cycle suppression, compared to the more fluctuating serum levels often observed in people using testosterone esters and testosterone gel.

Although prospective changes in serum testosterone levels were not correlated to prospective changes in serum oestradiol levels in the entire study population, a positive correlation between prospective changes in serum testosterone and oestradiol levels was observed in contraceptive users over the first three months of testosterone use. The main contraceptive 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 oestradiol values comparable to levels observed in early follicular menstrual phase (30). We hypothesize that addition of testosterone therapy resulted in aromatization of testosterone, resulting in increased serum oestradiol levels in contraceptive users. This is in accordance with the observed increase in AFAB people who underwent hystero-oophorectomy in the current study.

Our study results may have been affected by several limitations. Follow-up in Amsterdam only consisted of visits at baseline and after 3, 6, 9, 12 and 36 months, leading to a decrease in sample size and power in the analyses of the 18^{th}, 24^{th} month. As a further consequence of this, along

with the fact that AFAB people included in the ENIGI study in Ghent exclusively received testosterone undecanoate injections, we were not able to prospectively evaluate serum oestradiol 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. This may have led to fluctuations in measured serum testosterone and oestradiol levels.

Despite these limitations, this study has a number of strengths. To our knowledge, this is the largest prospective study to date in which serum oestradiol levels in AFAB 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 oestradiol values.

Conclusion

Testosterone administration in AFAB transgender people results in lower serum oestradiol levels. Therefore, there is no evidence that testosterone therapy in AFAB people could be a risk factor for gynaecological cancers or the cause of undesired oestrogenic effects. However, the oestradiol levels after initiation of testosterone therapy in this population remain higher than those observed in the male control group.

Although several possible causes have been investigated in the current study, the exact underlying mechanism remains unclear. We conclude from previous research that the observed lower serum oestradiol levels do not lead to adverse outcomes, as in hypogonadal females.

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Nederlandse samenvatting

Lager serum oestradiolgehalte bij transmannen na start van testosterontherapie

Inleiding

De rol van het oestradiolgehalte bij transmannen onder behandeling met testosteron is nog niet ten volle opgehelderd. Het is onduidelijk of het serum oestradiolgehalte verandert na start van testosterontherapie en of deze waarden überhaupt gemonitord moeten worden. Het effect van gewijzigde oestradiolgehaltes bij transmannen op gezondheidsgerelateerde outcomes werd nog niet beschreven.

Methoden

Deze prospectieve corhortstudie was een onderdeel van de European Network for the Investigation of Gender Incongruence (ENIGI). Serum oestradiolgehaltes werden prospectief geëvalueerd bij 746 transmannen gedurende een opvolgingsperiode van drie jaar, vanaf het opstarten van hormonale therapie. De gegevens werden cross-sectioneel en prospectief geanalyseerd.

Resultaten

Het oestradiolgehalte daalde van 45.49 [24.00 – 102.15] pg/mL (baseline) tot 42.77 [30.24 – 59.38] pg/mL (na drie maanden hormonale therapie) (mean - 17.13, 95% CI -23.82 – -10.56, P<0.001), bleef stabiel gedurende het volgende jaar met een geleidelijke toename tussen 18 en 36 maanden (mean +7.47, 95% CI 2.16 – 12.77, P<0.001). Serum oestradiol was lager was bij patiënten die contraceptiva gebruikten bij baseline en na drie maanden, in vergelijking met zij die geen contraceptiva gebruikten (P<0.001). In vergelijking met oestradiol bij de mannelijke controlegroep was het oestradiolgehalte hoger bij transmannen (P<0.001).

Prospectieve veranderingen van serum oestradiol waren positief gecorreleerd met prospectieve veranderingen van LH ($\rho = 0.107$, $P < 0.001$) en negatief gecorreleerd met wijzigingen van FSH ($\rho = -0.167$, $P < 0.001$) en BMI ($\rho = -0.082$, $P < 0.001$). Er was geen correlatie tussen veranderingen in oestradiolgehalte en testosterongehalte en total body fat percentage.

Conclusie

Testosterontoediening bij transmannen resulteert in een lager oestradiolgehalte. Er is dan ook geen evidentie dat testosterontherapie bij transmannen een risicofactor zou kunnen zijn voor gynaecologische carcinomen of ongewenste oestrogeeneffecten zou kunnen veroorzaken. Het oestradiolgehalte na start van de hormonale therapie in deze populatie was nog steeds hoger dan bij de mannelijke controlegroep. We besluiten uit eerdere studies dat het lagere oestradiolgehalte niet leidt tot adverse outcomes.