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SERUM HORMONE CONCENTRATIONS IN TRANSGENDER INDIVIDUALS RECEIVING GENDER AFFIRMING HORMONE THERAPY: A LONGITUDINAL RETROSPECTIVE COHORT STUDY

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Running title: Serum hormone concentrations in transgender people

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Abstract

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Objective: To examine the association of various gender affirmation hormone therapy (GAHT) regimens with blood hormone concentrations in transgender individuals.

Methods: This retrospective study included transgender persons receiving GAHT between January 2000 and September 2018. Data on patient demographics, laboratory values, and hormone dose and frequency were collected. Non-parametric tests and linear regression analyses were used to identify factors associated with serum hormone concentrations.

Results: Overall 196 subjects (134 trans women, 62 trans men) with a total of 941 clinical visits were included into this study. Trans men receiving transdermal testosterone had a significantly lower median value of total serum testosterone when compared to those who were receiving injectable preparations (326.0 vs. 524.5 ng/dL respectively, p=0.018). Serum total estradiol concentrations of trans women was higher in those receiving intramuscular estrogen compared to those receiving oral and transdermal estrogen (366.0 vs. 102.0 vs. 70.8 pg/mL respectively, p<0.001). A dose dependent response in hormone levels was observed for oral estradiol (p<0.001) and injectable testosterone (p=0.018), but not for intramuscular estradiol and not for transdermal formulations. Older age and history of gonadectomy in both trans men and women were associated with significantly higher concentrations of serum gender-affirmed hormone.

Conclusion: In trans men, all routes and formulations of testosterone appear to be equally effective in achieving concentrations in the male range. Intramuscular injections of estradiol resulted in the highest serum concentrations of estradiol whereas transdermal estradiol resulted in the lowest concentration. Dose was directly related to hormone levels for oral estradiol and injectable testosterone.

Keywords: transgender, gender affirming hormone therapy, serum testosterone level, serum estradiol level

Abbreviations:
TGGNB = Transgender and gender non-binary; **GAHT =** gender affirming hormone therapy, **SD =** standard deviation, **BMI =** body mass index, **IQR =** interquartile range, **VTE =** venous thromboembolism, **HDL =** high-density lipoprotein, **TG =** triglycerides, **LDL =** low-density lipoprotein, **SHBG =** sex hormone binding globulin.

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Introduction

Many transgender and gender non-binary people (TGGNB) people receive gender affirming hormone therapy (GAHT) to align their gender identity with their secondary sex characteristics [1]. Other ways that TGNB people affirm their gender identity include social transitioning, voice therapy, and gender affirmation surgery [2]. The goal of GAHT is to closely mirror the sex steroid concentrations found within the reference range of the affirmed gender [3]. Over a period of 2-3 years, GAHT typically results in physical changes expected for the affirmed gender. In transfeminine individuals, GAHT produces increased volume of breast tissue, redistribution of subcutaneous fat, and changes in skin and hair. In transmasculine individuals, GAHT causes deepening of the voice, an increase in muscle mass, redistribution of subcutaneous fat, and increased facial and body hair [4-6].

Although GAHT is considered safe under medical supervision [7-11], evidence indicates that TGNB people may have potential adverse effects, such as polycythemia secondary to testosterone administration, and venous thromboembolism due to estrogen use [9, 12]. The Endocrine Society guidelines suggest monitoring and adjusting hormone medications to maintain hormone levels within the desired sex-specific physiologic range of the affirmed gender to minimize these risks [1, 2]. However, published data on hormone dosing and corresponding blood concentrations are limited in the literature [7, 13, 14]. It is important for clinicians to have a better understanding of the impact of the dose of the hormone preparation, route of administration and frequency of dosing on blood hormone levels to ensure safety of GAHT regimens [11, 15].

The purpose of this study was to examine the effect of various GAHT regimens on blood hormone concentrations in transfeminine and transmasculine individuals receiving care at a single

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center. We included all subjects who were receiving GAHT over a 15 year period and had data on the details of the hormone regimen and hormone concentrations.

Methods

Study population

This is a retrospective study of patients identifying as transgender who received GAHT at the Emory Clinic and Emory University Hospital between January 1, 2000, and September 6, 2018. The protocol for this study was approved by the Emory Institutional Review Board. All subjects were treated in accordance with the Endocrine Society clinical practice guidelines for the treatment of gender-dysphoric/gender-incongruent persons [2, 11]. Trans women received estrogen in various types of preparations, including oral, intramuscular, and transdermal routes. Some trans women also received testosterone lowering agents such as spironolactone. Trans men were prescribed testosterone which in most instances was administered as an intramuscular injection.

Data collection

The information abstracted from the medical records included patient demographic characteristics (gender identity, current age, age at hormone initiation and race/ethnicity), clinical and general health-related variables (body mass index and history of gonadectomy) as well details of GAHT (medication type, dose, frequency, and route of administration). Transdermal preparations of sex steroid hormones included patches and gels. All subjects had regular hormone testing during outpatient visits. Serum total estradiol was performed by tandem mass spectrometry as a send out test to ARUP Laboratories (Salt Lake City, Utah) and testosterone

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analyses were performed by tandem mass spectrometry by Emory Medical Laboratories in both trans men and women. According to the guidelines of the Endocrine Society, blood testing was performed every 3-6 months in subjects initiating on GAHT and approximately 6-12 months for subjects who have already been on a stable regimen after 2 years from initiation of GAHT. The dates of hormone level testing and test results were obtained from the laboratory reports.

Statistical analysis

The data were analyzed by using SPSS Statistics version 20 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used to summarize demographic data. Means ± standard deviations (SD) and medians ± interquartile ranges (IQR) were used to describe the distributions continuous variables respectively, while categorical variables were characterized in terms of frequencies and proportions. Differences between groups were compared using $\chi 2$ test for categorical variables. Due to skewed distributions of hormone levels non-parametric Kruskal-Wallis or Mann-Whitney U tests were used to compare serum concentrations of testosterone across categories of trans men and estradiol concentrations across categories of trans women. In addition, simple linear regression models were used to examine the relation between GAHT dose and serum sex hormone levels. The dose-response analyses were performed separately for each route of hormone administration. A two-sided *p*-value of less than 0.05 was considered as evidence of statistical significance.

Results

Study subjects

The study dataset included 244 patients; however, 48 patients had missing data thus leaving 196 subjects in the final cohort. These subjects included 134 trans women and 62 trans

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men. After excluding visits in which subjects were not on GAHT, the outpatient clinic visits were divided into two groups: 647 transfeminine hormone treatment visits and 294 transmasculine hormone treatment visits (Figure 1).

Table 1 presents baseline demographic characteristics of the study participants. Trans men were younger than trans women with 40% vs. 11% under the age of 21 years and 24% vs 43% over the age of 35 years in the two respective groups (p=0.001). The race/ethnicity distributions (60% non-Hispanic whites in both groups, p=0.927) and the percentages of subjects undergoing gonadectomy in trans women and trans men were similar (21.6% vs 29.0%, p=0.283). Compared to trans women, trans men included a greater proportion of participants with BMI of 30kg/m_2 of greater (42% vs. 30%), but the difference was not statistically significant (p=0.095).

Factors influencing blood sex steroid hormone concentrations

As shown in Table 2 more advanced age and a history of gonadectomy were associated with higher sex hormone levels in both trans men and trans women. For example, median serum testosterone concentration in trans men who did and did not have gonadectomy were 603 (IQR: 437-936) ng/dL and 481 (IQR: 276-686) ng/dL, respectively (p=0.030). The corresponding median (IQR) estradiol concentrations were 186 (114-359) for trans women who underwent gonadectomy and 116 (63-276) for trans women who did not have the procedure (p<0.001) There was also a statistically significant difference in serum estradiol concentrations in trans women on anti-androgen therapy compared to the levels in trans women not taking anti-androgen therapy (120 pg/mL vs 163 pg/mL, p=0.006).

Gender Affirming Hormone Therapy Regimens

The majority of blood test values came from trans men taking injectable testosterone with approximately half of the trans men using the equivalent dose of higher than 75 mg/week (Table

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3). Injectable testosterone represented both intramuscular testosterone in 97.5% of the observations and subcutaneous testosterone in 2.5%. Trans men receiving transdermal GAHT had a significantly lower median concentration of total serum testosterone when compared to those who were receiving injectable preparations (326.0 vs 524.5 ng/dL respectively). Higher doses of injectable testosterone were associated with higher hormone concentrations with median (IQR) estimates of 442.5 (257.5 – 644.3) for ≤50 mg/week; 483.0 (317.8 – 645.3) for 51-75 mg/week and 588.0 (380.3 – 840.3) for >75 mg/week (p for trend = 0.018). By contrast, the difference between levels associated with lower and higher doses of transdermal testosterone levels was not statistically significant (p=0.66).

Table 4 summarizes serum concentrations of total estradiol according to route and dose of GAHT among trans women. Oral and intramuscular preparations of estradiol were more common among trans women compared to transdermal route (n= 366, 242, and 39, respectively). Among trans women taking oral estradiol, the majority of the subjects received a dose of 15-30 mg/week (45.6%) or greater than 30 mg/week (36.3%). Among trans women taking intramuscular estradiol, the majority received a dose of 10 mg/week or more. There were 488 visits (75.9%) in trans women using anti-androgenic agents, most of whom were taking spironolactone alone (n=468, 72.8%) or in addition to progesterone (n=43, 6.7%). Very few trans women were taking GnRH analogues (n=4, 0.7%).

Among trans women, serum total estradiol concentrations were higher in those receiving intramuscular estradiol compared to those receiving oral or transdermal estradiol (366.0 vs. 102.0 vs. 70.8 pg/mL respectively, p<0.001). Women with a higher oral dose of estradiol had a higher concentration of serum estradiol in a dose dependent fashion with median (IQR) estimates of 58.0 (47.6 - 104), 90.7 (58.7 - 138.8), and 140.0 (91.6 - 215.5) for doses of ≤14 mg/week, 15-30 mg/week, and >30 mg/week, respectively (p for trend <0.001). The same analyses showed little

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evidence of dose-response for intramuscular (p for trend =0.481) and transdermal (p for trend = 0.157) routes of estradiol administration (Table 4)

We examined daily regimens of estradiol and their corresponding serum estradiol concentrations in three commonly prescribed ranges for trans women (Figure 2). There was again a dose dependent increase in serum estradiol concentrations with increasing dose of estradiol. A total daily dose of estradiol in the range of 4-5 mg resulted in estradiol concentrations near the minimum suggested level for trans women, 93.5 pg/mL (IQR 58.6 – 146.8) (Figure 2).

Discussion

This study reports data on GAHT types and doses and the corresponding blood hormone concentrations among transgender individuals receiving care at a large specialized clinic in the United States. In both trans men and women, injectable GAHT was associated with significantly higher serum hormone levels when compared to oral or transdermal preparations. In both transgender men and women, factors that resulted in higher serum estradiol and testosterone concentrations included greater age (>35years) and a history of gonadectomy.

In our study, intramuscular and subcutaneous testosterone administration resulted in in the median serum total testosterone concentration of approximately 525 ng/dL. This level of serum total testosterone is consistent with levels found in other cohorts of trans men [13, 14, 16, 17]. Pelusi et. al reported even higher serum concentrations of total testosterone in trans men receiving intramuscular and transdermal formulations (median = 739.0 and 589.0 ng/dL respectively) [18]. Higher serum testosterone concentrations found in that study could be explained by the higher median age of their population and lower BMI compared to our study. In our study, we found that greater age was associated with higher serum testosterone concentrations. The relationship between serum total testosterone concentration and BMI remains unclear. One previous study reported that patients with a lower BMI had significantly

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higher serum testosterone concentrations [19]. In contrast, our data seems to suggest a V-shaped curve whereby persons with a BMI in the 25-29.9 kg/m_2 range had higher hormone concentrations than those whose BMI was less than 25 kg/m_2 and those with the BMI of 30 kg/m_2 or greater.

Serum estradiol concentrations of trans women in our study are also comparable with those reported in previous studies conducted in Europe [7, 13, 20]. One important distinction between studies conducted in the US and studies based in Europe is the use oral estradiol in combination with cyproterone, which is common in European clinical practice but not in the US. Cyproterone has been shown to increase sex hormone binding globulin and serum estradiol concentrations [21]. Some studies have reported a negative correlation between total testosterone level and sex hormone binding globulin [22, 23], while spironolactone is not known to have any impact on serum estradiol concentrations. Interestingly, serum estradiol concentrations in trans women receiving testosterone lowering agents were significantly lower compared to trans women not taking anti-androgen therapy. This is consistent with Leunig et al study of oral estradiol [24]. The likely explanation for a higher estradiol concentration in trans women not taking anti-androgen therapy is that the majority of trans women not taking anti-androgen therapy had previously undergone gonadectomy.

Monitoring sex steroid hormone concentrations in transgender people receiving GAHT is recommended by the Endocrine Society to decrease the risk of potential complications. Venous thromboembolism (VTE) and ischemic stroke are serious adverse outcomes in transgender individuals receiving GAHT, especially in transgender females taking oral estradiol [27, 28]. The reported incidence of VTE in trans women ranges from 1- 6% [9, 10, 29, 30]. One previous study reported transgender females taking oral estradiol had a 3-times higher rate of VTE and a 2-times higher rate of ischemic stroke, compared to cisgender male referents of the same age and race/ethnicity [31]. Although no studies have examined the association between serum estradiol

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dose and risk of VTE in trans women, it is presumed that higher hormone concentrations would increase risk. In trans men, GAHT is associated with an increase in hematocrit, a decrease in HDL, an increase in TG, LDL and inflammatory parameters [32-35]. However, clinical outcomes such as VTE, cardiovascular disease and cerebrovascular accidents do not appear to be increased in this population [31]. Most studies report that receiving GAHT under the supervision of medical providers is safe [7, 8]. One possible explanation for this safety is that serum hormone concentrations of TGNB persons are routinely monitored and kept within the recommended ranges. The precise serum hormone levels that produce adequate gender-affirming results (both physical and psychological) with the lowest risk of complications are still unknown [15]. However, most guidelines recommend keeping serum hormone levels within physiologic ranges of the affirmed gender [1, 2].

We found that serum estradiol levels increased in a dose dependent fashion according to oral estradiol dose in trans women. We also observed that doses of estradiol between 4 and 5 mg/day primarily in combination with spironolactone resulted in a median concentration at the minimum of recommended range of 100-200 pg/mL and doses of 2 mg/day might not be sufficient to achieve target concentrations[2]. Clinicians may consider starting at lower doses of oral estradiol and up titrate to 4-5 mg daily to reach recommended therapeutic levels of estradiol. Higher doses of estradiol (6 mg and above) may be reserved for those who still do not reach target levels but may result in supraphysiologic levels greater than 200-300 pg/mL Injectable estradiol and transdermal estradiol did not result in a dose dependent increase in estradiol concentrations. This may be since injectable estradiol leads to larger fluctuations of estradiol making timing of the measurement important and since transdermal estradiol may have significant variability in skin absorption between individuals. We also found that intramuscular injections of estradiol lead to mean estradiol concentrations above the reference range when given in doses of more than 5 mg weekly. In addition, trans women who underwent gonadectomy had higher

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serum estradiol concentrations which supports the common belief that estradiol dose can be decreased following removal of gonads. Although oral estradiol has many advantages, including ease of administration and low cost, current evidence suggests that oral estradiol may lead to a higher risk of thrombotic events compared to transdermal estradiol [9, 10, 29, 30]. Our observation that serum estradiol concentrations in trans women using transdermal estradiol were significantly lower when compared to other routes of administration may partly explain this difference in risk.

All routes of testosterone are equally effective in raising serum hormone concentrations into the male reference range; however, injectable testosterone appeared to be more likely to raise median testosterone concentrations into the range of 400-700 ng/dl recommended by the Endocrine Society. Intramuscular testosterone was the most popular route among trans men in our clinic. Testosterone doses of 100-200 mg every 1 or 2 weeks resulted in target concentrations. Transdermal preparations resulted in lower serum testosterone concentrations that were not in the recommended range.

The strengths of this study include the relatively large number of different observations with various hormone preparations and resultant hormone concentrations. Some limitations include variability of the timing of the hormone concentrations. In practice, this clinic prefers measuring the trough hormone concentrations in patients taking intramuscular injections to assess the efficacy of the hormone regimen; however, some subjects may not have had blood testing at that time point. We did not have enough numbers of lab tests done in trans men taking subcutaneous testosterone, so we are unable to compare serum testosterone levels to men taking intramuscular testosterone. Also, serum estradiol concentrations may vary according to the time of day when the oral estradiol dose is ingested. Our cohort primarily used twice daily dosing when estradiol doses were greater than 2 mg daily. This may be very important when comparing studies in trans women on once daily to twice daily dosing of estradiol. Furthermore, some trans women may take estradiol sublingually as opposed to orally without informing their physician. We do not

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have any information regarding this route in our study. Another important point is that we do not have any information on sex hormone binding globulin (SHBG). Although measurement of SHBG is not recommended by Endocrine Society, administration of sex steroids will alter SHBG concentrations. Testosterone therapy will lower SHBG which may result in more bioavailable testosterone whereas estradiol therapy will increase SHBG which may result in less bioavailable estradiol. Using SHBG as a guide to adjust GAHT should be evaluated in future studies. Almost all our subjects were adults and thus these findings may not apply to transgender youth. Finally, these results may differ from findings in Europe as the main anti-androgen in the United States is spironolactone as opposed to cyproterone.

In conclusion, several routes and formulations of sex steroid hormones used in the United States produced target hormone concentrations in our patient population. In trans men, all routes and formulations of testosterone appear to be equally as effective in achieving target hormone concentrations. In trans women, there was a dose dependent increase in serum estradiol concentration with increasing oral dose of estradiol with a dose of at least 5 mg daily appearing to be effective in achieving adequate estradiol concentrations. Intramuscular injections of estradiol resulted in the highest serum concentrations of estradiol whereas transdermal estradiol resulted in the lowest concentration of estradiol. Trans women undergoing bilateral orchiectomy had higher serum estradiol concentrations which confirms the expectation that estradiol dose can be lowered after gonadectomy.

Conflict of interest

This study was not supported by any funding. All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

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Figure 1. Flow diagram of study participants

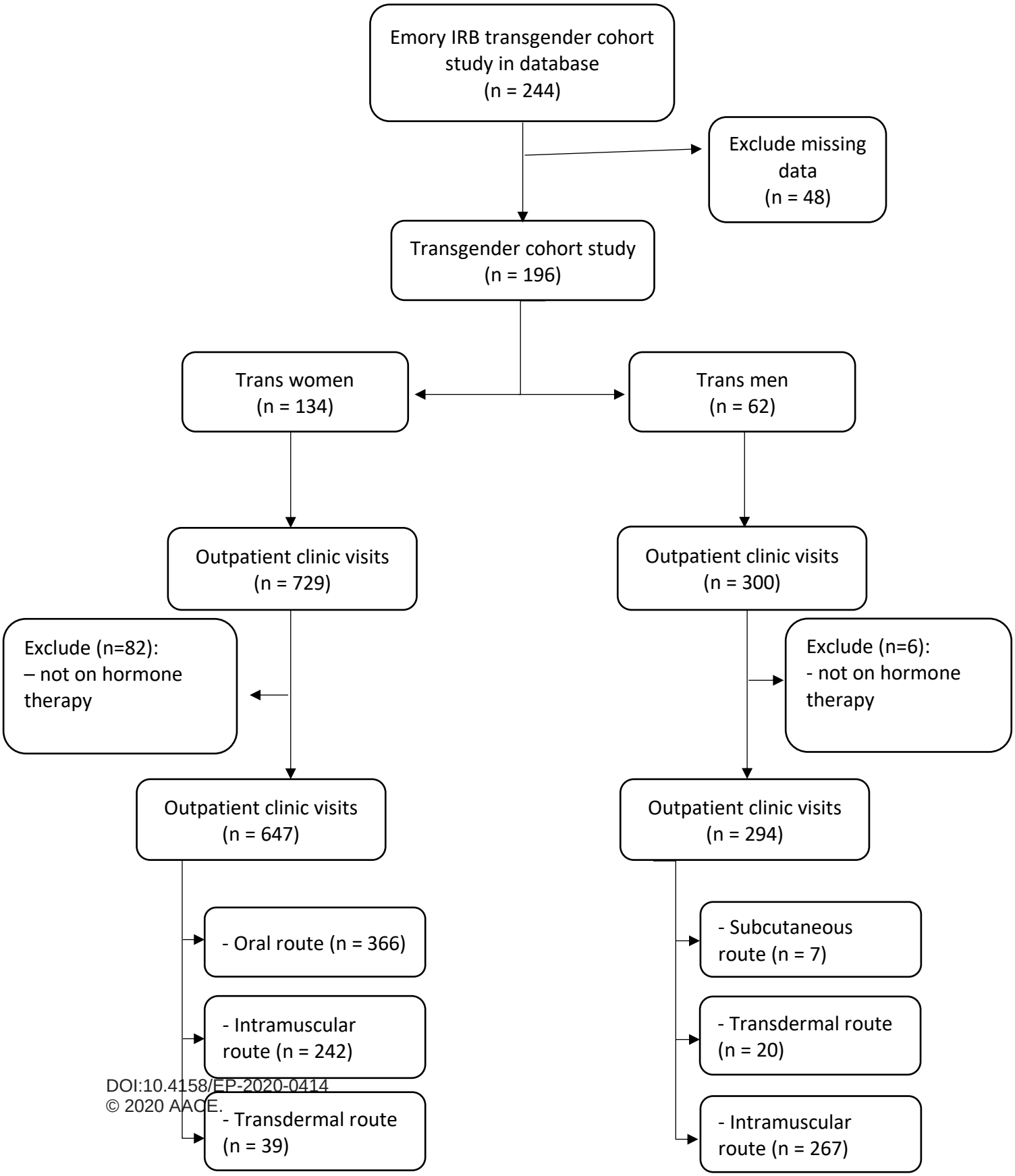

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Figure 2

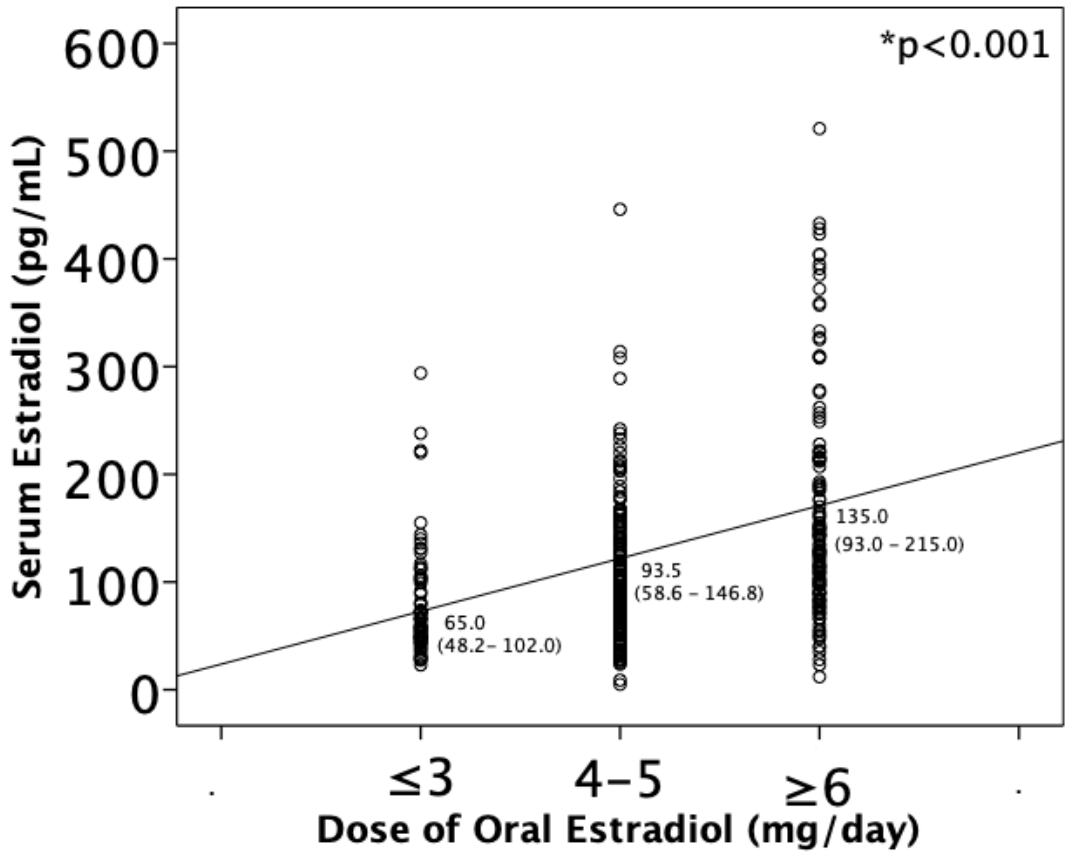

Figure 2 Total daily dose of oral estradiol and the corresponding serum estradiol concentration in trans women taking gender affirming hormone therapy Serum estradiol concentrations demonstrated a dose dependent increase with increasing total daily dose of oral estradiol ($p < 0.001$, Kruskal-Wallis). A total daily dose of between 4 and 5 mg daily resulted in a median estradiol concentration of 93.5 pg/mL (IQR 58.6 – 146.8), which is near the minimum recommended therapeutic range for trans women.

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Table 1 Characteristics of transgender subjects at cohort entry

Participant Characteristics	Trans men (n=62) N (%)	Trans women (n=134) N (%)	P-value*
Age (years)			
<21	19 (40.6%)	15 (11.3%)	0.001
21- 34	28 (45.2%)	62 (46.3%)	
≥35	15 (24.2%)	57 (42.9%)	
BMI category (kg/m2)			
<25 (normal/underweight)	21 (33.8%)	63 (47.0%)	0.095
25-29.9 (overweight)	13 (21.0%)	27 (20.1%)	
≥ 30 (obese)	26 (41.9%)	40 (29.9%)	
Race/ethnicity			
Non-Hispanic White	37 (59.7%)	80 (59.7%)	0.927
African American	11 (17.7%)	27 (20.1%)	
Other/Unknown	14 (22.6%)	26 (20.1%)	
History of gonadectomy			
Yes	18 (29.0%)	29 (21.6%)	0.283
No	44 (31.0%)	105 (78.4%)	

* Chi-square test

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Table 2. Sex Steroid Hormone Concentrations in trans men and women according to demographic characteristics.

Participant Characteristics	Testosterone levels in trans men Median (IQR)	P-value*	Estradiol levels in trans women Median (IQR)	P-value*
Age (years)				
<21	446.5 (255.7 – 652.0)	0.027	76 (50.2 – 153.5)	0.001
21- 34	508.0 (314.0 – 685.5)		123.0 (70.7 – 294.0)	
≥35	563.0 (364.0 – 864.0)		146.0 (74.0 – 309.0)	
BMI category (kg/m2)				
<25 (normal/underweight)	496.0 (340.0 - 731.0)	0.857	145.0 (70.7 - 327.0)	0.171
25-29.9 (overweight)	515.0 (284.0 - 729.0)		153.0 (74.0 - 281.5)	
≥ 30 (obese)	495.0 (309.5 - 668.5)		119.5 (66.2 - 234.5)	
Race/ethnicity				
Non-Hispanic White	515.0 (288.0 - 755.0)	0.160	135.5 (74.9 - 309.0)	0.097
African American	582.0 (398.5 - 832.0)		119.0 (52.3 - 466.0)	
Other/Unknown	956.0 (352.25 - 1378.25)		55.8 (42.3 - 77.5)	
History of gonadectomy				
Yes	603.0 (437.00 - 936.00)	0.030**	186.0 (114.0 - 359.0)	<0.001**
No	481.0 (276.25 - 686.25)		116.0 (63.00 - 276.00)	

*Kruskal-Wallis test
**Mann-Whitney U test

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Table 3 Serum concentrations of total testosterone in trans men by route of administration and dose*

Regimen characteristics	**Level (ng/dL) Median (IQR)**	**Route-specific P-value** by dose**
Injectable (n=274)	524.5 (333.8 – 756.0)	
≤50 mg/week (n=80)	442.5 (257.5 – 644.3)	0.018
51-75 mg/week (n=50)	483.0 (317.8 – 645.3)	
>75 mg/week (n=144)	588.0 (380.3 – 840.3)	
Transdermal (n=20)	326.0 (85.5 - 441.0)	
≤50 mg/week (n=13)	303 (61.5 – 442.0)	0.660
51-75 mg/week (n= 0)	-	
>75 mg/week (n=7)	349.0 (188.0 - 447.0)	
P-value for all routes, by dose	0.006 **	
P-value for all doses, by route	<0.001***	

* Based on individual visits
**Simple linear regression analysis
***Mann-Whitney U test

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Table 4 Serum concentrations of total estradiol in trans women by route of administration and dose*

Regimen characteristics	**Level (ng/dL) Median (IQR)**	**Route-specific P-value** by dose**
Oral (n=366)	102.0 (61.8 - 155.0)	
≤14 mg/week (n=66)	58.0 (47.6 - 104)	<0.001
15-30 mg/week (n=167)	90.7 (58.7 - 138.8)	
>30 mg/week (n=133)	140.0 (91.6 - 215.5)	
Intramuscular (n=242)	366.0 (159.5 - 629.0)	
≤5 mg/week (n=24)	155.5 (62.28 - 605.0)	0.481
6-9 mg/week (n=29)	371.0 (227.5 - 550.0)	
≥10 mg/week (n=189)	365.0 (165.3 - 633.0)	
Transdermal (n=39)	70.8 (38.1 -119.0)	
≤2 mg/week (n=24)	71.5 (35.1 – 115.0)	0.157
2.1-5 mg/week (n=9)	105.0 (50.9 – 159.5)	
>5 mg/week (n=6)	50.3 (28.9 – 83.6)	
P-value for all doses, by route	<0.001***	

* Based on individual visits
** Simple linear regression analysis
***Kruskal-Wallis test

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