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Sublingual Estradiol Is Associated with Higher Estrone Concentrations than Transdermal or Injectable Preparations in Transgender Women and Gender Nonbinary Adults

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Abstract

Purpose: Serum hormone profiles among different feminizing gender-affirming hormone therapies (GAHT) are poorly characterized. To address this gap, we described the serum estrogen profiles of three 17β-estradiol preparations, taken with or without an antiandrogen, using a novel liquid chromatography–mass spectrometry (LC-MS/MS) assay in adults taking feminizing GAHT.
Methods: This was a secondary analysis of 93 healthy transgender women and gender nonbinary adults taking feminizing GAHT in a prospective cross-sectional study. Eligible participants took 17β-estradiol (sublingual tablet, transdermal patch, or intramuscular/subcutaneous injection) with or without oral spironolactone for ≥12 months before study entry. We determined serum estrone and estradiol concentrations for each hormone preparation and described the association between estrone and (1) clinically relevant estradiol concentration ranges (≤200 and >200 pg/mL) and (2) antiandrogen use. To achieve our objectives, we described our protocol for developing an LC-MS/MS assay to measure estrone and estradiol concentrations.
Results: Estrone concentrations were higher among participants taking sublingual 17β-estradiol tablets compared with transdermal or injectable preparations ($p < 0.0001$). Estradiol concentrations were higher for injectable versus transdermal preparations ($p = 0.0201$), but both were similar to sublingual tablet concentrations ($p > 0.05$). Estradiol >200 pg/mL (vs. ≤200 pg/mL) was associated with higher estrone concentrations among participants taking sublingual 17β-estradiol, but not transdermal or injectable 17β-estradiol. We observed no association between spironolactone and estrone concentrations ($p > 0.5$).
Conclusion: Estrone concentrations were higher among transgender women and gender nonbinary adults taking sublingual 17β-estradiol compared with transdermal or injectable preparations. The role of estrone in clinical monitoring and the influence of other antiandrogens (e.g., cyproterone acetate) on the estrogen profile remain to be determined.

Keywords: estrone, estradiol, gender-affirming hormone therapy, transgender, transgender women

Introduction

Approximately 0.5% of the global population is transgender.[1] Within this population, gender-affirming hormone therapy (GAHT) is a common first-line medical intervention for gender-affirming medical care.[2] Transgender women, individuals with a female gender identity who were assigned male at birth, may take 17β-estradiol (with or without an antiandrogen) as feminizing GAHT to align their physical characteristics with their affirmed gender identity.[2,3] Gender nonbinary individuals who wish to feminize may also seek GAHT.[4,5] Several feminizing GAHT

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regimens are available,[3,5] but associated estrogen profiles (estradiol and estrone concentrations) are poorly characterized, limiting our ability to understand how to monitor safety and effectiveness across GAHT regimens.

Estrone, the predominant active metabolite of 17β-estradiol, may influence safety outcomes during GAHT, but clinical data on transgender or gender nonbinary adults are limited. Data from cisgender adults suggest that different hormone preparations may influence safety outcomes. For example, estrone is associated with increased thrombin generation in postmenopausal women taking oral, but not transdermal, estrogen therapy.[6] Estrone concentrations are often supraphysiologic during oral estrogen replacement therapy in cisgender populations,[7–11] suggesting a concentration-dependent relationship between estrone and thrombin generation. However, transgender and gender nonbinary adults take various feminizing GAHT regimens (doses, hormone preparations, administration routes, and adjunctive agents), limiting our ability to extrapolate available estrone data from cisgender populations to transgender and gender nonbinary individuals. Furthermore, limited clinical data suggest that spironolactone, a common antiandrogen in the United States,[12] increases endogenous estrone concentrations in cisgender men,[13] but its impact on estrone during feminizing GAHT is unclear.

We hypothesized that transdermal and injectable 17β-estradiol, when taken by transgender women and gender nonbinary adults for feminizing GAHT, would be associated with lower serum estrone concentrations than sublingual 17β-estradiol and that oral spironolactone would be associated with conversion of 17β-estradiol to estrone for all 17β-estradiol preparations. To this end, our primary objective was to compare estrone and estradiol concentrations associated with different 17β-estradiol preparations (sublingual tablet, transdermal patch, or intramuscular/subcutaneous injection) in a U.S. cohort of transgender and gender nonbinary adults by developing a novel, highly sensitive, and specific liquid chromatography–mass spectrometry (LC-MS/MS) assay. Our secondary objective was to compare estrone concentrations associated with clinically significant estradiol concentration ranges (>200 and ≤200 pg/mL) and with or without an antiandrogen (oral spironolactone) across 17β-estradiol preparations.

Methods

Study design and participants

This was a secondary analysis of a prospective cross-sectional study of 93 healthy transgender women and gender nonbinary adults (≥18 years).[14] Participants took feminizing GAHT prescribed by their clinical provider either in Seattle, WA, or Iowa City, IA, for at least 12 months.[14] Individuals with body–mass index (BMI) >30 mg/kg^2 or current tobacco use were excluded from the primary study.[14] In the current analysis, we included participants with complete GAHT records (dose/administration route) and evaluable estrone and estradiol serum concentration data. One participant was excluded due to incomplete 17β-estradiol dosing history (injectable estradiol). This study was conducted in accordance with the Declaration of Helsinki and approved by the Western Institutional Review Board (IRB) (Approval number: 1179338; Seattle, WA) and the University of Iowa IRB (Approval number: 201710702; Iowa City, IA). All participants provided written informed consent.

Sample collection and serum estrogen and testosterone analysis

Venous whole blood was collected into a 5-mL gold-top serum separator tube for quantification of estrone, estradiol, and total testosterone. Serum was separated and stored at −80°C until LC-MS/MS analysis. Detailed analytical methods for estrone and estradiol determination are included in Supplementary Method and Assay Performance Characteristics (Supplementary Data). Assay analytical parameters are described in Supplementary Table S1. The functional sensitivity at coefficient of variation <20% was 10 and 4 pg/mL for estrone and estradiol, respectively. Estrone and estradiol concentrations were quantified at Seattle Children's Hospital, WA; total testosterone was quantified at Northern California Kaiser Permanente, CA, using the LC-MS/MS method as previously described.[15] Both laboratories are accredited by the College of American Pathologists.

Statistical analysis

Participant demographics and hormone concentrations were summarized descriptively using the median (interquartile range) for continuous variables and frequencies (percentages) for binary variables. Based on sample sizes in this secondary analysis, we performed nonparametric statistical tests using the Mann–Whitney U test or Kruskal–Wallis test with Dunn's test for multiple comparisons for continuous data or Fisher's exact test for categorical data. Based on expert consensus for estradiol concentrations during feminizing GAHT,[3] we assessed estradiol concentrations ≤200 and >200 pg/mL and associated estrone concentrations. We assessed the association between spironolactone and total testosterone concentrations after excluding participants who underwent orchiectomy/vaginoplasty. A two-sided p value <0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism, version 8, for MacOSX (GraphPad Software, La Jolla, CA) and SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Study participants

In total, 92 participants with complete GAHT records (dose/administration route/duration of therapy) were included in this analysis (Table 1). Demographic and clinical data for the original cohort have been described elsewhere.[14,15] Fifty-four participants took sublingual 17β-estradiol tablets (tablet subgroup), 9 took transdermal 17β-estradiol patches (patch subgroup), and 29 took intramuscular or subcutaneous 17β-estradiol injections (injection subgroup). Across subgroups, 31%–50% of participants took oral spironolactone, the majority of whom were in the tablet subgroup. The remaining participants either did not take an antiandrogen (between 30% [tablet subgroup] and 59% [injection subgroup]) or had undergone orchiectomy/vaginoplasty (between 10% [injection subgroup] and 22% [patch subgroup]). The injection subgroup was older than the tablet subgroup ($p = 0.0013$), but remaining characteristics were similar across subgroups.

Table 1. Participant Characteristics and Sex Hormone Concentrations Among Transgender and Gender Nonbinary Adults Taking Feminizing Gender-Affirming Hormone Therapy

Parameter	*Sublingual tablet* (n = *54*)	*Transdermal patch* (n = *9*)	*Injection* (n = *29*)	*Overall* p *value*[a]
Transgender women, *n* (%)[b]	53 (98)	9 (100)	28 (97)	0.284
Age, years	29 (24–36)	31 (26–59)	39 (32–48)	0.002[c]
GAHT duration, years	2.5 (1.6–4.0)	2.9 (2.0–3.9)	3.5 (1.7–11.8)	0.558
17β-Estradiol dose (total dose: median, range)	6 mg daily, 2–8 mg	175 mcg/daily, 100–200 mcg/daily	7 mg weekly, 3–10 mg	—
Spironolactone, *n* (%)	27 (50.0)	3 (33.3)	9 (31.0)	0.211
Spironolactone dose (total daily dose, mg: median, range)	100 (25–400)	200 (150–200)	150 (50–300)	0.486
Progestogen, *n* (%)	8 (14.8)	0	4 (13.8)	0.725
Finasteride, *n* (%)	2 (3.7)	0	1 (3.4)	1.000
Orchiectomy/vaginoplasty, *n* (%)	11 (20.4)	2 (22.2)	3 (10.3)	0.542
Estrone, pg/mL	693.0 (457.3–1147.5)	58.6 (32.7–69.6)	67.5 (55.9–113.1)	<0.0001[d,e]
Estradiol, pg/mL	154.4 (103.4–217.1)	80.1 (52.1–102.2)	192.6 (128.0–243.3)	0.024[f]
Estradiol concentration ≤200 pg/mL, *n* (%)	38/54 (70)	8/9 (89)	16/29 (55)	0.144
Estrone/estradiol[g]	4.3 (2.6–6.4)	0.4 (0.3–0.8)	0.4 (0.3–0.4)	<0.0001[d,e]
Total testosterone, ng/dL[h,i]				
−spironolactone	18 (13–205), *n* = 16	14 (11–20), *n* = 4	11 (9–18), *n* = 17	0.046[j]
+spironolactone	11 (6–35), *n* = 27	12 (10–20), *n* = 3	10 (9–11), *n* = 9	0.809
Proportion, suppressed testosterone (≤50 ng/dL), *n* (%)[h]	32/43 (74)	7/7 (100)	23/26 (88)	0.230

Data reported as median (interquartile range) unless otherwise noted. To convert sex hormone concentrations to SI units, multiply estrone by 3.699, estradiol by 3.67, and total testosterone by 0.0347.

[a]Binary and categorical characteristics were compared using Fisher's exact test; continuous variables were compared using the Kruskal–Wallis test with Dunn's test.

[b]Nonbinary participants: $n = 1$, 17β-estradiol sublingual group; $n = 1$, 17β-estradiol injection group.

[c]Dunn's test: Sublingual versus injectable, $p = 0.0013$.

[d]Dunn's test: Sublingual versus transdermal, $p < 0.0001$.

[e]Dunn's test: Sublingual versus injectable; $p < 0.0001$.

[f]Dunn's test: Transdermal versus injectable, $p = 0.0201$.

[g]Estrone/estradiol is presented as the median (interquartile range) of individual participants' estrone/estradiol ratios.

[h]Excludes participants who underwent orchiectomy/vaginoplasty before study enrollment.

[i]Mann–Whitney *U* test: Sublingual group with or without spironolactone, $p = 0.031$; all other within-group comparisons: $p > 0.05$.

[j]Dunn's test: Sublingual versus injectable, $p = 0.0282$.

GAHT, gender-affirming hormone therapy.

Serum estrone, estradiol, and estrone/estradiol concentrations across subgroups

Estrone concentrations were significantly higher in the tablet subgroup than either the patch or injection subgroup ($p < 0.0001$) (Table 1). When considering estradiol concentrations, the tablet subgroup had similar concentrations to both the patch and injection subgroups, but concentrations were lower in the patch subgroup than in the injection subgroup ($p = 0.0201$). The estrone/estradiol ratio was significantly higher in the tablet subgroup than in either the patch or injection subgroup ($p < 0.0001$).

Estradiol concentrations ≤200 and >200 pg/mL and associated estrone concentrations

Within the tablet subgroup, estradiol concentrations >200 pg/mL were associated with significantly higher estrone concentrations than ≤200 pg/mL ($p = 0.0064$) (Fig. 1). We observed no difference in estrone concentrations within the injection subgroup ($p = 0.308$). The patch subgroup was too small to perform within-group statistical testing. Despite low estrone concentrations associated with estradiol ≤200 pg/mL within the tablet subgroup, estrone concentrations were significantly higher than in either the patch or injection subgroup ($p < 0.0001$).

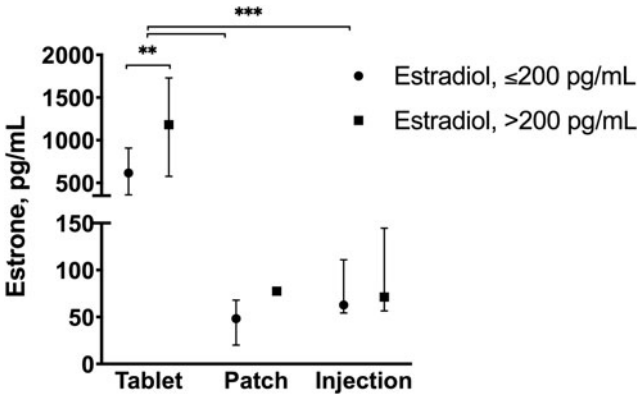

FIG. 1. Median (interquartile range) serum estrone concentrations associated with serum estradiol concentration ranges ≤200 pg/mL (●) and >200 pg/mL (■). Kruskal–Wallis and Dunn's tests: ***$p < 0.0001$. Among participants in the tablet subgroup, estradiol >200 pg/mL was associated with higher median estrone concentrations than estradiol ≤200 pg/mL (Mann–Whitney *U* analysis: **$p = 0.0064$). Within-group estrone concentrations were similar in the patch and injection subgroups. Subgroup sample sizes: ●$n = 38$ (tablet), 8 (patch), and 16 (injection); ■$n = 16$ (tablet), 1 (patch), and 13 (injection).

Spironolactone association with estrone and estradiol concentrations, estrone/estradiol ratio, and total testosterone across subgroups

For the tablet and injection subgroups, within-group estrone and estradiol concentrations were similar with and without spironolactone therapy (both preparations: $p > 0.05$) (Fig. 2A, B). In the patch subgroup, estradiol concentrations were significantly higher among participants taking spironolactone versus those who were not ($p = 0.0238$) (Fig. 2B), although within-group estrone/estradiol ratios were similar across all subgroups (Fig. 2C). Within-group total testosterone was numerically lower among participants taking spironolactone than those not taking spironolactone for all subgroups (Table 1), although this was only significant in the tablet subgroup (Mann–Whitney U test: $p = 0.031$).

Discussion

To our knowledge, this study is the first to compare estrone and estradiol concentrations in transgender and gender nonbinary adults taking sublingual, transdermal, or injectable 17β-estradiol. We used a novel LC-MS/MS method to quantify serum estrone and estradiol concentrations in this study because this is a sensitive and specific method for determining sex hormone concentrations.[16] Estrone concentrations were significantly higher among participants in the tablet subgroup than in either the patch or injection subgroup. These findings are similar to a prospective cohort in Europe that reported higher estrone concentrations among transgender women taking oral estradiol tablets (as valerate ester) than transdermal patches, with up to a 12- and 3-fold increase, respectively, from baseline (GAHT-naïve).[17,18] Our study suggests that estrone is higher among participants taking sublingual 17β-estradiol compared with transdermal or injectable preparations, but longitudinal studies with standardized dosing and blood sampling are needed to characterize estrone concentrations over time for each preparation.

Since estrone is converted from estradiol during hormone therapy,[19] we estimated the estrone/estradiol ratio for all participants. Participants in the tablet subgroup had significantly higher estrone/estradiol ratios than in either the patch or injection subgroup. This finding is driven by higher estrone concentrations measured in the tablet subgroup, although the magnitude of the between-group difference was unexpected. Sublingual administration bypasses intestinal and hepatic 17β-hydroxysteroid dehydrogenase,[20–23] avoiding substantial metabolism relative to standard oral dosing. However, lymphatic tissues in the neck region may metabolize sublingual 17β-estradiol.[24,25] While our study lacked an oral dose comparator, data from postmenopausal women suggest that sublingual 17β-estradiol yields lower estrone/estradiol concentrations than oral administration, although outcomes vary based on sampling strategy and study design (Table 2).[8–11,21,25–27] Additional studies with standardized sampling are needed to understand the clinical significance of estrone/estradiol ratios and to compare these ratios with historical data from cisgender adults.

To explore whether the hormone dose was associated with the estrogen profile in the tablet subgroup, we performed a *post hoc* Spearman rank analysis between the total daily sublingual 17β-estradiol dose and estrone, estradiol, and estrone/estradiol concentrations. We observed no correlation between estrone and dose ($p = 0.105$) (Fig. 3A), although estradiol concentrations were moderately positively correlated with the daily dose ($p = 0.013$) (Fig. 3B). Estrone/estradiol and dose were not correlated ($p = 0.936$) (Fig. 3C). Since we measured hormones from a single blood sample collected within an 8–12-hour dosing interval, future pharmacokinetic studies should use timed, serial blood sampling to better characterize the relationship between standardized sublingual dosing (dose, frequency, and duration) and the estrogen profile.

To determine whether age influenced our findings, we conducted a *post hoc* Spearman rank analysis between age and estrone, estradiol, and estrone/estradiol for all

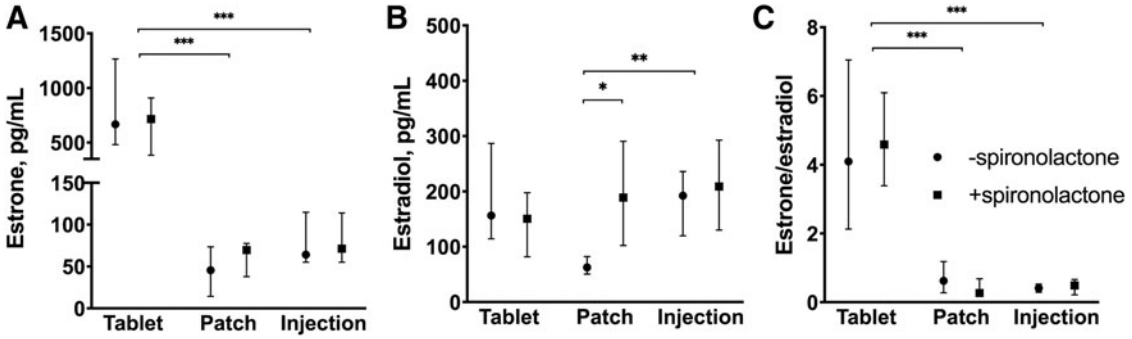

FIG. 2. Median (interquartile range) estrone and estradiol concentrations following ≥12 months of 17β-estradiol therapy without (●) and with (■) oral spironolactone. **(A)** Within-subgroup estrone concentrations were similar for all preparations, regardless of spironolactone use (all $p > 0.05$). Overall estrone concentrations were higher among participants in the tablet subgroup than in the patch or injection subgroup (Kruskal–Wallis and Dunn's tests: $^{***}p < 0.0001$). **(B)** Within-subgroup estradiol concentrations were similar in the tablet and injection subgroups, regardless of spironolactone use (both: $p > 0.05$). In the patch subgroup, estradiol was significantly lower among participants not taking spironolactone (Mann–Whitney U analysis: $^{*}p = 0.0238$) and was significantly lower than the overall injection subgroup estradiol concentration (Mann–Whitney U analysis: $^{**}p = 0.002$). **(C)** Within-subgroup estrone/estradiol ratios were similar regardless of spironolactone use for all preparations. Estrone/estradiol ratios were higher among participants in the tablet subgroup than in the patch or injection subgroup (Kruskal–Wallis and Dunn's tests: $^{***}p < 0.0001$). Subgroup sample sizes: ●$n = 27$ (tablet), 6 (patch), and 20 (injection); ■$n = 27$ (tablet), 3 (patch), and 9 (injection).

Table 2. Summary of Estrone/Estradiol Serum Concentration Ratios by Administration Route in Postmenopausal Cisgender Women

Hormone preparation, reference	*Participants,* n	*Dose, duration*	*Estrone/estradiol*[a]
Sublingual tablets			
Burnier et al.[25]	5	0.5 mg, single dose	0.4–4.6[b]
Fiet et al.[26]	8	0.5 mg, single dose	0.5–1.6[b]
Price et al.[27]	6	1.0 mg, single dose	2.0–3.0[b]
Oral tablets			
Devissaguet et al.[8]	36	2.0 mg, single dose	4.0 (1.9–6.8)[b,c]
Järvinen et al.[21]	12	2.0 mg (as estradiol valerate), 14 days	5.9
Price et al.[27]	6	1.0 mg, single dose	3.6[b]
Powers et al.[9]	14	2.0 mg, 3 days	5.0
Scott et al.[10]	13	2.0 mg, 14 days	5.0
Yen et al.[11]	9	2.0 mg, single dose	3.0–5.9
Transdermal patches			
Devissaguet et al.[8]	36	50 mcg/daily, single dose	0.8 (0.3–2.1)[b,c]
Järvinen et al.[21]	15	50 mcg/daily, 14 days	0.8
Powers et al.[9]	14	25 mcg/daily, 21 days	1.4
		50 mcg/daily, 21 days	1.1
		100 mcg/daily, 21 days	0.8
Scott et al.[10]	12	50 mcg/daily, 14 days	1.1

[a]All ratios are reported as mean serum concentrations of estrone and estradiol over the dosing interval unless otherwise indicated. Measures of variability are reported where available.
[b]Estimated by comparing the estrone and estradiol areas under the plasma concentration–time curves over 24 hours postestradiol dose.
[c]Data reported as geometric mean (range).

subgroups. Age did not correlate with these variables (data not shown). Similarly, in postmenopausal women taking oral estrogen replacement therapy (mean age: 60.8 years), age similarly was not correlated with either estrone or estradiol concentration.[28]

We visually compared our estrone/estradiol ratios with published data from postmenopausal women taking estrogen replacement therapy (Table 2).[8–11,21,25–27] Our tablet subgroup had numerically higher estrone/estradiol compared with postmenopausal women taking sublingual 17β-estradiol, whereas our patch subgroup had numerically lower estrone/estradiol compared with postmenopausal women taking patches.[8–10,21] Although injectable 17β-estradiol data in postmenopausal women are limited, data from premenopausal women taking intramuscular 17β-estradiol (single-dose 5 mg valerate or cypionate) suggest that the estrone/estradiol ratio is $\sim$0.5,[19] whereas the estrone/estradiol ratio measured in our injection subgroup was 0.4. These findings are likely influenced by pharmacologic estrogen doses taken for GAHT within our cohort, and further studies are needed to determine whether actual differences in estrogen metabolism exist.

We observed no association between spironolactone and the estrogen profile. Spironolactone has estrogenic-like effects in cisgender adults,[29] and a small study of cisgender men taking spironolactone (for primary hypertension) reported increased endogenous estrone compared with baseline concentrations (spironolactone-naïve, $p < 0.01$).[13] Unlike endogenous estrone, which is produced through androstenedione aromatization,[19,30] estrone is converted from estradiol by 17β-hydroxysteroid dehydrogenase during estrogen therapy. Thus, different estrone formation pathways

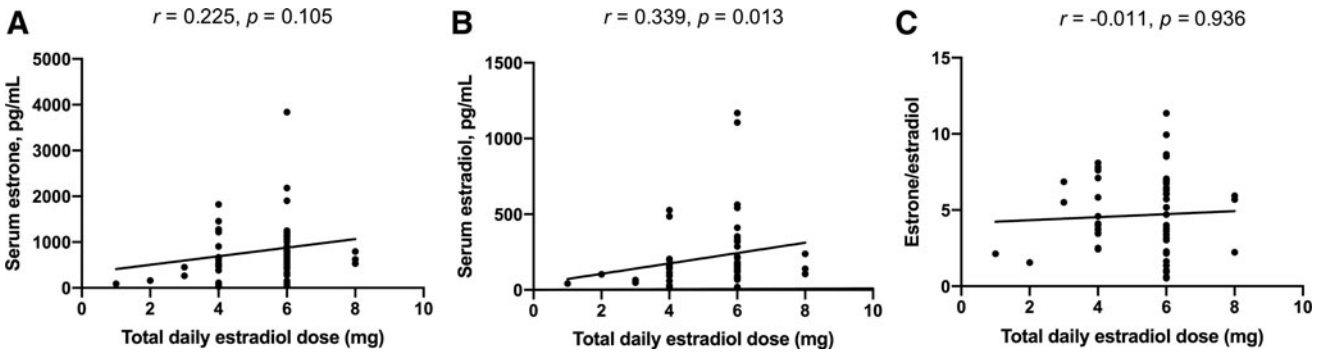

FIG. 3. Serum estrone and estradiol concentration correlations versus sublingual 17β-estradiol total daily dose (mg). **(A)** Serum estrone concentration was not correlated with the total daily 17β-estradiol dose: Spearman rank correlation, $p = 0.105$. **(B)** Serum estradiol concentration was moderately correlated with the total daily 17β-estradiol dose: Spearman rank correlation, $p = 0.013$. **(C)** Estrone/estradiol was not correlated with the total daily 17β-estradiol dose: Spearman rank correlation, $p = 0.936$.

may have contributed to the lack of an observed association in our study. Furthermore, given interindividual variability across subgroups, our sample sizes may have been too small to detect a difference. Outside the United States, providers of gender-affirming care have access to several adjunctive therapies for feminizing GAHT (including oral cyproterone acetate in Europe or gonadotropin-releasing hormone analogs used in the United Kingdom),[12] and comparative studies should be considered to determine the impact of these agents on the estrogen profile for appropriate clinical monitoring.

Since clinicians in the United States prescribe spironolactone as an antiandrogen,[12] we assessed total testosterone concentrations with and without spironolactone within subgroups. We excluded participants who underwent orchiectomy/vaginoplasty because testosterone would presumably be suppressed regardless of the GAHT regimen. In the tablet subgroup, total testosterone was significantly lower among participants taking spironolactone (vs. those who were not). Prospective[31,32] and retrospective[33–35] studies suggest that spironolactone suppresses total testosterone concentrations in conjunction with oral estrogens, but few studies have included participants taking either transdermal[32,35] or injectable 17β-estradiol,[32] and an independent effect of spironolactone on testosterone concentrations across 17β-estradiol preparations remains to be demonstrated.[33] In our cohort, the majority of participants in the patch subgroup had serum hormone concentrations within recommended ranges for GAHT (estradiol: ≤200 pg/mL; total testosterone: ≤50 ng/dL).[3] Although it is tempting to speculate that transdermal 17β-estradiol influences these findings, our patch subgroup was small, and comparative studies using a standardized patch and antiandrogen are needed.

Limitations

This was a secondary analysis of participants enrolled in a prospective clinical study at two U.S. sites, allowing us to evaluate participants from diverse geographic locations. Conversely, this secondary analysis had limitations. Participants took GAHT for ongoing gender-affirming care and regimens (dose, frequency, and duration) varied. Participants may have switched between hormone preparations during the year before enrollment, although all participants took hormone preparations for several months before hormone measurement in our study. Sublingual versus oral administration was not distinguished in earlier publications of this cohort,[14,15] but as per clinical protocols at both study sites, clinicians counsel all patients to dissolve micronized 17β-estradiol tablets under the tongue and assess the administration technique during follow-up visits. These protocols are based on successful sublingual 17β-estradiol use in transgender adults[32] and limited data suggesting that the absorption rate and extent are higher following sublingual than oral administration ($p < 0.05$).[27] In our study, hormone doses were not directly observed, and the extent to which tablets were absorbed sublingually versus swallowed may have varied. Future studies should account for this source of variability.

To minimize selection bias, the primary study used prespecified inclusion criteria,[14] but certain factors (e.g., body composition and BMI) were unavailable in this secondary analysis. BMI is positively associated with serum estradiol in postmenopausal women taking oral 17β-estradiol ($p < 0.01$), but not with estrone.[28] In retrospective studies among transgender women taking oral estradiol, BMI and estradiol data are conflicting.[33,36,37] Since a considerable proportion of U.S. transgender adults are either overweight or obese,[38,39] future studies should explore the association between estrone, estrone/estradiol, and BMI during feminizing GAHT.

Finally, this was not a pharmacokinetic study. Blood sampling was based on routine gender-affirming care, and the exact timing of the last hormone dose before sample collection was unavailable. Based on existing clinical protocols at participating sites, clinicians measured estradiol concentrations either before the next dose (injectable estradiol) or without regard to the timing of the last dose (sublingual or transdermal). The dosing frequency was within the ranges of the elimination half-lives for each preparation (e.g., weekly 17β-estradiol injections [elimination half-life: 120 to 240 hours][40,41] and twice- or thrice-daily sublingual 17β-estradiol [elimination half-life: 11–14 hours][42]), and samples were collected at presumed steady-state concentrations (≥12 months of continuous GAHT), which collectively help minimize variability in measured hormone concentrations. The exact proportion of participants taking intramuscular versus subcutaneous injections was unavailable, but at participating sites, 50%–90% of transgender or gender nonbinary patients use subcutaneous administration when taking injectable 17β-estradiol.

Recommendations for future research

Our study was not designed to evaluate the safety or efficacy of estrone in transgender and gender nonbinary adults. Future studies should investigate clinical implications of elevated estrone, including safety parameters such as thrombogenicity and cardiovascular outcomes, particularly for understudied administration routes such as sublingual and injectable 17β-estradiol.[32] We did not measure hydroxylated estrone compounds in our study, but these metabolites increase during oral 17β-estradiol ($p < 0.001$ vs. transdermal)[43] administration and are associated with breast cancer in postmenopausal cisgender women.[19] Thus, these factors should be included as part of a long-term safety evaluation among transgender and gender nonbinary people taking feminizing GAHT.

Longitudinal studies are needed to determine whether estrone or the estrone/estradiol ratio enhances feminization in transgender and gender nonbinary adults, and if so, whether this outcome is commensurate with the safety profile of respective 17β-estradiol preparations (e.g., tablets versus transdermal or injectable preparations). Data from adolescents with Turner syndrome or ovarian failure suggest that the extent of feminization is similar with either oral or transdermal 17β-estradiol,[7,43–45] but these studies used physiologic 17β-estradiol replacement doses that are often lower or dosed less frequently than feminizing GAHT,[3] impacting our ability to extrapolate these findings to transgender and gender nonbinary adults. The current study was not designed to determine whether estrone (or the estrone/estradiol ratio) should be measured during routine clinical care, and further studies are needed to associate estrone and the estrone/estradiol ratio with long-term safety and efficacy during feminizing GAHT.

Conclusion

Sublingual 17β-estradiol was associated with higher estrone concentrations compared with transdermal or injectable preparations during feminizing GAHT. Spironolactone was not associated with conversion of estradiol to estrone. Further studies are needed to determine the clinical relevance of these findings and to understand whether estrone or estrone/estradiol ratios are predictive of safety outcomes and feminization in transgender or gender nonbinary adults.

Authors' Contributions

L.R.C. and D.N.G. designed the study. G.W.M., J.R., K.S., J.C.D., M.D.K, J.A.D., and D.N.G. contributed to data collection. All authors analyzed and interpreted the data. L.R.C. and D.N.G. drafted the manuscript. All authors reviewed, critically revised, and approved the final manuscript.

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Author Disclosure Statement

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Supplementary Material

Supplementary Data
Supplementary Table S1

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