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Original Article

Pharmacokinetics of Sublingual Versus Oral Estradiol in Transgender Women

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ABSTRACT

Objective: To investigate the pharmacokinetics of 17β-estradiol (E2) administered orally versus those of 17β-E2 administered sublingually in transgender women.
Methods: Single doses of 17β-E2 were administered orally (1 mg) to 10 transgender women and then sublingually (1 mg) after a 1-week washout period. Blood samples were collected at baseline (0 hour) and at 1, 2, 3, 4, 6, and 8 hours after dosing. The samples were frozen and analyzed using liquid chromatography mass spectrometry (LC-MS/MS) and immunoassay.
Results: The results demonstrated that sublingual E2 had a significantly higher peak serum E2 concentration of 144 pg/mL, measured using LC-MS/MS, compared with an oral E2 concentration of 35 pg/mL, measured using LC-MS/MS ($P = .003$). Sublingual E2 peaked at 1 hour and oral E2 peaked at 8 hours, as measured using LC-MS/MS. The area under the curve (AUC) (0-8 hours) for sublingual E2, measured using LC-MS/MS, was 1.8-fold higher than the AUC (0-8 hours) for oral E2, measured using LC-MS/MS. Additionally, sublingual E2 was found to have an increased E2-to-estrone ratio at all time points (1.1 ± 1.0 vs 0.7 ± 0.4, $P \leq .0001$), the clinical significance of which is unclear.
Conclusion: Oral E2 administered sublingually has a different pharmacokinetic profile, with higher serum E2 levels and AUC (0-8 hours) than traditionally administered oral E2. Multidaily dosing may be necessary to suppress testosterone levels with sublingual E2. The appropriate dosing, efficacy, and safety of sublingual E2, compared with those of other E2 preparations, are unknown.

Introduction

Transgender women may use estrogens for the purpose of developing female secondary sex characteristics and decreasing undesired endogenous testosterone production. The route of estradiol (E2) administration affects its metabolism, resulting in distinct pharmacokinetic profiles. Transgender women are often prescribed oral E2 because it is the most cost-effective option for feminization. It has been found to be effective in achieving the desired 17β-E2 levels while also achieving secondary sex characteristic development.[1–4] Unfortunately, transgender women on oral estrogen replacement therapy have been found to experience increased venous thromboembolism and ischemic stroke rates compared with cisgender women and cisgender men (ie, women and men who identify with their sex assigned at birth).[5] Patients with a higher average dose of E2 over the first 2 years of gender-affirming hormone therapy (GAHT) have been found to have a higher risk of adverse events.[5]

Transdermal estrogen has a lower risk of adverse events than oral estrogen but has limited dosing options, which often require transgender women to wear multiple patches in order to achieve the desired E2 levels.[1,6–11] There is paucity of data on injectable

Abbreviations: AUC, area under the curve; BMI, body mass index; E1, estrone; E2, estradiol; GAHT, gender-affirming hormone therapy; LC-MS/MS, liquid chromatography mass spectrometry.

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estrogens. Sublingual administration of E2 tablets may prove to be a good alternative, with sublingual E2 having similar pharmacodynamics to transdermal E2—because sublingual absorption also bypasses much of first-pass metabolism—while maintaining the ease of use that is comparable with that of oral E2. Sublingual dosing entails holding the oral tablet under the tongue and allowing it to dissolve, where its absorption is enhanced by rich vascularization under the tongue. This method results in direct entry of the drug into the circulation, bypassing enteral processing and resulting in more rapid absorption.[12,13] However, sublingual dosing may not entirely bypass first-pass metabolism because some portion of the drug may be swallowed following dissolution in the saliva.

Orally administered E2 limits bioavailability to approximately 2% to 10%.[14] In contrast, the area under the curve (AUC) for 24 hours with sublingual E2 (in cisgender postmenopausal women) has been found to be 2.6-fold higher than that with oral dosing, demonstrating increased drug exposure and higher peak serum concentrations.[12] The investigations of sublingual E2 in transgender women are limited, although none have specifically studied the pharmacokinetics of sublingual E2 in transgender women. It is necessary to illuminate the basic kinetics of sublingual E2 in this population before appropriate dosing, efficacy, and safety studies can be conducted for sublingual E2 administration. Because this patient population may be on hormone therapy for most of their lifetime, it is imperative to gain a better understanding of the merits and limitations of optimal dosing options and methods of administration.

Methods

Study Design

This study was approved by the Medical College of Wisconsin Institutional Review Board and registered on ClinicalTrials.gov as identifier NCT04036500. Subject participation was required for a total of 2 days, and the subjects were not required to fast. Blood samples were drawn at baseline at 0 hour via a percutaneous intravenous catheter. Then, the subjects were provided with a 1 mg E2 tablet (Mayne Pharma) to be taken orally. Additional blood samples were drawn at 1, 2, 3, 4, 6, and 8 hours after taking the oral E2. A minimum 7-day washout period was completed prior to the second study day in order to ensure clearance of the oral dose because the half-life of 1 mg of oral 17β-E2 has been estimated to be 20.1 ± 14.2 hours.[12] On day 2, the subjects were provided with a 1 mg E2 tablet to be taken sublingually and instructed to hold the tablet under the tongue until it dissolved entirely. Blood was drawn at identical time points. Subjects who used tobacco abstained from smoking on the morning of the visit to ensure that the absorption of sublingual E2 was not affected by the vasoconstrictive effects of smoking. At the end of day 2, the subjects were administered a brief one page survey regarding their experience with sublingual administration.

Study Participants

Ten transgender women were recruited through the Froedtert and Medical College of Wisconsin Inclusion Health Clinic. The inclusion criteria required the participants to be English-speaking transfeminine individuals, aged ≥18 years, and new to hormone therapy. The exclusion criteria included a history of GAHT, orchiectomy, venous thromboembolism or pulmonary embolism, arterial thromboembolic disease, breast cancer, liver disease, bleeding disorders, and needle phobia. A medication list was obtained from each subject for review by our research team pharmacist, who determined whether any potential drug interactions were likely via cytochrome P450 3A4 induction or inhibition.

Laboratory Measurements

The blood samples were centrifuged; serum or plasma aliquots were frozen at −20 °C and then sent to Wisconsin Diagnostic Laboratories for E2 testing using immunoassay and to ARUP Laboratories for analysis using LC-MS/MS. Immunoassay testing was performed using Roche Elecsys Estradiol III assay, operated on a cobase801 platform, standardized against CRM 6004a via isotope dilution CG-MS. The limit of detection of this method was 5 pg/mL (18.4 pmol/L). Analytical specificity information from the supplier showed no appreciable cross-reactivity with other endogenous steroid hormones at typical in vivo concentrations. Additionally, both E2 and estrone (E1) were quantified using LC-MS/MS, wherein these were measured after derivatization with 2-fluoro-1-methylpyridinium-*p*-toluenesulfonate using LC-MS/MS with Agilent 6495C triple-quadrupole instrument. The limit of detection and quantification was 2 pg/mL for both the analytes, and the coefficients of variation ranged from 10% to 15%, with the mean concentrations ranging from 4 to 837 pg/mL.

Data Analysis

The independent variables in this study were time points (0, 1, 2, 3, 4, 6, and 8 hours), route of E2 administration (oral and sublingual), obesity, and blood analysis method (LC-MS/MS and immunoassay). The primary dependent variable was serum drug level, measured in picogram per milliliter. Each of the 10 participants had their serum E2 levels measured using LC-MS/MS and immunoassay at all 7 time points. E1 was also measured using LC-MS/MS for each condition and at each time point. Descriptive tables, bar graphs, and line graphs were created to summarize the drug levels across the time points and within the E2 administration and blood analysis groups. Mean and SDs were calculated for the descriptive tables. Paired *t* tests (or 2-sample *t* tests for obesity comparisons) were performed for 2 group comparisons, and repeated-measure analysis of variance was performed to compare ≥3 groups. To determine where the significance lies in the repeated-measure analysis of variance, multiple pairwise *t* tests were performed as post hoc tests. For the post hoc tests, *P* values were adjusted using Bonferroni correction. All the statistical analyses were performed using R (version 4.0.3), and a *P* value of <.05 was considered statistically significant.

Results

Subject Demographics

Demographically, the age of the 10 subjects ranged from 18 to 43 years, with a mean age of 24 ± 8 years and a median age of 20 years. Eight participants were nonsmokers, and 2 were regular smokers. Eight participants were White, 1 was Hispanic, and 1 was Black. The mean body mass index (BMI) of the 10 subjects was 33 ± 13 kg/m^2, with a median BMI of 32 kg/m^2. A majority of the subjects were obese ($n = 6$; BMI > 30 kg/m^2).

Oral Versus Sublingual E2

The mean baseline E2 level at 0 hour was 24 ± 8 pg/mL, as measured using LC-MS/MS (reference range for cisgender men: 10-42 pg/mL). There was no significant difference in the starting level of E2, measured using LC-MS/MS, between the sublingual

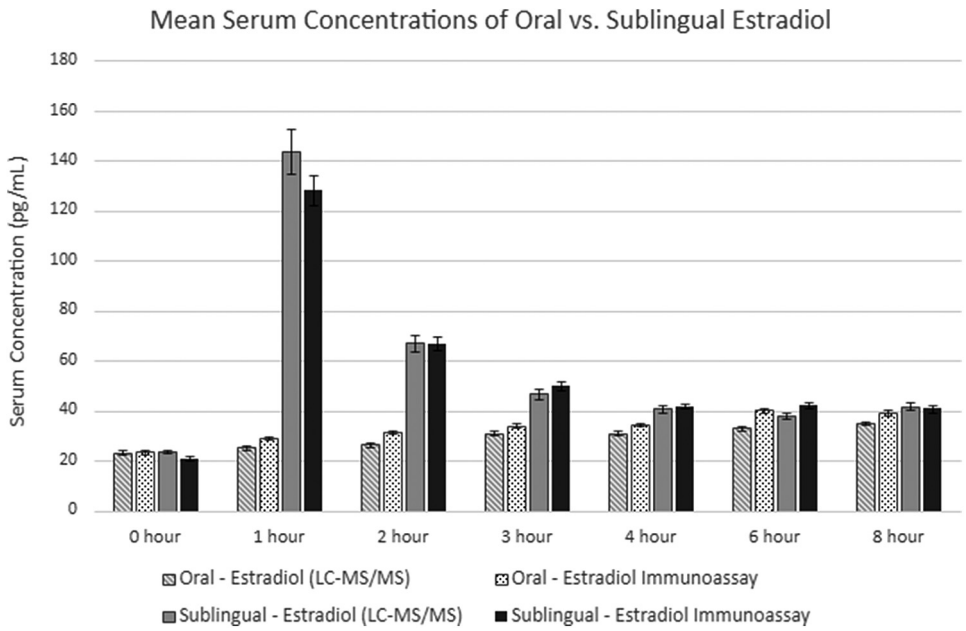

Fig. 1. Comparison of serum estradiol concentrations over time following oral and sublingual administration, measured using LC-MS/MS and immunoassay in both the conditions ($N = 70$ per series). Error bars represent SEs. *LC-MS/MS* = liquid chromatography mass spectroscopy.

obese and nonobese groups ($P = .876$) and between the oral obese and nonobese groups ($P = .802$).

The peak sublingual E2 serum concentration, measured using LC-MS/MS, was 144 pg/mL at 1 hour, whereas the peak oral E2, measured using LC-MS/MS, was 35 pg/mL at 8 hours (Fig. 1). Overall, sublingual administration resulted in a 4-fold higher peak E2 level at 1 hour, compared with the peak oral E2 level at 8 hours, although a higher level of intersubject variability was noted in the sublingual group at the peak-concentration time point. At 1 hour, the oral and sublingual E2 levels, measured using LC-MS/MS, were significantly different (25 ± 7 pg/mL vs 144 ± 90 pg/mL, respectively, $P = .003$); however, there was no difference in the end concentrations at 8 hours (35 ± 8 pg/mL vs 42 ± 14 pg/mL, respectively, $P = .179$). Additionally, the AUC (0-8 hours) for sublingual E2 was 1.8-fold higher than the AUC (0-8 hours) for oral E2 (Fig. 2).

In the oral dosing group, a statistically significant difference was observed in the E2 concentration at 0 hour and 8 hours (23 ± 9 pg/mL vs 35 ± 8 pg/mL, respectively, $P \leq .0001$), indicating that 1 mg of oral E2 significantly increased the E2 levels from that at the baseline to that at 8 hours. When the oral E2 levels at 1, 2, 3, 4, 6, and 8 hours were compared, the only significant difference was observed between 1 hour and 8 hours (25 ± 7 pg/mL vs 35 ± 8 pg/mL, respectively, $P = .024$), suggesting that oral E2 is relatively stable over time (Fig. 2). Within the sublingual group, the E2 concentration remained elevated at 8 hours compared with that at the baseline (24 ± 8 pg/mL at 0 hour vs 42 ± 14 pg/mL at 8 hours, $P = .0008$). During the 8 hours, there was a significant difference at 1 hour (144 ± 90 pg/mL, peak concentration) compared with that at 2 (67 ± 33 pg/mL, $P = .00003$), 3 (47 ± 21 pg/mL, $P \leq .001$), 4 (41 ± 14 pg/mL, $P \leq .001$), 6 (38 ± 12 pg/mL, $P \leq .001$), and 8 hours (42 ± 14 pg/mL, $P \leq .001$).

LC-MS/MS Versus Immunoassay

We found a statistical difference between LC-MS/MS and immunoassay in the oral E2 group when all the time points were grouped together (29 ± 9 pg/mL vs 33 ± 10 pg/mL, $P \leq .0001$, determined using paired *t* test). However, there was only a statistical difference between the assays at 1 hour (25 ± 7 pg/mL vs 29 ± 8 pg/mL, respectively, $P = .036$) and 6 hours (33 ± 8 pg/mL vs 40 ± 11 pg/mL, respectively, $P = .024$). There were no differences between oral E2 measured using LC-MS/MS and that measured using immunoassay at any other time point. In the sublingual group, there was no statistical difference between the E2 levels measured using LC-MS/MS and those measured using immunoassay when all the time points were grouped together (57 ± 52 pg/mL vs 56 ± 42 pg/mL, respectively, $P = .423$, determined using paired *t* test). When analyzed at each time point, a statistical difference between the methods was observed only at 6 hours (38 ± 12 pg/mL vs 42 ± 14 pg/mL, respectively, $P = .044$).

E2 Versus E1

The E2:E1 ratio was increased across all the time points with sublingual administration compared with oral administration (1.1 ± 1.0 pg/mL vs 0.7 ± 0.4 pg/mL, respectively, $P \leq .0001$) (Fig. 3). In the sublingual E2 group, the E2:E1 ratio was the highest at the 1-hour peak E2 concentration, which differed significantly from the ratios seen at 0, 2, 3, 4, 6, and 8 hours. Therefore, although the E2:E1 ratio was significantly higher in the sublingual group than in the oral group, it was also significantly higher at 1 hour than at any other time point within the sublingual group. This finding, along with Figure 3, suggests that the highest proportion of E2:E1 ratio with sublingual dosing occurs at the peak serum E2 concentration, then decreases, and plateaus by 8 hours, possibly a result of peripheral conversion of E2 to E1. In the oral group, the E2:E1 ratio was significantly lower at 4, 6, and 8 hours compared with that at the baseline, which demonstrates that E1 increases over time following oral E2 administration, further lowering the E2:E1 ratio.

Administration Method Survey

Results of a brief survey administered to the subjects at the end of their participation showed sublingual administration to be a

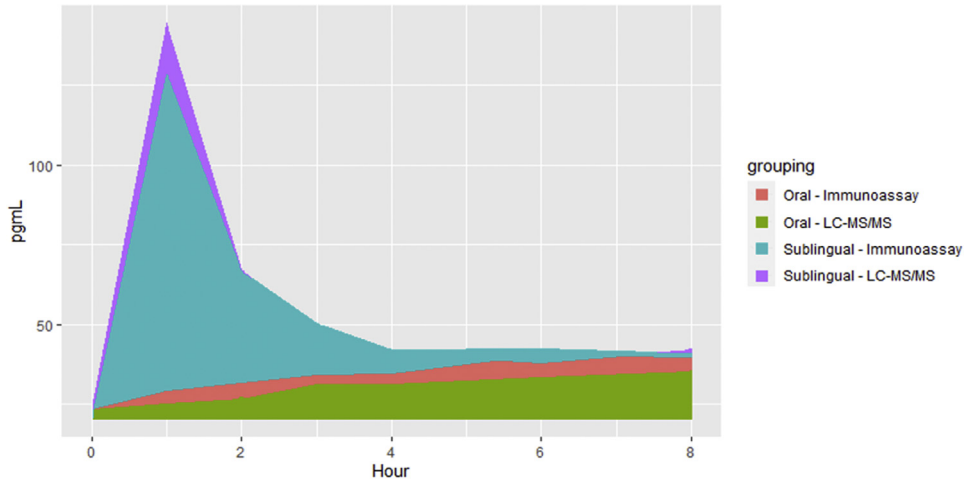

Fig. 2. Area under the curve (0-8 hours) for concentration (pg/mL) versus time (hours) of exposure to estradiol, compared based on the method of administration and the type of assay (ie, immunoassay or LC-MS/MS). *LC-MS/MS* = liquid chromatography mass spectroscopy.

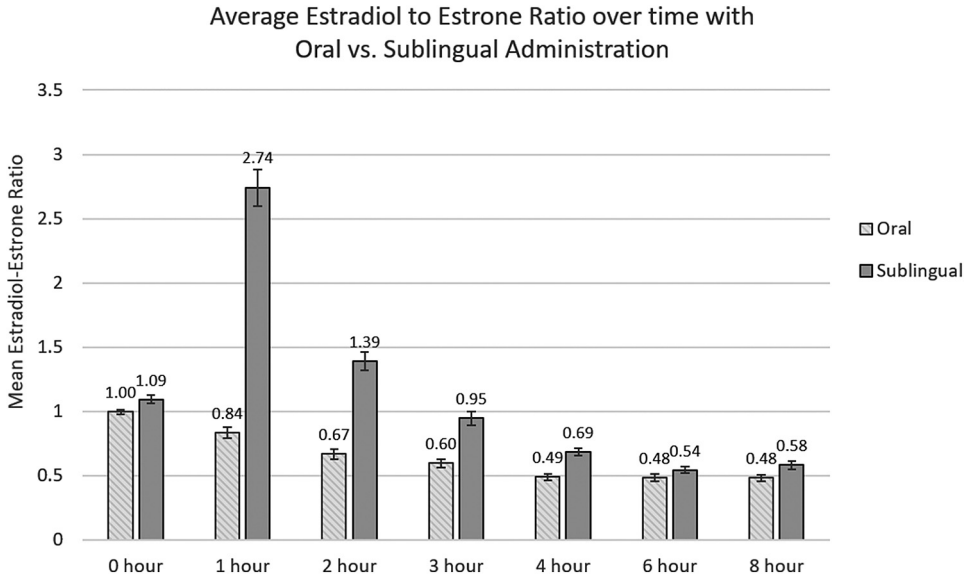

Fig. 3. Comparison of the estradiol:estrone ratio over time using oral or sublingual administration, measured using LC-MS/MS ($N = 70$ per series). Error bars represent SEs.

feasible dosing method. The subject descriptions of sublingual administration included a chalky taste or sensation (40%), and 30% of the subjects reported uncertainty in knowing when the tablet was dissolved because of its small size. No negative effects were reported. All the subjects indicated that they would be willing and able to consistently take GAHT sublingually.

Discussion

As previously discussed, one of the critical potential benefits of sublingual E2 is its avoidance of first-pass metabolism. Instead, it is absorbed transmucosally via passive diffusion into the sublingual vascular bed and then distributed into the systemic circulation. The small volume of saliva present in the oral cavity is usually sufficient to fully disintegrate sublingually administered tablets, although it is certainly possible that a portion of the dissolved drug is swallowed via the saliva.[15] In this study, the subjects reported that the tablet dissolved within approximately 1 to 2 minutes. Sublingual administration was well tolerated, suggesting that this could be a nonburdensome dosing method for patients.

There was a prominent difference between the pharmacokinetics of sublingual E2 and those of oral E2 despite the tablets not being formulated specifically for sublingual administration. We observed that the oral E2 levels, measured using LC-MS/MS, gradually increased, without dramatic fluctuation at any time points, suggesting a steady-state level. Sublingual E2 resulted in a more rapid peak at 1 hour, after which it plateaued at a similar (nonsignificantly different) concentration as oral E2 by 8 hours. This suggests that sublingual E2 did not decrease to a level lower than oral E2 levels after reaching a plateau. Therefore, sublingual E2 not only reached a dramatically higher peak concentration in the initial hours after administration but also maintained comparable elevated serum levels with oral E2 at 8 hours, following its plateau (Fig. 2). These results mirror that of Price et al,[12] who reported rapid, burst-like absorption with significantly higher E2 levels following sublingual dosing in postmenopausal women.[12] The elimination half-life of sublingual E2 has been previously reported to be between 11 to 14 hours.[16] In conjunction with our data, this points toward a multidose daily schedule. Twice-daily dosing may not be ideal for some patients who prefer once-daily medications,

and this may be a barrier to compliance compared with compliance with once-daily options.

Although the decline from the peak concentration of sublingual E2 was steep, we found that the AUC (0-8 hours) was 1.8-fold higher than that for oral E2 after accounting for the baseline E2 levels. The consequence of such an increased AUC is more extensive exposure to the drug. However, when considering the more gradual rise in E2 that we observed with oral administration, with the reported half-life of oral E2 being 20.1 ± 14.2 hours, it is likely that the AUC was underestimated by the 8-hour time point of sample collection.[12] Because of the 8-hour limitation, neither the oral E2 half-life nor oral clearance was calculated. A greater AUC in the setting of lower levels at later time points, as seen with sublingual dosing, cannot be assumed to be beneficial. It is unknown whether feminizing benefits relative to thrombotic risk are maximized with a higher peak concentration and AUC or a higher steady-state level.

A major contrast point between oral and parenteral methods is that with oral E2 dosing, the E1 levels increase proportionally more than the E2 levels because of the interconversion of E2 to E1 by 17 β-hydroxysteroid dehydrogenase 2 during first-pass metabolism.[16] E2 and E1 are the 2 main biologically active estrogens in nonpregnant humans. Although E2 and E1 interconvert, E1 is a weak estrogen, with only 4% of the estrogenic activity of E2.[16] There is greater conversion to E1 by 17 β-hydroxysteroid dehydrogenase 2 when E2 is orally administered, leading to a low E2:E1 ratio of about 1:5.[16] In comparison, sublingual administration avoids much of the extensive transformation to E1 that occurs in the intestinal tract and liver, increasing the E2:E1 ratio to 3:1.[16]

This distinction in the estrogen ratio was observed in our data: the mean E2:E1 ratio observed with oral E2 was significantly lower than that observed with sublingual E2, indicating relatively higher E1 levels. The metabolism of E1 is less straightforward compared with that of sublingual E2, although investigations have been limited. A recent cross-sectional study based on prospective data of transgender women demonstrated that sublingual E2, not transdermal or injectable E2, results in a higher E1:E2 ratio, a finding that we did not anticipate given our study.[17] The authors did not compare parenteral methods with oral methods of E2 administration. Nevertheless, it is interesting that sublingual E2 was found to undergo more conversion to E1 than transdermal or injectable E2, when one might have thought them to be similar. The authors mentioned metabolism by lymphatic tissues in the neck as a potential explanation for the increased conversion to E1.[17] Overall, the clinical significance of increasing the E2:E1 ratio is yet to be clearly established.

We measured E2 using both LC-MS/MS and immunoassay because LC-MS/MS is the gold standard, but immunoassay is cheaper and more widely available. Mass spectrometry-based assays are preferred for the measurement of steroid hormone end points in high-quality endocrine research, particularly because immunoassay methods can lack selectivity and demonstrate cross-reactivity between estrogens.[18] However, clinical monitoring of E2 levels may not require such extensive rigor. We found that E2 measured using LC-MS/MS in the oral group was statistically different from E2 measured using immunoassay in the oral group, although further analysis demonstrated a statistical difference at only 2 of the 7 time points. In the sublingual group, a difference in the E2 level, measured using immunoassay and LC-MS/MS, was seen only at 1 time point. In both the cases, the absolute difference between the values was small and unlikely to be clinically significant (eg, impact on treatment decisions such as dose adjustment). Further comparison of these techniques on a larger scale may be useful in determining whether more readily available immunoassays are adequately accurate for monitoring E2 levels in a clinical setting because this study is not powered to determine this.

The limitations of this study are not insignificant because of the low power conferred by the sample size of 10 subjects. As such, rather than firmly establishing the pharmacokinetics of sublingual E2 in transgender women, the results presented here are intended to inform future studies of sublingual GAHT. However, the initial data offer evidence that the lower bioavailability and increased conversion to E1 are limitations to the potency achievable through the oral administration of E2, limitations by which sublingual E2 may be less constrained. This comes at the expense of a more stable steady-state concentration.

Additional possible limitations to this investigation involve subject demographics. Most participants were obese, and adiposity is well-established to be correlated with increased circulating estrogens. More specifically, plasma E2 levels have been found to positively correlate with adiposity measurements (E2 levels: 3.26 ± 1.24 pg/mL, 4.55 ± 2.81 pg/mL, and 8.00 ± 3.53 pg/mL in normal-weight, overweight, and obese cisgender women, respectively [$P \leq .0001$]).[19] However, the subjects included in our study did not begin the study with higher-than-normal E2 levels despite their obesity (mean E2: 24 ± 9 pg/mL at 0 hour), and no significant difference in baseline E2 was seen between obese (BMI > 30 kg/m^2) and nonobese subjects. Retrospective studies on transgender women have shown that BMI does not correlate with E2 levels achieved ($r = -0.063$, $P = .413$).[20] However, where an effect of BMI on the E2 levels was seen, the variance attributable to BMI was small ($R^2 = 0.056$).[3] Studying whether BMI significantly influences sublingually administered E2 would be a valuable future investigation.

Conclusion

Our results show that sublingually dosing oral E2 tablets results in changes in its pharmacokinetics. Sublingual E2 reaches higher serum E2 levels and AUC (0-8 hours) than oral E2, has an early, significant peak, and then decreases. This periodicity may make multidaily dosing of sublingual E2 necessary to suppress testosterone, and this may not be an ideal regimen for every individual seeking transfeminine hormone therapy. Our pharmacokinetic data also demonstrate the importance of providers to interpret E2 levels of patients taking E2 sublingually in the context of the timing of the last dose. Moreover, the appropriate dosing, efficacy, and safety of administering E2 sublingually versus those of administering E2 orally are unknown. Larger-scale investigations on the differences in estrogen administration in transgender women are needed.

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Disclosure

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