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Clinical Research Article

Effect of Spironolactone and Cyproterone Acetate on Breast Growth in Transgender People: A Randomized Clinical Trial

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Abstract

Context: Transgender people with sex recorded male at birth desiring feminization commonly use cyproterone acetate or spironolactone as antiandrogens with estradiol, but the optimal antiandrogen is unclear.

Objective: We aimed to assess the effect of antiandrogens on breast development. We hypothesized this would be greater in those treated with cyproterone acetate than spironolactone due to more potent androgen receptor antagonism and suppression of serum total testosterone concentrations.

Methods: A randomized clinical trial was conducted between 2020-2022 at an outpatient endocrinology clinic. Transgender people aged 18+ years old commencing feminizing gender affirming hormone therapy were included. The intervention was standardized estradiol therapy plus either spironolactone 100 mg daily or cyproterone acetate 12.5 mg daily for 6 months. The primary outcome was breast development as measured by the breast–chest distance. Secondary outcomes included estimated breast volume, suppression of serum total testosterone concentration <2 nmol/L, and Gender Preoccupation and Stability Questionnaire (GPSQ).

Results: Sixty-three people (median age 25 years) were enrolled, randomized, and included in intention to treat analysis (cyproterone acetate n = 32, spironolactone n = 31). At 6 months, there was no between-group difference in breast–chest distance (mean difference 0.27 cm, 95% CI −0.82 to 1.35, $P = .6$) or estimated breast volume (mean difference 17.26 mL, 95% CI −16.94 to 51.47, $P = .3$). Cyproterone acetate was more likely to suppress serum testosterone concentration to <2 nmol/L (odds ratio 9.01, 95% CI 1.83 to 4.44, $P = .008$). Changes in GPSQ were similar between groups.

Conclusion: Antiandrogen choice should be based on clinician and patient preference with consideration of side effects. Further research is needed to optimize breast development in transgender people.

Key Words: antiandrogen, transgender, feminization, testosterone

Abbreviations: ALT, alanine transaminase; BMI, body mass index; GPSQ, Gender Preoccupation and Stability Questionnaire; PHQ-9, Patient Health Questionnaire 9.

People with a female or nonbinary gender whose sex was recorded male at birth commonly use antiandrogens with estradiol to induce physical changes such as breast development, body fat redistribution, and decreased facial and body hair (1-3). Antiandrogens decrease the effects and/or synthesis of testosterone through androgen receptor antagonism, inhibition of synthetic enzymes, and/or suppression of gonadotrophins. Common antiandrogens include spironolactone and cyproterone acetate, but use of nonsteroidal androgen receptor antagonists (eg, bicalutamide), gonadotrophin-releasing hormone analogues, progestogens, and 5-alpha-reductase inhibitors is well described (4).

Through potent progestogenic suppression of hypothalamic–pituitary–gonadal axis and moderate androgen receptor antagonism, cyproterone acetate is associated with greater suppression of serum testosterone than spironolactone but the effects on feminization remain unclear (5). A systematic review of antiandrogens in transgender people identified low-quality studies which suggested that cyproterone acetate, progestogens and gonadotrophin-releasing hormone analogues resulted in greater suppression of serum total testosterone concentrations than spironolactone or estradiol alone (4). Subsequently, a 12-week single-blinded randomized trial in transgender people on estradiol showed that those using cyproterone acetate resulted in lower median serum total testosterone concentrations than spironolactone (0.26 nmol/L vs 14.23 nmol/L, $P < .001$) (6). These findings supported cross-sectional data (7) but unfortunately did not include clinically relevant outcomes of feminization.

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In the absence of robust evidence on comparative efficacy and safety of antiandrogens, prescribing practices vary significantly worldwide reflecting local differences in cost, availability, and experience. In this study of transgender people commencing feminizing hormone therapy, we aimed to assess the effects of cyproterone acetate and spironolactone on breast development, a commonly desired goal of feminization. We hypothesized that use of cyproterone acetate would result in greater breast development than spironolactone due to greater suppression of serum total testosterone due to its progestogenic effects and more potent antagonism of the androgen receptor.

Materials and Methods

This study was reported using the CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials (8).

Trial Design

This was an investigator-initiated, double-blind, 6-month randomized trial conducted at a single tertiary referral hospital in Melbourne, Australia. Ethics approval was obtained from Austin Health Human Research Ethics Committee (HREC/44503/Austin-2018) and LGBTIQ+ community-controlled organization Thorne Harbour Health's Community Research Endorsement Panel (THH/CREP 20-002). Participants provided written informed consent. The trial was prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12620000339954).

Participants

Transgender people aged over 18 years newly commencing estradiol were eligible for enrollment. Participants were recruited from the Austin Health Gender Clinic, and local primary care and endocrine clinics.

Exclusion criteria included commencement of estradiol >6 weeks prior to enrollment, preexisting hypogonadism (serum total testosterone concentration <10 nmol/L prior to commencing estradiol), previous treatment with antiandrogens, contraindications to treatment with spironolactone or cyproterone acetate, and planned orchidectomy during the study period.

Interventions and Trial Procedures

Participants were randomized to treatment with oral cyproterone acetate 12.5 mg daily or oral spironolactone 100 mg daily for 6 months, with administration recommended in the morning. Medications were prepared as identical capsules in appearance and taste and provided to participants at 0 and 3 months. In the absence of dose-finding studies with clinically meaningful outcomes to determine the optimal dose, the doses of spironolactone and cyproterone acetate used were derived from the median doses used in a cohort of transgender people, reflecting real-world clinical practice (7).

Standardized estradiol therapy was commenced with oral estradiol valerate 4 mg daily or transdermal dose equivalent based on participant and clinician preference. Estradiol doses were subsequently titrated at months 1, 2, and 3 to achieve a serum estradiol concentration of 250 to 600 pmol/L, in line with Australian consensus guidelines (1).

Outcomes and Safety Measures

Primary outcome

Change in breast–chest distance. The primary outcome was the change in breast–chest distance measured at 0, 3, and 6 months, derived by subtracting the chest/underbust circumference from the breast/overbust circumference as described by de Blok et al (9). This was chosen as a measurement of breast development due to the availability of existing data in a cohort of transgender people at the time of trial conceptualization and design.

Secondary outcomes

Change in estimated breast volume. Using a digital camera, standardized frontal and lateral view clinical photographs were taken of the left and right breast, which included placement of a 28-mm piece of blue tape on the skin to provide a known distance for scale. The Breast Idea Volume Estimator application (10) was used to estimate volume by 2 independent researchers at baseline and 6 months using a new module for people assigned male at birth that was validated against a 3D scanner gold standard (11).

Serum total testosterone concentration. The percentage of participants with serum total testosterone concentration <2 nmol/L (typical cisgender female range) was measured at 0, 1, 2, 3, and 6 months. Total testosterone was measured by after liquid/liquid extraction by liquid chromatography tandem mass spectrometry on the Shimadzu HPLC connected to AbSciex 5500 mass spectrometer. The assay range was 0.1 to 34.0 nmol/L with precision of 4.8% at 0.4 and 2.4% at 12 nmol/L.

Gender preoccupation and stability questionnaire. Gender dysphoria was measured with the Gender Preoccupation and Stability Questionnaire (GPSQ) (12); a validated 14-item scale measuring gender-related distress was performed at 0, 3, and 6 months. Scores >28 indicate clinically significant gender dysphoria, and changes in score >11 indicate reliable change in gender dysphoria (12). We hypothesized that there may be a between-group difference in the GPSQ if there was a between-group difference in clinical feminization. However, we acknowledge that not all transgender people, including those taking gender-affirming hormone therapy, experience gender dysphoria.

Safety Measures

Serum potassium, urea and creatinine, alanine transaminase (ALT), and prolactin concentrations were measured at 0, 1, 2, 3, and 6 months given theoretical concerns of spironolactone-related hyperkalemia, renal impairment, and cyproterone acetate–related hepatotoxicity and hyperprolactinemia. Electrolytes, urea, creatinine, and liver function tests were measured using the Beckman Coulter AU5800 series clinical chemistry analyzer. Prolactin was measured using the Beckman Coulter DxI 800 analyzer, which employs an immunoassay (Beckman Coulter Cat# 33530, RRID:AB_2750985). Blood pressure was recorded at 0, 3, and 6 months due to concerns regarding spironolactone-related hypotension. The Patient Health Questionnaire 9 (PHQ-9) (13) was administered at 0, 3, and 6 months given reported associations with cyproterone acetate and worsening of depression.

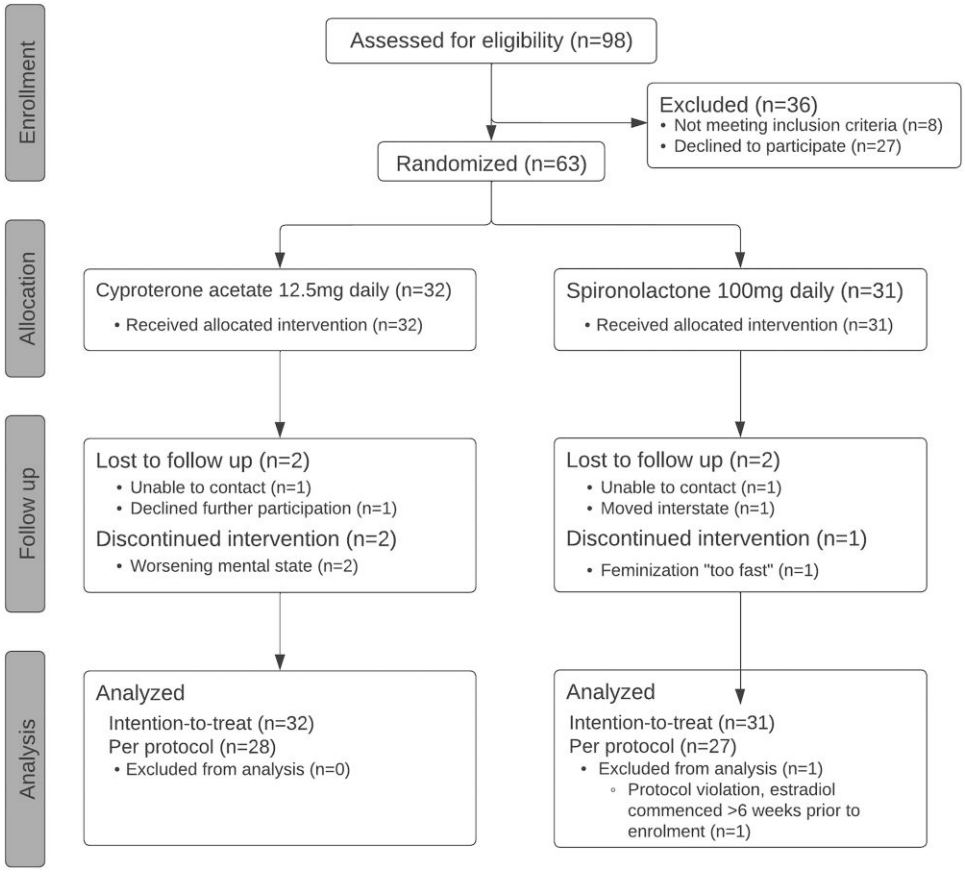

Figure 1. CONSORT participant flow diagram.

Sample Size

Sample size calculation was based on the percentage of participants that suppressed serum total testosterone <2 nmol/L in a previously published cross-sectional study, in the absence of preliminary data of breast–chest distance by antiandrogen (7). To detect a difference of 40%, a sample size of 25 per group was required to achieve power 0.9 and level of significance .05. Using a conservative dropout of rate of 20%, we estimated that 64 participants would need to be recruited.

Randomization

Randomization was performed by an independent trials pharmacist with participants allocated in a 1:1 ratio according to a computer-generated randomization procedure, stratified in blocks of 2 for baseline age (<27 or ≥27 years) and body mass index (BMI) (<24.7 or ≥24.7 kg/m^2). The age and BMI cutoffs to define groups and medication doses were based on median values from previous analysis of our clinic data (7).

Blinding

Participants, care providers, and investigators were blinded to treatment allocation until data collection and data analysis were complete.

Statistical Methods

Statistical analysis was performed using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria) on an intention to treat and per protocol basis (14). Normally distributed continuous data were reported with mean and SD, and median and interquartile range (IQR) were reported for non-normally distributed data. Frequency and percentage were reported for categorical data. A linear mixed effects model was fitted for breast chest distance over time, with treatment group, time, and treatment by time interaction term. All models were adjusted for age and BMI. Group differences at each timepoint were compared using prespecified post hoc contrasts adjusted for multiple comparisons. The main outcomes were reported as mean difference and corresponding 95% CI, along with estimated marginal means across each time point. For the analysis of secondary outcome serum testosterone concentration at 6 months, the effect of treatment group on the odds of achieving <2 nmol/L was estimated using logistic regression with Firth's penalized likelihood adjusting for age, BMI, and serum testosterone concentration at baseline. Further exploratory analysis was performed using linear regression to investigate the effect of serum estradiol concentration on breast volume adjusted for age, BMI, and breast volume at baseline. Self-rated breast satisfaction across time was analyzed using a cumulative link mixed model adjusted for age and BMI. A 2-sided $P < .05$ were deemed to be statistically significant.

Results

Participant Flow

Participant flow is illustrated in Fig. 1. Ninety-eight individuals were screened for eligibility. Of these, 35 did not meet

Table 1. Baseline participant characteristics

	Cyproterone acetate	Spironolactone
N	32	31
Age—years	25.3 (22.0-29.0)	25.1 (21.9-27.8)
Body mass index—kg/m^2	25.7 (21.5-30.0)	24.6 (21.1-31.9)
Country of birth		
Australia—n (%)	27 (84.4)	27 (87.1)
Race—n (%)		
White	31 (96.9)	27 (87.1)
Aboriginal and Torres Strait Islander	1 (3.1)	1 (3.2)
Asian	2 (6.3)	4 (12.9)
African	0 (0)	2 (6.5)
Overbust/breast circumference—cm	97.3 (90.1-105.0)	92.7 (89.2-108.7)
Underbust/chest circumference—cm	89.0 (83.0-98.3)	88.7 (83.3-100.8)
Breast–chest distance—cm	6.0 (5.1-8.2)	5.8 (4.9-7.0)
Left breast volume—mL	29.5 (14.0-62.4)	27.1 (12.9-61.9)
Right breast volume—mL	27.1 (13.2-60.7)	29.6 (10.5-82.2)
Averaged breast volume—mL	29.7 (13.4-58.8)	28.3 (10.9-69.7)
Serum estradiol—pmol/L	117.0 (93.5-178.5)	127.5 (96.5-276.5)
Serum total testosterone—nmol/L	17.6 (12.0-22.5)	15.9 (12.9-21.4)

Values are presented as median (interquartile range) unless otherwise stated.

inclusion criteria (n = 8) or declined participation (n = 27). Following randomization, participants were allocated to treatment with cyproterone acetate (n = 32) or spironolactone (n = 31). In the cyproterone acetate group, 2 participants were lost to follow-up due to being uncontactable (n = 1) or declined further participation due to perceived low estradiol doses (n = 1) and 2 discontinued the intervention due to worsening of mental state (n = 2). In the spironolactone group, 2 participants were lost to follow-up due to being uncontactable (n = 1) or moving interstate (n = 1), and 1 participant discontinued the intervention due to feminization occurring "too fast" (n = 1). One participant in the spironolactone group was excluded from analysis due to commencing estradiol >6 weeks prior to enrollment (protocol violation). Ultimately, 63 participants were included in intention to treat analysis (cyproterone acetate group n = 32, spironolactone group n = 31) and 55 participants were included in per protocol analysis (cyproterone acetate group n = 28, spironolactone group n = 27).

Recruitment

Recruitment occurred between August 2020 and March 2022 and was stopped once the target of 25 participants completing the study per group had been achieved. Data collection was completed in September 2022. There were minor disruptions related to the COVID-19 pandemic and associated restrictions in Melbourne, Australia.

Baseline Characteristics

Baseline group characteristics including age, BMI, country of birth, race, breast–chest distance, calculated cup size, estimated breast volume, serum estradiol, and serum total testosterone concentrations were comparable between groups and are presented in Table 1.

Outcomes

Key outcomes are presented in Table 2 and Fig. 2.

Breast development

At 6 months, the mean (SD) breast–chest distance was 9.2 cm (3.0) in the cyproterone acetate group and 8.2 cm (2.7) in the spironolactone group with no between-group difference (mean difference 0.27 cm, 95% CI −0.82 to 1.35, $P = .6$). The corresponding mean calculated cup size was AAA cup in both groups, with range AAA to B cup.

Using the Breast Idea Volume Estimator application, the mean averaged estimated breast volume was 190.2 mL (158.6) in the cyproterone acetate group and 158.5 mL (110.0) in the spironolactone group (mean difference 17.26 mL, 95% CI −16.94 to 51.47, $P = .3$).

Notably, there was significant interindividual variation within groups in the change in breast volume over the study period with range −0.77 to 479.79 mL (Fig. 3). Exploratory analyses showed an association of change in averaged estimated breast volume with age (3.49 mL for every 1 year increase with age, 95% CI 0.60 to 6.37, $P = .02$) and serum estradiol concentration (0.11 mL for every 1 pmol/L increase in serum estradiol concentration, 95% CI 0.04 to 0.18, $P = .003$) (Fig. S2 (15)).

Participants also self-rated breast satisfaction using a 5-point Likert scale (completely unsatisfied, unsatisfied, neither satisfied or dissatisfied, satisfied, completely satisfied) at 6 months. Overall, 34 (53.97%) were satisfied and 3 (4.76%) were completely satisfied with breast development with no between-group difference ($P = .5$).

Serum total testosterone

Mean serum total testosterone level was 1.48 nmol/L (3.45) in the cyproterone acetate group and 4.15 nmol/L (5.38) in the spironolactone group at 6 months but the mean difference of −3.33 nmol/L was not statistically significant. Suppression of serum total testosterone <2 nmol/L was 9-fold more likely in the cyproterone acetate group than the spironolactone group.

Gender Preoccupation and Stability Questionnaire

Overall, the mean difference GPSQ total score was −6.44 (95% CI −8.12 to −4.76, $P < .001$) over 6 months, with no between-group difference.

Safety measures

Serum prolactin concentration was higher in the cyproterone acetate group than the spironolactone group, which is less than twice the upper limit of normal when interpreted using the female reference range and of unclear clinical relevance. While the between-group mean difference in serum urea, creatinine, and ALT was statistically significant, all remained within the normal reference range and are unlikely to be clinically relevant. There was no between-group difference in serum potassium concentration, and systolic or diastolic blood pressure.

Overall, the mean PHQ-9 total score at 6 months was 11.0 (6.7) and did not significantly change throughout the trial period. There was no between-group difference.

Table 2. Results from intention to treat analysis

Outcome	Cyproterone acetate (n = 32)	Spironolactone (n = 31)	Mean difference (95% CI)	*P*	*P* (interaction)
Estradiol therapy at 6 months					
Formulation—n (%)					
Missing[a]	4 (12.5)	3 (9.7)			
Gel	6 (18.8)	1 (3.2)			
Patch	8 (25.0)	12 (38.7)			
Tablet	14 (43.8)	15 (48.4)			
Dose					
Gel—mg/day	2.5 (2.0-3.0)	2.0 (2.0-2.0)			
Patch—µg/day	150.0 (137.5-162.5)	175.0 (150.0-200.0)			
Tablet—mg/day	6.0 (4.0-6.0)	4.0 (4.0-6.0)			
Anthropometric measurements of breast development					
Overbust/breast—cm					
Baseline	98.8 (12.8)	98.0 (13.3)			
3 months	98.4 (11.8)	97.2 (12.2)	1.24 (−0.60, 3.07)	.2	.06
6 months	100.1 (12.9)	99.1 (12.6)			
Underbust/chest—cm					
Baseline	92.2 (12.2)	91.6 (12.7)			
3 months	90.3 (11.2)	89.1 (13.0)	0.97 (−0.86, 2.80)	.3	.1
6 months	91.0 (11.6)	90.9 (12.7)			
Breast–chest distance—cm					
0 months	6.6 (2.2)	6.4 (2.5)			
3 months	8.0 (2.2)	8.1 (3.2)	0.27 (−0.82, 1.35)	.6	.3
6 months	9.2 (3.0)	8.2 (2.7)			
Calculated cup size at 6 months—n (%)					
AAA	17 (53.1)	18 (58.1)			
AA	5 (15.6)	6 (19.4)	N/A	.92	N/A
A	5 (15.6)	3 (9.7)			
B	1 (3.1)	1 (3.2)			
Estimated breast volume using Breast Idea Volume Estimator application					
Left breast volume—mL					
Baseline	54.5 (73.3)	48.5 (57.0)	16.68 (−17.78, 51.14)	.3	.2
6 months	187.3 (152.5)	156.7 (113.4)			
Right breast volume—mL					
Baseline	57.7 (75.8)	53.0 (59.3)	17.86 (−17.98, 53.71)	.3	.2
6 months	193.2 (168.8)	160.3 (111.2)			
Averaged breast volume—mL					
Baseline	56.1 (73.9)	50.7 (57.3)	17.26 (−16.94, 51.47)	.3	.2
6 months	190.2 (158.6)	158.5 (110.0)			
Biochemistry					
Total testosterone—nmol/L					
Baseline	17.30 (7.53)	17.02 (8.10)			
3 months	0.81 (0.40)	6.75 (6.60)	−6.19 (−9.78, −2.60)	.0008	<.0001
6 months	1.48 (3.45)	4.15 (5.38)	−3.33 (−6.99, 0.32)	.07	
Suppression <2 nmol/L—n (%)					
Baseline	0 (0.0)	1 (3.2)	OR 9.01 (1.83, 44.44)	.008	N/A
6 months	22 (68.8)	14 (45.2)			
Sex hormone binding globulin—nmol/L					
Baseline	27.42 (15.26)	34.35 (19.84)			
3 months	42.07 (28.73)	49.63 (33.48)	−6.23 (−17.60, 5.13)	.3	.7
6 months	44.76 (27.13)	48.04 (34.43)			

(*continued*)

Table 2. Continued

Outcome	Cyproterone acetate (n = 32)	Spironolactone (n = 31)	Mean difference (95% CI)	*P*	*P* (interaction)
Estradiol—pmol/L					
Baseline	152.03 (86.42)	218.50 (205.44)			.03
3 months	340.36 (181.61)	347.97 (154.99)	59.12 (−103.65, 221.89)	.5	
6 months	515.93 (351.19)	354.38 (186.99)	228.38 (62.45, 394.31)	.007	
Luteinizing hormone—IU/L					
Baseline	4.01 (1.39)	4.30 (1.73)			<.0001
3 months	0.44 (0.94)	2.99 (2.11)	−2.42 (−3.56, −1.28)	<.0001	
6 months	0.44 (0.94)	2.52 (2.53)	−1.83 (−3.00, −0.66)	<.002	
Prolactin—mIU/L					
Baseline	231.42 (76.36)	220.19 (68.17)			<.0001
3 months	626.19 (243.18)	255.85 (88.08)	365.67 (288.10, 443.25)	<.001	
6 months	609.88 (201.42)	292.46 (134.81)	316.73 (237.35, 396.12)	<.001	
Potassium—mmol/L					
Baseline	3.95 (0.27)	4.05 (0.33)			
3 months	3.98 (0.31)	3.96 (0.35)	−0.08 (−0.20, 0.03)	.2	.5
6 months	3.95 (0.28)	4.03 (0.31)			
Urea—mmol/L					
Baseline	4.67 (1.13)	5.19 (1.10)			
3 months	4.14 (1.03)	5.34 (1.47)	−0.82 (−1.29, −0.34)	.001	.07
6 months	3.95 (1.23)	4.86 (1.31)			
Creatinine—μmol/L					
Baseline	76.28 (10.44)	80.27 (10.57)			
3 months	70.90 (9.28)	77.61 (9.79)	−6.78 (−11.72, −1.83)	.008	.2
6 months	68.57 (8.92)	75.63 (13.38)			
Alanine transferase (ALT)—IU/L					
Baseline	27.31 (12.86)	30.83 (19.21)			
3 months	21.07 (9.27)	29.54 (17.51)	8.09 (0.84, 15.35)	.03	.3
6 months	27.19 (17.35)	24.04 (10.03)			
Questionnaires					
Gender Preoccupation Stability Questionnaire—total score					
Baseline	44.9 (6.6)	44.2 (6.1)	−0.71 (−4.04, 2.61)	.7	.2
3 months	38.5 (8.3)	39.9 (7.3)			
6 months	37.9 (9.5)	38.0 (7.5)			
Patient Health Questionnaire 9—total score					
Baseline	10.2 (5.5)	11.4 (6.)	−0.93 (−3.65, 1.79)	.5	.8
3 months	10.2 (5.8)	10.3 (6.2)			
6 months	11.1 (6.4)	10.9 (7.0)			
Other					
Systolic blood pressure—mmHg					
Baseline	123.3 (10.5)	123.7 (12.7)	0.89 (−4.00, 5.78)	.7	.3
6 months	124.0 (11.1)	120.3 (11.0)			
Diastolic blood pressure—mmHg					
Baseline	79.8 (8.7)	80.1 (6.8)	0.32 (−2.85, 3.50)	.8	.7
6 months	78.3 (9.8)	77.4 (7.1)			

Key outcomes, presented as mean (SD) unless otherwise stated by group at 6 months and mean difference between treatment groups (95% CI). Mean difference and OR (95% CI) are adjusted for age and BMI. N/A, not applicable. $P < .05$ deemed statistically significant.
[a]Seven participants did not complete the final study visit and hence do not have an estradiol formulation recorded.

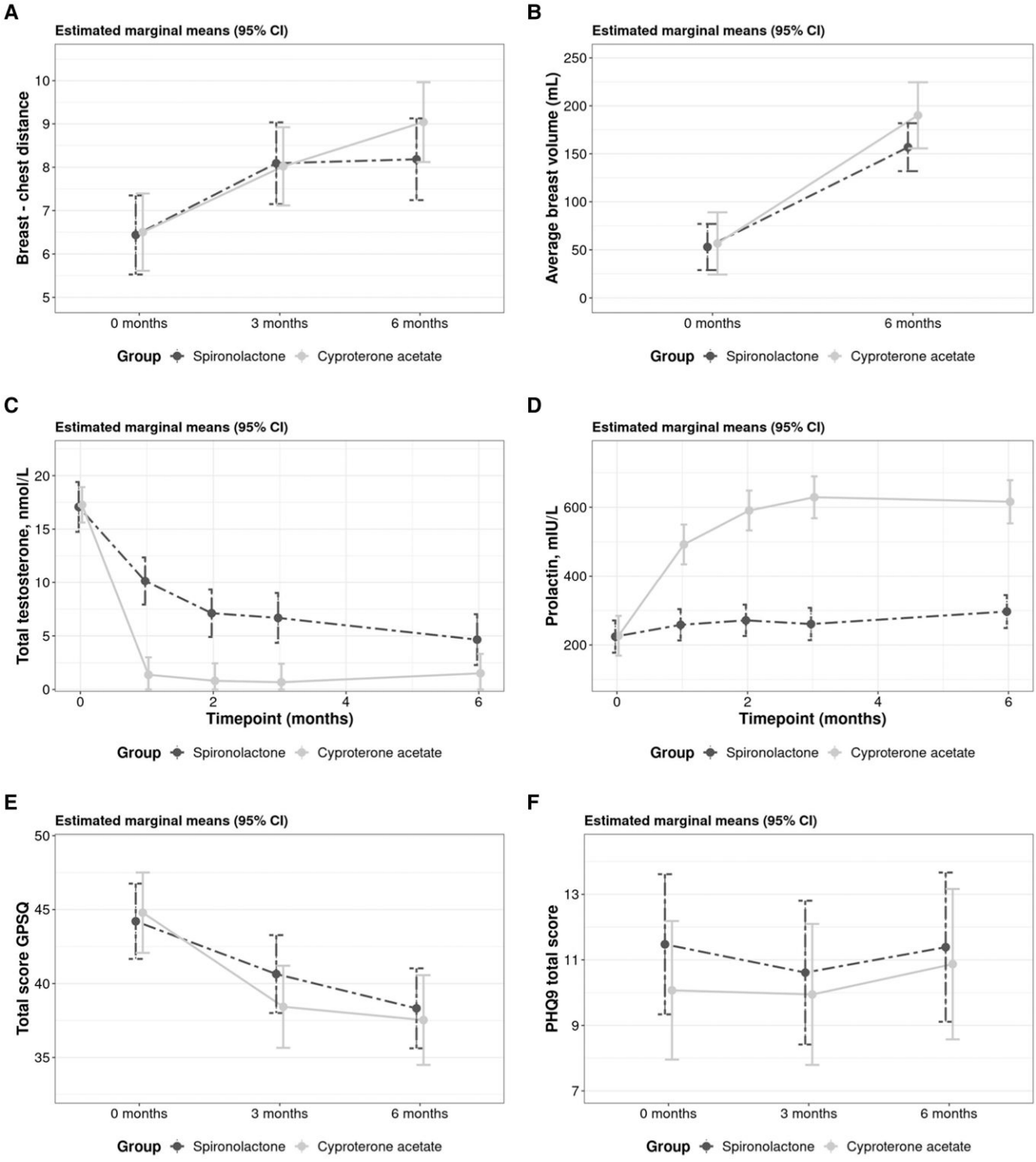

Figure 2. Plot of primary and secondary outcomes over time by treatment group. (A) Breast–chest distance; (B) averaged estimated left breast volume; (C) serum total testosterone concentration; (D) serum prolactin concentration; (E) Gender Preoccupation Stability Questionnaire—total score; (F) Patient Health Questionnaire 9—total score.

Discussion

In this randomized clinical trial comparing antiandrogen agents cyproterone acetate with spironolactone in transgender people newly commencing feminizing hormone therapy with estradiol, there was no between-group difference in breast–chest distance, estimated breast volume, GPSQ, depression severity, or serum potassium concentration. There was significant interindividual variation in breast development and 59% were satisfied with their breast development over 6 months. Exploratory analyses found a higher estimated breast volume with increasing age and increasing serum estradiol concentration achieved at 6 months. Cyproterone acetate was more likely to result in serum total testosterone <2 nmol/L, and higher serum prolactin and ALT concentrations while spironolactone was more likely to result in higher serum urea

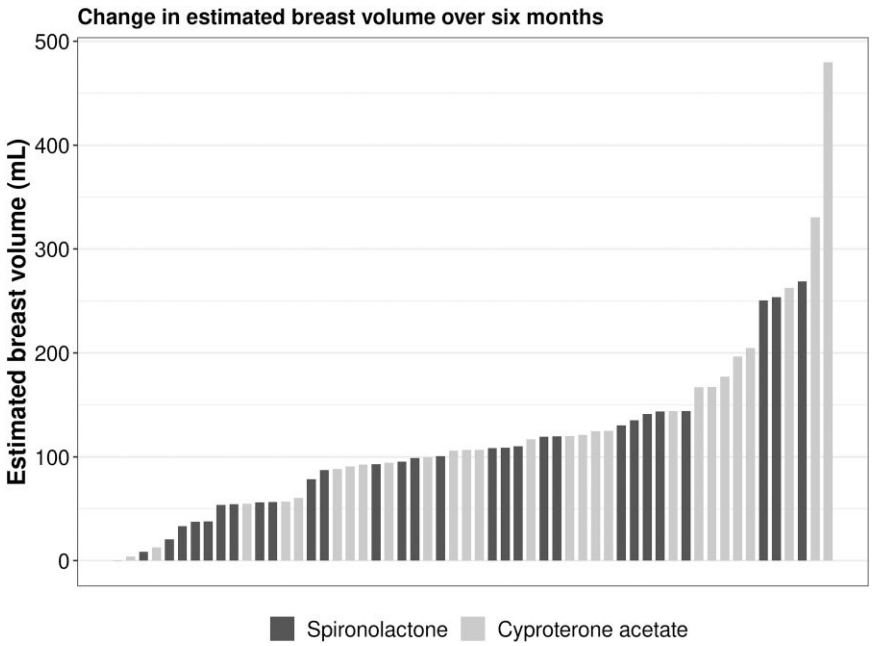

Figure 3. Waterfall plot of change in estimated breast volume by participant over 6 months using per protocol analysis.

and creatinine concentrations, although these remained within normal reference ranges.

Breast development is one of the most desired aspects of feminizing hormone therapy (16). The breast–chest distance was chosen as the primary endpoint measure of breast development due to the availability of comparative data from a cohort of transgender people commencing feminizing hormone therapy (9). These data, which followed participants over 12 months, showed that the peak rate of change in breast–chest distance occurred in the first 6 months, which informed the duration of intervention in our clinical trial (9). However, when the breast–chest distance was later compared with estimated breast volume derived from a 3D scanner over 36 months, the breast–chest distance appeared to plateau after 9 months of therapy, despite further apparent increases in 3D volume (17). As such, it is important to highlight limitations of the breast–chest distance as a measure of breast development. This includes limited data validating its use as a measure of breast development and potential interindividual and intra-individual variation in measurement. Additionally, adipose tissue and muscle mass contribute to the breast–chest distance and would be expected to change with the commencement of feminizing hormone therapy. This inability to distinguish breast tissue from adipose and muscle is true for most techniques used to estimate breast volume (eg, 3D scanning, thermoplastic casting, water displacement), with the exception of magnetic resonance imaging. In an attempt to mitigate these factors, measurements were performed by 2 researchers, the average of 3 measurements was recorded at each timepoint, we focused on the change in these measures over time rather than absolute values, and additional measures of breast volume were used.

We used the BIVE application to estimate breast volume from frontal and lateral view clinical photographs at baseline and 6 months as an additional measure of breast development. This tool had previously been validated in cis-gender women (10) but not in transgender people who had undergone testosterone-mediated puberty prior to commencing feminizing hormone therapy. This is noteworthy given differences in chest anatomy that occur during testosterone-mediated puberty, such as a wider sternum, larger distance between nipple areolar complexes, shorter nipple–intramammary fold distance, broader shoulders, and more prominent pectoral muscles (18). As part of a validation study, BIVE measurements were compared with breast volume estimated by a low-grade 3D scanner in a subset of participants at baseline and 6 months. This showed that measurements using the BIVE application were highly accurate and reliable when compared with 3D scanning (11).

There has been recent community interest in the role of progesterone as part of feminizing hormone therapy to aid breast development (19). Estradiol-mediated puberty typically results in ductal proliferation, branching, and growth while progesterone is thought to assist with maturation of the terminal ductal-lobular unit, particularly in pregnancy in preparation for breastfeeding (20, 21). While some studies report subjective benefits in those using progestogens such as medroxyprogesterone acetate (22), there are currently no high-quality published data using objective and reliable measures of breast development. If progesterone played a key role in breast development, one may have expected a between-group difference to emerge given cyproterone acetate's potent progestogenic effect, acknowledging that cyproterone acetate was used from commencement of estradiol therapy and for a relatively short intervention time of 6 months.

Despite the relatively modest breast development observed during this study, almost two-thirds of participants were "satisfied" or "very satisfied" with breast development, similar to findings from another study (17). Notably, there was significant interindividual variation in the observed change in breast volume over 6 months. Interestingly, post hoc analyses suggested an association between the change in breast volume with increasing age and serum estradiol. This contrasts with other studies, which found no such association between serum estradiol and breast–chest distance after 12 months (9). While serum estradiol concentration samples

were analyzed using liquid chromatography tandem mass spectrometry and collected under standardized conditions, these are notoriously variable in clinical practice with oral or transdermal estradiol therapy and further research is needed to determine whether higher dose estradiol therapy or higher serum estradiol concentrations improve breast development. The observed between-group difference in serum estradiol concentrations was an unexpected finding (Fig. S3 (15)) given both groups were treated with standardized estradiol therapy. We speculate that the observed differences reflect differences in estradiol formulations and timing of blood collection relative to administration. Notably, post hoc analyses of breast–chest distance and estimated averaged breast volume adjusting for serum estradiol remained nonstatistically significant.

Cyproterone acetate was more likely to result in suppression of serum total testosterone to the typical female range than spironolactone. The observed decline in serum total testosterone was rapid in the cyproterone acetate group and paralleled changes in luteinizing hormone, while the decline of serum total testosterone concentration was more gradual in the spironolactone group. This may be explained by the negative feedback exerted by estradiol on the hypothalamic pituitary testicular axis as estradiol dose was titrated throughout the study period given spironolactone does not appear to decrease luteinizing hormone when used as monotherapy (23).

The optimal dose of cyproterone acetate and spironolactone for gender affirmation is not known, with the Endocrine Society Clinical Practice Guidelines recommending 25 to 50 mg daily and 100 to 300 mg daily, respectively (2). In the absence of dose-finding pharmacodynamic studies using meaningful clinical outcomes, serum total testosterone concentration has historically been used as a surrogate marker of effect, but does not account for androgen receptor antagonism—important mechanisms of action for both cyproterone acetate and spironolactone (4). The doses used in this clinical trial were derived from the median dose used in a cohort of transgender people and reflect real-world practice (7).

In terms of safety, cyproterone acetate was associated with higher serum prolactin concentrations than spironolactone, though this was less than twice the upper limit of normal when interpreted using a female reference range (60-280 mIU/L in males, 70-570 mIU/L for females <50 years old). This is consistent with previous studies which have shown a reversible and dose-dependent effect of cyproterone acetate on serum prolactin concentrations (24). Notably, large observational studies have suggested an association with high-dose cyproterone acetate (>50 mg daily) and higher cumulative cyproterone acetate exposure (>10 g cumulative dose) and meningioma (25, 26), prompting the European Medicines Agency Safety Committee to recommend using alternatives to cyproterone acetate where practicable and avoiding doses ≥10 mg daily (27). In Australia, cyproterone acetate is available commercially only in 50 or 100 mg strength tablets, hence the chosen dose for this study of 12.5 mg daily, which commenced recruitment in 2020 prior to publication of these data. However, we note that lower doses of cyproterone acetate (eg, 10 mg daily, or 12.5 mg twice weekly) can be effective in maintaining suppression of serum total testosterone concentrations in transgender people (24, 28). While cyproterone acetate is not FDA-approved for use in the United States, it remains commonly used in Europe and Australia and further research is needed to assess this risk when used for gender affirmation.

While there was an observed decline in GPSQ over 6 months, there was no between-group difference. This is perhaps intuitive given we did not observe any between-group difference in feminization, also acknowledging that not all trans people experience gender dysphoria. Similarly, there was no between-group difference in depression severity using the PHQ-9 total score. This is somewhat reassuring given cyproterone acetate has historically been associated with mood disturbance and depression. The lack of improvement in depression over the first 6 months of feminizing hormone therapy in contrast to the beneficial effects of masculinizing hormone therapy (29) may be explained by the inability to reverse some secondary sex characteristics induced by male puberty (ie, voice, facial features) and greater social stigma experienced by transgender women relative to transgender men (30).

Strengths of this study include the double-blind, randomized trial design, inclusion of participants newly commencing feminizing hormone therapy, low dropout rates, and clinically meaningful measures of breast development. Limitations include a short intervention period of 6 months, unknown optimal dosing of spironolactone and cyproterone acetate, use of the breast–chest distance as a measure of breast development, limited generalizability given our participants were predominantly young, White race, and free of comorbidity, and that the study may have been underpowered to detect breast volume changes given power calculations were based on serum total testosterone levels in the absence of preliminary data on breast development. Post hoc power calculation indicates that the current sample size achieved 80% power for the difference observed in breast–chest distance.

In conclusion, in this randomized clinical trial of cyproterone acetate vs spironolactone in transgender people newly commencing feminizing hormone therapy with estradiol, there was no observed difference in breast development, GPSQ or depression at 6 months. Serum estradiol concentrations were positively associated with estimated breast volume, which requires further evaluation. Cyproterone acetate was more likely to suppress serum total testosterone concentrations to the female range and increase serum prolactin concentrations. As such, antiandrogen medication choice should be individualized and made through a shared decision-making approach with consideration of the side effects of each drug. Further research is required to optimize feminization in transgender people.

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Author Contributions

L.M.A.: conceptualization, methodology, investigation, writing—original draft, writing—review and editing; S.L.: methodology, formal analysis, writing—review and editing, visualization; A.K.T.: methodology, software, writing—review and editing; MM: methodology, software, writing—review and editing; J.D.: methodology, resources, writing—review and editing; J.D.Z.: supervision, writing—review and editing; A.S.C.: conceptualization, methodology, resources, writing—review and editing, supervision, funding acquisition.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request for projects that will benefit the transgender community.

Disclosures

L.M.A. has previously received speaking honoraria and medical advisory board fees from Kyowa Kirin Australia, unrelated to this manuscript. A.S.C. has received product from Besins Healthcare for investigator-initiated clinical studies using estradiol and progesterone. No monetary support from Besins Healthcare was received for these studies and Besins Healthcare have had no input into the design, analysis or writing of any manuscripts. S.Y.L., A.K.T., M.M., J.D., and J.D.Z. have nothing to disclose.

Clinical Trial Information

Australian New Zealand Clinical Trials Registry (ACTRN12620000339954, registered March 11, 2020), anzctr.org.au.

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