ORIGINAL ARTICLE

Subcutaneous Testosterone Is Effective and Safe as Gender-Affirming Hormone Therapy in Transmasculine and Gender-Diverse Adolescents and Young Adults: A Single Center's 8-Year Experience

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Abstract

Purpose: To describe our Center's 8-year experience with subcutaneous testosterone (SC-T) as gender-affirming hormone therapy (GAHT) in transmasculine and gender-diverse (TM/GD) youth.

Methods: An Institutional Review Board (IRB)-approved retrospective study for 119 TM/GD subjects who started SC-T at age 13–19 and received SC-T for >6 months between 2012 and 2020.

Results: SC-T was typically started at 25–50 mg biweekly and dose was escalated at provider's discretion. Over 96% of subjects were on 100–320 mg monthly (divided weekly or biweekly) at last follow-up. There was an overall increase in mean total and free testosterone (T) over the dose range ($p = 0.003$), with mean total and free T levels of 460 ng/dL and 92 pg/mL, respectively, at a monthly SC-T dose of 200 mg. For subjects on SC-T without additional menstrual suppression, 54% had cessation of menses at 140 mg monthly and 97% at 200 mg monthly. On average, menses stopped 4.7 (standard deviation 3.0) months after starting SC-T. There was a decrease in high-density lipoprotein and increase in hematocrit from baseline to follow-up. Body mass index Z-scores did not change significantly with treatment. Mild acne was common; severe acne and significant injection site reactions were uncommon. Sustained hypertension, transaminitis, and dyslipidemia were infrequent.

Conclusions: SC-T is well tolerated and effective in reaching recommended T levels and stopping menses in TM/GD youth. Occurrence of serious adverse effects is low and inability to tolerate injections is very uncommon. SC-T is a safe and effective alternative to intramuscular testosterone in initiation and maintenance of GAHT in TM/GD youth.

Keywords: adolescent; subcutaneous; testosterone; transmasculine; young adult

Background

Testosterone (T) replacement therapy is utilized in hypogonadal cismales and as gender-affirming hormone therapy (GAHT) for transmasculine and gender-diverse (TM/GD) adults and youth.[1,2] T replacement can be given as intramuscular (IM) injection, transdermally (patch or gel), orally, or as an implant. In addition to higher treatment cost often associated with transdermal formulations, patches may irritate skin and are unavailable in small/gradually incrementing doses, while T-gel carries the risk of inadvertent transfer to close contacts. Other T formulations considered less accessible or desirable include oral (not readily available for use in the United States) and implantable subcutaneous pellets (dose cannot be quickly adjusted as pellets are in place for several months and carry risk of dermatitis, infection, or extrusion[3]). Therefore, it is often preferred to use an injectable form of T as GAHT. Subcutaneous testosterone (SC-T) injections are approved for T enanthate administration for adults

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only using a costly self-injector device. Studies in adult transgender patients have suggested that SC-T is well tolerated with stable and therapeutic serum T levels.[4,5] Studies in hypogonadal adult cisgender males also indicate that SC-T is safe and well tolerated with steady pharmacokinetic profile.[6–8] Although one study using injectable depot T undecanoate (formulation not used in our Center) showed some preferred IM route over SC,[9] another study using T cypionate and enanthate (the two formulations most commonly used in our center) showed all participants who had utilized both routes preferred SC administration.[10]

Thus, interest in SC-T for TM/GD youth has increased, but extensive pediatric data are lacking. Olson et al.[11] showed good efficacy and tolerability of SC-T with favorable adverse effect profile in transgender youth and young adults (aged 13–24). The study limitations were a relatively small cohort (36 subjects) and short (6-month) follow-up. Since our clinic was established in 2012, we have almost exclusively had patients administer T-SC as opposed to IM. Patients at our center are started on biweekly (every 2 weeks) injections and at follow-up are offered to switch to weekly SC-T. Although some choose to stay on biweekly injections, the majority prefer weekly dosing especially if experiencing mood changes related to peak or nadir of biweekly SC-T dosing. Our large patient population has received SC-T over many years, which enables us to further characterize the efficacy, safety, and tolerability of SC-T in TM/GD youth. In our center, SC-T is typically initiated around age 14 or older, after multidisciplinary assessment of readiness, in youth with gender dysphoria who desire GAHT, and have no contraindications. We use a gradually increasing dose schedule in keeping with published guidelines.[1,2]

Objectives

Our primary objective was to assess the effectiveness and safety of SC-T in achieving recommended T levels and cessation of menses in TM/GD youth. As the two providers in our center differ in recommendations for timing of blood draws for T levels (Provider A: midway between injections; Provider B: just before the next injection is due/trough level), a secondary objective was to describe differences in T levels at different time points since the previous injection and when given weekly or biweekly. In addition, we assessed the average SC-T dose and time since starting SC-T, at which cessation of menses occurred, and the frequency of adverse events.

Methods

This retrospective, single-center study to evaluate SC-T as GAHT in TM/GD youth was conducted at Rady Children's Hospital San Diego (RCHSD), California, between August 2012 and February 2020. All 119 subjects were younger than the age of 21 years when starting SC-T and received SC-T for a minimum of 6 months. Study subjects consented (assented when applicable) into an Institutional Review Board (IRB)-approved endocrine database. Subjects were assessed by a mental health professional for readiness to start SC-T and met diagnostic criteria for gender dysphoria by WPATH Standards of Care version 7 guidelines.[2] All subjects (and parents/guardians if <18) signed informed consent for T start. Subjects were taught and supervised by a nurse how to self-administer first SC-T injection (using an 18-gauge needle to draw and a 25-gauge to inject).

Age at first presentation to clinic and at SC-T start, gender identity, race, and ethnicity were documented. Baseline and follow-up body mass index (BMI; kg/m^2) and BMI Z-score (for subjects younger than the age of 20 years, using CDC age 2–20 standards for cisgender females[12]) were documented to assess for change over treatment period. We collected baseline and on-treatment T levels during the follow-up period (defined as the date of T start to date of the most recent T level check). We included four subjects with laboratory follow-up <6 months, but office follow-up 6 months or more. When multiple levels were checked on the same SC-T dose, the final value obtained on a given dose was recorded. The majority (close to 90%) of T levels were measured using liquid chromatography with dual mass spectrometry (LC-MS/MS) assay (most at Quest Diagnostics, remainder at ARUP). A minority of T levels were performed at LabCorp using electrochemiluminescence immunoassay (ECLIA).

Data on menstrual patterns included time to cessation of menses and SC-T dose at which this occurred. The effect of SC-T on cessation of menses did not include subjects treated with gonadotropin-releasing hormone (GnRH) agonists or other forms of menstrual suppression (e.g., combined oral contraceptive or progesterone-only pills) or who were premenarchal before SC-T start.

We recorded baseline and follow-up values of ultrasensitive estradiol, AST, ALT, hematocrit, and lipids: total cholesterol, high-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. Presence of any injection-site reaction and circumstances were

recorded. We documented subjects with systolic blood pressure (SBP) or diastolic blood pressure (DBP) >95th percentile for age and height if <18 years, or SBP >140, or DBP >90 mm/Hg if >18, for three consecutive visits. Presence of acne at baseline, progression during SC-T therapy, and treatment of acne including whether a referral was made to dermatology were noted.

De-identified data from the eligible subjects were entered into the Research Electronic Data Capture (REDCap) database securely hosted at RCHSD, including date of birth as approved by the IRB. REDCap is a secure, web-based software platform designed to support data capture for research studies.[13,14] For statistical analysis, we used two-sample t-test and chi-square test to compare continuous variables and categorical variables, respectively, between groups. We used paired t-test to analyze changes in various laboratory values within each group. When there were more than two groups, analysis of variance was used to compare differences between groups.

Results

Baseline characteristics

At presentation to our center, the majority of subjects had a transmale/male gender identity (Table 1); others identified as nonbinary or had other gender-diverse identities. The majority of them were Caucasian and approximately one-quarter identified as Latinx/Hispanic. Mean baseline BMI Z-score was normal at 0.55. Nineteen subjects (16%) were overweight (BMI ≥85% and <95%, Z-score 1.05–1.64) and 24 subjects (20.2%) were obese (BMI ≥95%, Z-score >1.64); 3 subjects (2.5%) were underweight (BMI <5%, Z-score less than −1.64). Average age at presentation to clinic was 16 years (range 10.1–19.8 years) and at the time of SC-T start was 16.5 years (range 13–19.9 years); six subjects started SC-T younger than the age of 14 years, per individualized approach. Nearly all subjects were started on 50–100 mg SC-T monthly divided into every 2 weeks (biweekly) doses; two subjects misunderstood instructions and started on 120–140 mg SC-T monthly.

Median follow-up was 1.9 years and ranged from 6 months to 5.5 years.

Final T levels

SC-T dose was increased typically every 3–6 months guided by serum T levels, patient's preference, and timing of follow-ups. The last available SC-T dose

Table 1. Demographic and Baseline Characteristics Before Starting Subcutaneous Testosterone

Total	119
Age at presentation in years (range)	16 (10.1–19.8)
Age at SC-T start in years (range)	16.5 (13–19.9)
Gender identity (%)	
Transmale/male	110 (92.5%)
Other[a]	6 (5%)
Nonbinary	3 (2.5%)
Race (%)	
Caucasian	79 (66.4%)
Asian	7 (5.9%)
Native American/Alaska Native	4 (3.4%)
African American	2 (1.7%)
Native Hawaiian/Pacific Islander	1 (0.8%)
Unknown/unavailable	20 (16.8%)
Other[b]	17 (14.3%)
Ethnicity (%)	
Non-Hispanic/Latinx	88 (73.9%)
Hispanic/Latinx	29 (24.4%)
Unknown	2 (1.7%)
BMI, kg/m^2 average (SD)	24.85 (7.26)
BMI Z-score average (SD)	0.55 (1.22)
BMI Z-score category (%)	
Obese	24 (20.2%)
Overweight	19 (16%)
Underweight	3 (2.5%)
On menstrual suppression before start SC-T (%)	
No	99 (83.2%)
GnRHa	12 (10.1%)
Other (progesterone only; combined oral contraceptive)	8 (6.7%)

[a]Other gender identities included gender queer (n=1), gender queer or fluid (n=1), gender fluid (n=1), agender (n=1), on the gender spectrum (n=1), no preferred gender identity, or pronoun expressed at first visit (n=1). Age at presentation and age at T start expressed as mean (range). BMI and BMI Z-score expressed as mean (SD).
[b]Other self-reported races included: Mexican (n=1), Peruvian (n=1), Puerto Rican (n=1), and Hispanic not otherwise specified (n=14).
BMI, body mass index; GnRHa, gonadotropin-releasing hormone agonist; SC-T, subcutaneous testosterone; SD, standard deviation; T, testosterone.

and corresponding total and free serum T levels were recorded (Fig. 1a, b). As free T measurements by ECLIA (LabCorp) were significantly lower than those measured by LC-MS/MS (Quest and ARUP), free T levels performed at LabCorp (representing a minority of subjects) were omitted from the free T analysis. The majority of subjects (n=94, 79%) were on 100–200 mg of SC-T monthly; an additional 21 subjects (18%) were on 240–320 mg of SC-T monthly. Across all final SC-T doses, 70% (n=83) were on weekly injections. In those that reached a monthly SC-T dose of 200 mg or more, 83% were on weekly injections. For those at 100 mg or more of SC-T monthly, mean total T level ranged from 274 to 534 ng/dL and mean free T levels 43–129 pg/mL. The most common final SC-T dose, taken by 49 (41%) subjects, was 200 mg monthly. Mean total and free T levels at 200 mg
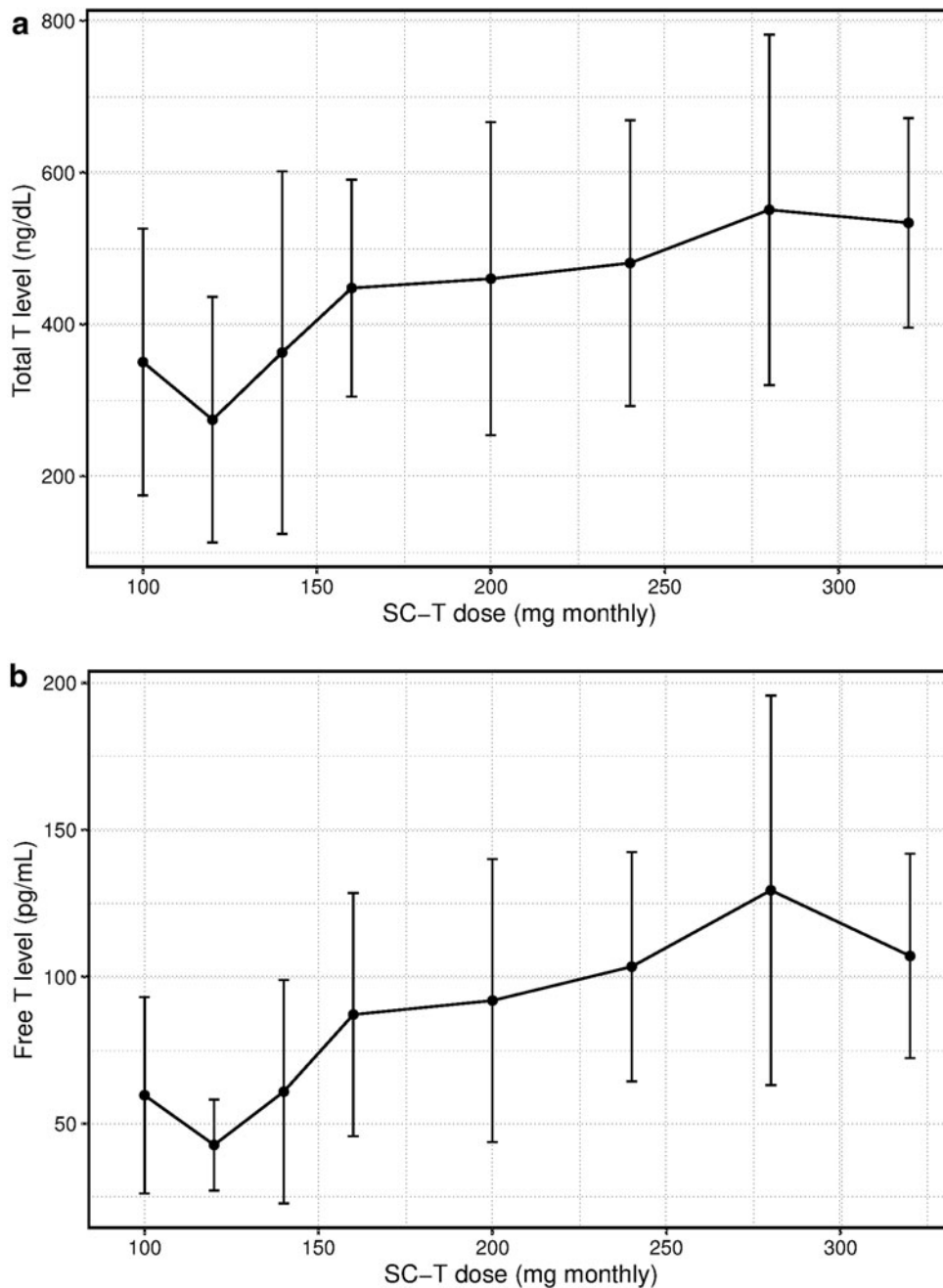
FIG. 1. **(a, b)** Mean total **(a)** and free testosterone level **(b)** at final testosterone doses. Bars represent standard error of the mean. SC-T, subcutaneous testosterone; T, testosterone.

monthly were 460 ng/dL and 92 pg/mL, respectively, and there was no difference in total and free T levels between the 38 on weekly dosing (458.5 ng/dL and 91.9 pg/mL, respectively) and the 11 on biweekly dosing (465.9 ng/dL and 91.8 pg/mL, respectively) at this dose.

Cessation of menses

Data on SC-T dose at which menses stopped were available for 78 subjects. The majority (76/78, 97.4%) of these subjects achieved cessation of menses on total monthly SC-T dose of 200 mg (Fig. 2). Over half
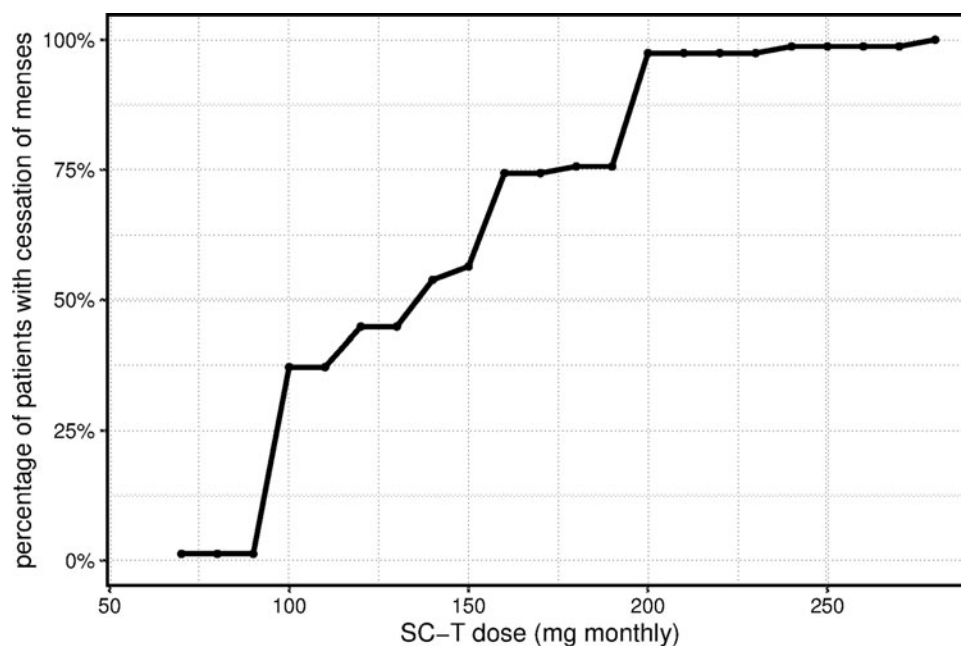
FIG. 2. Cumulative percentage of subjects with cessation of menses by final SC-T dose.

(53.9%) had cessation of menses when monthly SC-T dose of 140 mg was reached.

Data on time from SC-T start to cessation of menses were available for 81 subjects. On average, menses stopped 4.7 (standard deviation [SD] 3.0) months after starting SC-T: 5.4 (SD 2.9) months if starting monthly dose was 50 mg and 3.9 (SD 3.0) months if it was 100 mg ($p = 0.025$). There were seven subjects (5.9%) in whom it took >400 days to stop menses; three of these had interruptions in adherence/supply or follow-up.

Differences in timing of the blood draws

We compared total and free T levels at various SC-T doses between two groups, those followed by Provider A (who recommends mid-injection blood draws) and those followed by Provider B (who recommends trough blood draws) (Fig. 3a, b). As the majority of subjects ($n = 83$, 70%) were on weekly dosing at final SC-T dose, we omitted those on biweekly dosing from the analysis. We did not observe statistically significant differences in mean total or free T levels between providers A and B.

Changes in BMI, BMI Z-score, and assessment of correlation with final SC-T dose

Mean follow-up BMI was 25.71 kg/m^2, an increase of 0.86 kg/m^2 from mean baseline BMI ($p < 0.001$). BMI

Z-scores at the start and end of study period were available for 95 subjects, as 24 subjects had turned 20 during study period. For those 95, mean BMI Z-score before SC-T start and at last follow-up was 0.56 (1 SD = 1.26) and 0.50 (1 SD = 1.26), respectively ($p = 0.334$). There was no statistically significant correlation between a higher final SC-T dose and final BMI Z-score.

Laboratory parameters for subjects on different SC-T doses

To assess effect of SC-T from baseline to follow-up on laboratory parameters, as well as the effect of increasing SC-T dose on changes in parameters, the cohort was subdivided into three dosing groups for further analysis: those on relatively low final SC-T dose (<160 mg monthly), those on an average dose of 160–240 mg monthly (the majority of subjects), and those on a relatively high dose of >240 mg monthly (Table 2). There were statistically significant increases in total and free T and decrease in estradiol from baseline to follow-up, as expected. The effect of increasing SC-T dose on final total T ($p = 0.003$) and free T ($p < 0.001$) was significant, and similarly, the changes in total and free T were also significant. The change in total and free T at each dosing group was significant ($p < 0.001$). There was a statistically and clinically significant decrease in HDL of 6.42 mg/dL from baseline to follow-up for the
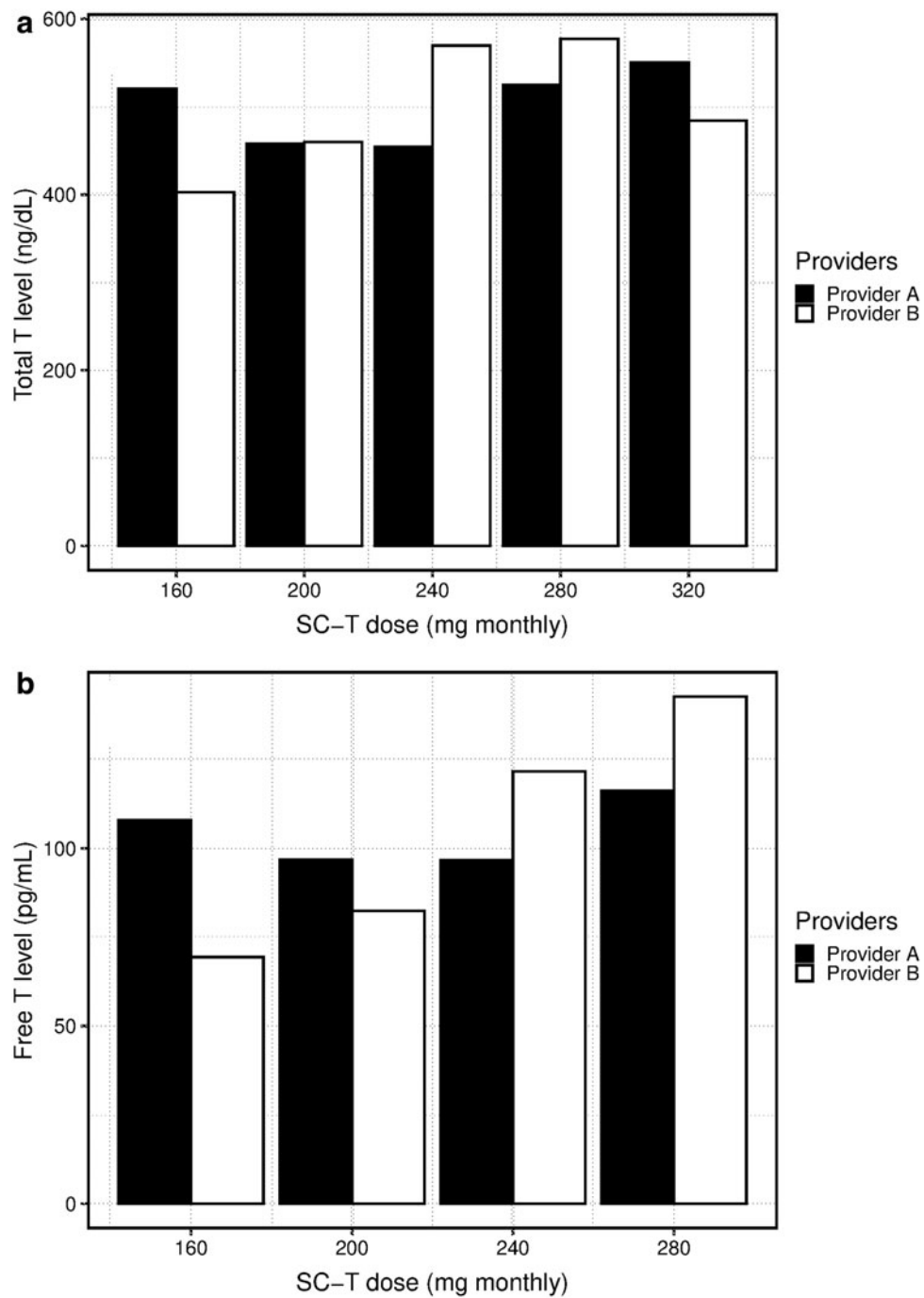
FIG. 3. **(a, b)** Testosterone levels based on timing of blood draw. **(a)** Bar plot of total T level for patients of Provider A and Provider B, on weekly dosing. **(b)** Bar plot of free T level for patients of Provider A and Provider B, on weekly dosing. Provider A, mid-dose level; Provider B, trough level.

Table 2. Laboratory Values at Baseline and on Final Subcutaneous Testosterone Dose, at and Across Three Dose Ranges

Parameter, mean (SD), [p value] for each Δ at each SC-T dose	SC-T/month, <160 mg (n=28)	SC-T/month, 160–240 mg (n=81)	SC-T/month, >240 mg (n=10)	Overall (n=119)	Trend across dose range: p-value
Baseline total T, ng/dL	29.2 (14.8)	31.6 (13.6)	37.8 (15.8)	31.6 (14.1)	0.269
Final total T, ng/dL	328.8 (185.5)	456.8 (190.6)	522.6 (162.6)	432.2 (195.4)	0.003
Δ total T, ng/dL	287.1 [a]	424.6 [a]	484.7 [a]	399.3 [a]	0.003
Baseline free T, pg/mL	3.8 (3.7)	3.6 (2.4)	4.5 (1.8)	3.7 (2.6)	0.619
Final free T, pg/mL	53.9 (30.9)	91.9 (45.3)	124.6 (47.9)	86.2 (46.5)	a
Δ free T, pg/mL	47.2 [a]	89.3 [a]	120.1 [a]	84.3 [a]	a
Baseline estradiol, pg/mL	81.3 (70.4)	79.6 (71.1)	68.6 (52.66)	78.9 (69.0)	0.894
Final estradiol, pg/mL	52.9 (31.2)	48.4 (50.3)	47.3 (28.6)	49.1 (45.7)	0.938
Δ Estradiol, pg/mL	−67.6 [0.036]	−30.7 [0.001]	−27.0 [0.293]	34.7 [a]	0.372
Baseline TC, mg/dL	161.0 (28.8)	156.2 (29.8)	165.2 (33.9)	158.0 (29.8)	0.575
Final TC, mg/dL	155.6 (28.0)	151.8 (32.3)	170.7 (25.5)	154.6 (31.1)	0.202
Δ TC, mg/dL	−5.4 [0.362]	−4.5 [0.182]	5.5 [0.452]	−3.5 [0.194]	0.497
Baseline HDL-C, mg/dL	50.4 (15.0)	50.2 (13.2)	47.6 (8.5)	50.0 (13.2)	0.833
Final HDL-C, mg/dL	45.6 (12.6)	43.5 (9.3)	39.5 (7.5)	43.4 (9.8)	0.301
Δ HDL-C, mg/dL	−3.7 [0.08]	6.7 [a]	−8.1 [0.016]	6.4 [a]	0.488
Baseline LDL-C, mg/dL	90.4 (22.6)	90.4 (25.4)	101.3 (28.8)	91.4 (25.1)	0.427
Final LDL-C, mg/dL	93.0 (26.2)	91.1 (24.5)	104.3 (22.2)	92.9 (24.7)	0.296
Δ LDL-C, mg/dL	0.4 [0.928]	−0.4 [0.899]	2.9 [0.602]	0.2 [0.922]	0.883
Baseline TG, mg/dL	96.9 (38.8)	87.6 (34.0)	87.5 (38.5)	89.6 (35.4)	0.541
Final TG, mg/dL	96.8 (42.5)	97.5 (59.3)	128.6 (86.4)	100.8 (60.5)	0.309
Δ TG, mg/dL	−1.3 [0.926]	9.0 [0.276]	41.1 [0.092]	11.4 [0.100]	0.237
Baseline hematocrit, %	39.4 (2.7)	39.1 (2.5)	40.1 (2.7)	39.2 (2.6)	0.48
Final hematocrit, %	43.1 (3.1)	44.1 (3.3)	46.5 (3.3)	44.1 (3.3)	0.024
Δ Hematocrit, %	3.7 [a]	5.1 [a]	6.4 [a]	4.9 [a]	0.054
Baseline AST, U/L	24.4 (10.3)	21.3 (7.3)	19.0 (4.6)	21.8 (8.0)	0.115
Final AST, U/L	20.8 (7.3)	23.6 (9.5)	23.4 (7.0)	23.0 (8.8)	0.349
Δ AST, U/L	−4.3 [0.024]	2.4 [0.060]	4.4 [0.078]	1.1 [0.290]	0.010
Baseline ALT, U/L	20.1 (11.2)	21.0 (9.7)	20.3 (10.5)	20.8 (10.0)	0.908
Final ALT, U/L	18.4 (9.6)	21.3 (13.8)	30.0 (9.7)	21.4 (12.9)	0.050
Δ ALT, U/L	−2.0 [0.353]	0.4 [0.824]	9.7 [0.041]	0.7 [0.600]	0.054

Δ is the change from baseline to final testosterone dose. Values in parentheses for each laboratory value is SD, and in brackets for the Δ it is the p-value indicating if that change is significant.

Statistical significance: [a]p<0.001.

HDL-C, high-density lipoprotein cholesterol; LDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

whole cohort ($p < 0.001$); however, the effect of increasing SC-T dose on change in HDL was not statistically significant. There was a significant increase in hematocrit from baseline to follow-up at each dosing group ($p < 0.001$), and there was a significant increase in hematocrit as SC-T dose increased ($p = 0.024$) and a tendency for the change in hematocrit from baseline to increase across the three groups ($p = 0.054$). However, final mean hematocrit remained in the normal range for males. There was a significant effect of increasing SC-T dose on change in AST ($p = 0.010$) since there was a drop at lower doses and increase on higher SC-T doses. The absolute increase in mean AST from baseline to follow-up of 1.11 U/L for the whole cohort was not clinically significant. The mean final ALT value compared across the dose range was borderline statistically significant ($p = 0.05$), but not clinically significant.

Absolute values of and changes from baseline to follow-up in other laboratory parameters and the effect of increasing dose on these values and changes were not statistically significant.

Clinical adverse events and changes in SC-T therapy

Local reaction. Mild injection site reactions during SC-T therapy were documented in 14 subjects (11.8%). The spectrum of reactions included skin lumps, pruritus, swelling, erythema, focal hair growth at the site (n=2, as previously reported[15]), decreased sensation (n=1, limited to a single site), and skin infection. Two cases of skin infection during SC-T therapy occurred: one subject had cellulitis after not properly cleaning skin before injection (without recurrence), and one subject had two abscesses. Neither subject with skin infection changed their SC-T therapy. In total, only 4/14 subjects with documented injection site reactions changed therapy in some way. Three subjects changed from SC-T cypionate to SC-T enanthate with resolution of symptoms, and one subject switched to T-gel because of pain and swelling with injections.

Hypertension. No subjects developed hypertension that was felt to be related to SC-T therapy or required

antihypertensive treatment. Six subjects had either SBP or DBP >95th percentile for age on three consecutive visits, which did not appear to be true hypertension; four of these subsequently had a blood pressure (BP) below this threshold. One subject had consistently elevated SBP and pulse both at T start and during therapy, thought to be related to anxiety, but did not have consistently elevated DBP and no intervention was taken to lower BP. Another subject had consistently elevated BP in our center, however, on outside assessment had normal BP and electrocardiogram.

Acne. Acne at baseline was noted for 61/106 (57.5%) subjects who had acne documentation; the majority of acne was mild to moderate. Progression of acne was documented for 77/119 subjects (64.7%); advanced acne management (oral treatment and/or referral to dermatology) was documented in 23/119 (19.3%). None stopped SC-T.

Descriptive review of adverse effects on laboratory parameters. No subjects developed transaminitis related to SC-T therapy.

One subject developed worsening of dyslipidemia while on SC-T. His baseline LDL was 120 mg/dL but increased to >160 mg/dL starting 6 months after SC-T start. Hyperlipidemia was treated with low-dose statin with good response. Of note, his BMI increased from normal to obese over follow-up.

No subjects developed hematocrit >55%. Six subjects (5%) had hematocrit >50% at some point. Highest recorded hematocrit in the cohort was 53.9% in a subject receiving SC-T 100 mg biweekly; hematocrit decreased subsequently without dose change. SC-T dose was transiently decreased due to high-normal T level (822 ng/dL) and mildly increased hematocrit (51.4%, hemoglobin 17.4 g/dL) for one subject on SC-T for 15 months. Dose was readjusted to 60 mg weekly at follow-up (hematocrit = 51%, hemoglobin = 16.9 g/dL, and corresponding T = 466 ng/dL). Another subject had slight SC-T dose decrease due to borderline high hematocrit = 49.8% in the setting of upper-normal free T = 131.7 (18–111 pg/mL).

Variance from prescribed dosing. Per chart review, at some point in their therapy, 31.1% of the cohort slightly deviated from the dose or schedule recommended by the provider: subjects giving minimally different doses than prescribed, or injections being occasionally delayed or missed due to interruption in supply, follow-up, or adherence.

Subjects who discontinued SC-T. In addition to the one subject who stopped SC-T and switched to T-gel because of pain/swelling with injections, three additional subjects without local/cutaneous reaction to SC-T chose to change treatment. One subject switched from SC-T to T-patch 8 months after SC-T start due to needle phobia; one subject switched from SC-T to T-gel after 1 year on SC-T due to pain and difficulty injecting himself; and one subject switched from adult dose of SC-T to T-gel after 3 years of SC-T therapy simply due to preference. Three subjects stopped SC-T altogether due to desire to end masculinizing therapy. Two stopped because they were satisfied with effects of SC-T achieved and one stopped within 6 months of starting after reassessing their gender identity. This subject started GAHT at age 16.5 years with SC-T dose of 25 mg biweekly, subsequently increased to 25 mg weekly. The subject decided to stop SC-T 6 months after starting due to concerns about body changes and potential impact on fertility. Unfortunately, this subject was lost to follow-up and no long-term outcome is available.

Conclusions/Discussion

This study, to date the largest pediatric study of SC-T, adds to the currently limited literature supporting the efficacy and safety of SC-T as an alternative to IM testosterone injections for GAHT in TM/GD youth. Dose of SC-T required to achieve mean total T levels in the goal range (400–700 ng/dL when measured mid-injection[1]) and achieve cessation of menses for the majority of our study subjects was 140–200 mg monthly, which is similar to or lower than those suggested by guidelines[1] as adult doses for IM or SC-T (100–200 mg every other week). Of note, a small subset of subjects did not achieve the ultimately intended dose due to limited follow-up and some subjects requested lower-than-typical doses.

We observed an overall increase in mean total and free T levels as SC-T dose increased, with slight deviations from this pattern noted in those individuals on a final T dose of 120 mg monthly and on 320 mg monthly (these subgroups were small, $n = 7$ and $n = 4$, which may have accounted for deviation from overall trend). There was a wide spread in the ranges of final total and free T levels obtained in different subjects on the same SC-T dose, and significant overlap

between the ranges of T levels obtained on different SC-T doses. This finding likely speaks to the significant interindividual variability in SC-T dose requirements and highlights the importance of monitoring T levels. An additional factor to consider is the challenge of imperfect adherence to SC-T therapy and recommendations for timing of serum T level checks. Indeed, chart review supported that nearly one-third of subjects took SC-T slightly differently than it was prescribed at some point during treatment course, although usually for short periods. We did not find the expected correlation of higher T levels when purportedly assessed as mid-injection compared to as a trough. This lack of an expected finding is most likely due to improper timing of the blood draw (i.e., deviating from providers' instructions).

Cessation of menses occurred on average 4.7 months after starting SC-T, and it took longer in those on 50 mg versus 100 mg as a monthly starting dose. Therefore, when faster cessation of menses is desired (and no concerns about acne or height), we suggest to start SC-T at 100 mg/month. While adherence or lack of follow-up can be contributing factors, some patients may need a GnRH agonist or progestin to achieve amenorrhea. If ongoing menses is a cause of significant dysphoria for an individual, an additional form of menstrual suppression should be offered right away.

We found that adverse effects of SC-T were uncommon. Progression of acne was common, as expected with T therapy, but severe acne was uncommon. Hypertension necessitating antihypertensive treatment was not observed. In our cohort, the absolute BMI increase from baseline to follow-up was due to the increase of BMI with increasing age, but there was no difference when comparing BMI Z-scores adjusted for age. Therefore, pediatric studies should only compare BMI Z-scores. Being on a higher SC-T dose did not affect the BMI Z-score.

Similar to a recent study of transgender male youth,[16] we found an increase in hematocrit that was statistically significant; however, mean final hematocrit was still in the normal range for males. While six subjects had hematocrit >50% at some point during SC-T therapy, only two required small dose adjustments. Some had transient hematocrit increase when fasting, due to hemoconcentration, which was corrected with nonfasting samples. Importantly, while some consider a hematocrit of 50% the upper limit of normal for adult males,[17] hematocrit up to 54% is considered high-normal by others.[18] Cessation of

SC-T therapy or other treatments for polycythemia were not required for any subjects.

Although increases in AST and ALT from baseline to final follow-up were statistically significant and borderline significant, respectively, those were not clinically significant and follow-up values for both parameters remained normal.

Dyslipidemia after SC-T start requiring additional treatment occurred in only one subject. He started a statin because of increased LDL cholesterol, which is not typically associated with T therapy, but likely due to obesity that developed over the follow-up period. HDL lowering, an expected effect of testosterone therapy, and lower estradiol levels, has been substantiated in recent pediatric studies.[16,19] We also observed a statistically and clinically significant decrease in HDL from baseline to follow-up in the overall cohort; this decrease in HDL did not seem to worsen on higher SC-T doses. Counseling about the importance of increased physical activity, while being sensitive to barriers to exercise in this population such as exacerbation of dysphoria or restricted exercise capacity due to chest binding, is crucial in helping raise HDL and can aid in improving mental health and weight management.

Injection site reactions occurred rarely; only 3/119 (2.5%) subjects were unable to tolerate SC-T.

Particular strengths of our study include the large size and diversity of this cohort and the long experience with using SC-T in a real-world setting. This is the largest study of SC-T use in the pediatric population published to date, with safety laboratories repeated over time and with a sufficient follow-up duration to adequately assess for adverse effects.

The primary limitations of the study are its retrospective nature and single-center cohort. Detailed information on exact timing of blood draws was not always possible to ascertain retrospectively, so separation of the cohort into mid-injection or trough T levels based on treating providers may not have been accurate in all cases. However, we feel that the size of the cohort is large enough for us to reasonably conclude that timing of the blood tests did not make a significant impact on serum T levels. Finally, imperfect adherence to therapy as prescribed was noted, but usually for brief periods. Adherence is a common challenge in adolescents in general, and we feel that inclusion of all subjects with reasonable adherence to treatment and follow-up was important, as it reflects real-world conditions.

As SC-T is not an FDA-approved route for T-cypionate, this publication is a valuable addition to

the medical literature relevant not only to TM/GD individuals but also to cisgender hypogonadal adolescents and young adults. We conclude that SC-T is effective in TM/GD youth in achieving adult male serum T levels and cessation of menses. No subjects had to stop SC-T because of serious adverse effects on BP, acne, transaminases, lipid profile, or hematocrit. Overall, tolerance of SC-T was very good. We recommend, based on our extensive experience, that providers consider SC-T as an excellent alternative to IM T for initiation and maintenance of GAHT in TM/GD youth.

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Abbreviations Used

BMI	body mass index
BP	blood pressure
DBP	diastolic blood pressure
ECLIA	electrochemiluminescence immunoassay
GAHT	gender-affirming hormone therapy
GnRH	gonadotropin releasing-hormone
HDL	high-density lipoprotein
IM	intramuscular
IRB	Institutional Review Board
LC-MS/MS	liquid chromatography with dual mass spectrometry
LDL	high-density lipoprotein
RCHSD	Rady Children's Hospital San Diego
REDCap	Research Electronic Data Capture
SBP	systolic blood pressure
SC-T	subcutaneous testosterone
SD	standard deviation
T	testosterone
TC	total cholesterol
TG	triglycerides
TM/GD	transmasculine and gender-diverse