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Insulin sensitivity, body composition and bone mineral density after testosterone treatment in transgender youth with and without prior GnRH agonist therapy

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ABSTRACT

Background: 1.8% of youth identify as transgender; a growing proportion are transgender male (female sex, male gender identity). Many receive gonadotropin releasing hormone agonist (GnRHa) therapy to suppress endogenous puberty and/or will start testosterone to induce secondary sex characteristics that align with gender identity.

Objective(s): To determine the effects of 12 months of testosterone on cardiometabolic health among transgender youth, including insulin sensitivity, body composition, and bone mineral density and whether changes in outcomes differ based on prior GnRHa treatment.

Methods: Participants (n = 19, baseline age 15.0 ± 1.0 years) were examined prior to and 12 months after testosterone therapy in a longitudinal observational study. Fasted morning blood draw, a 2-hour 75-gram oral glucose tolerance test, body composition and bone mineral density (dual-energy X-ray absorptiometry) were assessed at baseline and 12 months. Insulin sensitivity was estimated by HOMA-IR and Matsuda index. Changes were compared with mixed linear regression models evaluating time (baseline, 12 months), group (GnRHa treatment yes/no), and their interaction.

Results: In the entire cohort, fasted insulin decreased (median [25,75 %ile]: −3 [-5, 0] mIU/L, p = 0.044) and 2-hour glucose increased (mean ± standard deviation): +18.5 ± 28.9 mg/dL, p = 0.013 from baseline after 12 months of testosterone therapy. There were no significant changes in HOMA-IR (p = 0.062) or Matsuda index (p = 0.096), nor by GnRHa status. Absolute (+6.2 [4.7, 7.5] kg, p = 0.016) and percent fat-free mass increased (+7.3 [5.4, 9.1] %, p = 0.003) and percent fat mass declined (−7.4 [-9.3, 5.3]%, p = 0.005) for the entire cohort. There were time*group interactions for absolute (p = 0.0007) and percent fat-free mass (p = 0.033). There were time*group interactions for bone mineral content (p = 0.006).

Conclusions: Twelve months of testosterone in transgender adolescents resulted in changes in body composition and bone mineral density, with baseline differences between the +/-GnRHa group and convergence after 12 months. There were no changes in insulin sensitivity over time or between groups.

Introduction

The number of youth who identify as transgender, meaning their gender identity differs from their sex at birth, is rising in the United States (U.S.) [1]. Treatment with a gonadotropin-releasing hormone agonist (GnRHa) is recommended, starting at Tanner 2 pubertal

Abbreviations: ALT, alanine aminotransferase (ALT); AR, androgen receptor; AST, aspartate aminotransferase (AST); DXA, dual energy x-ray absorptiometry; GnRHa, gonadotropin releasing hormone agonist; HDL, high-density lipoprotein; LDLc, low-density lipoprotein cholesterol; LH, luteinizing hormone; FSH, follicle stimulating hormone; OGTT, oral glucose tolerance test; SHBG, sex hormone-binding globulin.

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development or later, to block endogenous pubertal changes that do not align with gender identity [2,3]. Later in adolescence, eligible adolescents (as defined in guidelines) or adults may receive treatment with testosterone or estradiol to induce development of secondary sex characteristics that align with gender identity [2,3]. Some individuals may not have received prior treatment with GnRHa therapy due to their age or pubertal stage at presentation to care, lack of insurance coverage, lack of access to gender-affirming providers, lack of family support, or other reasons [4]. Despite a growing body of research on the cardiometabolic effects of testosterone and estradiol among transgender and gender diverse individuals [5–8], there is a lack of research on the cardiometabolic effects of past or current GnRHa therapy, particularly in youth.

There are known sex differences in cisgender (gender identity aligns with sex at birth) adult populations, with cisgender women having higher total and subcutaneous adipose tissue mass, lower skeletal muscle mass and lower visceral adipose tissue mass than cisgender men [9]. There are also sex differences in lipid parameters, with cisgender women having higher high-density lipoprotein (HDL), and lower low-density and very-low-density lipoprotein cholesterol, and higher free fatty acids than cisgender men [9]. Finally, there are differences in glucose homeostasis with cisgender adult women having higher insulin sensitivity, lower fasted glucose and lower type 2 diabetes prevalence than cisgender men [9]. Cisgender adult men treated with 4 weeks of a GnRH antagonist have lower insulin sensitivity in some studies [10], but not others [11]. Low concentrations of sex steroids (either from gonadal insufficiency or in response to acute sex steroid deprivation in studies) confer higher cardiometabolic risk and higher risk of metabolic syndrome in cisgender men and women [9,12].

However, it is not clear if the effects of GnRHa therapy are the same in youth. Of note, the sex differences seen in type 2 diabetes prevalence in adolescence, is opposite that in adults, with nearly two-thirds of recent diagnoses seen in cisgender girls vs. boys [13]. The onset of puberty marks a divergence in body composition between sexes [14] and, in both sexes, insulin sensitivity decreases in parallel with increased growth hormone secretion, returning to baseline in most youth at puberty completion [15,16]. It is unclear if GnRHa treatment prevents or exacerbates the physiologic insulin resistance of puberty. Preliminary, cross-sectional data from our group showed that insulin sensitivity is lower in transgender youth on GnRHa compared to age-, sex-, and body mass index (BMI)-matched control youth [17]. Therefore, we aimed to fill the gap in the literature by evaluating a cohort of individuals assigned female at birth who had clinically either received or not received GnRHa therapy, and determine whether changes in insulin sensitivity, body composition and peak aerobic exercise capacity changed after 12 months of testosterone therapy, and whether the changes were different between those who received and continued GnRHa compared with those who did not.

Materials and methods

Participants

Nineteen adolescent transgender participants assigned female at birth were enrolled in a longitudinal observational study, evaluating the effects of standard of care testosterone therapy with or without GnHRa and metabolic profile (NCT03557268). Participants were selected based on self-decision to start gender affirming hormonal treatment. Eight participants were receiving GnRHa (7onleuprolideinjections,oneonahistrelinimplant) and 11 were not receiving GnRHa treatment. Participants were recruited from 6/2018 to 8/2019 from a pediatric multidisciplinary gender clinic on the University of Colorado Anschutz Medical Campus (CU-AMC). Participants were excluded if they had cognitive, psychiatric, or physical impairment resulting in inability to tolerate study procedures, type 1 or type 2 diabetes, weight $>$ 181 kg (due to limits of the DXA), or hypertension with a blood pressure $\geq$ 140/90 mmHg), were taking antipsychotic medications, or receiving exogenous estrogen and/or progesterone. All participants were clinically prescribed subcutaneous testosterone cypionate injections with a dose escalation schedule over the course of the 12 months. Participants on a GnRHa continued it throughout the duration of the study. No medications were given as a part of the research study. The research study was approved by the Colorado Multiple Institutional Review Board (#17–2328) and all participants and their guardian provided assent and consent, respectively.

Research visits

Study visits occurred prior to and 12 months after exogenous testosterone treatment. All participants had a research visit in the morning in the Clinical Translational Research Center (CTRC). The study visit occurred in the follicular phase of the menstrual cycle (for those having menses) and at a testosterone trough (for the 12-month time point). Blood was drawn for laboratory evaluation following an overnight 8-hour fast. Pubertal staging was performed by a pediatric endocrinologist using the standards of Tanner and Marshall [18]. Height was measured on a Harpenden stadiometer and weight on a digital electronic scale. Height and weight were recorded to the nearest 0.1 cm and kilogram, respectively. Pediatric CDC norms for BMI of sex assigned at birth were used (percentile, where 5th to $<$ 85th percentile is normal weight, 85th to $<$ 95th percentile is overweight, and $\geq$ 95th percentile is obese) [19]. Waist (at minimal waist) and hip (at widest part of the buttocks) measurements were taken, and the waist-to-hip ratio was calculated. Participants filled out a demographic questionnaire and all study data were managed using REDCap electronic data capture tools (hosted at CU-AMC) [20].

Procedures

All participants had a 2-hour oral glucose tolerance test (OGTT) after a 75 g glucose load. Blood glucose and insulin concentrations were drawn at 0, 30, 60, 90 and 120 min following ingestion.

Body composition was measured by total body dual energy x-ray absorptiometry (DXA, Hologic Horizon W, Apex 5.6.05). Absolute fat-free mass was defined as lean mass (in g or kg, not including bone mineral content) and relative fat-free mass the percent of lean mass (g), defined as lean mass divided by the total mass (lean mass, fat mass, bone mineral content) *100.

Laboratory analysis

Glucose, insulin, lipid panel, free fatty acids, leptin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin A1c, hematocrit, estrone, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex hormone-binding globulin (SHBG), and direct measurement of low-density lipoprotein cholesterol (LDLc) were processed at the CU-AMC CTRC lab and UC Health Clinical Lab using standard laboratory techniques. Glucose was measured by enzymatic UV testing (AU480 Chemistry Analyzer, Beckman Coulter, Brea, CA). Insulin was measured by radioimmunoassay (EMD Millipore, Darmstadt, Germany). Leptin was measured by radioimmunoassay (Millipore, Darmstadt, Germany). SHBG was measured at the CU-AMC CTRC lab and free and total testosterone by LC-MS/MS at the Brigham Research Assay Core lab (Boston, MA, no norms established by Tanner stage, adult female reference range 15–70 ng/dL). Percent free testosterone was calculated and provided by the BRAC lab.

Calculations

To assess the post-prandial glucose response, the Matsuda index was calculated as: [10,000/square root of (fasting glucose x fasting insulin) x (mean glucose x mean insulin during OGTT), which is highly correlated

with the rate of whole-body glucose disposal during the hyperinsulinemic euglycemic insulin clamp [21].

To better understand fasting insulin sensitivity, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as (fasting insulin)*(fasting glucose)/405 with concentrations measured in mIU/L for insulin and mg/dL for glucose.

Statistical analysis

Descriptive statistics were calculated, including n and percent for categorical variables, mean (standard deviation) for normally distributed continuous variables, and median (interquartile range) for non-normally distributed continuous variables. Normality was determined using the Shapiro-Wilk's test. Demographic differences were compared using Fisher's Exact tests for categorical variables, T-tests for normally distributed variables, and Mann Whitney U tests for two timepoints if not normally distributed. Within group changes from baseline to 12 months were assessed using mixed linear regression models with time (baseline, 12 months) and GnRHa (treatment yes/no) interactions, accounting for a random intercept for subject. Non-normally distributed variables were log transformed prior to modeling. Within group changes from baseline to 12 months were assessed using paired T-tests and Mann Whitney U tests. We evaluated correlations with Spearman's correlation tests. As this study was primarily exploratory in nature, no adjustments for multiple comparisons were made. Statistical analyses were performed in R, version 4.2.2, and figures were produced in GraphPad Prism 10.0 (Boston, Massachusetts).

Results

Baseline demographics are provided in Table 1 for the entire cohort (n = 19) and separately for those on a GnRHa (n = 8) and not on a GnRHa (n = 11). One participant (no GnRHa group) did not complete the 12-month assessment. There were statistically significant differences in age, pubertal stage, age of gender identity and disclosure, and menstrual status between those who were on a GnRHa vs. not. There were non-statistically significant differences in prevalence of depression/anxiety and past inpatient psychiatric hospitalization between those who were on a GnRHa vs. not. At 12 months, individuals on a GnRHa were on an average of 185 mg/month of testosterone (dosing range 30–60 mg subcutaneously every 7 days), and individuals not on a GnRHa were on an average of 196 mg/month of testosterone (range 40–60 mg subcutaneously every 7 days).

Glycemia and insulin sensitivity

Glucose and insulin values are shown in Table 2 and Fig. 1. In the entire cohort, after 12 months of testosterone therapy, fasted insulin decreased (12-month minus baseline median difference [25, 75 %ile]: −3 (−5, 0) uIU/mL, p = 0.044), there were no differences in fasting glucose, and there were no differences between the groups by GnRHa treatment. In the OGTT, the 30-minute glucose was significantly higher after 12 months of testosterone therapy only among those on a GnRHa (change 21.7 ± 25.5 mg/dL, p = 0.018), but there was no significant time*group interaction. In the OGTT, the 2-hour glucose was significantly higher for the entire cohort (18.5 ± 28.9 mg/dL, p = 0.013) and in the GnRHa + group (23.7 ± 20.6 mg/dL, p = -0.047) after 12 months of testosterone, with no significant time*group interaction. After 12 months of testosterone, there were no significant changes in fasting glucose, 2-hour insulin, Matsuda Index, HOMA-IR or hemoglobin A1c overall or between groups (Table 2).

There was a positive correlation between HOMA-IR (higher values indicating worse insulin sensitivity) and % fat (r = 0.66 [0.43, 0.81], p < 0.001) and a negative correlation between Matsuda index (lower values indicating worse insulin sensitivity) and % fat (r = -0.6, [-0.78, −0.33], p < 0.001]). There was no correlation between visceral adipose tissue (VAT) or the android to gynoid ratio and HOMA-IR or Matsuda Index (data not shown). There was no correlation between 2-hour glucose, HOMA-IR or Matsuda index and fat-free mass.

Table 1

	All (n = 19)	GnRHa+ (n = 8)	GnRHa- (n = 11)
Age (years)	15.0 ± 1.0	14.4 ± 0.8	15.5 ± 0.9*
Length of time on GnRHa (months)	–-	25.3 ± 13.1	–-
Chest/breast Tanner stage	5 (3.5, 5)	3 (2.75, 4)	5 (5,5)***
Pubic hair Tanner stage	5 (3.25, 5)	2.75 (2.3, 4.25)	5 (5,5)**
BMI (kg/m^2)	22.8 ± 4.9	21.7 ± 4.0	23.6 ± 5.5
BMI (%ile)	70 (41,95)	56.3 (43.8, 90.6)	73.4 (34.2, 94.9)
Gender identity†			
Male or transgender male	18 (95)	8 (100)	10 (91)
Agender	1 (5)	0 (0)	1 (9)
Gender expression†			
Mostly masculine	16 (84)	7 (88)	9 (82)
Sometimes masculine	2 (11)	1 (13)	1 (9)
Androgynous/neither	4 (21)	1 (13)	3 (27)
Sometimes feminine	1 (5)	0 (0)	1 (9)
Mostly feminine	0 (0)	0 (0)	0 (0)
Age at which they first identified current gender	11 (8,13)	10 (4.75, 11)	13 (12,13) *
Age at which they told someone about their gender identity	12 (10.5, 13)	10.5 (8.5, 11)	13 (12.5, 13)**
Race†			
White	14 (74)	5 (63)	9 (82)
Black	1 (5)	1 (13)	0 (0)
Asian	1 (5)	1 (13)	0 (0)
Native American	1 (5)	0 (0)	1 (9)
More than one race	2 (11)	1 (13)	1 (9)
Hispanic or Latino Ethnicity	5 (26)	2 (25)	3 (27)
Depression	7 (37)	2 (10)	5 (45)
Anxiety	4 (21)	1 (13)	3 (27)
In behavioral health care			
Current	15 (79)	6 (75)	9 (82)
Past	4 (21)	2 (25)	2 (18)
Past inpatient psychiatric admission	3 (16)	0 (0)	3 (27)
Menarche (yes)	16 (84)	5 (63)	11 (100)
Current menses	11 (58)	0 (0)	11 (100) ***
Age of menarche (years)	12 (11.75, 12)	12 (12,12)	12 (11,12)
Menses distress (1 = no distress, 10 = worst)	7 (6,10)	–-	7 (6,10)
Family history†			
Hypertension	9 (47)	4 (50)	5 (46)
Hypercholesterolemia	8 (42)	5 (63)	3 (27)
Type 2 diabetes	7 (37)	3 (38)	4 (36)
Myocardial infarction	6 (32)	2 (25)	4 (36)
Stroke	5 (26)	1 (13)	4 (36)
Depression	10 (53)	3 (38)	7 (64)
Anxiety	12 (63)	3 (38)	9 (82)

Data are shown as n (%), mean ± standard deviation if normally distributed or median and interquartile range if not normally distributed, as determined by Shapiro Wilk's test. P-values are determined using Fisher's Exact tests for categorical variables, T-tests for normally distributed continuous variables, and Kruskal-Wallis tests for non-normally distributed continuous variables. †multiple pick, *p < 0.05, **p < 0.01, ***p < 0.0001 and reflect statistically significant differences between those on a GnRHa or not. GnRHa = gonadotropin releasing hormone agonist.

Metabolic assays

There were no significant differences overall, by group or in the time*group interaction for any of the measured lipid parameters (Table 2). Leptin was significantly lower after 12 months of testosterone therapy in the entire cohort (−24.5 [-36.6, −14.4] ng/mL, p < 0.001),

Table 2
Laboratory measures at baseline and 12-months by group.

	Baseline (n = 19) GnRHa+ (n = 8) GnRHa- (n = 11)	12 months (n = 18) GnRHa+ (n = 8) GnRHa- (n = 10)	Baseline – 12-month difference	Within group p-value	Between group p-value
Glycemic markers and insulin sensitivity					
Fasting insulin (uIU/mL)					0.397
GnRHa+	8.0 (6.5, 9.75)	6.5 (6.0, 7.0)	−2 (−5, 0.5)	0.335	
GnRHa-	9.0 (7.0, 10.0)	5.0 (4.3, 5.8)	−3.5 (−5, −0.25)	0.103	
Overall	9 (6.5, 10)	6 (5,7)	−3 (−5, 0)	**0.044**	
2-hour Insulin (uIU/mL)					0.246
GnRHa+	35 (29.5, 43.5)	41 (37.5, 55)	5 (−1.5, 14)	0.406	
GnRHa-	59 (38,84)	36.5 (30.25, 67.5)	−17 (−28.25, 0.5)	0.341	
Overall	43.5 (31.25, 69.5)	40 (31,59)	−5 (−22, 12)	0.644	
Fasting glucose (mg/dL)					0.560
GnRHa+	85.4 ± 7.7	85.8 ± 4.6	0.4 ± 10.2	0.916	
GnRHa-	89.8 ± 8.8	87.3 ± 6.9	−0.8 ± 8	0.573	
Overall	88.0 ± 8.5	86.6 ± 5.8	−0.3 ± 8.8	0.648	
30-minute glucose (mg/dL)					0.312
GnRHa+	132.1 ± 13.1	153.9 ± 17.0	21.7 ± 25.5	**0.018**	
GnRHa-	131.09 ± 21.7	139.2 ± 19.5	8.5 ± 34.4	0.503	
Overall	131.5 ± 18.4	145.24 ± 19.4	13.9 ± 30.9	0.051	
2-hour glucose (mg/dL)					0.536
GnRHa+	93.3 ± 17.4	117.0 ± 19.7	23.7 ± 20.6	**0.047**	
GnRHa-	102.2 ± 18.6	116.9 ± 25.3	14.9 ± 34.2	0.231	
Overall	98.7 ± 18.2	116.9 ± 22.5	18.5 ± 28.9	**0.013**	
Matsuda index					0.390
GnRHa+	5.89 (4.58, 7.16)	5.64 (5.42, 7.45)	1.12 (−1.88, 2.46)	0.620	
GnRHa-	5.11 (3.69, 5.29)	7.63 (5.04, 9.1)	1.88 (0.93, 3.98)	0.173	
Overall	5.14 (4.18, 6.05)	6.46 (5.23, 8.88)	1.49 (−0.16, 3.7)	0.096	
HOMA-IR					0.401
GnRHa+	1.80 (1.31, 2.16)	1.34 (1.24, 1.52)	−0.49 (−1.17, 0.08)	0.328	
GnRHa-	1.82 (1.45, 2.36)	1.02 (0.92, 1.31)	−0.77 (−1.14, −0.02)	0.132	
Overall	1.82 (1.39, 2.28)	1.22 (1, 1.48)	−0.65 (−1.16, 0.03)	0.062	
Hemoglobin A1c					0.818
GnRHa+	5.38 ± 0.25	5.36 ± 0.21	−0.01 ± 0.22	0.915	
GnRHa-	5.19 ± 0.29	5.2 ± 0.24	0.01 ± 0.19	0.859	
Overall	5.27 ± 0.29	5.27 ± 0.24	0 ± 0.2	0.939	
Lipid parameters					
Total cholesterol (mg/dL)					0.589
GnRHa+	149.3 ± 24.4	134.8 ± 30.5	−14.5 ± 19.3	0.318	
GnRHa-	127.82 ± 8.9	120.7 ± 33.9	−6.7 ± 35.1	0.259	
Overall	136.8 ± 19.9	126.9 ± 32.3	−10.2 ± 28.7	0.331	
Triglycerides (mg/dL)					0.925
GnRHa+	94.3 ± 42.6	75.0 ± 14.8	−19.3 ± 42.2	0.528	
GnRHa-	88.6 ± 39.7	72.2 ± 33.1	−19.6 ± 37.9	0.324	
Overall	91.0 ± 39.9	73.4 ± 25.9	−19.4 ± 38.8	0.181	
HDL-C (mg/dL)					0.390
GnRHa+	45 (44,48)	41 (37,44)	−9 (−12, −4.5)	0.124	
GnRHa-	39 (37,46)	41 (32,43)	−3.5 (−5, −2)	0.548	
Overall	44 (37.5, 45.5)	40.5 (34.75, 43.75)	−5 (−10, −2)	0.148	
LDL-C (mg/dL)					0.838
GnRHa+	89.9 ± 22.0	94.0 ± 29.7	4.1 ± 17.5	0.798	
GnRHa-	74.8 ± 9.5	76.3 ± 24.4	2.4 ± 26.4	0.573	
Overall	81.2 ± 17.2	84.2 ± 27.6	3.2 ± 22.3	0.964	
Other metabolic markers					
Leptin (ng/mL)					0.271
GnRHa+	43 (34,58)	9.2 (6.9, 19.5)	−29.7 (−46.9, −24.6)	**0.002**	
GnRHa-	31 (19,47)	7.7 (6.6, 23.7)	−21.6 (−27.1, −11.5)	**0.016**	
Overall	40.5 (23.4, 51.2)	7.95 (6.6, 19.6)	−24.5 (−36.6, −14.4)	**<0.001**	
Free fatty acids (uEq/L)					0.877
GnRHa+	556 (490,719)	628 (334,865)	–23.5 (−219.5, 305.5)	1.000	
GnRHa-	565 (421,758)	580 (413,865)	27 (−300.5, 234.5)	0.809	
Overall	565 (477.5, 758)	595 (378, 887.75)	−5 (−293, 276.5)	0.893	
AST (U/L)					0.272
GnRHa+	18.8 ± 5.4	19.8 ± 7.1	1 ± 6.1	0.874	
GnRHa-	18.6 ± 3.2	23.9 ± 7.6	5 ± 9.2	0.083	

(*continued on next page*)

Table 2 (*continued*)

	Baseline (n = 19) GnRHa+ (n = 8) GnRHa- (n = 11)	12 months (n = 18) GnRHa+ (n = 8) GnRHa- (n = 10)	Baseline – 12-month difference	Within group p-value	Between group p-value
Overall	16.7 ± 4.1	22.1 ± 7.5	3.2 ± 8.0	0.166	
ALT (U/L)					0.260
GnRHa+	11.5 (9.7, 13.2)	11.5 (10.5, 13.8	0 (−1, 1.25)	0.958	
GnRHa-	13 (9.5, 14.5)	11.5 (8.3, 21.3)	1 (−0.75, 6)	0.750	
Overall	12 (9.5, 14)	11.5 (9,16)	0 (−1, 2.75)	0.819	
Hematocrit (%)					0.445
GnRHa+	41.4 ± 1.4	45.5 ± 2.3	4.1 ± 2.8	**0.004**	
GnRHa-	42.6 ± 2.4	45.8 ± 2.4	3.0 ± 2.6	**0.012**	
Overall	42.1 ± 2.1	45.6 ± 2.3	3.5 ± 2.7	**<0.001**	
SHBG (nmol/L)					0.862
GnRHa+	41.4 ± 23.5	22.4 ± 9.7	−19.0 ± 17.9	0.156	
GnRHa-	41.4 ± 22.2	20.5 ± 9.5	−20.0 ± 15.6	**0.020**	
Overall	41.4 ± 22.1	21.3 ± 9.3	−19.6 ± 16.2	**0.008**	
Gonadotropins and sex steroids					
LH (mIU/mL)					0.173
GnRHa+	0.70 (0.48, 0.72)	0.5 (0.3, 0.6)	−0.1 (−0.25, 0.05)	0.223	
GnRHa-	5.3 (2.8, 7.8)	7.0 (4.2, 8.8)	0.05 (−1.42, 2.65)	0.460	
Overall	2.3 (0.7, 5.75)	2.45 (0.6, 7.22)	−0.1 (−0.77, 0.35)	0.891	
FSH (mIU/mL)					0.508
GnRHa+	2.34 ± 0.87	0.51 ± 0.26	−1.82 ± 0.83	**<0.001**	
GnRHa-	6.42 ± 1.21	5.25 ± 2.72	−1.34 ± 2.4	0.231	
Overall	4.7 ± 2.32	3.14 ± 3.13	−1.56 ± 1.84	**0.046**	
Estradiol (pg/mL)					**0.0001**
GnRHa+	4.3 (3.4, 5.5)	14 (11.3, 18.5)	10.03 (6.73, 14.27)	**<0.001**	
GnRHa-	33.4 (25.9, 42.9)	25.4 (19.3, 33.2)	−7.5 (–23.7, 8.38)	0.132	
Overall	24.8 (4.94, 33.75)	19.3 (13.3, 25.85)	6.57 (−10.05, 13.02)	0.893	
Estrone (pg/mL)					0.110
GnRHa+	35.0 (20.3, 41.5)	78.0 (62.5, 81.5)	56 (42.5, 58)	**0.024**	
GnRHa-	42.0 (37.5, 52.5)	77.0 (68.0, 82.0)	24 (11,42)	**0.006**	
Overall	41 (31.5, 46)	77.5 (63, 82.75)	35.5 (11.75, 49.25)	**<0.001**	
Progesterone (ng/mL)					0.703
GnRHa+	0.30 (0.18, 0.32)	0.30 (0.20, 0.65)	0.1 (0, 0.22)	0.486	
GnRHa-	0.30 (0.10, 0.50)	0.35 (0.20, 0.78)	0.05 (−0.17, 0.1)	0.373	
Overall	0.3 (0.1, 0.4)	0.3 (0.2, 0.78)	0.05 (−0.07, 0.2)	0.247	
Total testosterone (ng/dL)					0.303
GnRHa+	13.6 (10.8, 18.1)	385 (304.5, 475.8)	374.78 (274.85, 462.39)	**<0.001**	
GnRHa-	25.1 (17.0, 31.9)	474 (442.5, 669)	461.18 (411.75, 641.55)	**<0.001**	
Overall	18.19 (13.55, 27.17)	445.5 (329, 624.25)	429.27 (300.99, 597.02)	**<0.001**	
Free testosterone (%)					0.366
GnRHa+	3.9 (3.2, 4.4)	4.3 (3.3, 5.9)	0.44 (−1.36, 2.51)	**<0.001**	
GnRHa-	3.3 (2.5, 3.9)	4.9 (3.2, 6.0)	1.55 (0.89, 2.26)	**0.043**	
Overall	3.72 (2.6, 4)	4.86 (3.2, 6)	1.55 (−0.93, 2.29)	0.078	

Data are shown as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables, as determined by Shapiro Wilks test. Within group p-value represents whether or not there is a change from baseline to 12 months within each group (+/-GnRHa); between group p-value represents the time*GnRHa interaction coefficient using the baseline and 12-month timepoint, evaluating whether change over time is different according to GnRHa status. Bolded values are statistically significant. Non-normally distributed variables were log transformed before modeling.

with a larger decrease in the GnRHa + group (−29.7 [-46.9, −24.6] ng/mL, p = 0.002) than the GnRHa- (−21.6 [-27.1—11.5] ng/mL, p = 0.016), group but no significant time*group interaction. There were no significant changes in free fatty acids after 12 months of testosterone. There was a statistically significant decline in SHBG for the entire cohort and the GnRHa- group.

Blood chemistries

There were no changes in AST or ALT after 12 months of testosterone. There was a rise in hematocrit in the entire cohort, and in the GnRHa + and GnRHa- groups after 12 months of testosterone (Table 2).

Gonadotropins and sex steroids

There were statistically significant increases in testosterone concentrations after 12 months of testosterone therapy. There were statistically significant increases in estrone in the entire cohort, the GnRHa + and GnRHa- group and increases in estradiol in the GnRHa + group after 12 months of testosterone therapy. There were also statistically significant declines in FSH in the entire cohort and the GnRHa + group (Table 2). There were no significant changes in LH or progesterone.

Body composition

Anthropometric measurements and body composition measures by

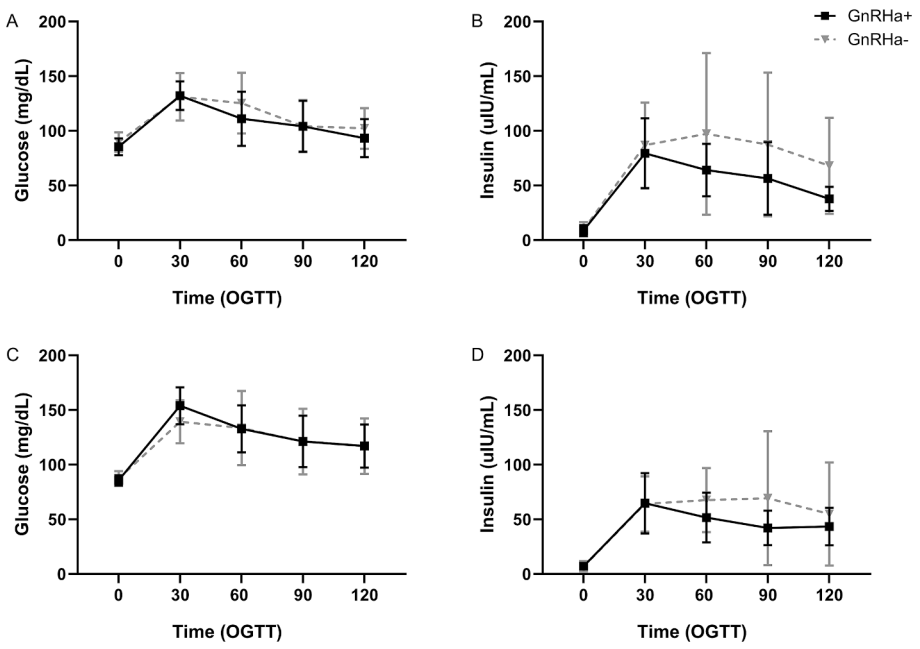

Fig. 1. Oral glucose tolerance test results. This figure shows the results of the oral glucose tolerance test including the glucose and insulin values at baseline (A and B, respectively) and 12 months after testosterone therapy (C and D) for individuals treated with a GnRHa (black squares and solid lines) and without a GnRHa (gray triangles and dotted lines). GnRHa = gonadotropin releasing hormone agonist, OGTT = oral glucose tolerance test.

DXA are shown in Table 3 and Fig. 2. There were no significant changes in weight or BMI after 12 months of testosterone therapy. After 12 months of testosterone therapy, there were no significant changes in absolute fat mass (in kg) but there were decreases in percent fat in all groups, although only statistically significant for the entire cohort and the GnRHa- group. There were statistically significant increases in % fat-free mass in all groups and absolute fat-free mass in the entire cohort and the GnRHa + group. There was a significant time*group interaction ($p = 0.0007$) with greater gains fat-free mass in the GnRHa + group (8.1 [6.7, 10.4] kg) than the GnRHa- group (4.9 [4.3, 6.1] kg) and a convergence of body composition with similar body composition between GnRHa groups at 12 months.

Measures of fat distribution were also evaluated (Table 3). There were significant differences in fat distribution for lean/height^2 and appendicular lean/height^2 but not the other measures.

Bone density

Bone density measures from DXA are shown in Table 4. There were significant differences in bone density measures in the mixed linear regression models with time*group for total bone density ($p = 0.028$), total (total body including head) bone mineral content ($p = 0.006$) and subtotal (total body less head) bone mineral content ($p = 0.002$). There were statistically significant changes in bone density measures after 12 months of testosterone therapy for the entire cohort or the GnRHa + or GnRHa- groups.

We ran a secondary analysis to test if serum testosterone concentration confounded any significant effect of GnRHa on laboratory or body composition measures, as serum testosterone was lower in the GnRHa group. Testosterone was not significant when it replaced GnRHa in the models of laboratory or body composition measures. While leptin and GnRHa were not significantly associated, lower testosterone values were predictive of higher leptin values ($p = 0.024$).

Discussion

Twelve months of testosterone therapy did not induce major changes in cardiometabolic health in transgender adolescents. We show differences in body composition and bone mineral density at baseline among adolescents treated with GnRHa compared to those who were not treated. Importantly, although individuals on a GnRHa had a less favorable body composition (higher percent fat and lower percent fat-free mass) and lower bone mineral density at baseline, these improved after 12 months of testosterone therapy. The two groups had similar bone density and body composition after 12 months of testosterone. While there were no significant changes in insulin sensitivity in either group, the group on a GnRHa did have higher 30-minute and 2-hour glucose values after testosterone therapy, an area that warrants more investigation.

Longitudinal studies in Europe that have followed individuals from the start of GnRHa therapy in adolescence through and beyond the start of testosterone or estradiol provide insights into the impact of these medications on cardiometabolic health. After initiation of GnRHa monotherapy, there are increases in BMI, total, HDL and LDL cholesterol [22]. At age 22, after having been on testosterone since about age 16, there were statistically significant increases in BMI, systolic and diastolic blood pressure, total and LDL cholesterol and triglycerides, and decreases in insulin and HDL cholesterol and improved insulin sensitivity by HOMA-IR. However, all individuals received GnRHa and there are limited available data comparing differences between those who did vs. did not receive GnRHa therapy. In the PEDSnet electronic health record database, individuals with a diagnosis of gender dysphoria and a testosterone prescription had increased risk of a diagnosis of overweight/obesity, dyslipidemia, liver dysfunction and hypertension [23]. Individuals with a testosterone and a GnRHa prescription had an increased risk of dyslipidemia and liver dysfunction [23]. In PEDSnet, individuals with a female sex listed in the chart had higher odds of overweight/obesity than female controls [23]. Unlike these studies, in our study there were no statistically significant changes in BMI or lipid parameters.

Glycemia and insulin sensitivity

Although we did not show any significant changes in estimates of insulin sensitivity, 30-minute and 2-hour glucose rose after 12 months of testosterone therapy in the group on a GnRHa, suggesting possible mild

Table 3
Body composition measures at baseline and 12-months by group.

	Baseline (n = 19) GnRHa+ (n = 8) GnRHa- (n = 11)	12 months (n = 18) GnRHa+ (n = 8) GnRHa- (n = 10)	Baseline – 12-month difference	Within group p-value	Between group p-value
Anthropometric Measurements					
Weight (kg)					0.289
GnRHa+	56.11 ± 13.03	62.4 ± 13.5	6.29 ± 3.46	0.442	
GnRHa-	65.14 ± 18.94	66.9 ± 19.71	4.06 ± 5.06	0.918	
Overall	61.34 ± 16.92	64.9 ± 16.91	5.05 ± 4.45	0.523	
Height (cm)					**<0.001**
GnRHa+	160.26 ± 5.91	163.81 ± 5.26	3.55 ± 1.67	0.189	
GnRHa-	165.2 ± 5.83	165.61 ± 5.54	1.18 ± 0.34	0.725	
Overall	163.12 ± 6.22	164.81 ± 5.34	2.23 ± 1.64	0.224	
BMI (kg/m^2)					0.704
GnRHa+	21.68 ± 4.01	23.11 ± 4.09	1.44 ± 1.15	0.442	
GnRHa-	23.59 ± 5.48	24.18 ± 6.05	1.17 ± 1.88	0.918	
Overall	22.79 ± 4.89	23.7 ± 5.16	1.29 ± 1.56	0.518	
BMI (%ile)					0.492
GnRHa+	56.3 (48.8, 86.1)	71.9 (51.5, 92.0)	3.75 (−0.55, 7.73)	0.721	
GnRHa-	73.4 (44.4, 94.8)	80.1 (47.1, 90.9)	0.55 (−2.15, 4.3)	1.000	
Overall	69.7 (46.3, 93.7)	74.8 (47.07, 91.15)	1.2 (−0.85, 7.03)	0.893	
Body composition measures					
Fat (%)					0.174
GnRHa+	36.8 (33.4, 45.9)	26.2 (25.0, 38.5)	−8.35 (−9.6, −7.4)	0.083	
GnRHa-	34.1 (31.1, 40.8)	29.1 (24.1, 32.9)	−5.55 (−7.4, −2.5)	**0.049**	
Overall	34.3 (31.7, 43.4)	28 (24.9, 37.3)	−7.4 (−9.3, −5.3)	**0.005**	
Fat-free mass (%)					**0.033**
GnRHa+	59.7 (51.3, 63.1)	70.5 (58.6, 71.6)	8.52 (7.31, 9.46)	**0.050**	
GnRHa-	62.1 (56.3, 65.2)	67.3 (63.8, 72.5)	5.64 (2.74, 7.28)	**0.043**	
Overall	62.03 (53.74, 64.81)	68.56 (59.69, 71.76)	7.26 (5.42, 9.1)	**0.003**	
Fat mass (kg)					0.636
GnRHa+	18.7 (15.1, 28.2)	14.9 (13.2, 27.6)	−3.1 (−3.5, −2.3)	0.279	
GnRHa-	18.9 (16.9, 31.3)	17.9 (12.9, 24.0)	−2.7 (−3.7, 0.6)	0.512	
Overall	18.9 (16.1, 30.1)	16.0 (12.7, 26.7)	−2.9 (−3.6, −1.2)	0.178	
Fat-free mass (kg)					**0.0007**
GnRHa+	32.8 (28.1, 35.0)	40.3 (37.9, 42.8)	8.1 (6.7, 10.4)	**0.007**	
GnRHa-	39.0 (33.1, 42.3)	41.8 (37.1, 46.5)	4.9 (4.3, 6.1)	0.314	
Overall	35.3 (30.7, 39.1)	40.3 (36.8, 45.8)	6.2 (4.7, 7.5)	**0.016**	
Fat distribution					
Fat Mass/Height^2 (kg/m^2)					0.393
GnRHa+	6.96 (6.46, 11.07)	5.60 (4.96, 10.25)	−1.28 (−1.67, −1.09)	0.093	
GnRHa-	6.78 (6.16 (11.4)	6.64 (4.89, 8.49)	−1.17 (−1.42, 0.19)	0.426	
Overall	6.78 (6.31, 11.15)	5.66 (4.83, 9.84)	−1.17 (−1.62, −0.71)	0.083	
Android/Gynoid ratio					0.532
GnRHa+	0.94 ± 0.14	0.99 ± 0.14	0.05 ± 0.06	0.371	
GnRHa-	0.91 ± 0.16	0.96 ± 0.18	0.07 ± 0.05	0.417	
Overall	0.92 ± 0.15	0.97 ± 0.16	0.06 ± 0.05	0.28	
% Fat Trunk/%Fat Legs					0.325
GnRHa+	0.86 ± 0.13	0.88 ± 0.16	0.03 ± 0.05	0.916	
GnRHa-	0.83 ± 0.17	0.86 ± 0.18	0.05 ± 0.04	0.777	
Overall	0.84 ± 0.15	0.87 ± 0.17	0.04 ± 0.05	0.819	
Trunk/Limb Fat Mass Ratio					0.937
GnRHa+	0.85 ± 0.23	0.89 ± 0.22	0.04 ± 0.06	0.713	
GnRHa-	0.9 ± 0.25	0.92 ± 0.24	0.04 ± 0.05	0.751	
Overall	0.88 ± 0.23	0.91 ± 0.22	0.04 ± 0.05	0.638	
Est. VAT Mass (g)					0.126
GnRHa+	373.5 ± 139.79	320.5 ± 117.05	−53 ± 56.56	0.431	
GnRHa-	376.45 ± 208.98	375.4 ± 235.86	11.7 ± 98.61	0.809	
Overall	375.21 ± 178.51	351 ± 189.43	−17.06 ± 89.95	0.475	
Est. VAT Volume (cm^3)					0.123
GnRHa+	404.12 ± 151.01	346.62 ± 126.78	−57.5 ± 61.07	0.431	
GnRHa-	406.73 ± 225.95	405.8 ± 255.16	12.9 ± 106.66	0.809	
Overall	405.63 ± 192.95	379.5 ± 204.95	−18.39 ± 94.09	0.475	

(*continued on next page*)

Table 3 (*continued*)

	Baseline (n = 19) GnRHa+ (n = 8) GnRHa- (n = 11)	12 months (n = 18) GnRHa+ (n = 8) GnRHa- (n = 10)	Baseline – 12-month difference	Within group p-value	Between group p-value
Est. VAT area (cm^2)					0.127
GnRHa+	77.46 ± 28.92	66.47 ± 24.33	−10.99 ± 11.62	0.442	
GnRHa-	78.1 ± 43.3	77.89 ± 48.99	2.48 ± 20.66	0.809	
Overall	77.83 ± 36.97	72.82 ± 39.35	−3.51 ± 18.14	0.48	
Lean/ Height^2 (kg/m^2)					**0.010**
GnRHa+	12.29 ± 1.37	14.91 ± 1.49	2.62 ± 0.8	**0.007**	
GnRHa-	13.83 ± 1.98	15.43 ± 2.26	1.8 ± 0.39	0.130	
Overall	13.18 ± 1.88	15.2 ± 1.92	2.17 ± 0.72	**0.005**	
Appen. Lean/ Height^2 (kg/m^2)					**0.013**
GnRHa+	5.27 ± 0.6	6.73 ± 0.75	1.46 ± 0.4	**0.003**	
GnRHa-	5.85 ± 1	6.82 ± 1.07	1.09 ± 0.14	0.051	
Overall	5.6 ± 0.89	6.78 ± 0.92	1.25 ± 0.34	**<0.001**	

Data are shown as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables, as determined by Shapiro Wilks test. Within group p-value represents whether or not there is a change from baseline to 12 months within each group (+/-GnRHa); between group p-value represents the time*GnRHa interaction coefficient using the baseline and 12-month timepoint, evaluating whether change over time is different according to GnRHa status. Bolded values are statistically significant. Non-normally distributed variables were log transformed before modeling.

beta cell dysfunction. There may be sex-specific effects of testosterone on β cell function. In cisgender men, testosterone's actions on the androgen receptor (AR) present on β cells have been shown to enhance glucose-stimulated insulin secretion by potentiating the insulinotropic action of glucagon-like peptide-1 [24]. In cisgender women, testosterone action via the AR on β cells has been shown to promote insulin hypersecretion leading to oxidative injury, which may predispose to type 2 diabetes [24]. Recent studies in other human cell types (dermal and lung fibroblasts) have demonstrated decreased bioenergetic capacity and inhibited cell proliferation among 46,XX cells treated with dihydrotestosterone and 46,XY cells treated with estradiol [25]. In transgender adults in the Netherlands, after 12 months of testosterone therapy, there were no changes in fasted glucose, insulin, nor glucose utilization evaluated with hyperinsulinemic euglycemic clamps, but peripheral insulin sensitivity at the high-dose insulin step of the clamp worsened [26]. In another study, during step 1 of the clamp, with insulin concentrations in the physiologic range, glucose utilization decreased after 4 months of testosterone administration [27]. Endogenous glucose release measured by a glucose isotope tracer was not impacted by hormone administration, so the authors concluded that the decline in glucose utilization was due to diminished peripheral uptake [27].

Overall, systematic reviews suggest that testosterone does not have an impact on insulin sensitivity in transgender adults [28], although more detailed investigation of testosterone on β cell function is needed. Several large studies show that transgender men in the U.S. and Europe do not have a higher risk of diabetes compared to cisgender men or women [29–31]. Finally, more recent studies have shown a correlation between both android and gynoid fat and lower insulin sensitivity by HOMA-IR in transgender adults [32], although we did not find any correlations in our cohort. Longer term follow-up studies are needed, particularly of those who received GnRHa in adolescence as they had higher glucose values after testosterone.

Metabolic assays and blood chemistries

In our study, there were statistically significant increases in hematocrit after testosterone therapy, which aligns with other studies in adults [33] and adolescents [34].

In our study, there were no significant differences in lipids or aminotransferases after testosterone therapy. This is in contrast to what has been shown in other studies of adolescents and adults. A longitudinal study of transgender youth after 24 months of testosterone therapy showed a statistically significant increases in systolic blood pressure (mean increase of + 12.4 mmHg), ALT (+10.6 U/L) and a decrease in HDL (mean of 7.3 mg/dL) [35]. Another study demonstrated that changes in HDL after 12 months of testosterone therapy are attenuated by obesity, with lower HDL values among individuals with obesity compared to those without obesity [36]. Systematic reviews suggest that adult transgender men have decreases in HDL cholesterol and increases in LDL cholesterol after testosterone therapy [33]. In a *meta*-analysis that accompanied the 2017 Endocrine Society guidelines, testosterone therapy in transgender adults was associated with increases in serum triglycerides (at 3–6 months and ≥ 24 months of therapy) and LDL (at 12 and ≥ 24 months) and a decrease in HDL at all timepoints [37]. Of note, studies in adults are reflective of long-term treatment with testosterone; thus, a 12-month time period may not fully demonstrate potential impacts of testosterone on metabolic health. On the other hand, adults are more likely to have underlying metabolic health complexity. The 2017 Endocrine Society guidelines recommend screening hemoglobin/hematocrit and lipids in transgender adolescents and adults starting testosterone therapy [2].

Finally, the increases in estrone and estradiol were likely secondary to aromatization from androgens. Individuals on GnRHa stayed on it throughout the entire study, and the small rise in estradiol on the GnRHa + group and the low LH value indicate continued suppression of the hypothalamic-pituitary gonadal axis.

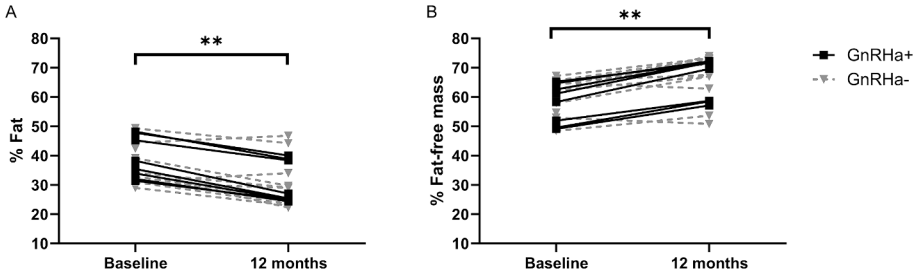

Fig. 2. Relative body composition changes. This figure shows percent fat (A) and percent fat-free mass (B) at baseline and 12 months after testosterone therapy for individuals treated with a GnRHa (black squares and solid lines) and without a GnRHa (gray triangles and dotted lines). GnRHa = gonadotropin releasing hormone agonist.

Table 4
Bone density measures at baseline and 12-months by group.

	Baseline (n = 19) GnRHa+ (n = 8) GnRHa- (n = 11)	12 months (n = 18) GnRHa+ (n = 8) GnRHa- (n = 10)	Baseline – 12-month difference	Within group p-value	Between group p-value
TOTAL Bone mineral density (g/cm^2)					**0.028**
GnRHa+	0.99 ± 0.06	1.04 ± 0.06	0.05 ± 0.03	0.083	
GnRHa-	1.09 ± 0.1	1.09 ± 0.09	0.01 ± 0.04	0.944	
Overall	1.05 ± 0.1	1.07 ± 0.08	0.03 ± 0.04	0.494	
SUBTOTAL Bone mineral density (g/cm^2)					0.330
GnRHa+	0.88 ± 0.06	0.94 ± 0.06	0.06 ± 0.03	0.130	
GnRHa-	0.97 ± 0.09	0.88 ± 0.06	0.04 ± 0.05	0.705	
Overall	0.93 ± 0.09	0.97 ± 0.08	0.05 ± 0.04	0.248	
TOTAL Bone mineral content (g)					**0.006**
GnRHa+	1,734.94 ± 223.48	1,905.66 ± 250	170.72 ± 68.47	0.130	
GnRHa-	2,074.53 ± 370.44	2,091.58 ± 331.55	74.19 ± 63.41	0.705	
Overall	1,931.54 ± 354.02	2,008.95 ± 304.91	117.09 ± 80.59	0.248	
SUBTOTAL Bone mineral content (g)					**0.002**
GnRHa+	1,359.28 ± 189.8	1,515.39 ± 209.17	156.12 ± 63.07	0.130	
GnRHa-	1,634.36 ± 319.75	1,639.07 ± 276.68	58.65 ± 52.64	0.756	
Overall	1,518.54 ± 300.47	1,584.11 ± 250.08	101.97 ± 74.76	0.298	
TOTAL Z-score compared to female norms					0.415
GnRHa+	−0.51 ± 0.94	−0.29 ± 0.85	0.22 ± 0.41	0.673	
GnRHa-	0.37 ± 1.08	0.39 ± 1	0.11 ± 0.35	0.972	
Overall	0 ± 1.09	0.09 ± 0.98	0.16 ± 0.37	0.903	
SUBTOTAL Z-score compared to female norms					0.131
GnRHa+	−0.54 ± 0.86	−0.1 ± 0.84	0.44 ± 0.46	0.430	
GnRHa-	0.33 ± 1.09	0.33 ± 0.94	0.13 ± 0.31	1.000	
Overall	−0.04 ± 1.07	0.14 ± 0.9	0.27 ± 0.4	0.594	
TOTAL Z-score compared to male norms					0.565
GnRHa+	−0.34 ± 1.03	−0.48 ± 0.88	−0.14 ± 0.3	0.916	
GnRHa-	0.2 ± 1.11	−0.08 ± 1	−0.23 ± 0.37	0.341	
Overall	−0.03 ± 1.08	−0.26 ± 0.94	−0.19 ± 0.34	0.386	
SUBTOTAL Z-score compared to male norms					0.233
GnRHa+	−0.61 ± 0.96	−0.66 ± 0.79	−0.05 ± 0.39	1.000	
GnRHa-	−0.2 ± 1.15	−0.53 ± 0.95	−0.27 ± 0.38	0.217	
Overall	−0.37 ± 1.07	−0.59 ± 0.86	−0.17 ± 0.39	0.345	

Data are shown as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables, as determined by Shapiro Wilks test. Within group p-value represents whether or not there is a change from baseline to 12 months within each group (+/-GnRHa); between group p-value represents the time*GnRHa interaction coefficient using the baseline and 12-month timepoint, evaluating whether change over time is different according to GnRHa status. Bolded values are statistically significant. Non-normally distributed variables were log transformed before modeling. Subtotal = total body less head.

Body composition

Like other studies, we show gains in fat-free mass and a decline in fat mass after testosterone therapy [38]. However, we uniquely show that individuals on a GnRHa started with higher % fat and lower % fat-free mass than those not on a GnRHa, and had greater gains fat-free compared to those not on a GnRHa, with a convergence of body composition with similar body composition between GnRHa groups at 12 months. There are limited data on the impact of GnRHa therapy on body composition. A cross-sectional study from our group showed that transgender youth on GnRHa monotherapy had higher percent body fat and leptin than age-, sex- and BMI-matched control youth. In the same study, youth on testosterone therapy had an intermediate body composition between age- and BMI-matched controls [39]. European longitudinal studies, following individuals who had started GnRHa followed by testosterone until age 22 years, similarly show an increase in fat-free mass and decline in fat mass, with a waist-to-hip ratio standard deviation score above the average for cisgender women and below the average for cisgender men [40]. Meta-analyses and systematic reviews of longitudinal studies evaluating effects of testosterone therapy in transgender adults on body composition consistently demonstrate increases in body weight and fat-free body mass and decreases in body fat [28,38]. Our results corroborate this, as the cohort had an increase in fat-free mass and a decrease in fat mass (although no changes in weight or BMI), with those on GnRHa treatment having higher fat mass and lower fat-free mass than those who were not treated with a GnRHa. Testosterone seems to "correct" the potentially negative body composition change seen with GnRHa treatment. Further studies are needed to see if this translates into different health outcomes over time.

Bone density

While we did not show any statistically significant changes in total or subtotal bone mineral density after 12 months of testosterone therapy, there were significant differences by group. The group on GnRHa treatment had lower total bone mineral density at baseline than the group not on GnRHa, but total bone mineral density was similar between

groups after 12 months of testosterone therapy. The group on GnRHa treatment also had greater gains in bone mineral content, suggesting a rapid "catch up" of bone mineral density. The Z-scores were around average or slightly below average. Our results are similar to other studies showing decreases in bone mineral density Z-scores after initiation with GnRHa therapy, as recently reviewed [41]. Longitudinal studies show that initiation of testosterone or estradiol in transgender youth improves bone mineral density [42] and a female sex assigned at birth is a positive predictor of bone mineral density Z-scores [43]. A recent study showed Z-scores in individuals treated with GnRHa and later testosterone catch up to pretreatment levels [44]. In transgender adults who did not receive GnRHa, testosterone therapy is not associated with significant changes in bone mineral density [45].

Strengths and limitations

This is the first observational, longitudinal study to evaluate differences between transgender male adolescents on the basis of GnRHa treatment in addition to 12 months of testosterone therapy. This study has several strengths, including a prospective design to evaluate individual changes after testosterone therapy, the comprehensive metabolic phenotyping of the participants, and assessment of body composition. The most significant limitation is the small sample size, and we may be underpowered to find significant differences over time between groups. Since GnRHa treatment status was not randomized, there may be other differences between these groups, including, but not limited to, insurance coverage, family support, age of disclosure of gender identity, and experiences of minority stress. Similarly, the groups were a convenience sample based on enrollment and were not matched on age or BMI. There were non-statistically significant differences in prevalence of depression/anxiety and past inpatient psychiatric hospitalization between those who were on a GnRHa vs. not.

Conclusion

Twelve months of testosterone therapy did not induce major changes in cardiometabolic health in than male adolescents. There were differences in body composition and bone mineral density among adolescents treated with GnRHa at baseline compared to those who were not treated. In this cohort, testosterone seemed to "correct" the potentially negative body composition change seen with GnRHa treatment. Larger studies with a longer duration of follow-up are needed to understand the long-term effects of testosterone therapy in transgender youth and if there are differences between those who received a GnRHa or not.

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CRediT authorship contribution statement

Natalie J. Nokoff: Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Samantha Bothwell:** Writing – review & editing, Visualization, Formal analysis, Data curation. **John D. Rice:** Data curation, Formal analysis, Visualization, Writing – review & editing. **Melanie G. Cree:** Writing – review & editing, Methodology, Conceptualization. **Megan M. Kelsey:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kerrie L. Moreau:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Philip Zeitler:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kristen J. Nadeau:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Natalie Nokoff reports a relationship with Neurocrine Biosciences Inc that includes: consulting or advisory and funding grants. Natalie Nokoff reports a relationship with Ionis Pharmaceuticals Inc that includes: consulting or advisory. Natalie Nokoff reports a relationship with World Athletics that includes: consulting or advisory. Melanie Cree reports a relationship with Pollie, Inc that includes: consulting or advisory. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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