The association of gender-affirming hormone therapy duration and body mass index on bone mineral density in gender diverse adults

Sean J. Iwamoto [a,b,c,*], John D. Rice [d], Kerrie L. Moreau [e,f], Marc-André Cornier [g], Margaret E. Wierman [a,b], Mary P. Mancuso [h], Amanuail Gebregzabheir [a], Daniel B. Hammond [a,c], Micol S. Rothman [a,c]

[a] *Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, 12801 E 17th Ave, 7103, Aurora, CO 80045, USA*
[b] *Endocrinology, Medicine and Research Services, Rocky Mountain Regional VA Medical Center, 1700 N Wheeling St, 111H, Aurora, CO 80045, USA*
[c] *UCHealth Integrated Transgender Program, University of Colorado Hospital, 1635 Aurora Court, Anschutz Outpatient Pavilion, 6th floor, Aurora, CO 80045, USA*
[d] *Department of Biostatistics, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI 48109, USA*
[e] *Division of Geriatrics, Department of Medicine, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, 12631 E 17th Ave, 8111, Aurora, CO 80045, USA*
[f] *Geriatric Research Education and Clinical Center, VA Eastern Colorado Healthcare System, Rocky Mountain Regional VA Medical Center, 1700 N Wheeling St, Aurora, CO 80045, USA*
[g] *Division of Endocrinology, Diabetes and Metabolic Diseases, Department of Medicine, Medical University of South Carolina, 96 Jonathan Lucas St, CSB 822, Charleston, SC 29425, USA*
[h] *Division of General Internal Medicine, Department of Medicine, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, 12631 E 17th Ave, Aurora, CO, 80045, USA*

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ABSTRACT

Introduction: Feminizing and masculinizing gender-affirming hormone therapy (fGAHT, mGAHT) results in bone mineral density (BMD) maintenance or improvement over time in transgender and gender diverse (TGD) adults. Mostly European TGD studies have explored GAHT's impact on BMD, but the association of BMI and BMD in TGD adults deserves further study.
Objective: To determine whether GAHT duration or BMI are associated with BMD and Z-scores among TGD young adults.
Methods: Cross-sectional study of nonsmoking TGD adults aged 18–40 years without prior gonadectomy or gonadotropin-releasing hormone agonist (GnRHa) therapy taking GAHT for > 1 year. BMD and Z-scores were collected from dual-energy x-ray absorptiometry. Associations between femoral neck, total hip, and lumbar spine BMDs and Z-scores and the predictors, GAHT duration and BMI, were estimated using linear regression.
Results: Among 15 fGAHT and 15 mGAHT, mean BMIs were 27.6 +/- standard deviation (SD) 6.4 kg/m^2 and 25.3 +/- 5.9 kg/m^2, respectively. Both groups had mean BMDs and Z-scores within expected male and female reference ranges at all three sites. Higher BMI among mGAHT was associated with higher femoral neck and total hip BMDs (femoral neck: $\beta = 0.019$ +/- standard error [SE] 0.007 g/cm^2, total hip: $\beta = 0.017$ +/- 0.006 g/cm^2; both p < 0.05) and Z-scores using male and female references. GAHT duration was not associated with BMDs or Z-scores for either group.
Conclusions: Z-scores in young, nonsmoking TGD adults taking GAHT for > 1 year, without prior gonadectomy or GnRHa, and with mean BMIs in the overweight range, were reassuringly within the expected ranges for age based on male and female references. Higher BMI, but not longer GAHT duration, was associated with higher femoral neck and total hip BMDs and Z-scores among mGAHT. Larger, prospective studies are needed to understand how body composition changes, normal or low BMIs, and gonadectomy affect bone density in TGD adults.

Introduction

Background and rationale

Transgender and gender diverse (TGD) people have a gender identity that differs from their sex assigned at birth. Recent data from the Williams Institute estimate that 0.5 % of adults and 1.4 % of youth aged 13–17 years in the U.S. identify as TGD [1]. According to the World Professional Association for Transgender Health, worldwide estimates for TGD people in the general population are 0.02–0.1 % for health systems-based studies and much higher for self-reported survey-based studies of adults (0.3–0.5 % for transgender-only adults and 0.3–4.5 % for TGD adults) [2]. Gender-affirming hormone therapy (GAHT) can help align gender identity with outward appearance and secondary sex characteristics. GAHT is sex steroid-based, with estradiol and anti-androgen therapy as the mainstays of feminizing GAHT (fGAHT) and testosterone of masculinizing GAHT (mGAHT).

It is well known that sex steroids play crucial roles in bone mineral density (BMD) accrual and maintenance, regardless of sex assigned at birth [3,4]. Additionally, many studies in TGD adults and youth have shown a higher prevalence of low BMD in persons taking fGAHT compared to cisgender comparators even prior to the initiation of GAHT [5,6]. The etiology is not clear, but differences in lifestyle factors and physical activity, body mass index (BMI) and lower 25-hydroxyvitamin D levels may play roles [5,7]. Transmasculine individuals, in contrast, generally have preserved BMD prior to GAHT initiation compared to cisgender comparators.

Studies from Europe have also shown that both fGAHT and mGAHT maintain lumbar spine BMD and increase Z-scores compared to baseline, even in the face of relative suppression of endogenous sex steroid production out to 10 years [3,4,6]. To date, hip BMD and fracture data are limited [6,8,9]. Additionally, studies in TGD adults have been limited to non-U.S. cohorts where rates of gonadectomy and tobacco use are higher, and body mass index (BMI) lower, than in the U.S. For example, participant characteristics from the largest European TGD cohort with adult bone data, the Amsterdam Cohort of Gender Dysphoria, included the following: 75.3 % and 83.8 % with history of gonadectomy, 34.9 % and 39.5 % currently smoking, and mean BMI 23.7 kg/m^2 and 25.6 kg/m^2, for fGAHT and mGAHT groups, respectively [6,10]. In contrast, the largest U.S. TGD cohort, the Study of Transition, Outcomes and Gender, has not yet published bone data but has the following characteristics described in its cohort profiles: 1.5 % and 11 % with history of gonadectomy, 15 % and 17–18 % currently smoking, and BMIs in the overweight and obesity categories combined (48–52 % and 56–57 %), for fGAHT and mGAHT cohorts, respectively [11,12].

Objectives and hypotheses

Given these differences in gonadectomy status, tobacco smoking, and BMI between European and U.S. TGD cohorts, and the lack of bone data among U.S. TGD adults, this study sought to evaluate the associations between GAHT duration and BMI with BMD and Z-scores in nonsmoking TGD adults aged 18–40 years without prior gonadectomy or gonadotropin-releasing hormone agonist (GnRHa) therapy, taking fGAHT (specifically estradiol + spironolactone for this study) or mGAHT (testosterone) for greater than one year. We hypothesized that 1) BMDs and Z-scores in fGAHT and mGAHT groups would be within both male and female reference ranges and 2) longer GAHT duration and higher BMI would be associated with higher BMDs and Z-scores at femoral neck, total hip, and lumbar spine.

Material and Methods

Study design

These data were part of parent hypothesis-generating pilot studies

approved by the Colorado Multiple Institutional Review Board that cross-sectionally evaluated the association of long-term fGAHT and mGAHT (defined as more than one year duration) on various cardiometabolic and bone health outcomes in TGD adults. The data on bone density and BMI are presented in this manuscript.

Setting

Participants attended study visits between 2019 and 2023 at the University of Colorado Anschutz Medical Campus, Colorado Clinical & Translational Sciences Institute, Clinical and Translational Research Center Outpatient Clinic. Dual-energy X-ray absorptiometry (DXA) was performed in the fasted state through the Colorado Nutrition Obesity Research Center's Energy Balance Core. Study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at University of Colorado Anschutz Medical Campus [13]. REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Study size and participants

Thirty healthy TGD adults aged 18–40 years were recruited from the Denver metropolitan area by word of mouth, informational flyers, and social media, without limitations on race, ethnicity, socioeconomic status, or education level. Inclusion criteria included self-identification as TGD (not cisgender; e.g., transgender, transfeminine, transmasculine, nonbinary) and current use of GAHT for greater than one year duration. Exclusion criteria included not identifying as TGD, GAHT use less than one year, prior or active neoplasms, acute liver or gallbladder disease, venous thromboembolism, active or overt hyperthyroidism, current smoking (or quit less than one year prior to enrollment), or illicit drug use. No participants had previously used a GnRHa. Potential participants were also excluded if they had a serious illness within the last six months and any who had a confirmed positive COVID-19 test and were ever hospitalized due to COVID-19 complications. If someone recently had COVID-19, but was not hospitalized, they were at least two weeks after the onset of the positive COVID-19 test and more than seven days without symptoms.

As part of the consent process, participants received written and verbal assurance that each study received a Certificate of Confidentiality from the National Institutes of Health Office of Extramural Research. Participants were also compensated for their participation in the study.

Variables

GAHT duration was calculated as time between self-reported month and year the participant initiated GAHT and the study visit date. BMI (kg/m^2) was calculated as measured weight (kg, using a scale [seca 644]) per measured height (m, using a stadiometer [seca 216]) at the study visit. The mean BMDs and Z-scores at the lumbar spine, femoral neck, and total hip were obtained from DXA scans (Horizon® W). Total body fat percent was also obtained from the DXA report. Serum estradiol was measured using chemiluminescent immunoassay (Beckman Coulter, Inc., USA) and serum total testosterone was measured using a 1-step competitive assay (Beckman Coulter, Inc., USA).

Statistical methods

The sample size of 30 was dictated by the parent pilot studies mentioned above; therefore, a separate power analysis was not conducted as part of this study. Group means ± standard deviations (SD) were calculated for participant demographics, GAHT duration, BMI,

body composition variables, and serum estradiol and total testosterone levels. Group means +/- SD for BMD and Z-scores (presented for female and male references) were also calculated. Associations between femoral neck, total hip, and lumbar spine BMDs and Z-scores and the predictors, GAHT duration and BMI, were estimated using linear regression and presented as group means +/- standard error (SE). $P < 0.05$ was considered statistically significant. Although multiple statistical tests were conducted to analyze the associations of GAHT duration and BMI with BMD and Z-scores, this study was intended to be hypothesis generating so we did not adjust formally for multiple comparisons. All analyses were performed in R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Participants

Fifteen fGAHT and 15 mGAHT had DXA scans available to analyze. Mean ages were 28.7 +/- 4.8 years for fGAHT and 28.5 +/- 5.7 years for mGAHT (See Table 1 for participant characteristics). Mean fGAHT and mGAHT durations were 3.1 +/- 2.1 years and 4.0 +/- 2.0 years, respectively.

For fGAHT, the mean serum estradiol level was within the guideline-recommended range (e.g., 100 to 200 pg/mL) but the mean serum total testosterone level was above the guideline-recommended range (e.g., <50 ng/dL) [2,14]. Two fGAHT had serum estradiol levels < 100 pg/mL (range 67 to 72 pg/mL) and five fGAHT had serum estradiol levels > 200 pg/mL (range 230 to 467 pg/mL); five fGAHT had serum testosterone levels > 50 ng/dL (range 82 to 358 ng/dL).

For mGAHT, the mean serum total testosterone level was within the guideline-recommended range (e.g., 400 to 700 ng/dL for mid-dose level if on injections or within the laboratory assay's reference range for men regardless of route of administration [for the assay used in this study: 260 to 816 ng/dL]) [2,14]. Six mGAHT had serum testosterone levels < 260 ng/dL (range < 17 to 254 ng/dL) and four mGAHT had serum testosterone levels > 816 ng/dL (range 837 to 1071 ng/dL). Current guidelines do not provide recommendations for serum estradiol level in mGAHT and do not suggest measuring it routinely, though a previous guideline iteration suggested a serum estradiol level < 50 pg/mL [2,14,15]. Among mGAHT, seven had serum estradiol levels > 50 pg/mL (range 56 to 87 pg/mL).

The fGAHT group reported various routes of administration of estrogen at the time of the study including oral estradiol (n = 7), intramuscular estradiol valerate (n = 5), sublingual estradiol (n = 2), and transdermal patch (n = 1). All fGAHT were currently taking spironolactone, 8 of 15 (53 %) taking progesterone, and 2 of 15 (13 %) taking cholecalciferol. Ten of 15 (67 %) reported never smoking and the remaining 5 (33 %) were former smokers who had quit more than one year prior to the study. The mGAHT group reported various routes of administration of testosterone at the time of the study including intramuscular testosterone cypionate (n = 9), subcutaneous testosterone cypionate (n = 3), and testosterone gel (n = 3). No mGAHT reported

taking cholecalciferol. Twelve of 15 (80 %) reported never smoking and the remaining 3 (20 %) were former smokers who quit more than one year prior to the study.

BMI and body composition

For fGAHT and mGAHT, mean BMIs were 27.6 +/- 6.4 kg/m^2 and 25.3 +/- 5.9 kg/m^2, respectively, total lean mass percentages (not including bone mineral content) were 63.0 +/- 6.8 % and 66.8 +/- 6.8 %, respectively, and total body fat percentages were 33.7 +/- 7.3 % and 29.8 +/- 7.1 %, respectively. Overall, total body fat percent was strongly correlated with BMI (R = 0.85, p < 0.01). For fGAHT, the total body fat percentage equated to total % fat percentiles of 51.7 +/- 29.2 and 87.9 +/- 16.5 compared to age-matched references for females and males, respectively. For mGAHT, the total body fat percentage equated to total % fat percentiles of 34.1 +/- 29.4 and 77.3 +/- 30.0, compared to age-matched references for females and males, respectively.

BMD and Z-scores

For both fGAHT and mGAHT, mean BMDs resulted in mean femoral neck, total hip, and lumbar spine Z-scores within the expected ranges for age using both female and male references (See Table 2). When assessing individual Z-scores by GAHT type, age, and site, only one fGAHT participant had low BMD for age (defined by Z-score less than or equal to −2.0) at the total hip and lumbar spine using the male reference only, and only one mGAHT participant had low BMD for age at the femoral neck by both female and male references (See Fig. 1).

Linear regression analyses

Higher BMI was associated with higher femoral neck and total hip BMDs and Z scores, and lumbar spine BMD, among mGAHT, not fGAHT (See Table 3 and Fig. 2). Specifically, for mGAHT, a 1 kg/m^2 higher BMI was significantly associated with a higher BMD by 0.019 +/- 0.007 g/cm^2, 0.017 +/- 0.006 g/cm^2, and 0.010 +/- 0.004 g/cm^2 at the femoral neck, total hip, and lumbar spine, respectively. A 1 kg/m^2 higher BMI among mGAHT was also significantly associated with higher Z-scores at the femoral neck and total hip using both male and female references, but not at the lumbar spine. In a sensitivity analysis, after removing the individual with BMI 34.7 kg/m^2 and the highest BMDs at all three sites, there was still a positive correlation between BMI and BMD (bivariate analysis, p = 0.05). Lumbar spine Z-scores were not associated with BMI among fGAHT. GAHT duration was not associated with BMDs or Z scores for either group.

Discussion

Although multiple studies have raised concerns about low baseline BMD in the TGD population, particularly in transfeminine individuals, the data from the present study provide reassuring results for a cohort of nonsmoking young adults taking mGAHT or fGAHT for several years on

Table 1
Participant characteristics.

Regimen	N	Age (years)	Race (n, % non-white)	Ethnicity (n, % Hispanic)	Never smoker (n, %)	GAHT duration (years)	Serum estradiol (pg/mL)	Serum total testosterone (ng/dL)	Body mass index (kg/m^2)	Total body fat (%)
fGAHT	15	28.7 +/- 4.8	0, 0	0, 0	10, 67	3.1 +/- 2.1	185 +/- 117	93 +/- 97	27.6 +/- 6.4	33.7 +/- 7.3
mGAHT	15	28.5 +/- 5.7	3, 20	3, 20	12, 80	4.0 +/- 2.0	47 +/- 24	545 +/- 333	25.3 +/- 5.9	29.8 +/- 7.1

Continuous variable data are presented as mean +/- standard deviation.
GAHT = gender-affirming hormone therapy.
fGAHT = feminizing gender-affirming hormone therapy.
mGAHT = masculinizing gender-affirming hormone therapy.

Table 2
Bone mineral density and Z-scores at the femoral neck, total hip, and lumbar spine.

Regimen	Femoral neck			Total hip			Lumbar spine		
	BMD (g/cm^2)	Z-score (Female reference)	Z-score (Male reference)	BMD (g/cm^2)	Z-score (Female reference)	Z-score (Male reference)	BMD (g/cm^2)	Z-score (Female reference)	Z-score (Male reference)
fGAHT	0.931 +/- 0.111	0.8 +/- 1.0	0.2 +/- 0.8	0.997 +/- 0.126	0.5 +/- 1.0	−0.2 +/- 0.8	1.102 +/- 0.122	0.6 +/- 1.1	0.1 +/- 1.1
mGAHT	0.902 +/- 0.186	0.4 +/- 1.3	−0.2 +/- 1.0	0.999 +/- 0.155	0.4 +/- 1.0	−0.3 +/- 0.8	1.06 +/- 0.105	0.2 +/- 0.8	−0.2 +/- 0.8

All data are presented as mean +/- standard deviation.
BMD = bone mineral density.
fGAHT = feminizing gender-affirming hormone therapy.
mGAHT = masculinizing gender-affirming hormone therapy.
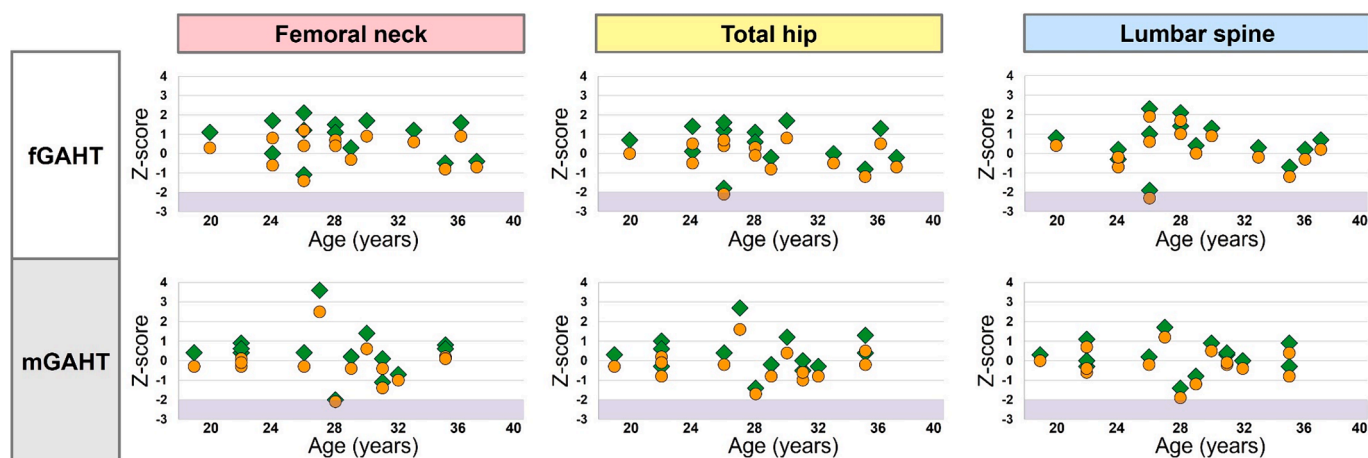
Fig. 1. Participants' Z-scores by gender-affirming hormone therapy regimen, age, and bone site. Each participant had Z-scores calculated using references for females (green diamonds) and males (orange circle). Nearly all Z-scores, regardless of the reference used, were within the normal range. Based on the standard definition of Z-score less than or equal to −2.0, one fGAHT participant (26-year-old) had low BMD for age at the total hip and lumbar spine by male reference only and one mGAHT participant (28-year-old) had low BMD for age at the femoral neck as calculated using both references for females and males. fGAHT = feminizing gender-affirming hormone therapy. mGAHT = masculinizing gender-affirming hormone therapy.

average and without a history of gonadectomy or GnRHa use. Cross-sectional mean Z-scores, using both male and female references, at the lumbar spine, femoral neck and total hip were all within normal limits after an average duration of GAHT of 3.1 years for fGAHT and 4.0 years for mGAHT. This study provides data from a small cohort in the U.S., where BMD data have not been well studied in TGD adults. In the U.S., BMI is typically higher and gonadectomy and smoking rates are lower than in Europe, from which most bone data in TGD adults have come.

Higher BMI among mGAHT was associated with greater femoral neck, total hip, and lumbar spine BMDs in our study. BMI was also associated with femoral neck and total hip Z-scores using both male and female references, but not lumbar spine Z-scores, for only the mGAHT group. The relationship between Z-scores and BMDs is found in the calculation of Z-score (Z-score = [patient's BMD – expected BMD] / population standard deviation, with expected BMD representing that for persons of the same age and gender [16]). These results are important given the fact that fracture risk increases by 1.5 to up to 2.5 times for every standard deviation drop in BMD [16–18].

Again, mean Z-scores using both male and female references were within normal ranges at all three bone sites for fGAHT and mGAHT. Determining Z-scores using both male and female references is important for research but also in clinical practice. The 2019 International Society for Clinical Densitometry guidelines provided updated clinical recommendations on Z-score utilization, stating that Z-scores should be calculated using the normative database that matches a person's gender identity, but DXA reports should include Z-scores calculated according to both male and female databases when requested.

In contrast to the present study, Van der loos *et al.* found a correlation between higher BMI and Z-scores at the femoral neck and total hip but not the lumbar spine in a cohort of 75 young adults on both fGAHT or mGAHT who had used puberty suppression in adolescence [19]. They and others have postulated that this may be related to differences in trabecular bone versus cortical bone; the spine is made of predominantly trabecular bone which may be more sensitive to sex steroids, while the hip has more cortical bone that is more affected by weight loading [20,21]. One difference in our study was that we documented this correlation in our mGAHT but not fGAHT cohort. Additional information on rates of physical activity and other factors that may have differed between the two groups is warranted to examine this difference in BMI association further.

The mean BMIs for both groups were in the overweight range and total fat body percentages were elevated with respect to age-matched reference ranges. Many studies have shown BMI to be positively associated with BMD [22]. However, there are concerns that this may not in fact provide protection against fracture, as many fractures occur in people with BMI in the overweight and obese range [23]. In studies of postmenopausal cisgender women with obesity, there are reports of increased risk of all-cause, vertebral, and upper and lower leg fractures, increased or similar risk of humerus fractures, decreased or similar risk of hip fractures, and decreased risk of pelvic fractures [23,24]. There are also concerns that elevated visceral fat, not examined in the current study, may be inflammatory in nature and negatively impact bone health [23]. BMI is relative to body weight and not body composition. U.S. data from general population adults aged 20–59 years revealed that

Table 3
Linear regression analyses to estimate the associations between femoral neck, total hip, and lumbar spine bone mineral densities and Z-scores and the predictors, body mass index and gender-affirming hormone therapy duration.

Regimen	Independent variable	Femoral neck			Total hip			Lumbar spine		
		BMD (g/cm²)	Z-score (Female reference)	Z-score (Male reference)	BMD (g/cm²)	Z-score (Female reference)	Z-score (Male reference)	BMD (g/cm²)	Z-score (Female reference)	Z-score (Male reference)
fGAHT	BMI	0.003 +/- 0.005	0.034 +/- 0.044	0.032 +/- 0.035	0.006 +/- 0.006	0.045 +/- 0.045	0.040 +/- 0.037	0.008 +/- 0.005	0.071 +/- 0.047	0.069 +/- 0.048
mGAHT		**0.019** +/- 0.007*	**0.138** +/- 0.046*	**0.112** +/- 0.038*	**0.017** +/- 0.006*	**0.114** +/- 0.035**	**0.090** +/- 0.029*	**0.010** +/- 0.004*	0.039 +/- 0.099	0.069 +/- 0.033
fGAHT	GAHT duration	-0.014 +/- 0.015	-0.124 +/- 0.132	-0.102 +/- 0.105	-0.013 +/- 0.017	-0.097 +/- 0.136	-0.085 +/- 0.109	-0.006 +/- 0.016	-0.062 +/- 0.141	-0.062 +/- 0.144
mGAHT		-0.025 +/- 0.020	-0.107 +/- 0.136	-0.085 +/- 0.112	-0.012 +/- 0.016	-0.022 +/- 0.103	-0.019 +/- 0.085	0.002 +/- 0.013	0.039 +/- 0.099	0.030 +/- 0.098

Data are presented as regression coefficient (β) +/- standard error.

*P < 0.05, **P < 0.01.

BMD = bone mineral density.

BMI = body mass index.

GAHT = gender-affirming hormone therapy.

fGAHT = feminizing gender-affirming hormone therapy.

mGAHT = masculinizing gender-affirming hormone therapy.

lean mass had a strong positive association with BMD, while fat mass had a moderate negative association with BMD, particularly among men in the highest quartile for fat mass index [24]. Given the high body fat percentages seen in the present study's cohort, consideration of risk factors for fracture beyond bone density should be considered when thinking about screening for low BMD in TGD individuals.

Our finding that duration of GAHT duration did not correlate with BMD is also consistent with previous studies from Europe that show gains in BMD during the first years on fGAHT followed by attenuation over time [25]. A *meta*-analysis/systematic review of 13 studies that included 392 individuals on fGAHT, all from Europe, found a statistically significant increase in lumbar spine BMD at 12 months (0.04 g/cm²; 95 % CI, 0.03 to 0.06 g/cm²) and 24 months (0.06 g/cm²; 95 % CI, 0.04 to 0.08 g/cm²) [8]. However, long-term data from the Amsterdam Cohort of Gender Dysphoria examining 102 transgender women found no significant change in BMD, but a significant change in Z-score after 10 years of fGAHT [6]. Longer term fracture risk has been less well investigated, although another recent study from the Amsterdam Cohort of Gender Dysphoria reported that adults over 50 years of age taking fGAHT had significantly higher fracture risk compared to age-matched reference men, younger adults taking fGAHT tended to have increased fracture risk compared to age-matched reference women, and young adults taking mGAHT had no increased fracture risk compared to either reference population [9].

Our fGAHT cohort had a mean serum estradiol level of 190 pg/mL which falls within the guideline recommended range and may have been additionally protective for bone health. Data from Amsterdam also demonstrated an association between serum estradiol level and lumbar spine BMD with those who had the highest tertile of estradiol (mean 443 pmol/L or 121 pg/mL) showing gains in lumbar spine BMD (+0.044 g/cm²; 95 % CI + 0.025 to + 0.063), while those in the lowest tertile of estradiol (mean 118 pmol/L or 32 pg/mL) had declines (−0.036 g/cm²; 95 % CI –0.044 to –0.009 g/cm²) [6]. Findings in cisgender women suggest that lower estradiol levels may be associated with increased fracture risk [26,27]. Data from the longitudinal Study of Women's Health Across the Nation examining cisgender women during the menopause transition around the U.S., reported a doubling of log estradiol was associated with a 10 % reduced risk of fracture independent of menopausal stage and other covariates [28]. Studies of premenopausal women on progesterone-based contraception suggest estradiol levels need to be maintained at 30–50 pg/ml for adequate suppression of bone turnover [29].

Our fGAHT population also had testosterone levels that were above guideline suggested range, despite being on estradiol along with spironolactone. The cohort had not been on GnRHa and did not have prior gonadectomy. Studies of TGD youth have shown those who received GnRH agonists had declines in Z-scores during treatment and may not fully "catch up" to age-matched peers even once GAHT is added, particularly for youth that initiated fGAHT [30]. More recent long-term data examining 25 young adults taking fGAHT with a mean age of 28.2 years and duration of treatment 11.6 years, found that Z-scores declined during GnRH agonist treatment, but did recover to pre-treatment levels after fGAHT at the hip but not in the lumbar spine [19]. They also observed a correlation between estradiol concentrations and lumbar spine BMD. Adequate estradiol levels and a lack of prior GnRH agonist therapy may have been protective for our cohort. Whether the non-suppressed testosterone, or the use of different antiandrogens in the U.S. (i.e., spironolactone) versus Europe (i.e., cyproterone acetate), would have any effect on bone is unknown and deserves further study.

We observed normal BMD Z-scores at all three sites measured in our mGAHT cohort. This is consistent with previous studies that suggest transmasculine individuals have baseline BMD in line with their peers that is not significantly altered after initiation of mGAHT. The *meta*-analysis and systematic review mentioned above looking at 247 transgender men did not find differences in BMD after 12 or 24 months of mGAHT, with one U.S. study in the analysis [8]. Amsterdam Cohort data
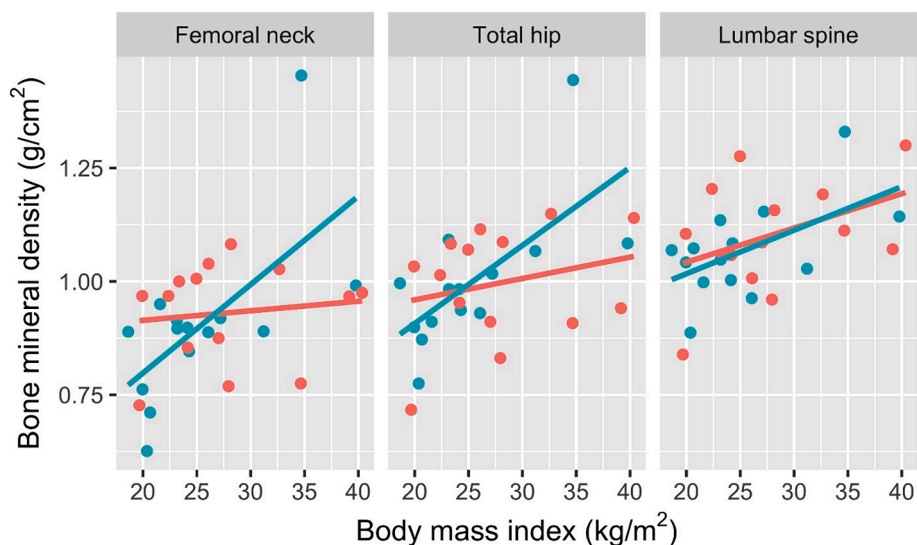
Fig. 2. The correlations between body mass index and bone mineral density at the femoral neck, total hip, and lumbar spine. Each participant was plotted on the graph for femoral neck (left), total hip (middle), and lumbar spine (right) using their body mass index (BMI) and the respective bone mineral density (BMD) at each site. For mGAHT (blue), a 1 kg/m^2 higher BMI was significantly associated with a higher BMD by 0.019 +/- SD 0.007 g/cm^2, 0.017 +/- 0.006 g/cm^2, and 0.010 +/- 0.004 g/cm^2 at the femoral neck, total hip, and lumbar spine, respectively. Higher BMI was not significantly associated with higher BMD in fGAHT (red). SD = standard deviation.

examining serial DXA in 70 adults taking mGAHT found similar BMD to baseline but an increase in L-spine Z-score at 10 years [6]. This effect was seen mainly among those who initiated mGAHT at the age of 40 years or older and had the lowest baseline E2 levels (perhaps being "perimenopausal" prior to the mGAHT initiation). It is well understood that in cisgender men, estradiol is crucial for bone health even in the face of physiologic testosterone levels [31]. Our mGAHT cohort had a mean estradiol level of 47 pg/mL which may have provided adequate protection for bone health. Additionally, mGAHT is known to alter body composition with increases in lean body mass and decreases in fat mass, which could positively impact bone health [32]. When compared to their affirmed gender, our mGAHT cohort had high total body fat percent (~30 %) as indicated by the group's mean percentile for total body fat compared to age-matched men (77th percentile). Given this was a cross-sectional study, we do not know how this mGAHT cohort's body composition changed after mGAHT initiation, though it does not appear to have had negative effects on BMD or Z-scores.

Limitations and future directions

This was a small, single site, cross-sectional study of young TGD adults without history of gonadectomy or use of GnRHa to suppress endogenous sex hormone production. This is a strength in that previous studies have not separated out bone outcomes between TGD adults with or without gonadectomy nor GnRHa use. This study population also reflects the gender-affirming surgical status and GnRHa use history of most of the TGD adults in the U.S. However, these data are not generalizable to the TGD population who has had history of gonadectomy and/or prior GnRHa use. Most participants were white, non-Hispanic and thus these data may not be applicable to non-white and Hispanic individuals. Research on the impacts of GAHT duration and BMI on BMD among racially and ethnically diverse TGD populations is warranted.

While we presented serum estradiol and total testosterone levels for study participants, the levels only capture one point in time (i.e., the level at the study visit day). Other hormone level-related factors that could contribute to BMD are difficult to assess and limit all TGD bone studies: account for different levels based on routes of administration, time from last dose of medication, history and duration of missed GAHT doses, mean hormone levels over time since GAHT initiation, cumulative hormone level exposure over time after GAHT initiation, time it

took for participants to achieve guideline-recommended hormone levels, and percent time a person has had guideline-recommended hormone levels. These are reasons why we did not calculate the associations between sex hormone levels and BMD but were more interested in GAHT duration.

Given the cross-sectional nature of the study, we did not have access to BMI, body composition, BMD, and Z-scores prior to GAHT initiation. We also cannot determine how changes in BMI and body composition from GAHT may have impacted BMD and Z-scores in our participants. Given the small sample, we are unable to make any statement about fracture risk but that is deserving of future study in TGD adults with an without history of gonadectomy and/or GnRHa use. Additionally, vitamin D and other metabolic bone laboratory assessments were not collected. Future prospective studies should include such measurements to evaluate their associations with BMD and Z-scores in a diverse sample of TGD adults. Details about diet and physical activity should also be collected in future studies assessing bone health and fracture risk in TGD adults. Additional research is also needed on the association of BMI and BMD with aging among older TGD adults on long-term GAHT and individuals who initiate GAHT at an older age.

Conclusions

BMD Z-scores in our small sample of young, nonsmoking TGD adults taking fGAHT or mGAHT for greater than one year, without prior gonadectomy or GnRHa, and with average BMIs in the overweight range, were reassuringly within the expected ranges for age based on both male and female references. Higher BMI, but not GAHT duration, was associated with higher femoral neck and total hip BMDs and Z-scores among mGAHT only. Larger, prospective studies are needed to understand the associations between body composition changes, BMI categories, and gonadectomy with BMDs and Z-scores among a diverse TGD population across the lifespan.

CRediT authorship contribution statement

Sean J. Iwamoto: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **John D. Rice:** Writing – review & editing, Software, Resources, Methodology, Formal analysis. **Kerrie L. Moreau:** Writing – review & editing, Conceptualization. **Marc-André Cornier:** Writing – review & editing, Conceptualization. **Margaret E. Wierman:** Writing – review & editing, Conceptualization. **Mary P. Mancuso:** Writing – review & editing, Project administration, Investigation, Data curation. **Amanuail Gebregzabheir:** Writing – review & editing, Project administration, Investigation, Data curation. **Daniel B. Hammond:** Writing – review & editing, Investigation, Data curation. **Micol S. Rothman:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sean Iwamoto reports financial support was provided by National Institute of Child Health and Human Development. 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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