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ORIGINAL ARTICLE

Effect of gender affirming hormone therapy on bone mineral density and bone marrow composition in transgender adults

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Abstract

Purpose The study was aimed at evaluating areal (a) bone mineral density (BMD) and bone marrow adipose tissue (BMAT) in Assigned Male at Birth (AMAB) and Assigned Female at Birth (AFAB) transgender adults before and during gender affirming hormone therapy (GAHT).

Methods The prospective observational pilot study was performed in 23 transgender adults (14 AFAB and 9 AMAB; mean age 24.1 and 27.1 years, respectively]. Testosterone in AFAB and 17-β estradiol, androgen antagonists in AMAB were administered as part of the clinical practice. Lumbar spine (L1-L4), femoral neck, total hip and distal radius aBMD were measured by Dual Energy X-ray Absorptiometry (iDXA, Lunar GE, USA) and BMAT by proton magnetic resonance spectroscopy (^{1}H-MRS) before (T0) and 12 months (T12) after initiating GAHT. L1-L4 trabecular bone score (TBS) was calculated by TBS iNsight (Medimaps, Switzerland). Vertebral fracture assessment was performed by DXA (iDXA, Lunar GE, USA).

Results AMAB had significant reduction in L1-L4 aBMD ($p<0.05$) and an increase in TBS ($p<0.05$) at T12. All-site aBMD and TBS did not significantly change over time in the AFAB group. No changes in BMAT values were observed in both groups. A negative correlation between BMAT and L1-4 aBMD was observed at T0 ($p<0.05$), which remained significant after adjustment for BMI and age.

Conclusion Lumbar spine aBMD declines while TBS increases in AMAB subject during GAHT. BMAT values are not influenced by a 12-month course of GAHT and their association with L1-L4 aBMD are significant before but not during treatment.

Keywords Bone · Bone marrow adipose tissue · AFAB · AMAB · Transgender people

Introduction

The term "transgender" describes a person whose gender identity is different from the sex assigned at birth. In Europe, gender incongruence has a prevalence of 0.2–0.6% in adults [1]. After psychological "diagnosis" and signing of an informed consent, gender affirming hormone therapy (GAHT) can be administered with the aim of modifying secondary sexual characteristics and adapt them to the individual desire. GAHT decreases serum levels of endogenous sex steroids, achieving sex steroid levels within the limits of the individual's gender identity [2]. Clinicians should personalize therapy according to age, health status, risk factors, lifestyle and individual desires [3]. GAHT significantly improves quality of life of patients suffering from gender dysphoria. Although it may be associated with potential

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adverse effects, including erythrocytosis in masculinizing regimen and venous thromboembolism in feminizing regimen, available evidence demonstrated that, when tailored to individual needs and administered under appropriate medical supervision, GAHT maintains a favourable safety profile and consistent therapeutic efficacy [4]; however, individual response to treatment may vary depending not only on therapy-related factors, such as type, dose, and route of administration, but also on inter-individual variability, including genetic and biological determinants [5]. The goal of GAHT in Assigned Female at Birth (AFAB) individuals is the induction of a full masculinization (if full male characteristics are desired). This can be achieved similarly to what is usually done in male hypogonadism with the administration of testosterone (intramuscular or transdermal), and with target serum testosterone levels typical of eugonadal men (320–1000 ng/dl; 11.1 to 34.7 nmol/L) [6, 7].

The feminizing hormonal treatment in Assigned Male at Birth (AMAB) individuals includes two types of drugs: estrogens that provide a feminizing process and androgen antagonists that block the action of the endogenously produced testosterone. Several estrogens can be used, most commonly oral and transdermal 17-β estradiol. Cyproterone acetate (oral 10–50 mg daily) and spironolactone (oral 10–200 mg daily) are among the most commonly used androgen antagonists in Italy [7]. While cyproterone acetate acts mostly at the pituitary level by decreasing FSH and LH production and eventually testosterone, and less at the peripheral level, spironolactone blocks directly the androgen receptor (AR) [6, 8, 9]. During treatment, serum estradiol levels should target the cis-women sex hormones levels (100–200 pg/ml), that should be obtained by the correct balance between doses and timing of administration [7]. Total testosterone levels should be maintained < 50 ng/dl (0.5 ng/ml) in subjects administered with cyproterone acetate [3, 5, 6].

Sex steroids can positively influence bone health in both genders, promoting osteoblastic activity while inhibiting osteoclasts [10]. As observed in other tissues, the effects of estrogens and androgens on bone are exerted through an high-affinity binding to the estrogen receptor (ER) α and β (also known as NR3A1 and NR3A2) and the AR (also known as NR3C4), respectively [11]. ERα, ERβ, and AR have been detected in several cell types along the differentiation process of mesenchymal and myeloid precursors to osteoblasts and osteoclasts, as well as in other bone marrow cell types or even in tissues distant from bone that may indirectly influence bone homeostasis [11]. Despite the abundance of data on the influence of sex hormones on the skeleton, consistent evidence on the effects of GAHT on bone metabolism is lacking because of challenges in designing and performing these types of studies [12]. Many studies assessed areal bone mineral density (aBMD) measured by Dual Energy X-ray Absorptiometry (DXA); GAHT was found to be protective on aBMD, which resulted normal or increased compared to controls [13, 14]. However, fracture rate associated with feminizing therapy is not yet clarified, as few, under-powered studies evaluated this outcome and assessed the alterations that could potentially lead to increased bone fragility in the transgender population [15].

Vertebral fracture assessment (VFA) is an accurate, DXA-based and safe technology commonly employed in clinical practice to obtain images of the lateral thoracic and lumbar vertebrae and assess the presence of fracture [16]. Several studies in adults, children and adolescence with different metabolic bone diseases successfully employed the VFA technology [16–18] Trabecular bone score (TBS) is a textural parameter derived from the DXA images of the lumbar spine and associated with the trabecular microarchitecture and with fracture risk [19]. Clinical studies in several metabolic bone disorders demonstrated that TBS may be reduced in association with altered bone quality and elevated fracture risk [20].

The overall evidence showed that GAHT could have an impact on bone microstructure and composition [21]. As changes in bone composition and microstructure could have a crucial role in the pathophysiology of bone fragility regardless of aBMD baseline values, the topic is of high interest in the definition of fracture risk, particularly in secondary, endocrine-related, forms of osteoporosis. Bone marrow adipose tissue (BMAT) is an endocrine regulator of bone metabolism, as it mediates the interaction between bone and adipose tissue; its increase was associated with impaired aBMD and bone quality in different metabolic disorders and during ageing [22]. BMAT represents a key component of the bone microenvironment, acting not only as an endocrine regulator but also as a direct modulator of bone metabolism through paracrine interactions with osteoblasts and osteoclasts [23, 24]. Specifically, BMAT modulates bone homeostasis via multiple paracrine mechanisms: it inhibits osteoblast differentiation by shifting mesenchymal stem cell fate toward adipogenesis; secretes osteoclastogenic factors such as RANKL and TNF-α that enhance bone resorption; releases saturated fatty acids that can impair osteoblast function; and produces soluble inhibitors of the Wnt/β-catenin pathway (e.g., sFRP1), thereby reducing osteoblastogenesis.

BMAT may be assessed by means of the gold standard, non-invasive technology, namely proton magnetic resonance spectroscopy (^{1}H-MRS); the overall evidence suggests a possible negative role played by BMAT on fracture risk [25–29]. To date, no studies have specifically investigated the association between bone marrow adiposity and

aBMD in transgender adults, nor the potential impact of GAHT on this relationship over time.

In this pilot study, we aimed to evaluate aBMD assessed by DXA and BMAT by ^{1}H-MRS in AMAB and AFAB transgender adults before and during GAHT. Our results will provide evidence on the effect of GAHT on the bone marrow composition, suggest the possible role of BMAT in the pathophysiology of bone fragility and fracture in transgender individuals, and potentially providing the preliminary mechanistic insights into hormone-induced skeletal adaptations.

Materials and methods

Participants

The study was conducted in accordance with the World Medical Associations Declaration of Helsinki-Ethical Principles for Medical Research involving Human Subjects and was approved by CET - Territorial Ethics Committee Lazio Area 1 (reference number: 6554, date of approval: November 24, 2021).

The observational pilot study prospectively assessed 23 transgender adult people (14 AFAB, mean age 24.1 ± 7.8; 9 AMAB, 27.1 ± 11.0 years) at the initiation (T0) of GAHT and after 12 months (T12). Participants were recruited consecutively from the Endocrinology and Andrology Outpatient Clinic, Sapienza University of Rome, and from the S.A.I.F.I.P. program (Service for the Alignment between Physical and Psychological Identity) at San Camillo-Forlanini Hospital, Rome, Italy. Inclusion of participants required a diagnosis of gender dysphoria, age over 18, the wish to start GAHT and the ability to understand and sign a written informed consent. Exclusion criteria were prior use of GAHT or any drug that could affect hormone levels or mineral metabolism, history of genetic, endocrine, coagulation, oncologic disorders or any metabolic bone disease. As a part of the clinical care, subjects whose serum 25-hydroxyvitamin D [25(OH)D] levels were < 30 ng/ml were treated with oral cholecalciferol 25,000 IU once or twice a month, as required. Data obtained at baseline and 12 months after initiation of GAHT included medical history and anthropometric measurements (weight, height, body mass index - BMI, waist circumference, hip circumference), glucose and lipid profile, serum sex hormone and 25(OH)D levels, aBMD, Trabecular Bone Score (TBS), Vertebral Fracture Assessment (VFA), and ^{1}H-MRS parameters. Any concomitant medication or new diagnosis emerging during follow-up was recorded in the case report form and reviewed; no cases meeting exclusionary criteria were retained in the analysis set. Physical activity level was systematically collected as part of the clinical history at baseline and follow-up visits. All participants reported no regular physical activity in the six months preceding enrolment, and physical activity status was therefore homogeneous and low across the cohort.

Gender affirming hormone therapy protocol

The hormone therapy regimen used in our center for AMAB individuals includes, for the first six months, cyproterone acetate at a daily dose of 25 mg in combination with estradiol valerate (2 mg daily) or spironolactone at the daily dose of 50–100 mg combined with either estradiol valerate (2 mg daily) or estradiol hemihydrate 2–4 mg daily. After six months, and in the absence of any controindication or adverse effects, the treatment is individualized according to specific clinical needs and participant's consent. Dose and formulation adjustments are made in order to ensure that estradiol and testosterone levels remain within the recommended reference ranges of 100–200 pg/mL and < 0.5 ng/mL, respectively [6, 7].

In AFAB, the hormone therapy protocol includes testosterone enanthate or testosterone esters at the intramuscular dose of 250 mg every 28 days, or daily transdermal testosterone (40–60 mg). The choice for a specific regimen was discussed with each individual, and both dose and formulation were tailored to achieve masculinizing characteristics ("binary") in line with their goals [6, 7]. Notably, none of the participants reported identifying as non-binary or gender-fluid.

At the 12-month follow-up, 44% of AFAB participants achieved total testosterone levels within the reference range for cisgender eugonadal men, while 71% of AMAB participants achieved estradiol levels within the reference range for cisgender eugonadal women.

Areal bone mineral density (aBMD)

Areal BMD was measured at the lumbar spine (L1-L4), femoral neck, total hip, distal 1/3, ultradistal and total radius by Lunar-iDXA densitometer (Lunar iDXA TM, GE Healthcare, Madison, WI, USA; enCORETM 2009 software version 13.20.033). Areal BMD data were expressed in grams per square centimeter (g/cm^2) and Z-score and T-score units were used, as appropriate. Coefficients of variation (CVs) are 1.0% at L1-L4, 1.3% at the femoral neck, 1.5% at the total hip, and 1.6% at the distal radius.

Trabecular bone score (TBS)

Trabecular bone score was calculated from the de-identified L1-L4 DXA scans using the TBS iNsight software (version 3.0.2.0; Medimaps Group, Geneva, Switzerland) as the

mean value of the individual measurements for the L1-L4 vertebrae. We calculated the sum of the grey-level between pixels differences in the DXA images at a given distance and angle; the slope of the log-log transformation of this variogram was then assessed for calculation [30].

The coefficient of variation was 1.8% for TBS and did not vary between the vertebrae measured. According to the meta-analysis by Harvey et al., we considered values above 1.31 as a normal pattern (low fracture risk), between 1.31 and 1.23 as a partially degraded microarchitectural pattern (intermediate fracture risk) and below 1.23 as a degraded pattern (high fracture risk) [31].

Vertebral fracture assessment (VFA)

Lateral images of the spine (from the vertebra T4 to L4) were acquired using dedicated software on the iDXA densitometer. The effective radiation exposure to the patient was ~ 12 µSv, as reported by the manufacturer. An expert skeletal radiologist identified the specific morphological signs of osteoporotic vertebral fractures (VF) on the DXA spine image according to the algorithm-based qualitative approach (ABQ) [32]. In particular, the endplate cortical fracture (ECF), and the endplate dip, were assessed [33, 34]. The enCORETM Software v13.5 was used to grade the VF. The software automatically inserts six points on each vertebral body to measure the anterior (Ha), middle (Hm) and posterior (Hp) heights. We applied the Genant's semi-quantitative method to classify VF as mild (20%-25% reduction), moderate (25%-40% reduction) or severe (> 40% reduction) [35] according to the vertebral height reduction.

Proton magnetic resonance spectroscopy (^{1}H-MRS)

Twelve AFAB and 9 AMAB subjects underwent lumbar spine MRI with a 3 Tesla scanner (GE Discovery 750; General Electric Healthcare, Milwaukee, WI) with a four-channel spine coil. Two AFAB subjects in the study did not undergo MRI because of claustrophobia; they did not differ from the rest of the cohort in terms of demographic and anthropometric characteristics (data not shown).

The MRI scan included a sagittal T2-weighted FSE sequence, which was used for visual assessment of the lumbar vertebrae and the spectral acquisition box. Single voxel ^{1}H-MRS was acquired in the vertebral bodies from L1 to L5 using the Point Resolved Spectroscopy (PRESS) sequence. The parameters of PRESS 1H-MRS were TE: 37ms, TR: 3000ms, 64 averages without water suppression, sweep width: 5000 Hz, data points: 4096; voxel size = 15 × 15 × 20 mm = 4.5 cm^3. The PRESS box was positioned in the middle of each lumbar vertebral body. Spectroscopy data were transferred to a workstation and analyzed using a software (SAGE Dev2 0017.1 GE). Four main peaks were obtained: unsaturated lipids (UL; oleofinic double bond –CH = CH protons at 5.3 ppm), water (W; 4.7 ppm), residual lipids (RL; CH2 methylene protons alpha to a double bond–CH = CHCH 2 at 2.0 ppm), and saturated lipids (SL; bulk CH2 methylene protons at 1.3 ppm). The peaks were fitted to obtain their line widths and areas (16, 17). The ^{1}H-MRS analyses were performed with automated software to obtain total bone marrow fat content (FC, %) for each vertebra and the L1-4 mean values for all subject 40,41.

FC,%= [(UL + SL + RL/(UL + SL + RL + water)] × 100%; unsaturation level (UL%) = [UL/(UL + SL + RL)] × 100%; saturation level (SL%) = [SL/(UL + SL + RL)] × 100%; residual lipids (RL, %) = [RL/(UL + SL + RL)] × 100% and water (W%) [36].

Biochemistry

Peripheral blood samples were collected from all subjects between 7.00 a.m. and 9.00 a.m. after an overnight fasting. Hormonal assays performed at the center were coded and validated using Antibody Research Resource Identifiers (RRIDs) to ensure standardisation and reproducibility. We quantified hormone levels by Chemiluminescent Microparticle Immunoassay, (CMIA, Architect System; Abbott Laboratories, Abbott Park, IL, USA) including follicle-stimulating hormone (FSH) RRID: AB_2813910 luteinizing hormone (LH) RRID: AB_2813909 testosterone (T) RRID: AB_2895254), estradiol (E) RRID: AB_2813911 and 25(OH)D RRID: AB_2924942.

The fasting plasma concentrations of glucose, insulin, total cholesterol, triglycerides, high and low-density lipoprotein cholesterol (HDL and LDL, respectively) were measured according to standard laboratory methods at clinical pathology laboratories.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 27.0 software (SPSS Inc., Chicago, USA). Parameters of interest were: aBMD, TBS, fat content. A formal a priori power analysis based on a two-way repeated measures design (time × group; α = 0.05, correlation between measures = 0.6) indicates that the final sample of 16 participants (9 AFAB and 7 AMAB completing 12 months) provides a statistical power of approximately 0.74 to detect large effect sizes (Cohen's f = 0.4). The Kolmogorov-Smirnov test was used to assess the normality of the variable's distribution. Continuous variables are described as median and interquartile range or mean ± standard deviation (SD), as appropriate.

Table 1 Anthropometric characteristics, laboratory and densitometric parameters in AFAB and AMAB subjects at baseline (T0)

Parameter	AFAB T0 ($n=14$)	AMAB T0 ($n=9$)
Age (years)	24.1±7.8	27.1±11.0
Weight (kg)	68.4±19.5	73±17.3
BMI (kg/m^2)	26.2±7	24.6±5.6
WCF (cm)	79.6±17.1	87.7±13.2
SBP (mmHg)	124.6±12.5	118.9±6
DBP (mmHg)	76.8±7.5	76.7±4.3
Glucose (n.r. 74–106 mg/dl)	86.8±11.0	85.3±7.0
Insulin (n.r. 2.6–24.9 µU/ml)	10.5±4.2	7.3±3.8
Tot Chol (n.r. 160–220 mg/dl)	162.4±28.5	181.8±46.3
HDL (n.r. 40–80 mg/dl)	56.1±17.0	50.1±8.3
LDL (n.r. < 130 mg/dl)	90.0±24.0	111.5±35.8
TG (n.r. 50–150 mg/dl)	79.2±35.6	112.4±60.4
TT (mg/ml)	0.5±0.5 **(n.r. 0.06–0.82)**	7.1±2.9 **(n.r. 2.9–11.0)**
E2 (n.r. 25–107 pg/ml)	96.1±85.2	27.8±9.3
FSH (n.r. 1.38–9.58 mUI/mL)	6.1±3.0	3.5±2.0
LH (n.r. 1.8–8.16 mUI/mL)	10.9±18.1	3.5±1.2
25(OH)D	19.5±7.4	17.6±7.3
L1-L4 aBMD (g/cm^2)	1.184 ± 0.115	1.064±0.152
Femoral Neck aBMD (g/cm^2)	1.040±0.146	0.956±0.180
Total Hip aBMD (g/cm^2)	1.051±0.118	0.956±0.174
1/3 Radius aBMD (g/cm^2)	0.827±0.075	0.766±0.297
Ultradistal Radius aBMD (g/cm^2)	0.420±0.076	0.431±0.091
Total Radius aBMD (g/cm^2)	0.609±0.099	0.671±0.087
TBS	1.284±0.55	1.11±0.63
Vertebral fracture (n)	0	0

AFAB, assigned female at birth; AMAB, assigned male at birth; GAHT, Gender Affirming Hormone Therapy; BMI, body mass index; WCF, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; n.r., normal values; Tot Chol, total cholesterol; TG, triglycerides; TT, testosterone; E2, estradiol; FSH, follicle-stimulating hormone; LH, lutenizing hormone; 25(OH)D, 25-hydroxy-vitamin D; aBMD, areal bone mineral density; TBS, trabecular bone score

The longitudinal within-group comparisons were analyzed using the Wilcoxon signed-rank test or Student's t-test for paired data, based on the distribution curve of the variable. The Spearman rank correlation test was used to evaluate the correlations between the variables considered. For some variables, the partial correlation coefficient was calculated to estimate the effect net of some variables (age, BMI). A two-tailed p-value of less than 0.05 was considered statistically significant.

Table 2 Anthropometric characteristics, laboratory and densitometric parameters in AFAB and AMAB subjects after 12 months (T12) of GAHT

Parameter	AFAB T12 ($n=9$)	AMAB T12 ($n=7$)
Age (years)		
Weight (kg)	73.9±22.9	71.4±16.0
BMI (kg/m^2)	27.7±8.4	23.9±4.6
WCF (cm)	84.7±18.9	84.1±12.2
SBP (mmHg)	120.8±7.6	117.5±4.6
DBP (mmHg)	80.0±10.0	78.6±3.5
Glucose (n.r. 74–106 mg/dl)	**77.8±12.0**[a]	86.1±12.2
Insulin (n.r. 2.6–24.9 µU/ml)	11.2±7.2	8.3±2.5
Tot Chol (n.r. 160–220 mg/dl)	158.8±32.8	196.1±57.2
HDL (n.r. 40–80 mg/dl)	48.7±19.9	55.4±8.7
LDL (n.r. < 130 mg/dl)	91.6±28.8	120.9±56.1
TG (n.r. 50–150 mg/dl)	92.3±45.7	110.6±50.6
TT (mg/ml)	**2.6±0.5**[a]	2.4±2.5
E2 (n.r. 25–107 pg/ml)	67.9±20.3	**72.6±30.5**[a]
FSH (n.r. 1.38–9.58 mUI/mL)	7.3±6.3	**1.2±1.0**[a]
LH (n.r. 1.8–8.16 mUI/mL)	6.8±4.6	7.2±11.1
25(OH)D	21.0±7.3	22.5±8.7
L1-L4 aBMD (g/cm^2)	1.175±0.144	**1.047±0.110**[a]
Femoral Neck aBMD (g/cm^2)	1.022±0.133	0.892±0.151
Total Hip aBMD (g/cm^2)	1.048±0.104	0.900±0.133
1/3 Radius aBMD (g/cm^2)	0.822±0.076	0.864±0.079
Ultradistal Radius aBMD (g/cm^2)	0.419±0.065	0.417±0.082
Total Radius aBMD (g/cm^2)	0.620±0.076	0.640±0.056
TBS	1.290±0.49	**1.23±0.61**[a]

AFAB assigned female at birth; AMAB assigned male at birth; aBMD, areal Bone Mineral Density; WCF, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; n.r., normal values; Tot Chol, total cholesterol; TG, triglycerides; TT, testosterone; E2, estradiol; FSH, follicle-stimulating hormone; LH, lutenizing hormone; 25(OH)D, 25-hydroxy-vitamin D; aBMD, areal bone mineral density; TBS, trabecular bone score; [a] p-value < 0.05 T0 vs. T12

Results

Baseline (T0) demographic, anthropometric, clinical, and laboratory data are summarised in Table 1. Table 2 shows aBMD and TBS data in the AFAB and AMAB groups at T12. No vertebral fractures were observed as assessed by ABQ and Genant's semi-quantitative method on images obtained by VFA (Table 1). Serum glucose levels were significantly lower in the AFAB group after 12 months of testosterone therapy, but without clinical relevance. As expected, statistically significant differences were observed after 12 months of GAHT in total testosterone (T0: 0.5±0.5 ng/ ml vs. T12: 2.6±0.5, $p<0.05$) in AFAB and estradiol levels (T0 27.8±9.3 pg/ml vs. T12: 72.6±30.5 pg/ml, $p<0.05$) and FSH (T0: 3.5±2 ng/ ml vs. T12: 1.2±1, $p<0.05$) in AMAB subjects. No significant changes were observed in other anthropometric, clinical and laboratory parameters. After 12 months of GAHT, there were no statistically significant

differences in aBMD and TBS compared to T0 in the AFAB group, whereas there was a statistically significant 1.6% decrease in L1-L4 aBMD (1.064±0.152 vs. 1.047±0.110; $p<0.05$) and a 10.8% increase in TBS compared to T0 (1.11±0.63 vs. 1.23±0.61; $p<0.05$) in the AMAB group (Table 2; Figs. 1 and 2).

After 12 months of GAHT, the FC values remained relatively stable in the AFAB group (Table 3).

Correlation analysis

We found a statistically significant negative association between L1-L4 aBMD and mean FC values at T0 (Table 4), but not at T12 (Table 5) in all subjects. However, no association was found between FC and any of the other anthropometric, glycometabolic parameters and TBS.

No association between FC and anthropometric, glycometabolic parameters was observed in AFAB both at T0 and at T12. Similar results were observed in the AMAB group (data not shown).

Discussion

To our knowledge, this is the first longitudinal observational pilot study exploring the relationship between BMAT and aBMD in transgender adults during GAHT. Lumbar spine aBMD tends to decrease and TBS to increase in AMAB adults during GAHT, while no changes in the BMAT are evident. Interestingly, baseline BMAT was significantly and negatively associated with lumbar spine aBMD.

In the last decade, much attention has been paid to the effects of GAHT on bone health, but few studies have been conducted that adequately evaluated the skeleton before and during hormone therapy [14, 37, 38]. The limited data available suggest a predominant protective role of estrogens in AMAB subjects treated with estrogens and

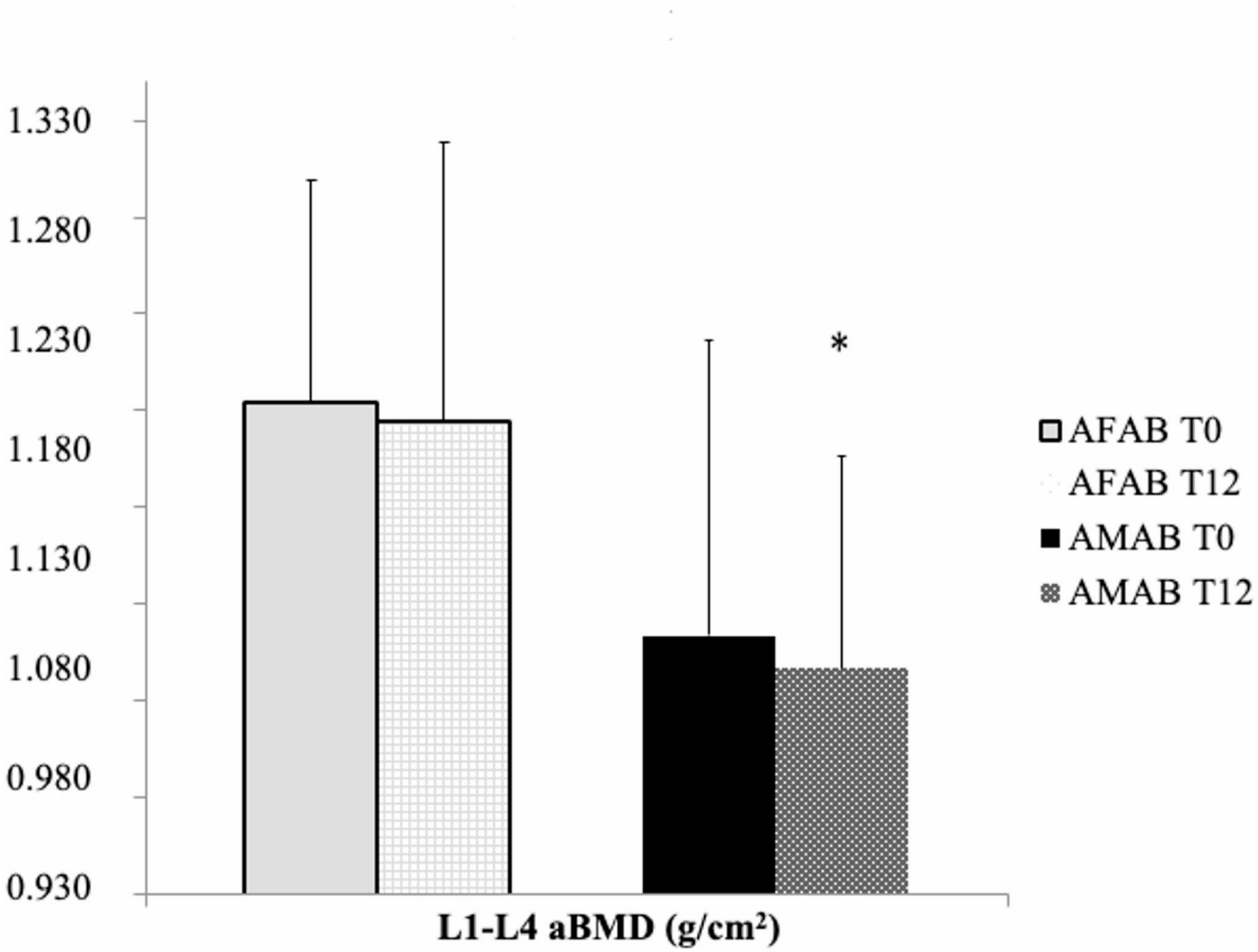

Fig. 1 L1-L4 aBMD values at baseline (T0) and after 12 months (T12) of GAHT in AFAB and AMAB adults; *p-value<0.05 AMAB T0 vs. AMAB T12

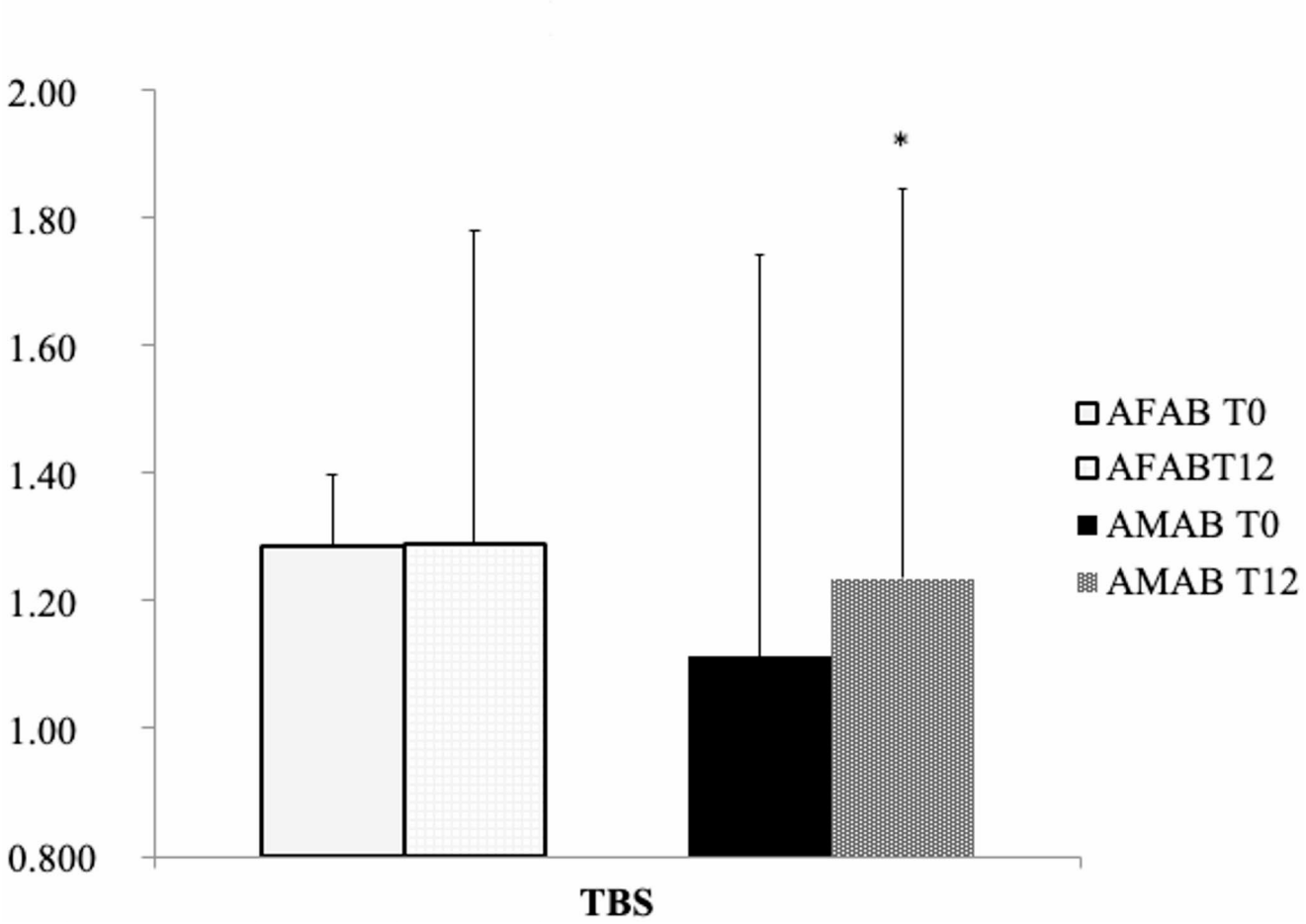

Fig. 2 TBS values at baseline (T0) after 12 months of GAHT (T12) in AFAB and AMAB adults; *p-value < 0.05 AMAB T0 vs. AMAB T12

Table 3 Fat content (%) values in AFAB and AMAB subjects before (T0) and after 12 months (T12) of GAHT

Parameter	AFAB T0 ($n=12$)	AFAB T12 ($n=7$)	p-value	AMAB T0 ($n=9$)	AMAB T12 ($n=5$)	p-value
FC L1 (%)	24.2 ± 7.0	29.3 ± 11.3	0.203	35.1 ± 9.7	33.6 ± 7.3	0.893
FC L2 (%)	26.9 ± 10.4	30.0 ± 12.4	0.864	35.6 ± 9.2	35.0 ± 8.5	0.785
FC L3 (%)	28.9 ± 10.6	32.4 ± 14.1	0.932	40.3 ± 7.5	35.4 ± 7.6	0.068
FC L4 (%)	30.1 ± 10.9	34.6 ± 13.0	0.301	42.8 ± 12.7	36.2 ± 8.4	0.216
Mean FC (%)	28.4 ± 9.4	32.3 ± 12.7	0.397	31.3 ± 9.3	35.6 ± 7.7	0.465

AFAB assigned female at birth; AMAB assigned male at birth; FC Fat Content

Table 4 Correlation analysis between the aBMD and the lumbar spine fat content (%) at T0

Parameter	FC% L1	FC% L2	FC% L3	FC% L4	Mean FC%
aBMD L1-4	-0.499	-0.516	-0.445	-0.525	-0.518
p-value	**0.021**	**0.017**	**0.043**	**0.014**	**0.016**

FC Fat Content; aBMD, areal Bone Mineral Density

Table 5 Correlation analysis between aBMD and the lumbar spine fat content (%) adjusted for BMI and age at T12

Parameter	FC% L1	FC% L2	FC% L3	FC% L4	Mean FC%
aBMD L1-4	-0.531	-0.569	-0.757	-0.651	-0.655
p-value	0.142	0.110	**0.018**	**0.057**	**0.056**
aBMD Neck	-0.430	-0.550	-0.727	-0.705	-0.640
p-value	0.248	0.125	**0.027**	**0.034**	0.063

FC Fat Content; aBMD, areal Bone Mineral Density

anti-androgens [6]. Nevertheless, our observation of a 1.6% decline in L1-L4 aBMD in this group suggests that the use of antiandrogens could potentially have a negative effect on bone that is not adequately compensated by the estrogen administration [21]. With reference to the effect of GAHT on aBMD, data from clinical studies have shown different results [10, 39–41]. Sosa et al. found higher aBMD values at the lumbar spine and femoral neck in transgender women who received non-surgical treatment over a period ≥ 3 years compared to controls [42]. A long-term study by Wiepjes

et al. showed no significant changes in lumbar aBMD in transgender women after 10 years of therapy [37]. Similar negative results regarding the effects of GAHT on aBMD have been documented in longer-term studies, while gender affirming surgery has been associated with a reduction in aBMD over time in several studies [40, 41].

According to our observations, there have been reports of no significant effect of testosterone treatment on aBMD and bone quality in the AFAB group [14]. Nonetheless, considering the numerically limited sample and the exploratory nature of the study, these observations must be regarded as preliminary. Larger longitudinal studies are required to validate these results and to clarify the long-term skeletal effects of GAHT in this population. The effect of testosterone on the skeleton may be due in part to the testosterone-induced increases in muscle mass and increased periosteal bone formation, as observed during puberty [14]. At a cellular level, testosterone promotes osteoblast proliferation, differentiation, extracellular matrix protein synthesis, and bone integrity [43]. The expression of the AR has been observed in osteoblasts, osteocytes and osteoclasts, and its activation increases the transcription of osteoprotegerin (OPG), with consequent inhibition of the osteoclast activity, while decreasing the expression of sclerostin, a potent inhibitor of the osteoanabolic pathway [44, 45]. In addition, the indirect effect of testosterone may be exerted through the aromatization process. As observed in women with endogenous hyperandrogenism and/or hirsutism in the setting of the polycystic ovary syndrome, there is a significant association between serum testosterone levels and lean mass, which in turn correlates with trabecular aBMD in the same region of interest [46].

Regarding TBS, there are few and inconsistent data in transgender individuals, and no specific association analysis between TBS and fracture risk has been evaluated [39, 47]. Recently, Andrade et al. showed no difference in the TBS in a group of 19 transgender men on GAHT for at least 6 months compared to 19 cisgender men [47]. Conversely, Wiepjes et al. observed increases and decreases in TBS values in transgender women and men during GAHT, respectively [37]. Accordingly, our results, although observed in a relatively small group of subjects, seem to suggest that estrogen administration could potentially improve trabecular microarchitecture in AMAB. These data are consistent with the beneficial effect of estrogen on TBS described in several studies using hormone therapy in different populations and clinical settings (e.g. as hormonal replacement therapy) [48]. We agree that there are other biological and methodological factors to consider in interpreting the observation of the dissociation between the effects of GAHT on aBMD and TBS. In this context, the ineffectiveness of estrogen in limiting aBMD decline during antiandrogen treatment may explain the discrepancy between the effect on TBS and aBMD in our cohort. Areal BMD and TBS reflect distinct aspects of bone properties: while aBMD quantifies mineral mass per projected area, TBS is a texture index derived from lumbar spine DXA images that better represents trabecular microarchitecture. Estrogenic therapy may preferentially enhance trabecular connectivity and texture, improving TBS even when aBMD appears reduced due to changes in bone geometry or body composition, such as lean mass loss. The dissociation between TBS and aBMD has been reported in longitudinal studies in transgender women undergoing GAHT and in other endocrine-related conditions [39, 49]. Additionally, body composition and technical artifacts can differentially affect DXA-based parameters. Variations in body weight, central adiposity, and soft-tissue thickness may influence aBMD and TBS in opposite directions. As highlighted by the ESCEO/IOF expert group, TBS should always be interpreted considering the least significant change (LSC) and adjustments for body composition and scanning conditions [19]. Bone marrow adipose tissue may contribute to this apparent paradox, as its expansion has been linked to reduced aBMD and impaired bone quality [50, 51]. Hormone-induced modulation of marrow lipid content and composition during GAHT could influence the DXA signal differently from microarchitectural texture, potentially explaining the divergent trends observed. Finally, these findings align with prior observations in both transgender cohorts and endocrine disorders showing variable aBMD changes but improved TBS with estrogen exposure [39, 49]. Nonetheless, our data should be regarded as preliminary, given the small sample size and exploratory nature of the study.

Clinical studies have shown that GAHT may have an effect on bone microstructure and composition [21], but there is little data on BMAT. There have been two pilot studies assessing bone marrow composition in transgender and gender non-conforming adolescents (< 18 years) after 12 months of therapy [52, 53]. Nasomyont et al. reported an increase in BMAT in the study group including both AMAB and AFAB and compared to cisgender controls, as well as an inverse association between BMAT and aBMD [52]. In AMAB adolescents, Guss et al. found no association between aBMD and BMAT [53]. The observations in our cohort seem to suggest that the bone marrow adiposity is not negatively affected by GAHT. The relationship between estrogens and BMAT has indeed been investigated in studies of cisgender individuals. Schellinger et al. showed that BMAT increases steadily throughout life, with a rapid increase in women associated with postmenopausal estrogen deficiency [54]. Accordingly, estrogen replacement therapy in postmenopausal women may reduce BMAT [55]. Therefore, it is possible that exogenous estrogens

administered to the AMAB adults in our cohort will play a role in the acquisition of the typical pre-menopausal cisgender female pattern of BMAT. Further longitudinal studies in larger cohorts may clarify whether BMAT is potentially reduced in AMAB during GAHT as an effect of estrogen therapy, as well as the potential influence of fat and lean mass distribution assessed by body composition analysis on bone-related outcomes. In particular, these studies will clarify how changes in BMAT may influence aBMD, which in our study decreased in AMAB at T12 without any association with BMAT at the same time point. The possibility that the small number of participants may have influenced these results cannot be excluded. The negative associations we observed between BMAT and L1-L4 aBMD at baseline confirm previous findings, which collectively demonstrate that bone marrow adiposity is a negative regulator of bone health. Studies in cohorts of individuals with various endocrinopathies (diabetes mellitus, obesity, primary hyperparathyroidism, hypoparathyroidism, and hypogonadism) have suggested an association between the age- and/or disease-related increases in bone marrow adiposity and loss of bone mass [22, 36, 56–58]. Longitudinal data from the SUPERB cohort demonstrated that higher bone marrow fat fraction (BMFF) predicts a greater risk of major osteoporotic fractures and partially mediates the association with lower femoral neck BMD [59]. The mechanisms involved are not yet fully understood. A complex process involving many growth and transcription factors regulates the differentiation of mesenchymal stem cells into adipocytes or pre-osteoblasts. The inhibition of osteoblasts differentiation by bone marrow adipocytes, which may act on multiple differentiation processes, or their negative influence on the function of mature osteoblasts or osteoclasts have been hypothesized [22, 60]. Eventually, we cannot rule out the possibility that other molecules, yet to be discovered, mediate the mutual cross-talk between bone marrow adipocytes and bone cells. In this context, early BMAT changes observed during GAHT could reflect dynamic bone remodeling before measurable aBMD alterations occur. However, our results should be interpreted with caution, as they derive from a pilot study with a limited sample size and short follow-up. Future long-term studies are warranted to confirm whether BMAT alterations may serve as early predictors of skeletal outcomes in transgender individuals.

Our study has some limitations. We acknowledge that the small number of subjects limits our analysis, and the design of the study with a 12-month follow-up excludes the possibility of an adequate assessment of fragility fractures. Although the study is not powered to identify small or moderate changes, it is adequately designed to capture clinically meaningful differences and to generate robust preliminary data for future, larger-scale trials. AMAB and AFAB subjects were not matched for age, BMI and other parameters that may influence bone metabolism; therefore, within-group comparisons were not performed because of their limited power. In this context, the inclusion of age- and BMI-matched control groups of cisgender individuals would be of great importance in future studies to best assess the role of bone marrow adiposity on bone health status in transgender individuals during GAHT. Given the exploratory and preliminary nature of this study, no formal correction for multiple comparisons was applied. Therefore, we acknowledge a possible inflation of type I error, and the results should be interpreted with caution and confirmed in larger cohorts.

In addition, although not part of the primary outcome of the study, testosterone, estradiol, and 25(OH)D were measured by cheiluminescence rather than the gold standard liquid chromatography-mass spectrometry (LC MS/MS). Finally, the subjects were treated with cholecalciferol during and not before the study and the possibility that vitamin D deficiency may have influenced the results cannot be excluded. Nevertheless, this is the first report of the effect of GAHT on aBMD, TBS and BMAT in adult AMAB and AFAB subjects. Further studies are needed to confirm the negative associations between L1-L4 aBMD and BMAT, as well as to investigate whether GAHT could positively influence BMAT and thus play a protective role in bone health by maintaining serum estradiol levels within the normal range for age.

In conclusion, our pilot study shows that BMAT negatively correlates with aBMD at the trabecular level in both AMAB and AFAB individuals before and during GAHT, the administration of which is not associated with a significant change in BMAT but rather with a small significant decrease in L1-L4 aBMD and an increase in TBS in AMAB. Our preliminary report highlights the need for further specifically designed studies to assess the possibility that the administration of hormone therapy to otherwise healthy AMAB subjects with no specific hormone deficiency has the potential to negatively affect quantitative parameters of bone (i.e. aBMD) while having a positive or no impact on those associated with bone quality and bone marrow adiposity.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Silvia Di Chiano, Davide Diacinti, Daniele Diacinti, Enrico Delli Paoli, Jessica Pepe, Francesco Lombardo, and Francesco Pallotti. The first draft of the manuscript was written by Silvia Di Chiano and Cristiana Cipriani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Some or all datasets generated during and/or analyzed during the current study are not publicly available but are

available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by CET - Territorial Ethics Committee Lazio Area 1 (reference number: 6554, date of approval: November 24, 2021).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Conflict of interests CC served as speaker, consultant and received travel reimbursement from Abiogen. She also served as consultant and in advisory board of Ascendis Pharma and IBSA and as speaker for Italfarmaco. The other authors have nothing to declare.

References

1. A. Joseph, C. Cliffe, M. Hillyard, A. Majeed, Gender identity and the management of the transgender patient: a guide for non-specialists. J. R Soc. Med. **110**(4), 144–152 (2017). https://doi.org/10.1177/0141076817696054
2. E. Coleman, A.E. Radix, W.P. Bouman, G.R. Brown, de A.L.C. Vries, M.B. Deutsch et al., Standards of care for the health of transgender and gender diverse People, version 8. Int. J. Transgend Health. **23**(Suppl 1), S1–S259 (2022). https://doi.org/10.1080/26895269.2022.2100644
3. A.D. Fisher, G. Senofonte, C. Cocchetti, G. Guercio, V. Lingiardi, M.C. Meriggiola et al., SIGIS-SIAMS-SIE position statement of gender affirming hormonal treatment in transgender and non-binary people. J. Endocrinol. Invest. **45**(3), 657–673 (2022). https://doi.org/10.1007/s40618-021-01694-2
4. A.D. Fisher, G. Castellini, J. Ristori, H. Casale, E. Cassioli, C. Sensi et al., Cross-Sex hormone treatment and Psychobiological changes in transsexual persons: Two-Year Follow-Up data. J. Clin. Endocrinol. Metab. **101**(11), 4260–4269 (2016). https://doi.org/10.1210/jc.2016-1276
5. F. Pallotti, G. Senofonte, A.C. Conflitti, A. Giancotti, A. Anzuini, E. Delli Paoli et al., Safety of gender affirming treatment in assigned female at birth transgender people and association of androgen and Estrogen beta receptor polymorphisms with clinical outcomes. Endocrine. **81**(3), 621–630 (2023). https://doi.org/10.1007/s12020-023-03421-8
6. W.C. Hembree, P.T. Cohen-Kettenis, L. Gooren, S.E. Hannema, W.J. Meyer, M.H. Murad et al., Endocrine treatment of Gender-Dysphoric/Gender-Incongruent persons: an endocrine society clinical practice guideline. J. Clin. Endocrinol. Metab. **102**(11), 3869–3903 (2017). https://doi.org/10.1210/jc.2017-01658
7. G. T'Sjoen, J. Arcelus, De A.L.C. Vries, A.D. Fisher, T.O. Nieder, M. Ozer et al., European society for sexual medicine position statement assessment and hormonal management in adolescent and adult trans People, with attention for sexual function and satisfaction. J. Sex. Med. **17**(4), 570–584 (2020). https://doi.org/10.1016/j.jsxm.2020.01.012
8. F.H. Schroder, Cyproterone acetate–mechanism of action and clinical effectiveness in prostate cancer treatment. Cancer. **72**(12 Suppl), 3810–3815 (1993). https://doi.org/10.1002/1097-0142(19931215)72:12+%3C3810::aid-cncr2820721710%3E3.0.co;2-o
9. T.V. Nguyen, M. Yao, C.J. Pike, Flutamide and cyproterone acetate exert agonist effects: induction of androgen receptor-dependent neuroprotection. Endocrinology. **148**(6), 2936–2943 (2007). https://doi.org/10.1210/en.2006-1469
10. C. Davidge-Pitts, B.L. Clarke, Transgender bone health. Maturitas. **127**, 35–42 (2019). https://doi.org/10.1016/j.maturitas.2019.05.002
11. D. Vanderschueren, M.R. Laurent, F. Claessens, E. Gielen, M.K. Lagerquist, L. Vandenput et al., Sex steroid actions in male bone. Endocr. Rev. **35**(6), 906–960 (2014). https://doi.org/10.1210/er.2014-1024
12. S. Kousteni, M. Almeida, L. Han, T. Bellido, R.L. Jilka, S.C. Manolagas, Induction of osteoblast differentiation by selective activation of kinase-mediated actions of the Estrogen receptor. Mol. Cell. Biol. **27**(4), 1516–1530 (2007). https://doi.org/10.1128/MCB.01550-06
13. C.M. Wiepjes, M.C. Vlot, M. Klaver, N.M. Nota, de C.J. Blok, de R.T. Jongh et al., Bone mineral density increases in trans persons after 1 year of hormonal treatment: A multicenter prospective observational study. J. Bone Min. Res. **32**(6), 1252–1260 (2017). https://doi.org/10.1002/jbmr.3102
14. Van E. Caenegem, K. Wierckx, Y. Taes, T. Schreiner, S. Vandewalle, K. Toye et al., Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). Eur. J. Endocrinol. **172**(2), 163–171 (2015). https://doi.org/10.1530/EJE-14-0586
15. M.S. Rothman, S.J. Iwamoto, Bone health in the transgender population. Clin. Rev. Bone Min. Metab. **17**(2), 77–85 (2019). https://doi.org/10.1007/s12018-019-09261-3
16. D. Diacinti, Del R. Fiacco, D. Pisani, F. Todde, M.S. Cattaruzza, D. Diacinti et al., Diagnostic performance of vertebral fracture assessment by the lunar iDXA scanner compared to conventional radiography. Calcif Tissue Int. **91**(5), 335–342 (2012). https://doi.org/10.1007/s00223-012-9643-0
17. D. Diacinti, D. Pisani, C. Cipriani, M. Celli, A. Zambrano, D. Diacinti et al., Vertebral fracture assessment (VFA) for monitoring vertebral reshaping in children and adolescents with osteogenesis imperfecta treated with intravenous neridronate. Bone. **143**, 115608 (2021). https://doi.org/10.1016/j.bone.2020.115608
18. C.M.S. Cipriani, J.P. Bilezikian, D. Diacinti, L. Colangelo, V. Piazzolla, M. Angelozzi, L. Nieddu, J. Pepe, D. Diacinti, Vertebral fracture assessment in postmenopausal women with postsurgical hypoparathyroidism. J. Clin. Endocrinol. Metab. **106**, 1303–1311 (2021). https://doi.org/10.1210/clinem/dgab076
19. E. Shevroja, J.Y. Reginster, O. Lamy, N. Al-Daghri, M. Chandran, A.L. Demoux-Baiada et al., Update on the clinical use of trabecular bone score (TBS) in the management of osteoporosis: results of an expert group meeting organized by the European society for clinical and economic aspects of osteoporosis, osteoarthritis and musculoskeletal diseases (ESCEO), and the international osteoporosis foundation (IOF) under the auspices of WHO collaborating center for epidemiology of musculoskeletal health and aging. Osteoporos. Int. **34**(9), 1501–1529 (2023). https://doi.org/10.1007/s00198-023-06817-4
20. L.C.C. Cianferotti, S. Corbetta, G. Corona, G. Defeudis, A.G. Lania, C. Messina, N. Napoli, G. Mazziotti, Bone quality in endocrine diseases: determinants and clinical relevance. J. Endocrinol. Invest. (2023). https://doi.org/10.1007/s40618-023-02056-w
21. I. Bretherton, A. Ghasem-Zadeh, S.Y. Leemaqz, E. Seeman, X. Wang, T. McFarlane et al., Bone microarchitecture in transgender adults: A Cross-Sectional study. J. Bone Min. Res. **37**(4), 643–648 (2022). https://doi.org/10.1002/jbmr.4497
22. T. Rharass, S. Lucas, MECHANISMS IN ENDOCRINOLOGY: bone marrow adiposity and bone, a bad romance? Eur. J. Endocrinol. **179**(4), R165–R82 (2018). https://doi.org/10.1530/EJE-18-0182
23. W.P. Cawthorn, E.L. Scheller, Editorial, Bone marrow adipose tissue: Formation, Function, and impact on health and disease.

Front. Endocrinol. (Lausanne). **8**, 112 (2017). https://doi.org/10.3389/fendo.2017.00112
24. C.S. Craft, E.L. Scheller, Evolution of the marrow adipose tissue microenvironment. Calcif Tissue Int. **100**(5), 461–475 (2017). https://doi.org/10.1007/s00223-016-0168-9
25. D. Ali, M. Tencerova, F. Figeac, M. Kassem, A. Jafari, The pathophysiology of osteoporosis in obesity and type 2 diabetes in aging women and men: the mechanisms and roles of increased bone marrow adiposity. Front. Endocrinol. (Lausanne). **13**, 981487 (2022). https://doi.org/10.3389/fendo.2022.981487
26. M.L. Mendonca, S.L. Batista, M.H. Nogueira-Barbosa, C.E. Salmon, F.J. Paula, Primary hyperparathyroidism: the influence of bone marrow adipose tissue on bone loss and of osteocalcin on insulin resistance. Clin. (Sao Paulo). **71**(8), 464–469 (2016). https://doi.org/10.6061/clinics/2016(08)09
27. C. McComb, A. Harpur, C. Yacoubian, C. Leddy, G. Anderson, S. Shepherd et al., MRI-based abnormalities in young adults at risk of adverse bone health due to childhood-onset metabolic & endocrine conditions. Clin. Endocrinol. (Oxf). **80**(6), 811–817 (2014). https://doi.org/10.1111/cen.12367
28. A. Cohen, W. Shen, D.W. Dempster, H. Zhou, R.R. Recker, J.M. Lappe et al., Marrow adiposity assessed on transiliac crest biopsy samples correlates with noninvasive measurement of marrow adiposity by proton magnetic resonance spectroscopy ((1)H-MRS) at the spine but not the femur. Osteoporos. Int. **26**(10), 2471–2478 (2015). https://doi.org/10.1007/s00198-015-3161-7
29. J. Tratwal, R. Labella, N. Bravenboer, G. Kerckhofs, E. Douni, E.L. Scheller et al., Reporting Guidelines, review of methodological Standards, and challenges toward harmonization in bone marrow adiposity research. Report of the Methodologies Working Group of the International Bone Marrow Adiposity Society. 2020 Front Endocrinol (Lausanne) 28: 11:65 https://doi.org/10.3389/fendo.2020.00065
30. F. Biamonte, J. Pepe, L. Colangelo, G. Desideri, E. Ettorre, L. Nieddu et al., Assessment of trabecular bone score (TBS) in the prediction of vertebral fracture in postmenopausal osteoporosis. Bone. **190**, 117307 (2025). https://doi.org/10.1016/j.bone.2024.117307
31. N.C. Harvey, C.C. Gluer, N. Binkley, E.V. McCloskey, M.L. Brandi, C. Cooper et al., Trabecular bone score (TBS) as a new complementary approach for osteoporosis evaluation in clinical practice. Bone. **78**, 216–224 (2015). https://doi.org/10.1016/j.bone.2015.05.016
32. G. Jiang, R. Eastell, N.A. Barrington, L. Ferrar, Comparison of methods for the visual identification of prevalent vertebral fracture in osteoporosis. Osteoporos. Int. **15**(11), 887–896 (2004). https://doi.org/10.1007/s00198-004-1626-1
33. Y.X.J. Wang, W.H. Liu, D. Diacinti, D.W. Yang, A. Iannacone, X.R. Wang et al., Diagnosis and grading of radiographic osteoporotic vertebral deformity by general radiologists after a brief self-learning period. J. Thorac. Dis. **12**(9), 4702–4710 (2020). https://doi.org/10.21037/jtd-20-2379
34. Y.X.J. Wang, D. Diacinti, M.P. Aparisi Gomez, F.R. Santiago, F. Becce, A.S. Tagliafico et al., Radiological diagnosis of prevalent osteoporotic vertebral fracture on radiographs: an interim consensus from a group of experts of the ESSR osteoporosis and metabolism subcommittee. Skeletal Radiol. (2024). https://doi.org/10.1007/s00256-024-04678-4
35. H.K. Genant, C.Y. Wu, van C. Kuijk, M.C. Nevitt, Vertebral fracture assessment using a semiquantitative technique. J. Bone Min. Res. **8**(9), 1137–1148 (1993). https://doi.org/10.1002/jbmr.5650080915
36. D. Diacinti, D.I.A. Diacinti, A. Pepe, L. Colangelo, L. Nieddu, E. Kripa, M. Orlandi, De V. Martino, S. Minisola, C. Cipriani, Bone marrow adipose tissue is increased in postmenopausal women with postsurgical hypoparathyroidism. J. Clin. Endocrinol. Metab. **108**(9), e807–e15 (2023). https://doi.org/10.1210/clinem/dgad116
37. C.M. Wiepjes, de R.T. Jongh, de C.J. Blok, M.C. Vlot, P. Lips, J.W. Twisk et al., Bone safety during the first ten years of Gender-Affirming hormonal treatment in transwomen and transmen. J. Bone Min. Res. **34**(3), 447–454 (2019). https://doi.org/10.1002/jbmr.3612
38. Van E. Caenegem, Y. Taes, K. Wierckx, S. Vandewalle, K. Toye, J.M. Kaufman et al., Low bone mass is prevalent in male-to-female transsexual persons before the start of cross-sex hormonal therapy and gonadectomy. Bone. **54**(1), 92–97 (2013). https://doi.org/10.1016/j.bone.2013.01.039
39. C.M. Wiepjes, M.C. Vlot, de C.J.M. Blok, N.M. Nota, de R.T. Jongh, den M. Heijer, Bone geometry and trabecular bone score in transgender people before and after short- and long-term hormonal treatment. Bone. **127**, 280–286 (2019). https://doi.org/10.1016/j.bone.2019.06.029
40. B. Lapauw, Y. Taes, S. Simoens, Van E. Caenegem, S. Weyers, S. Goemaere et al., Body composition, volumetric and areal bone parameters in male-to-female transsexual persons. Bone. **43**(6), 1016–1021 (2008). https://doi.org/10.1016/j.bone.2008.09.001
41. M. Dobrolinska, van der K. Tuuk, P. Vink, van den M. Berg, A. Schuringa, A.G. Monroy-Gonzalez et al., Bone mineral density in transgender individuals after gonadectomy and Long-Term Gender-Affirming hormonal treatment. J. Sex. Med. **16**(9), 1469–1477 (2019). https://doi.org/10.1016/j.jsxm.2019.06.006
42. M. Sosa, E. Jodar, E. Arbelo, C. Dominguez, P. Saavedra, A. Torres et al., Bone mass, bone turnover, vitamin D, and Estrogen receptor gene polymorphisms in male to female transsexuals: effects of Estrogenic treatment on bone metabolism of the male. J. Clin. Densitom. **6**(3), 297–304 (2003). https://doi.org/10.1385/jcd:6:3:297
43. J.F. Chen, P.W. Lin, Y.R. Tsai, Y.C. Yang, H.Y. Kang, Androgens and androgen receptor actions on bone health and disease: from androgen deficiency to androgen therapy. Cells. **8**(11) (2019). https://doi.org/10.3390/cells8111318
44. T. Yoshida, J. Wang, P.H. Stern, Gonadal hormones and bone. Handb. Exp. Pharmacol. **262**, 65–91 (2020). https://doi.org/10.1007/164_2019_327
45. Di A. Nisio, De L. Toni, E. Speltra, M.S. Rocca, G. Taglialavoro, A. Ferlin et al., Regulation of sclerostin production in human male osteocytes by androgens: experimental and clinical evidence. Endocrinology. **156**(12), 4534–4544 (2015). https://doi.org/10.1210/en.2015-1244
46. T. Douchi, T. Oki, H. Yamasaki, R. Kuwahata, M. Nakae, Y. Nagata, Relationship of androgens to muscle size and bone mineral density in women with polycystic ovary syndrome. Obstet. Gynecol. **98**(3), 445–449 (2001). https://doi.org/10.1016/s0029-7844(01)01450-8
47. S.R.L. Andrade, Y.M. Mucida, J.D.C. Xavier, L.N. Fernandes, R.O. Silva, F. Bandeira, Bone mineral density, trabecular bone score and muscle strength in transgender men receiving testosterone therapy versus cisgender men. Steroids. **178**, 108951 (2022). https://doi.org/10.1016/j.steroids.2021.108951
48. G. Papadakis, D. Hans, E. Gonzalez-Rodriguez, P. Vollenweider, G. Waeber, P.M. Marques-Vidal et al., The benefit of menopausal hormone therapy on bone density and microarchitecture persists after its withdrawal. J. Clin. Endocrinol. Metab. **101**(12), 5004–5011 (2016). https://doi.org/10.1210/jc.2016-2695
49. N. Chan Swe, S. Ahmed, M. Eid, L. Poretsky, E. Gianos, N.E. Cusano, The effects of gender-affirming hormone therapy on cardiovascular and skeletal health: A literature review. Metabol Open. **13**, 100173 (2022). https://doi.org/10.1016/j.metop.2022.100173
50. J. Li, X. Chen, L. Lu, X. Yu, The relationship between bone marrow adipose tissue and bone metabolism in postmenopausal

osteoporosis. Cytokine Growth Factor. Rev. **52**, 88–98 (2020). https://doi.org/10.1016/j.cytogfr.2020.02.003
51. F. Ferrau, S. Giovinazzo, Y. Alessi, A. Catalano, A. Tessitore, E. Mormina et al., Trabecular bone score, bone marrow fat and vertebral fractures in Cushing syndrome. Endocrine. **80**(2), 441–447 (2023). https://doi.org/10.1007/s12020-023-03318-6
52. N. Nasomyont, A.R. Meisman, K. Ecklund, S. Vajapeyam, K.M. Cecil, J.A. Tkach et al., Changes in bone marrow adipose tissue in transgender and gender Non-Conforming youth undergoing pubertal suppression: A pilot study. J. Clin. Densitom. **25**(4), 485–489 (2022). https://doi.org/10.1016/j.jocd.2022.06.006
53. C.E. Guss, R. Eiduson, S. Vajapeyam, K. Ecklund, R. Mulkern, S.E. Dahlberg et al., Bone density and bone marrow composition in transgender girls prior to pubertal blockade: A case series. Bone. **162**, 116454 (2022). https://doi.org/10.1016/j.bone.2022.116454
54. D. Schellinger, C.S. Lin, D. Fertikh, J.S. Lee, W.C. Lauerman, F. Henderson et al., Normal lumbar vertebrae: anatomic, age, and sex variance in subjects at proton MR spectroscopy–initial experience. Radiology. **215**(3), 910–916 (2000). https://doi.org/10.1148/radiology.215.3.r00jn42910
55. F.A. Syed, M.J. Oursler, T.E. Hefferanm, J.M. Peterson, B.L. Riggs, S. Khosla, Effects of Estrogen therapy on bone marrow adipocytes in postmenopausal osteoporotic women. Osteoporos. Int. **19**(9), 1323–1330 (2008). https://doi.org/10.1007/s00198-008-0574-6
56. J. Paccou, S. Badr, D. Lombardo, H. Khizindar, V. Deken, S. Ruschke et al., Bone marrow adiposity and fragility fractures in postmenopausal women: the ADIMOS Case-Control study. J. Clin. Endocrinol. Metab. **108**(10), 2526–2536 (2023). https://doi.org/10.1210/clinem/dgad195
57. E.W. Yu, L. Greenblatt, A. Eajazi, M. Torriani, M.A. Bredella, Marrow adipose tissue composition in adults with morbid obesity. Bone. **97**, 38–42 (2017). https://doi.org/10.1016/j.bone.2016.12.018
58. L. Xu, M. Min, X. Li, G.M. Blake, K. Zhao, X. Ruan et al., Marrow adipose tissue is increased in overweight and obese women with PCOS independently of hyperandrogenism related obesity and metabolic disorders. Front. Endocrinol. (Lausanne). **14**, 1168806 (2023). https://doi.org/10.3389/fendo.2023.1168806
59. M. Zoulakis, K.F. Axelsson, H. Litsne, L. Johansson, M. Lorentzon, Bone marrow adiposity assessed by HRpQCT is related to fracture risk and bone mineral density in older Swedish women. J. Clin. Endocrinol. Metab. (2025). https://doi.org/10.1210/clinem/dgaf216
60. W. Li, C. Wang, M. Zhang, J. Wu, Y. Gu, Y. Deng et al., Young and old adipocytes have differential influence on the development of osteoblasts. Obes. Res. Clin. Pract. **12**(6), 520–527 (2018). https://doi.org/10.1016/j.orcp.2018.06.006