

# Preservation of volumetric bone density and geometry in trans women during cross-sex hormonal therapy: a prospective observational study

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## Abstract

**Summary** Although trans women before the start of hormonal therapy have a less bone and muscle mass compared with control men, their bone mass and geometry are preserved during the first 2 years of hormonal therapy, despite of substantial muscle loss, illustrating the major role of estrogen in the male skeleton.

**Purpose** The aim of this study is to examine the evolution of areal and volumetric bone density, geometry, and turnover in trans women undergoing sex steroid changes, during the first 2 years of hormonal therapy.

**Methods** In a prospective observational study, we examined 49 trans women (male-to-female) before and after 1 and 2 years of cross-sex hormonal therapy (CSH) in comparison with 49 age-matched control men measuring grip strength (hand dynamometer), areal bone mineral density (aBMD), and total body fat and lean mass using dual X-ray absorptiometry (DXA), bone geometry and volumetric bone mineral density, regional fat, and muscle area at the forearm and calf using peripheral quantitative computed tomography. Standardized treatment regimens were used with oral estradiol valerate, 4 mg daily (or transdermal 17- $\beta$  estradiol 100  $\mu$ g/

24 h for patients >45 years old), both combined with oral cyproterone acetate 50 mg daily.

**Results** Prior to CSH, trans women had lower aBMD at all measured sites (all  $p < 0.001$ ), smaller cortical bone size (all  $p < 0.05$ ), and lower muscle mass and strength and lean body mass (all  $p < 0.05$ ) compared with control men. During CSH, muscle mass and strength decreased and all measures of fat mass increased (all  $p < 0.001$ ). The aBMD increased at the femoral neck, radius, lumbar spine, and total body; cortical and trabecular bone remained stable and bone turnover markers decreased (all  $p < 0.05$ ).

**Conclusions** Although trans women, before CSH, have a lower aBMD and cortical bone size compared with control men, their skeletal status is well preserved during CSH treatment, despite of substantial muscle loss.

**Keywords** Bone transsexual gender dysphoria sex steroids prospective

## Introduction

Sex steroids determine bone geometry during puberty. Men develop larger bones than women with a greater periosteal (outer) and endosteal (inner) circumference of the cortex, resulting in the sexual dimorphism of bone. This difference is mainly due to periosteal apposition during puberty on which testosterone has a positive influence [1]. Mechanical loading, through muscle mass and physical activity, has been shown to be an important factor in the acquisition of bone geometry in adulthood [2, 3]. Estrogens could increase the sensitivity of bone for mechanical stimuli, and mechanical stimuli are in their turn modulated by androgens, depending on GH-IGF1 action. However, there is conflicting data on this interaction of estrogens with the periosteal surface with evidence for both stimulatory and inhibitory effects [4, 5].

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Trans women (male-to-female trans persons) undergo hormonal treatment reducing the endogenous testosterone and inducing higher estrogen levels, which is referred to as cross-sex hormonal therapy (CSH). This way, masculine characteristics, e.g. hair growth, are suppressed and feminization is induced. In addition, sex reassignment surgery can be performed, which includes vaginoplasty and orchiectomy. The hormonal treatment reduces muscle mass and increases fat mass [6]. These changes during CSH in trans women are a model that may help to understand the differential effects of mechanical loading, fat and muscle mass, and sex steroid milieu in the bone. In an earlier cross-sectional study of our group, bone geometry was assessed in trans women after median 8 years of CSH and sex reassignment surgery (SRS). We found a lower areal and volumetric bone mineral density (aBMD and vBMD, respectively) and smaller bone size related to lower muscle mass and strength compared with male controls [7, 8]. Moreover, in a second study in trans women, at the age of peak bone mass and prior to any kind of hormonal treatment, we observed a lower bone mineral density and a smaller cortical bone area and thickness in relation to lower muscle mass compared with age-matched control males [9]. Prospective studies on the effects of CSH and CSH-induced muscle loss in bone geometry are still lacking. Other research using classical dual X-ray absorptiometry (DXA) found a maintained aBMD after 2 or more years of CSH in trans women [10–18]. In the latter studies, a variety of treatment regimens was used, some of which are no longer applied for safety reasons [19], e.g., ethinyl estradiol [11, 14, 18]. Also, treatment regimens with or without anti-androgens were described which hampers clear comparisons (cyproterone acetate in [14, 18] or GnRH-analogues in [10, 12, 13]).

In this prospective observational study, we examine bone mass using DXA and bone geometry using peripheral quantitative CT-scan (pQCT) in trans women before the start of CSH and after 1 and 2 years of CSH in relation to body fat, lean mass, and physical activity. This study is an extension on former research [9]: we doubled the group of trans women and followed all persons prospectively. Cross-sex hormonal therapy was initiated with a standardized treatment protocol with anti-androgens and estrogens.

## Materials and methods

### Study design and population

All trans women were diagnosed with gender dysphoria (DSM-5, 302.85; ICD-10, F64.0) and were recruited from the center for sexology and gender problems at the Ghent University Hospital, Belgium. All were treated following the World Professional Association for Transgender Health standards of care [20]. This research is part of the ‘European Network for the Investigation of Gender Incongruence’

(ENIGI), a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Hamburg, and Oslo), a study group created to obtain more transparency in diagnostics and treatment of gender dysphoria [21].

Between February 2010 and August 2012, all patients diagnosed with gender dysphoria and referred to our departments were invited to participate in this prospective study (trans women;  $n=87$ ). After screening by thorough medical history and determination of serum sex steroids, 37 persons were excluded resulting in a total population of 50 trans women. Reasons for exclusion were previous hormonal therapy ( $n=22$ ), unwilling ( $n=10$ ), medical (gastric bypass;  $n=1$ ), and other ( $n=4$ ). One patient had subclinical hypergonadotrophic hypogonadism due to a primary testicular problem and was excluded from further analyses. A final number of 49 trans women who had never used any kind of cross-sex hormonal treatment nor anti-androgen therapy, and thus before SRS, was included. All participants were Caucasian. A male control population was used, matched for age ( $\pm 2$  years, median=1 year). These were healthy men recruited from communities around Ghent or who responded on posters spread at the Ghent University Hospital and on its website and in schools.

All participants were in good physical health and completed questionnaires about previous illness and medication use, current and past smoking habits, and physical activity by recording the weekly frequency of sports, recreational, and/or working activities (using Baecke’s questionnaire [22]). At the start of the study, three trans women used a 5-alpha reductase inhibitor to reduce hair loss (finasteride 5 mg,  $n=2$ ) and for benign prostate hypertrophy (dutasteride 0.5 mg,  $n=1$ ). All three had serum testosterone within the normal ranges and only the latter had slightly elevated FSH and LH (11 and 16 U/l, respectively).

The trans women were evaluated after 1 and 2 years. In the meantime, structured clinical visits were conducted at 3, 6, 9, and 18 months. After 1-year follow-up, three trans women dropped out: two were lost to follow-up and one decided to stop hormones. The use of 5-alpha reductase inhibitors was ceased at baseline visit. One person was not compliant, with testosterone levels remaining within the normal male range, and was excluded for the 1- and 2-year evaluation. One trans woman skipped the bone examinations at 1 year (not the clinical evaluation), but did manage to do the visit at 2 years. This data is part of a large prospective study (ENIGI); at time of this analysis, all 49 subjects had completed 1-year follow-up and 29 had completed 2-year follow-up.

Standardized treatment regimens were started after the baseline visit with oral estradiol valerate, 4 mg daily ( $n=34$ ) (Prognova<sup>®</sup>, Bayer, Germany) or transdermal 17- $\beta$  estradiol 100  $\mu\text{g}/24$  h for patients older than 45 years old ( $n=15$ ) (Dermestril<sup>®</sup>, Besins, Belgium), both combined with oral cyproterone acetate 50 mg daily (Androcur<sup>®</sup>, Bayer, Germany). Transdermal estrogens were used in older trans women as this would have a lower thromboembolic risk [23]. When

psychologically indicated, trans women started with cyproterone acetate 50 mg alone, without estrogens, and estrogens were then associated after a median of 25 weeks (IQR 16–31). The latter group will be referred to as the “IAAM”-group (initial anti-androgens monotherapy) ( $n=18$ ), whereas trans women who received combined anti-androgens with estrogens from the start are referred to as the “AA + E”-group (anti-androgens and estrogens combined) ( $n=31$ ). Vitamin D supplements were used by a single trans woman at the baseline, but not by the control persons. None of the subjects used calcium supplements or bone-active drugs like bisphosphonates or SERMs at the baseline. The study protocol was approved by the ethics review board of the Ghent University Hospital, registered with clinicaltrials.gov (identifier: NCT01072825) and all participants gave written informed consent.

#### Body composition, muscle strength, and areal bone mineral density

Body weight and anthropometrics were measured in light indoor clothing without shoes. Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain, Ltd., Crymch, UK).

Grip strength at the dominant hand was measured using an adjustable hand-held standard grip device (JAMAR hand dynamometer, Sammons and Preston, Bolingbrook, IL, USA). The maximum strength of three attempts was assumed to best reflect the current status and history of their musculoskeletal adaptation and was expressed in kilograms (kg).

Body fat and lean mass, bone mineral content (BMC), bone area, and areal bone mineral density (aBMD) at the whole body, lumbar spine, non-dominant forearm, and left proximal femur (total hip and femoral neck region) were measured using dual X-ray absorptiometry (DXA) with a Hologic Discovery device (Software Version 11.2.1, Hologic, Inc., Bedford, MA, USA). The coefficient of variation for both spine and whole-body calibration phantoms was less than 1 %, as calculated from daily and weekly measurements, respectively.

#### Volumetric bone parameters and cross-sectional muscle and fat area

A pQCT device (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany) was used to evaluate the cortical volumetric bone parameters at the dominant midradius and tibia (at 66 % of bone length) and trabecular bone parameters at the metaphysis (at 4 % of bone length) of the dominant radius. Over 90 % of the scans were performed by a single operator. Procedure details were as described previously [5]. Scans with large movement artifacts ( $n=2$ ) and suspected position error (>10 % variation of total bone area at radius 4 %) ( $n=1$ ) were excluded.

#### Biochemical determinations

Venous blood samples were obtained between 08.00 and 10.00 h after overnight fasting. All samples were stored at  $-80$  °C until analysis.

Testosterone (T), estradiol (E2), estrone (E1), and androstenedione were determined using tandem mass spectrometry on an AB Sciex 5500 triple-quadrupole mass spectrometer (AB Sciex, Toronto Canada). Serum limit of quantification was 0.3 pg/ml for E2 and 0.5 pg/ml for E1, and the inter-assay CV was 4 % at 21 pg/ml for E2 and 7.6 % at 25 pg/ml for E1. Serum limit of quantification was 1 ng/dl (35 pmol/l) for T, and the inter-assay CV was 6.5 % at 3 ng/dl [24]. Commercial immunoassays kits were used to determine serum concentrations of sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle stimulating hormone (FSH), 25-hydroxyvitamin D (25(OH)D) (Modular, Roche Diagnostics, Mannheim, Germany), and dehydroepiandrosterone sulfate (DHEAS), markers of bone turnover viz. C-terminal telopeptides of type I collagen (CTX) (bone resorption), and procollagen 1 aminoterminal propeptide (P1NP) and osteocalcin (OC) (bone formation) (Cobas 411, Roche Diagnostics, Mannheim, Germany). Radio-immunoassays were used for leptin (Bio-connect diagnostics, Huissen, the Netherlands) and insulin-like growth factor 1 (IGF1) (Cisbio bioassays, Codolet, France). The intra- and inter-assay coefficients of variation for all assays were less than 10 %.

#### Statistical analysis

Descriptives are expressed as mean and standard deviation or median (first to third quartile), when criteria for normal distribution were not fulfilled.  $P$  values <0.05 were considered to indicate statistical significance; all tests were two-tailed. Comparison of general, anthropometric, biochemical, and hormonal determinations, bone and body composition before cross-sex hormonal therapy, between trans women and age-matched controls and between trans women of the AA + E and the IAAM group was performed using an independent sample  $t$  tests (or Mann–Whitney  $U$  test, when criteria for normal distribution were not fulfilled). Repeated measurements in trans women before and after 1 and 2 years of treatment were analyzed using mixed models to allow inclusion of all data from each subject despite missing values. Dependents in these models were the bone and body composition parameters. Study visit (defined as a categorical variable) was the main independent variable. Other independent variables, fixed factors (hormonal treatment protocol AA + E or IAAM) or covariates (e.g., age, weekly sports activity, muscle CSA, T, E2, PTH, 25(OH)D), were added to the model in specific questions. The  $p$  value for the fixed effect of the independent variable “study visit” is given in Tables 3 and 4. The  $p$  value for the fixed effect of the other independent variables,

covariates, is given in the results section “Influence of covariates on bone evolution during CSH”.

## Results

Before hormonal therapy: Comparison with age-matched controls

### *General characteristics, hormonal and biochemical determinations*

Trans women at inclusion were median 30 years old with a wide range (minimum 17 and maximum 67) (Table 1). Mean body weight, height, and BMI were similar in trans women and control men. There were 22 % active smokers in this group compared with 16 % in controls (n.s.) and the amount of pack years was similar.

The T, E2, E1, LH, and SHBG were comparable in both groups. We observed a slightly higher FSH in trans women versus control men, which remained borderline significant after exclusion of 5-alpha reductase users ( $n=3$ ). All participants had T levels within normal male range and all were clinically euthyroid.

### *Bone turnover markers, 25(OH) vitamin D status and leptin*

Trans women had a significantly lower serum 25(OH)D and higher PTH than control men, which remained significant after adjustment for fat mass and month of visit (Table 1). Vitamin D insufficiency (defined as 25(OH)D < 20 ng/ml) was prevalent in 67 % of trans women versus 35 % control males (chi-square test  $p=0.001$ ). Hyperparathyroidism (PTH > 65 ng/l) was found in four vitamin D-deficient trans women versus none in the control group (chi-square test  $p=0.033$ ).

### *Areal bone mineral density using DXA*

Trans women had a significantly lower aBMD at the lumbar spine, hip, femoral neck, and radial forearm compared with the control men (Table 2). The prevalence of osteoporosis based on male reference ranges (following ‘classical’ WHO-criteria as originally proposed for postmenopausal women: defined as a T-score  $\leq 2.5$  SD) was 18 % at the lumbar spine and 11 % based on female reference ranges (as suggested to define osteoporosis in men [25]) in trans women versus 4 and 2 %, respectively, in the male control group (chi-square test,  $p=0.025$ ).

### *Volumetric bone parameters at the upper and lower limb using pQCT*

At both the radius and the tibia, the periosteal, outer cortical, circumference was smaller and the cortical bone area and

thickness ( $p=0.002$ ) were smaller compared with control men (Table 3, Fig. 1). Trans women had a lower trabecular vBMD at the radius ( $p=0.013$ ), and a markedly lower polar strength strain index (SSI, as a measure of bone strength) ( $p=0.017$ ) was observed at both cortical sites and controls.

### *Body composition and physical activity*

Trans women presented with a significantly lower body lean mass (−4 %) and a lower grip strength and muscle mass, reflected by the muscle cross-sectional area (CSA) at the forearm compared with control men (Table 4). We also observed a tendency towards higher measures of fat mass despite the lower mean total body weight (n.s.) in trans women. The weekly sports activity was significantly lower in trans women compared with control men.

## During hormonal therapy: Follow-up after 1 and 2 years of CSH

Follow-up and changes in sex steroids and hormonal levels

Serum T decreased and E2 increased significantly in trans women and were both within the normal female ranges at the 1- and 2-year time points of CSH (Table 1). A higher serum E2 (114 vs. 57 pg/ml) and lower E1 (107 vs. 344 pg/ml) were measured in transdermal estrogen users versus oral estrogen users at year one and two of CSH. Furthermore, there were no significant differences between the transdermal and oral estrogen users (after adjustment for age). SHBG was significantly increased at the 1-year time point and further increased at the 2-year treatment time point. Precursor androgens, androstenedione, and DHEAS decreased as well during treatment. Gonadotropines were significantly decreased at 1 year and remained suppressed after 2 years of treatment in those who did not undergo SRS during year two. In trans women who underwent SRS ( $n=11$ ) and quit cyproterone acetate treatment afterwards, gonadotropines were higher and T lower in year two compared with trans women who did not undergo SRS yet ( $n=17$ ) (Mann–Whitney  $U$  test  $p_{\text{FSH, LH}} < 0.001$  and  $p_{\text{T}} = 0.029$ ). Leptin was increased at 1 year and remained like this after 2 years. Body fat mass was a significant positive predictor of serum leptin levels at both time points (mixed model, dependent serum leptin and independent fixed factors, visit and body fat mass; body fat mass  $p < 0.001$ ). After 1 year, two active smokers quit their tobacco use and four ex-smokers took up smoking again during the first ( $n=3$ ) and second ( $n=1$ ) year.

**Table 1** General characteristics, hormones, and bone turnover markers in control men and trans women before and after 1 and 2 years of CSH

	Control men ( <i>n</i> =49)	Trans women		
		before CSH ( <i>n</i> =49)	1 year CSH ( <i>n</i> =44)	2 years CSH ( <i>n</i> =29)
Age (years)	33±12	33±12	–	–
Weight (kg)	78.3±10.8	74.7±14.3*	76.5±14.2	80.8±15.9
Height (cm)	178.9±5.6	178.4±5.9	–	–
Pack year	0 (0–8)	19.11	–	–
Alcohol (drinks/week)	10 (3–16)***	2 (0–7)	1 (0–4)	3 (0–5)
Fracture prevalence(%) <sup>a</sup>	16	22	–	–
Sport index <sup>b</sup>	3.1±1.1*	2.6±1.1	2.5±1.2	2.5±1.1
Leisure time index <sup>b</sup>	2.9±0.7	3.1±0.6	3.0±0.7	3.1±0.7
Work index <sup>b</sup>	2.6±0.7	2.7±0.8	2.5±0.8	2.6±0.9
Total physical activity <sup>b</sup>	8.7±1.5	8.3±1.6	8.0±1.6	8.1±1.9
Testosterone (ng/dl)	515 (445–616)	546 (451–643)***	13 (10–15)	13 (10–24)
Estradiol (pg/ml)	21.0 (15.8–25.1)	20.8 (17.2–28.3)***	61.1 (46.5–85.8)	52.4 (36.2–75.1)
Estrone (pg/ml)	33.7 (27.9–44.5)	35.0 (26.5–44.3)***	262.1 (72–379.6)	193.2 (67.9–343.2)
LH (U/l) <sup>c</sup>	4 (3–6)	5 (4–7)***	<0.1 (<0.1–<0.1)	1 (<0.1–12)
FSH (U/l) <sup>d</sup>	3 (2–5)*	4 (3–6)***	<0.1 (<0.1–<0.1)	2 (<0.1–11)
SHBG (nmol/l)	35.6±(30.5–42.3)	38.5±(26.1–48.6)***	46.8±22.2	55.1±23.4
Androstenedione (ng/dl)	89 (76–115)	108 (93–125)***	61 (53–81)	61 (47–88)
DHEAS (μg/dl)	353±144	333±138**	274±125	309±180
TSH (mU/l)	2.3±1.1	2.4±1.3	2.4±1.3	2.3±1.1
Leptin (ng/ml)	4.2±3.2	5.9±4.2***	13.1±10.6	13.0±7.9
25(OH)D (ng/ml)	23±7***	16±8**	19±10	21±8
PTH (pg/ml)	36±12***	44±15*	39±14	40±1
P1NP (μg/l)	64.1±31.1	61.3±34.1	56.6±31.2	47.8±26.7
Osteocalcin (ng/ml)	23.1±7.0	25.7±10.3**	23.9±10.8	20.7±9.1
CTX (ng/ml)	0.52±0.26	0.49±0.25***	0.45±0.22	0.32±0.18
IGF1 (ng/ml)	251±90	240±78	275±74	238±66***

Descriptives are expressed as mean±SD or as median (1st–3rd quartile) when not normally distributed

Variables were compared between control men and trans women before CSH using independent *t* tests or Mann–Whitney U tests when not normally distributed. Repeated measures in trans women were tested using mixed models, the *p* value for the fixed effect of study visit (considering both years) is given

Conversions of conventional units to SI units: testosterone nmol/l; multiply ng/dl by 0.0347, estradiol nmol/l; multiply pg/ml by 3.671, estrone nmol/l; multiply pg/ml by 3.699, androstenedione nmol/l; multiply ng/dl by 0.0349, DHEAS nmol/l; multiply μg/dl by 27.14, leptin nmol/l; multiply ng/ml by 0.0625. 25(OH) vitamin D nmol/l; multiply ng/ml by 2.496, PTH ng/l; multiply pg/ml by 1, P1NP nmol/l; multiply μg/l by 0.0286, CTX ng/l; multiply ng/ml by 1000, IGF1 μg/l; multiply ng/ml by 1

<sup>a</sup> Using chi-square test

<sup>b</sup> Measured by the Baecke questionnaire on physical activity [22]

<sup>c</sup> Detection limit LH-assay=0.1 U/L

<sup>d</sup> Detection limit FSH-assay=0.1 U/L

Repeated measures in trans women were tested using mixed models, the *p* value for the fixed effect of study visit is given (\*)

\*\*\**p*≤0.001

\*\*0.001<*p*≤0.01

\*0.01<*p*≤0.05

Bone turnover markers, 25-hydroxyvitamin D status and IGF1

CTX, P1NP, and OC decreased after the first and second year (mean decrease at year two was P1NP –18 %,

OC –12 %, and CTX –25 %) (Table 1). Parathyroid hormone decreased after 1 year and 25(OH)D status ameliorated significantly between the 1- and 2-year visit. Vitamin D supplements were used by 11 and 5 participants at year one and two, respectively, which

**Table 2** Areal bone parameters in control men and trans women before and after 1 and 2 years of CSH

	Control men ( <i>n</i> =49)	Trans women		
		before CSH ( <i>n</i> =49)	1 year CSH ( <i>n</i> =44)	2 years CSH ( <i>n</i> =29)
<b>Lumbar Spine</b>				
Bone Area (cm <sup>2</sup> )	70±7	67±6	68±7	68±7
BMC (g)	73±11***	64±13***	66.7±14.2	67±14
aBMD (g/cm <sup>2</sup> )	1.040±0.113**	0.952±0.150***	0.983±0.156	0.982±0.136
<b>Total Body</b>				
Bone Area (cm <sup>2</sup> )	2,340±150*	2,258±181***	2,282±183	2,315±200
BMC (g)	2,823±375***	2,466±362***	2,500±384	2,562±399
aBMD (g/cm <sup>2</sup> )	1.205±0.102***	1.088±0.086**	1.090±0.090	1.100±0.090
<b>Femoral Neck</b>				
Bone Area (cm <sup>2</sup> )	6±0.3	6±0.4	6±0.4	6±0.4
BMC (g)	5±1***	5±1*	5±1	5±0.9
aBMD (g/cm <sup>2</sup> )	0.928±0.154***	0.795±0.119***	0.807±0.129	0.807±0.123
<b>Total Hip</b>				
Bone Area (cm <sup>2</sup> )	44±4	43±4	43±4	43±4
BMC (g)	48±8***	41±8	41±8	42±9
aBMD (g/cm <sup>2</sup> )	1.089±0.153***	0.947±0.134	0.952±0.141	0.955±0.145
<b>Radius</b>				
Bone Area (cm <sup>2</sup> )	29±3***	17±3.9	17±1.7	17±1.8
BMC (g)	19±2***	11±2.3***	10±1.7	10±1.8
aBMD (g/cm <sup>2</sup> )	0.639±0.052*	0.614±0.058**	0.621±0.057	0.622±0.062

Descriptives are expressed as mean±SD or as median (first–third quartile) when not normally distributed

Variables were compared between control men and trans women before CSH using independent *t* tests or Mann–Whitney U tests when not normally distributed. Repeated measures in trans women were tested using mixed models, the *p* value for the fixed effect of study visit (considering both years) is given

\*\*\**p*≤0.001

\*\*0.001<*p*≤0.01

\*0.01<*p*≤0.05

**Table 3** Volumetric bone parameters at the tibia in control men and trans women before and after 1 and 2 years of CSH

	Control men ( <i>n</i> =49)	Trans women		
		before CSH ( <i>n</i> =49)	1 year CSH ( <i>n</i> =44)	2 years CSH ( <i>n</i> =29)
<b>Tibia</b>				
Cortical vBMD (mg/cm <sup>3</sup> )	1,112±32	1,111±24	1,113±24	1,116±28
Cortical bone area (mm <sup>2</sup> )	364±49***	330±46	329±48	321±48
Cortical thickness (mm)	4.4±0.7**	4.1±0.5	4.1±0.5	4.0±0.5
Periosteal circumference (mm)	97±8*	94±7	93±6	94±8
Endosteal circumference (mm)	69±11	68±7	68±7	69±8
Polar SSI (mm <sup>3</sup> )	3,033±495***	2,671±536	2,624±513	2,595±546

Descriptives are expressed as mean±SD or as median (first–third quartile) when not normally distributed

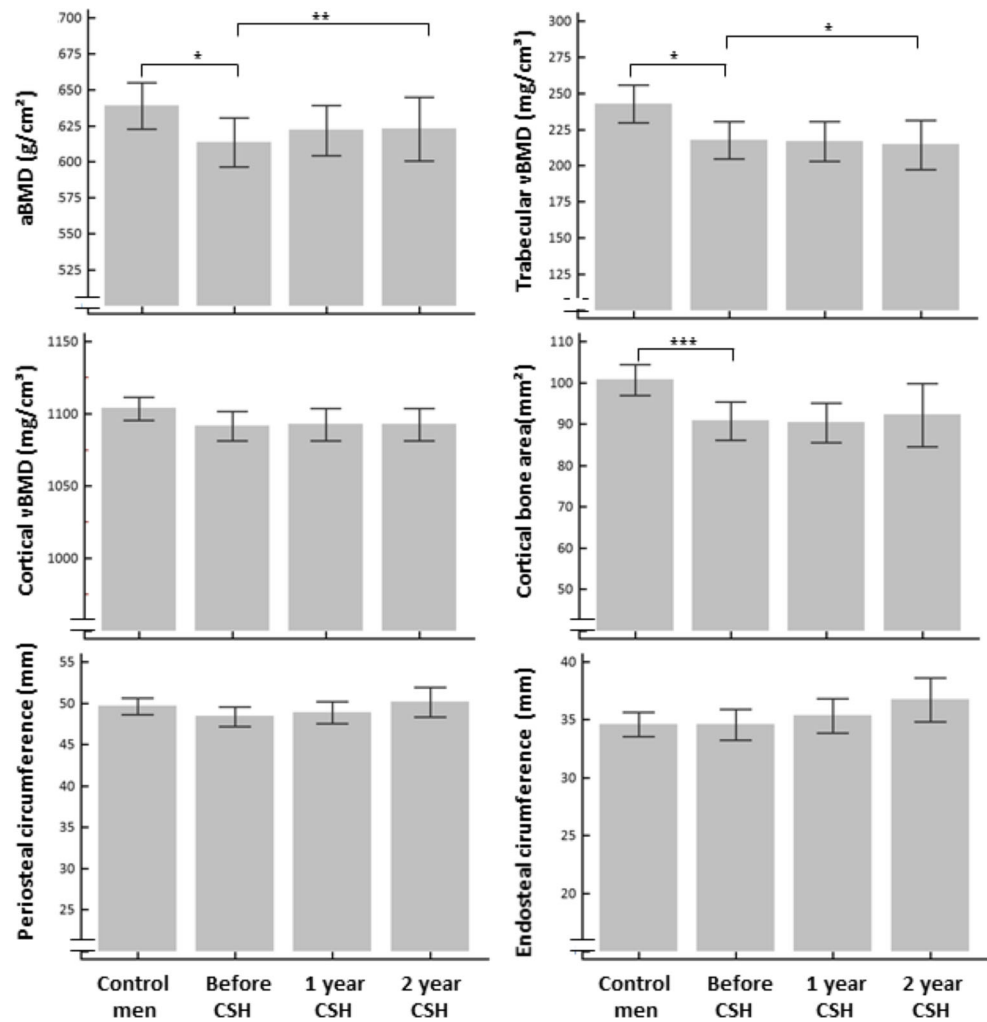
Variables were compared between control men and trans women before CSH using independent *t* tests or Mann–Whitney U tests when not normally distributed. Repeated measures in trans women were tested using mixed models, the *p* value for the fixed effect of study visit (considering both years) is given

\*\*\**p*≤0.001

\*\*0.001<*p*≤0.01

\*0.01<*p*≤0.05

**Fig. 1** Areal and volumetric bone parameters at the radius in trans women and control men



were combined with calcium in respectively four and three trans women. Bisphosphonates or other bone-active drugs were not used during the 2 years of CSH. IGF1 increased significantly after 1 year, but decreased again after the first year. The type of estrogen (oral vs. transdermal) did not have an impact on the evolution of IGF1 over time with and without adjustment for age (data not shown,  $p=n.s.$ ).

#### Areal bone mineral density using DXA

We observed an increase in aBMD at the femoral neck, lumbar spine, radius, and whole body after 1 and 2 years of hormonal therapy (+1.8, +3.2, +1.1, and +0.8 % at year one, respectively) (Table 2). At the total hip, no changes were observed. In a sensitivity analysis, we corrected for fat mass by normalizing the measured aBMD at each site for the corresponding regional fat (aBMD divided by the measured fat mass by DXA in that region). This showed similar results (data not shown).

#### Volumetric bone parameters at the upper and lower limb using pQCT

No changes in cortical bone parameters over time were noted (Table 3, Fig. 1). Also, for the ratio of endosteal over periosteal circumference, no differences were observed at the 1- or 2-year time points (data not shown). Trabecular vBMD appeared to decrease over time (Fig. 1), although trabecular area remained stable.

#### Body composition and physical activity

All measures of fat mass, including subcutaneous fat mass, increased during the first and second year of cross-sex hormonal treatment (+25 % fat body mass in year one) (Table 4). Trans women lost lean body mass (−4 % in year one), grip strength and muscle CSA of the forearm and calf mainly during the first year. Hip circumference augmented significantly during the first year and consequently WHR decreased. Waist circumference remained stable. Weekly total physical activity including sports,

**Table 4** Body composition in control men, trans women before and after 1 and 2 years of CSH

	Control men ( <i>n</i> =49)	Trans women		
		before CSH ( <i>n</i> =49)	1 year CSH ( <i>n</i> =44)	2 years CSH ( <i>n</i> =29)
Measures of fat mass				
Body Mass Index (kg/m <sup>2</sup> )	24.5±3.4	23.5±4.2***	24±4.3	25.0±4.3
Waist circumference (cm)	86±9	85±12	83±11	85±11
Hip circumference (cm)	98±6	97±8***	100±8	101±8
Waist-hip ratio	0.9±0.1	0.9±0.1***	0.8±0.1	0.8±0.1
Fat body mass (kg) <sup>a</sup>	14.1±5.7	14.9±6.6***	18.9±6.9	21.1±7.4
Fat percentage (%) <sup>a</sup>	17.6±5.4	19.1±5.6***	24.2±5.0	25.6±4.9
Forearm fat CSA (mm <sup>2</sup> ) <sup>b</sup>	769±426	795±462***	1,245±496	1,382±490
Calf fat CSA (mm <sup>2</sup> ) <sup>b</sup>	1,436±503	1,687±883***	2,228±766	2,391±762
Measures of muscle mass				
Lean body mass (kg) <sup>a</sup>	61.3±6.8*	57.4±8.7***	55.1±8.7	57.1±9.8
Lean percentage (%) <sup>a</sup>	78.5±5.1	77.5±5.3***	72.5±4.6	71.7±4.1
Forearm muscle CSA (mm <sup>2</sup> ) <sup>b</sup>	4,512±579***	3,999±746***	3,664±783	3,825±867
Calf muscle CSA (mm <sup>2</sup> ) <sup>b</sup>	8,233±1,498	7,742±1,361**	7,623±1,479	7,448±1,390
Grip strength (N/kg)	49±6***	42±9***	39±9	38±10

CSA cross-sectional area. <sup>a</sup> Measured with DXA. <sup>b</sup> measured with pQCT

Descriptives are expressed as mean±SD or as median (first–third quartile) when not normally distributed

Variables were compared between control men and trans women before CSH using independent *t* tests or Mann–Whitney U tests when not normally distributed. Repeated measures in trans women were tested using mixed models, the *p* value for the fixed effect of study visit (considering both years) is given

\*\*\**p*≤0.001

\*\*0.001<*p*≤0.01

\*0.01<*p*≤0.05

physical activity during leisure time and at work, did not change significantly over time.

#### Influence of covariates on bone evolution during CSH

Adjusting the mixed models for age, BMI, body fat mass, or leptin did not alter any of the observed changes in bone or body composition over 2 years of CSH. E2 and T (at year one) were not associated with the evolutions in bone or body composition.

Including the weekly sports activity or muscle CSA at the forearm or calf after 1 year of therapy in the model did not alter any of the observed changes over time in bone or body composition. Weekly sports activity at year one was however positively associated with trabecular vBMD, cortical bone area, bone size (periosteal and endosteal circumference), and polar SSI at the radius and tibia and nearly all areal bone parameters at all measured sites and the body lean mass, grip strength, and muscle area at the forearm and calf (all *p*<0.05). The latter muscle mass parameters decreased less in time with a higher weekly sports activity. No interactions of sports activity with year of visit were noted. Positive relationships were also found between muscle CSA at the forearm and calf at 1 year of therapy and areal and volumetric bone parameters (all *p*≤0.004).

Adjusting for the 25(OH)D status and PTH (at year one) did not alter the observed effects in bone over time. All bone turnover markers at year one were negatively associated with aBMD at the spine, whole body, and total hip (all *p*≤0.026) as well as cortical bone area, cortical thickness, and SSI (radius and tibia, all *p*≤0.038).

Fat body mass after the first year was significantly associated with BMC and aBMD at the femoral neck, total hip and radius, whole body BMC (all *p*<0.05), but adjustment for fat mass did not change the observed evolution in any of the bone parameters.

The effect of initial anti-androgens in monotherapy for a short period (IAAM) versus anti-androgens plus estrogens from start (AA + E)

The group who had anti-androgens in monotherapy for a median of 25 weeks before the association of estrogens (IAAM, *n*=18) were a median of 10 years older than those without (AA + E, *n*=31) (median age 27 vs. 37 years, *p*=n.s.). Body weight, height, BMI, areal and volumetric bone parameters, and body composition were similar in both groups at baseline (data not shown). Total hip aBMD, P1NP, CTX, IGF1, and 25(OH)D were higher in the younger subjects of



AA + E-group (all  $p < 0.05$ ), which remained significant after adjustment for age, pack years, serum LH, and T.

In the first year of CSH, bone turnover markers increased in the IAAM-group, whereas a decrease was observed in the AA + E-group (significant interaction of type of treatment protocol and P1NP,  $p = 0.012$ ) (Fig. 2). Body fat mass ( $p = 0.040$ ), calf fat CSA ( $p = 0.006$ ), SHBG ( $p = 0.017$ ), and leptin ( $p = 0.035$ ) had a greater increase in the AA + E-group (Fig. 2). Apart from the latter differences, adjustment for treatment protocol did not influence the described changes in body composition and bone parameters under cross-sex hormonal therapy (data not shown).

## Discussion

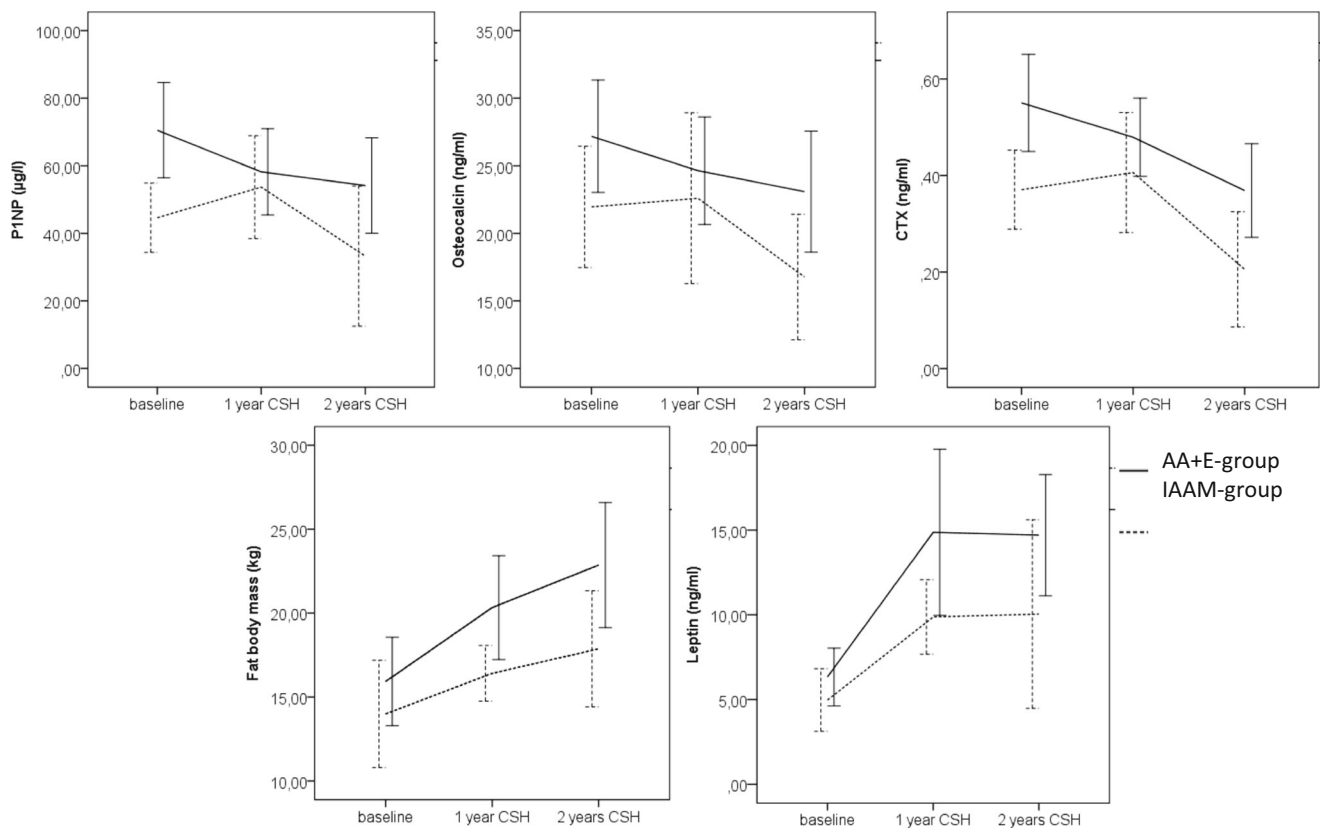
To our knowledge, this is the first prospective study on bone geometry in trans women, using pQCT, before and after 1 and 2 years of CSH. Studies in trans women provide a unique opportunity to examine the effects of sex steroids independent of sex chromosome determinants. We found that trans women, before any kind of hormonal therapy and sex reassignment surgery, already have a lower aBMD, a smaller bone size, and a lower muscle mass compared with age-matched control men. During the first 2 years of CSH, the bone turnover markers decreased and aBMD increased significantly. Trabecular and cortical bone parameters and bone size remained mainly stable during CSH in trans women.

The lower aBMD and lean body mass and higher fat mass in trans women before the start of any kind of therapy is in line with in a Norwegian study [11] and confirms our earlier report on trans women before CSH, which consisted of a smaller subgroup (only trans women at the age of peak bone mass) of the currently included trans women [9]. The lower weekly sports activity could have contributed to the lower muscle mass and strength and to a lower peak bone mass of trans women and both are important factors in building stronger bones during childhood [26] and adolescence [3, 27]. In addition, the lower 25(OH)D status and higher PTH could have contributed to the lower aBMD in trans women versus control males prior to CSH [28, 29]. The negative influence of smoking in bone [30] is not likely to explain the differences in bone between trans women and control men as the prevalence of active smokers and pack years is similar in both groups. Earlier research from our group showed lower aBMD, trabecular vBMD, and smaller bone size in relation to lower muscle mass and strength after a median of 8 years of CSH and SRS compared with male controls [7, 8]. One could hypothesize that the observed smaller bone geometry and lower aBMD and vBMD versus control men might have been present before the start of CSH and SRS due to differences in lifestyle.

Our prospective results are in line with the previous research using classical bone densitometry, which also found an maintained aBMD after 2 or more years of CSH [11, 13, 14, 18] or increase in aBMD at the lumbar spine after a minimum of 2 years of CSH [10, 12, 15–17]. In the latter studies, higher dosages of estrogens were used than the currently used protocol: 17-beta estradiol valerate 6 mg daily orally [12], ethinyl estradiol 35–100  $\mu\text{g}$  daily orally [16], 10 mg estradiol valerate IM/10 days [13], or combinations [17] with [12, 13, 16] or without anti-androgens or gonadectomy [15].

A first explanation for the stable bone geometry and increased aBMD at the lumbar spine, femoral neck, radius, and whole body can be found in estrogen therapy, as estrogens are known to slow down bone resorption through direct and indirect effects on osteoclast formation, activity, and lifespan and inhibition of osteocyte apoptosis and maintain bone formation through direct effects on osteoblasts [31]. Lower bone resorption was indeed observed after 1 and 2 years of CSH in our group of trans women and in other cohorts [11, 14, 17, 18]. In prostate cancer patients with or without androgen deprivation therapy (ADT), estrogen (but not testosterone) was inversely associated with bone turnover markers [32] and estrogen therapy seemed to protect bones and reduce bone turnover [33, 34]. Furthermore, treatment with selective estrogen-modulator toremifene in prostate cancer patients on ADT reduced fracture incidence and bone turnover rates [35]. We also observed a decreased P1NP after 2 years of CSH, which was also previously observed in trans women using CSH [7, 11, 18]. The decreased bone turnover markers in trans women after 2 years is in agreement with a short-term experiment with sex steroid suppression in adult men followed by selective replacement of either estrogen, testosterone, or both [36]. Increased bone resorption in the absence of the two hormones and no changes in men receiving both hormones was observed [36], and this is also supported by the increased CTX and P1NP after 1 year in the IAAM-group of trans women who underwent a short period of hypogonadism without estrogen replacement. When replacing separately both hormones in the trial, the reduction in bone resorption was much larger with estrogen than with testosterone. Therefore, it was concluded that it is primarily estrogen rather than testosterone that regulates the process of bone resorption [36], which is in line with our results. We hypothesize that estrogen therapy induced a lower bone turnover leading to an increase in aBMD in trans women (by estrogen-mediated filling of the remodeling space), as supported by the observed inverse association of the bone turnover markers at year one with the changes in aBMD during CSH.

Secondly, we observe a preserved bone mass and bone geometry from baseline, despite the hypoandrogenic status and drastic loss of muscle mass during treatment. Weekly sports activity in spite of the net muscle loss still had a clear positive influence on areal and volumetric bone parameters and bone size and even on the increase in aBMD after 1 year at the total body.



**Fig. 2** Evolution of bone turnover markers, leptin, and total body fat mass in the AA + E-group and the IAAM-group after 1 and 2 years of CSH

Furthermore, the increased 25(OH)D and decreased in PTH is mostly likely due to counseling during treatment and prescription of vitamin D supplements. Recently, vitamin D supplementation in adults has been found to have a weighted mean difference of  $-0.3$  to  $0.8$  % change in aBMD [37], which is lower than the observed increases in aBMD in our study. No associations with 25(OH)D, PTH, and bone parameters were found, so it seems unlikely that merely vitamin D supplementation would responsible for the observed increased aBMD in trans women.

Based on observations of positive associations of endogenous estrogen levels with vBMD and negative associations with the endosteal circumference in radius and/or tibia in large male cohorts, one might have expected an estrogen-mediated increase in cortical vBMD or a narrowing of the endosteal cortex [5, 38]. We found, however, stable volumetric bone measurements over time in trans women. Several explanations can be put forward. Firstly, the follow-up might have been too short to detect bone geometry changes. Although pQCT is considered to have high reproducibility [39], a recent paper assessing monitoring time intervals for longitudinal studies in postmenopausal women suggested a minimum interval of 2–6 years for changes at the radius and tibia [40]. Secondly, the group might have been too small to detect bone geometry changes. Positioning is extremely important in pQCT measurements, and special care has been taken to account for this (single operator, thorough positioning, scout view), although

this could introduce extra variability, which is less in aBMD [41]. Another explanation might be that classical bone densitometry using DXA is two-dimensional and can be influenced by soft tissue superposition, whereas pQCT is not. Trans women indeed gain fat mass during follow-up. Large changes in body weight, BMI, and fat percentage can induce artefacts [42]. Adjustment of our models for total body fat mass did however not change the observed increase in aBMD over time and aBMD normalized for the respective regional fat mass showed a similar increase after 1 and 2 years of CSH. Bone turnover markers were also correlated with aBMD changes, suggesting a true effect on bone mass.

The stable areal and volumetric bone parameters support that the current treatment protocol with a lower dosage of estrogens and physiological female serum estradiol levels is a safe protocol for bone protection even with the combined use of cyproterone acetate 50 mg daily. Furthermore, treatment with anti-androgens alone for a short period before the combination with estrogen therapy does not seem to have an impact on bone compared with combined anti-androgens and estrogen treatment. Nonetheless, bone turnover markers did increase during the first year in the IAAM-group, whereas a decrease was observed in the AA + E-group. Given the observed high prevalence of osteoporosis before hormonal therapy and detrimental effects of long-term hypogonadism [32], prolonged used of anti-androgens in monotherapy is not

advisable. The higher bone turnover markers and IGF1 in treatment protocol AA + E versus IAAM at baseline were probably due to the age difference [29, 43].

IGF1 was higher in the first year of CSH in trans women in both transdermal as oral estrogen users. This increase seemed estrogen-modulated as increased IGF1 has also been described in orchidectomized mice using estradiol, whereas no changes were seen in orchidectomized mice with or without DHT treatment [44]. Serum IGF1 levels were independent of the route of administration of estrogens, in contrast with the previously described effects in postmenopausal women on estrogen therapy which could also be contributed to the type of estrogens used [45].

The observed body composition changes with increased fat mass and decreased muscle mass and strength are in line with the previous results in trans women using MRI [6] and DXA [7, 13] and pQCT [7] as is the increased serum leptin [46]. In particular, we observed more subcutaneous fat mass and an increased hip circumference, already after 1 year of CSH. We also observed a slower increase in fat mass and serum leptin in trans women who initially received cyproterone acetate for a short period alone, indicating the role of estrogens in the accrual of fat mass. A recently published short-term trial in adult men using sex steroid suppression followed by the replacement of testosterone with or without inhibition of estrogen synthesis by aromatase inhibitors however showed that estrogen deficiency contributed to the increased fat mass, independent of the dose of testosterone substitution [47]. A potential explanation of our findings may be found in the higher serum estradiol levels compared with males as divergent effects of estrogens depending on estrogen dosage cannot be ruled out.

Our study has several limitations. Firstly, gender dysphoria is a rare condition, and large-scaled samples are difficult to obtain with implications for power. Moreover, we describe trans women of a broad age range: some might not have fully reached peak bone mass, while others are already middle-aged. Adjustment for age did, however, not alter any of the results. Secondly, the effect of aging cannot be ruled out as we did not follow the control group prospectively. The changes in markers of bone turnover are however greater than expected during aging [43, 48]. Thirdly, trans women use pharmacological doses of estrogen and the observed effects can differ of those of endogenous estrogen in males. The strengths of our study are the standardized treatment protocol, the state of the art methods to measure sex steroids (tandem mass spectrometry), and the relatively large sample despite the rarity of the condition.

## Conclusion

We conclude that whereas trans women have a lower aBMD and cortical bone size compared with control men before any

kind of hormonal treatment, probably related to a different, more sedentary lifestyle, their skeletal status is well preserved during CSH treatment. During 2 years of CSH treatment, bone turnover decreased, aBMD increased, and bone geometry was stable in trans women, despite a substantially decreased muscle mass and strength, which is a further illustration of the major role of estrogens for preservation of the integrity of the male skeleton. Nevertheless, a longer follow-up might be needed to further detail the effects of CSH treatment on bone geometry.

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**Conflicts of interest** Eva Van Caenegem, Katrien Wierckx, Youri Taes, Thomas Schreiner, Sara Vandewalle, Kaatje Toye, Jean-Marc Kaufman, and Guy T'Sjoen declare that they have no conflict of interest.

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