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Bone Mineral Density Increases in Trans Persons After 1 Year of Hormonal Treatment: A Multicenter Prospective Observational Study

Chantal M Wiepjes,[1] Mariska C Vlot,[1,2] Maartje Klaver,[1] Nienke M Nota,[1] Christel JM de Blok,[1] Renate T de Jongh,[1] Paul Lips,[1] Annemieke C Heijboer,[2] Alessandra D Fisher,[3] Thomas Schreiner,[4] Guy T'Sjoen,[5] and Martin den Heijer[1]

[1]Department of Internal Medicine and Center of Expertise on Gender Dysphoria, VU University Medical Center, Amsterdam, the Netherlands
[2]Department of Clinical Chemistry, VU University Medical Center, Amsterdam, the Netherlands
[3]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical, and Biomedical Sciences, University of Florence, Florence, Italy
[4]Department of Endocrinology, Oslo University Hospital, Oslo, Norway
[5]Department of Endocrinology, Center for Sexology and Gender, Ghent University Hospital, Ghent, Belgium

ABSTRACT

Sex steroids are important determinants of bone acquisition and bone homeostasis. Cross-sex hormonal treatment (CHT) in transgender persons can affect bone mineral density (BMD). The aim of this study was to investigate in a prospective observational multicenter study the first-year effects of CHT on BMD in transgender persons. A total of 231 transwomen and 199 transmen were included who completed the first year of CHT. Transwomen were treated with cyproterone acetate and oral or transdermal estradiol; transmen received transdermal or intramuscular testosterone. A dual-energy X-ray absorptiometry (DXA) was performed to measure lumbar spine (LS), total hip (TH), and femoral neck (FN) BMD before and after 1 year of CHT. In transwomen, an increase in LS (+3.67%, 95% confidence interval [CI] 3.20 to 4.13%, $p < 0.001$), TH (+0.97%, 95% CI 0.62 to 1.31%, $p < 0.001$), and FN (+1.86%, 95% CI 1.41 to 2.31%, $p < 0.001$) BMD was found. In transmen, TH BMD increased after 1 year of CHT (+1.04%, 95% CI 0.64 to 1.44%, $p < 0.001$). No changes were observed in FN BMD (–0.46%, 95% CI –1.07 to 0.16%, $p = 0.144$). The increase in LS BMD was larger in transmen aged ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, $p = 0.001$) compared with transmen aged <50 years (+0.68%, 95% CI 0.19 to 1.17%, $p = 0.007$). In conclusion, BMD increased in transgender persons after 1 year of CHT. In transmen of postmenopausal age, the LS BMD increased more than in younger transmen, which may lead to the hypothesis that the increase in BMD in transmen is the result of the aromatization of testosterone to estradiol. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: SEX STEROIDS; DXA; TRANSGENDER; OSTEOPOROSIS; CROSS-SEX HORMONAL TREATMENT

Introduction

Gender dysphoria (GD) is defined as the sufferance related to an incongruence between one's experienced and one's assigned gender of a duration of at least 6 months.[1] Persons who experience gender-related distress might desire gender-affirming treatment with sex steroids. Sex steroids are also important determinants of bone acquisition and bone homeostasis.

In natal men, testosterone stimulates the process of periosteal apposition, leading to a greater cortical bone size and wider bones than in women.[2,3] Men with aromatase deficiency have been found to have lower bone mass, indicating that estrogen is an important regulator of bone acquisition in men.[4–6] However, the effect of testosterone on bone mineral density (BMD) is less clear.

In natal women, estrogen inhibits periosteal apposition but stimulates endosteal bone formation.[7] Loss of estrogen at menopause leads to an increased osteoclastic activity and therefore accelerated bone loss.[8,9] In women with polycystic ovary syndrome with hyperandrogenism, an increased trabecular BMD has been found, even after adjustment for body mass.[10] In addition, women with complete androgen insensitivity syndrome (46,XY karyotype) have been found to have lower BMD,[11] which might indicate that testosterone also regulates BMD in women.

Cross-sex hormonal treatment (CHT) in transgender persons causes changes in gonadal hormone levels in order to achieve desired body changes. Transwomen (male-to-female transgenders) receive estrogen and anti-androgens, whereas transmen (female-to-male transgenders) are treated with testosterone. Although a few studies have investigated the

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Address correspondence to: Martin den Heijer, MD, PhD, Department of Internal Medicine, Section Endocrinology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail: m.denheijer@vumc.nl
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effects of CHT on BMD, most of these studies were cross-sectional or had small sample sizes with inconclusive or contradictory results.[12–19] The aim of this study is therefore to investigate, in a large multicenter prospective cohort, whether CHT influences BMD in transwomen and transmen during the first year of treatment.

Materials and Methods

Study design and population

This study is part of the European Network for Investigation of Gender Incongruence (ENIGI) study, a multicenter prospective observational study performed for its endocrine part in Ghent (Belgium), Oslo (Norway), Florence (Italy), and Amsterdam (the Netherlands).[20,21] Trans persons aged 18 years and older who started CHT between 2010 and April 2016 after a confirmed GD diagnosis[1] were asked to participate in the study. Exclusion criteria were prior cross-sex hormone use, psychological vulnerability, the occurrence of protocol deviations (eg, the use of gonadotropin-releasing hormone agonist or spironolactone), or insufficient knowledge of the native language. For the present analysis, only persons who completed the first year of CHT were included. Because the participating centers used different types of dual-energy X-ray absorptiometry (DXA) scanners (Ghent and Amsterdam: Hologic Discovery A [Hologic Inc, Bedford, MA, USA]; Florence: Hologic Delphi A; Oslo: Lunar [GE Lunar, Madison, WI, USA]), non-comparable BMD values were obtained. Consequently, only persons with BMD measurements performed in either Ghent or Amsterdam were included in the analyses. Persons who did not have a baseline DXA scan or had a baseline DXA scan outside the window of 3 months before or 1 month after the start of CHT were excluded. In addition, persons who did not have a follow-up DXA scan or had a follow-up scan before 10 or after 14 months after the start of CHT were excluded.

All trans persons were treated according to the Standards of Care Guidelines of the World Professional Association for Transgender Health (WPATH).[22] Transwomen received cyproterone acetate (50 to 100 mg daily) combined with oral estradiol valerate (2 to 4 mg daily) or an estradiol patch (50 to 100 μg twice a week). Transmen were treated with testosterone gel (50 mg daily), intramuscular testosterone esters (250 mg every 2 weeks), or intramuscular testosterone undecanoate (1000 mg every 12 weeks).

This study was conducted in accordance with the Declaration of Helsinki, and the overall study protocol was approved by the Ethical Review Board of the Ghent University Hospital, Belgium. In the other participating centers, approval for participation was also obtained by the local ethical committees. Study participants gave informed consent according to institutional guidelines.

Clinical data collection

During the first year of treatment, trans persons visited the outpatient endocrine unit once every 3 months, where they reported their medical history, medication use, smoking habits (in cigarettes per day), and alcohol use (in units per week). Physical examination was performed by measuring body weight (in kilograms) and height (in meters) in indoor clothing without shoes.

Biochemical assessment

Blood samples were drawn at baseline, after 3 months, and after 12 months of CHT, all after overnight fasting. In Amsterdam, estradiol was measured using a competitive immunoassay (Delfia, PerkinElmer, Turku, Finland) with an interassay coefficient of variation (CV) of 10% to 13% and a lower limit of quantitation (LOQ) of 20 pmol/L until July 2014. After July 2014, estradiol was measured using a LC-MS/MS (VUmc, Amsterdam, the Netherlands) with an interassay CV of 7% and a LOQ of 20 pmol/L. For conversion of the Delfia values, the formula LC-MS/MS = 1.60 * Delfia−29 was used. Testosterone was measured using a radioimmunoassay (RIA) (Coat-A-Count, Siemens, Los Angeles, CA, USA) with an interassay CV of 7% to 20% and a LOQ of 1 nmol/L until January 2013. Thereafter, testosterone was measured using competitive immunoassay (Architect, Abbott, Abbott Park, IL, USA) with an interassay CV of 6% to 10% and a LOQ of 0.1 nmol/L. The RIA values were converted to the competitive immunoassay values. For testosterone levels below 8 nmol/L, the formula Architect = 1.1 * RIA + 0.2 was used; for testosterone levels above 8 nmol/L, the formula Architect = 1.34 * RIA − 1.65 was used. 25-hydroxyvitamin D (25(OH)D) was measured using LC-MS/MS as described previously.[23]

In Ghent, estradiol was measured using a E170 Modular (Gen II, Roche Diagnostics, Mannheim, Germany) until March 19, 2015. Thereafter, estradiol was measured using a E170 Modular (Gen III, Roche Diagnostics), with an interassay CV of 3.2% and a LOQ of 25 pg/mL (92 pmol/L). For conversion of estradiol values measured before March 19, 2015, the formula Gen III = 6.687940 + 0.834495 * Gen II was used. E170 Modular (Roche Diagnostics, Germany) was used to measure testosterone (Gen II) and 25(OH)D, with an interassay CV of 2.6% and a LOQ of 10 ng/dL (0.4 nmol/L) for testosterone, and an interassay CV of 6.7% and a LOQ of 5 ng/mL (12.5 nmol/L) for 25(OH)D.

Bone mineral density

DXA was performed at baseline and after one year CHT. In both Ghent and Amsterdam, a Hologic Discovery A was used (Hologic Inc). In Ghent, software version 12.7.3.1 was used. In Amsterdam, the software version was updated from 13.3 to 13.5.3 in July 2015. The Hologic Statement of Equivalency allowed for comparison of absolute BMD values between the different scan dates and times. Absolute BMD values were obtained for lumbar spine (LS, L_1 to L_4), non-dominant total hip (TH), and femoral neck (FN).

Statistical analysis

The baseline characteristics of both transwomen and transmen are reported as medians (interquartile range [IQR]) or percentages. Differences between included and excluded persons were analyzed using independent *t* tests (or Wilcoxon rank-sum test in case of non-normal distribution) or chi-square tests. Difference between BMD values obtained in Amsterdam and Ghent were analyzed using an independent *t* test. Data were log-transformed before further analysis in case of non-normal distribution. For all analyses, individuals with missing values were excluded. Percentage changes in LS, TH, and FN BMD after 1 year of CHT were calculated for every person, and as these were normally distributed continuous variables, linear regression analyses were performed to generate the mean percentage changes with corresponding 95% confidence intervals (CI) and *p* values.

Analyses were stratified for age groups (18–20, 21–29, 30–49, and ≥50 years) in order to stratify for accrual of peak bone mass, peak bone mass, age-related decrease in bone mass, and, in transmen, for postmenopausal state. Analyses were stratified for the use of vitamin D supplementation, as persons with vitamin D deficiency were treated with vitamin D supplementation. For analyses on different estradiol or testosterone administration routes, only transmen and transwomen who used the same administration route and dose during the entire year were included. Difference between age groups, vitamin D supplementation, or administration routes were analyzed using linear regression analyses with percentage change in BMD as outcome variable and age groups, vitamin D supplementation, or administration routes as categorical independent variables, respectively. To adjust for possible mediating factors, linear regression analyses were performed between percentage change in BMD and change in body weight, and between percentage change in BMD and change in cigarette and alcohol use. The change in alcohol or cigarette use was calculated as the percentage difference in number of cigarettes or units of alcohol between baseline and the 12-month visit. To investigate the influence of the serum concentrations of sex steroids on BMD change, linear regression analyses were performed between the change in BMD and the mean serum estradiol or testosterone levels after 3 to 12 months of CHT, and were analyzed separately for Amsterdam and Ghent, as no conversion formulas were available for the sex steroid assays. Sensitivity analyses were performed by repeating the analyses after exclusion of all persons with comorbidities or medication use with possible influence on BMD. Analyses were performed with STATA Statistical Software (StataCorp, College Station, TX, USA), version 13.1.

Results

General characteristics

The flowchart of the inclusion of participants in both centers is shown in Fig. 1. In total, 231 transwomen and 199 transmen were included for analyses. The baseline characteristics are shown in Table 1. Except for a younger age in excluded transmen (median age 22 years, IQR 20 to 27) compared with included transmen (median age 24 years, IQR 21 to 31, difference $p = 0.004$), no baseline differences were found in weight, BMI, ethnicity, smoking habits, alcohol use, estradiol levels, testosterone levels, and vitamin D levels between persons excluded and included for analyses.

Bone mineral density

The mean time between two DXA scans was 12 months (range 10 to 14 months). No differences in baseline BMD and 12-month BMD between the two included centers were found (Table 2).

In transwomen, 1-year CHT increased BMD of the LS (+3.67%, 95% CI 3.20 to 4.13%, $p < 0.001$), TH (+0.97%, 95% CI 0.62 to 1.31%, $p < 0.001$), and FN (+1.86%, 95% CI 1.41 to 2.31%, $p < 0.001$). In transmen, 1-year CHT increased LS and TH BMD (+0.86%, 95% CI 0.38 to 1.35%, $p = 0.001$, and +1.04%, 95% CI 0.64 to 1.44%, $p < 0.001$, respectively). No changes were

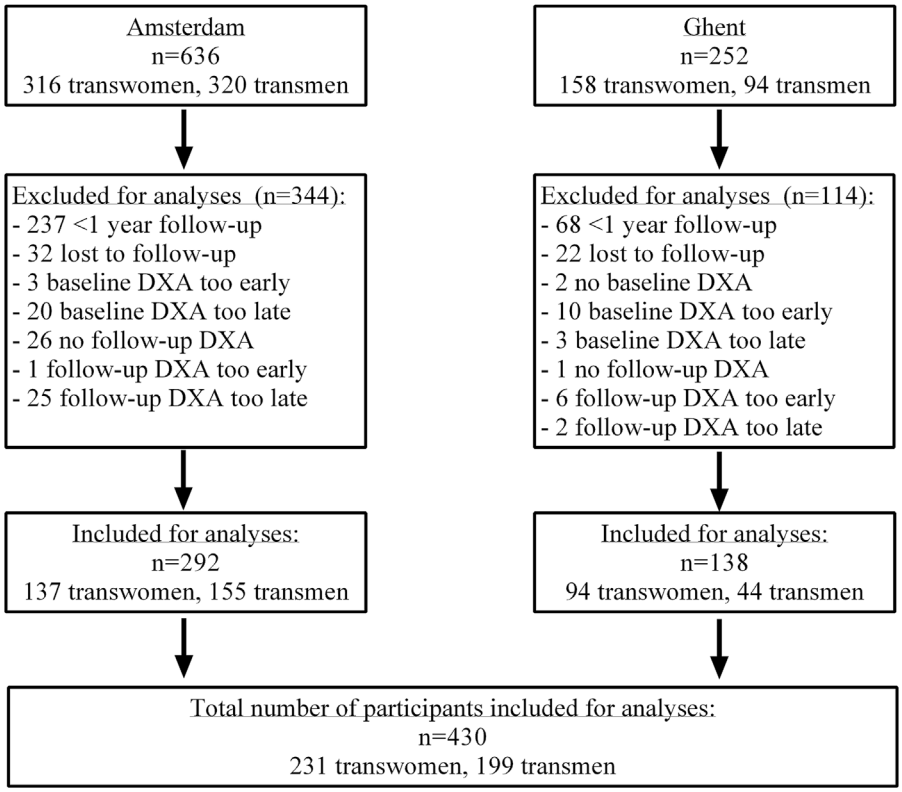

Fig. 1. Flowchart of the inclusion of participants in the present study. DXA = dual-energy X-ray absorptiometry.

Table 1. Baseline Characteristics of Transwomen and Transmen

	Transwomen ($n = 231$)	Transmen ($n = 199$)
Age (years [IQR])	28 (23 to 42)	24 (21 to 31)
Ethnicity (% white)	97.4	94.0
BMI (kg/m^2 [IQR])	22.5 (20.5 to 26.1)	23.9 (21.3 to 28.8)
Tobacco use (% yes)	23.5	29.3
Cigarettes per day (IQR)	10 (5 to 12)	10 (5 to 15)
Alcohol use (% >7 units per week)	6.1	4.6
Biochemical results Amsterdam (IQR)[a]		
Estradiol levels (pmol/L)	105 (82 to 133)	169 (61 to 377)
Testosterone levels (nmol/L)	18.5 (14.0 to 23.0)	1.3 (1.0 to 1.7)
25(OH) vitamin D levels (nmol/L)	38 (24 to 57)	54 (31 to 77)
Biochemical results Ghent (IQR)[b]		
Estradiol levels (pmol/L)	114 (95 to 135)	155 (114 to 301)
Testosterone levels (nmol/L)	19.0 (13.5 to 22.2)	1.1 (0.7 to 1.3)
25(OH) vitamin D levels (nmol/L)	34 (22 to 52)	54 (36 to 72)

IQR = interquartile range; BMI = body mass index.
Data are expressed as median (interquartile range) or percentages.
[a]Transwomen $n = 135$; transmen $n = 137$.
[b]Transwomen $n = 93$; transmen $n = 41$.

observed in FN BMD (–0.46%, 95% CI –1.07 to 0.16%, $p = 0.144$) (Fig. 2).

Effects of weight change on BMD change

Transwomen had a mean weight increase of 2.4 kg (95% CI 1.5 to 3.2 kg, $p < 0.001$). The gain in LS BMD did not change after adjustment for change in body weight (+3.64%), but an attenuation of TH (+0.65%) and FN (+1.52%) BMD change was found. Transmen had a mean weight increase of 2.0 kg (95% CI 1.2 to 2.8 kg, $p < 0.001$). After adjustment for change in body weight, the mean increase of LS (+0.90%) and FN (–0.87%) BMD did not change, but an attenuation of TH (+0.86%) BMD change was found.

Effects of change in cigarette or alcohol use on BMD change

A total of 52.1% and 27.5% of the transwomen who either smoked cigarettes or drank alcohol at baseline quit smoking and stopped using alcohol, respectively. Adjusting the analyses for percentage change in cigarette or alcohol use did not change the results of LS (+3.80%), TH (+0.92%), or FN (+1.91%) BMD change. Of the transmen, 45.6% and 28.7% who

Table 2. Baseline and 1 Year BMD in Lumbar Spine, Total Hip, and Femoral Neck for Transwomen and Transmen, Stratified per Center

	Amsterdam	Ghent	Total	p Values
Transwomen	($n = 137$)	($n = 94$)	($n = 231$)	
Lumbar spine BMD				
Baseline	0.966 (0.138)	0.983 (0.143)	0.972 (0.140)	0.362
1 year	1.001 (0.137)	1.015 (0.143)	1.007 (0.139)	0.470
Total hip BMD				
Baseline	0.938 (0.133)	0.939 (0.135)	0.938 (0.134)	0.942
1 year	0.948 (0.133)	0.946 (0.138)	0.947 (0.135)	0.905
Femoral neck BMD				
Baseline	0.798 (0.124)	0.799 (0.133)	0.799 (0.128)	0.960
1 year	0.814 (0.127)	0.811 (0.135)	0.813 (0.130)	0.834
Transmen	($n = 155$)	($n = 44$)	($n = 199$)	
Lumbar spine BMD				
Baseline	1.030 (0.124)	1.022 (0.114)	1.028 (0.121)	0.705
1 year	1.039 (0.126)	1.027 (0.108)	1.037 (0.122)	0.554
Total hip BMD				
Baseline	0.954 (0.113)	0.945 (0.130)	0.952 (0.116)	0.646
1 year	0.963 (0.114)	0.958 (0.129)	0.962 (0.117)	0.815
Femoral neck BMD				
Baseline	0.837 (0.116)	0.833 (0.112)	0.836 (0.115)	0.805
1 year	0.831 (0.116)	0.834 (0.117)	0.832 (0.116)	0.908

BMD = bone mineral density.
Numbers represent absolute bone mineral density in g/cm^2 (standard deviation). Independent t tests were performed between the Amsterdam and Ghent data.

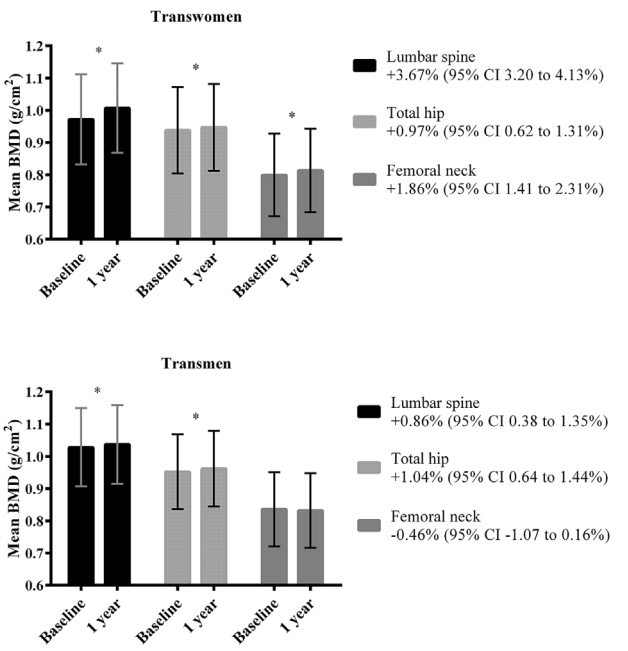

Fig. 2. Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen after 1 year of cross-sex hormonal treatment. Data represent mean baseline and mean 1-year BMD with standard deviation. Percentage changes in BMD were calculated for every person, and because these were normally distributed continuous variables, linear regression analyses were performed to generate the mean percentage changes with corresponding 95% confidence intervals (CI), which are shown in the legends on the right. $^{*}p \leq 0.001$. BMD = bone mineral density.

either smoked cigarettes or drank alcohol at baseline quit smoking and stopped using alcohol, respectively. No changes were observed in LS (+0.93%), TH (+1.24%), and FN (–0.50%) BMD change after adjustment for change in cigarette or alcohol use.

The effect of age on BMD change

As shown in Fig. 3, the change in LS, TH, or FN BMD in transwomen did not vary in different age groups, except for a larger increase in LS BMD in transwomen of aged 18 to 20 years compared with transwomen of 30 to 49 years. In transmen, LS BMD increased more in persons aged ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, $p = 0.001$) compared with persons younger than 50 years (+0.68%, 95% CI 0.19 to 1.17%, $p = 0.007$). The geometric mean estradiol levels increased from 14 pmol/L to 150 pmol/L (+949%, 95% CI 304 to 2629%, $p < 0.001$) in persons aged ≥50 years compared with no increase in persons younger than 50 years (158 pmol/L to 194 pmol/L; +22%, 95% CI –2 to 53%, $p = 0.078$).

The effect of vitamin D supplements on BMD change

As shown in Fig. 4, LS and FN BMD increased more in transwomen who used vitamin D supplements compared with those who did not use supplements. No differences were found in TH BMD change. In transmen, no differences in LS, TH, and FN BMD change were found between persons with or without vitamin D supplementation.

Correlation of BMD change with sex hormone levels

After 3 to 12 months of CHT, the estradiol levels of transwomen in Amsterdam were correlated with LS (per 100 pmol/L: +0.95%, 95% CI 0.34 to 1.56%, $p = 0.003$), TH (per 100 pmol/L: +0.48%, 95% CI 0.04 to 0.93%, $p = 0.034$), and FN (per 100 pmol/L: +0.83%, 95% CI 0.31 to 1.36%, $p = 0.002$) BMD change. The estradiol levels after 3 to 12 months in transwomen in Ghent were correlated with LS (per 100 pmol/L: +0.87%, 95% CI 0.27 to 1.47%, p=0.005), but not with TH (per 100 pmol/L: +0.40%, 95% CI –0.12 to 0.92%, $p = 0.126$) or FN (per 100 pmol/L: +0.09%, 95% CI –0.67 to 0.85%, $p = 0.814$) BMD change. In transmen in Amsterdam and Ghent, estradiol levels after 3 to 12 months were not correlated with BMD change. Because testosterone levels after 3 to 12 months were suppressed in transwomen, no correlation analyses could be performed. In transmen, testosterone levels after 3 to 12 months were not correlated with LS, TH, and FN BMD change.

The effect of estradiol or testosterone administration routes on BMD change

LS, TH, or FN BMD change did not differ between transdermal estradiol or oral estradiol valerate use in transwomen (Fig. 4). Serum estradiol levels were comparable between transdermal estradiol (ref) and oral estradiol valerate (difference –7 pmol/L, 95% CI –50 to 36 pmol/L, $p = 0.754$).

In transmen, no differences in LS and TH BMD change was observed between testosterone gel, testosterone esters, or testosterone undecanoate. FN BMD change was higher in testosterone undecanoate compared with testosterone gel (Fig. 4). Testosterone levels were comparable between testosterone gel (ref) and testosterone undecanoate (–3.5 nmol/L, 95% CI –12.9 to 5.9 nmol/L, $p = 0.459$), whereas testosterone esters provided higher testosterone levels than testosterone gel (+13.0 nmol/L, 95% CI 6.2 to 19.9 nmol/L, $p < 0.001$).

Sensitivity analyses

Repeating the analyses after exclusion of all persons with bone-influencing medication (use of diuretics [$n = 6$], anti-epileptics [$n = 3$], antidepressants [$n = 58$], antipsychotics [$n = 9$], corticosteroids [$n = 15$], or bisphosphonates [$n = 1$]) or comorbidities (eating disorder [$n = 4$], alcohol abuse [$n = 4$], thyroid disease [$n = 5$], diabetes mellitus [$n = 5$], gastrointestinal disease [$n = 8$], malignancy [$n = 2$]) did not change the effect sizes (data not shown).

Discussion

This study showed that after 1 year of CHT the mean BMD increased in both transwomen and transmen, especially in lumbar spine and in transwomen. In transmen of postmenopausal age, the LS BMD increased more than in younger transmen. In both transwomen and transmen, the change in BMD could not be completely explained by a change in body weight, a change in cigarette or alcohol use, or by vitamin D supplementation.

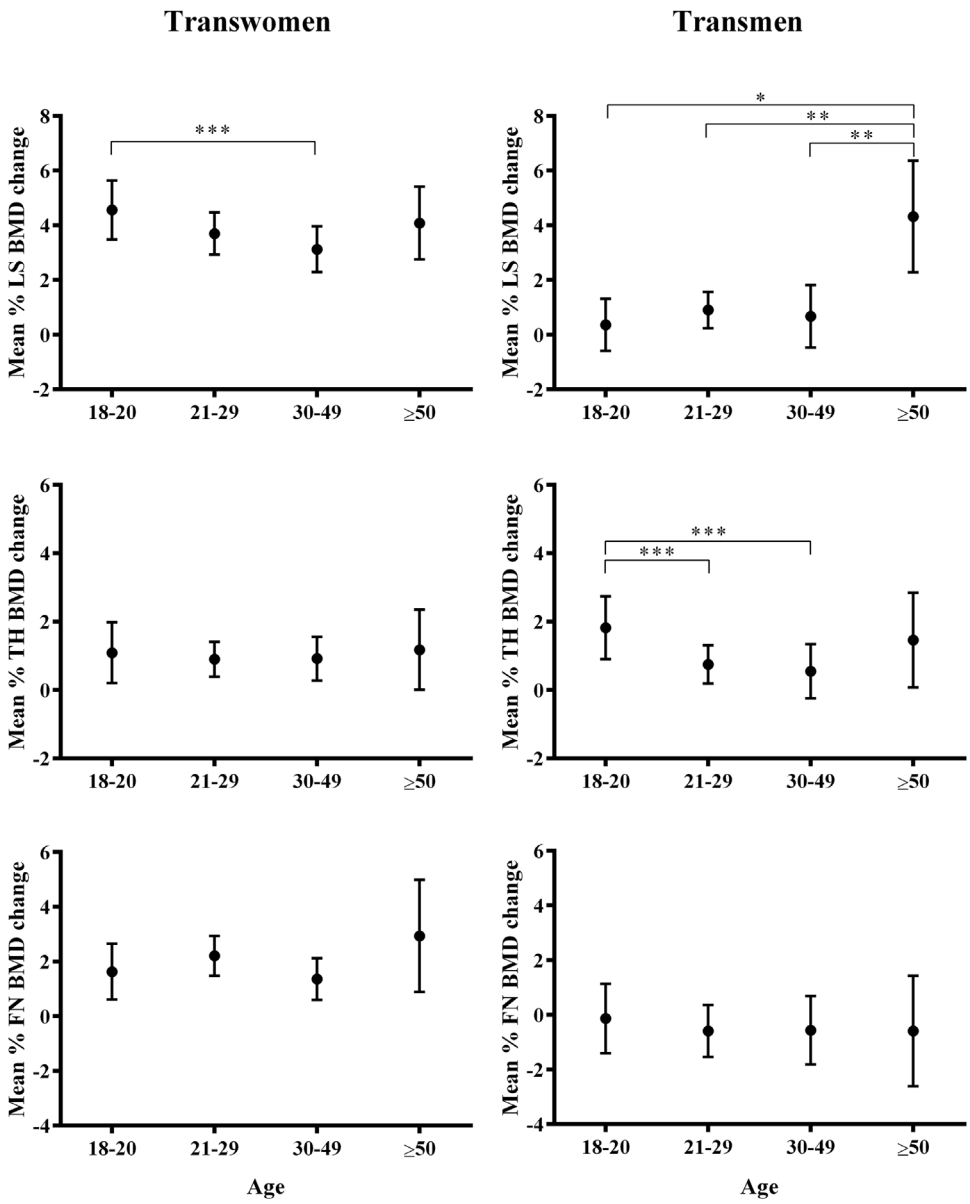

Fig. 3. Change in bone mineral density of lumbar spine, total hip, and femoral neck in transwomen and transmen, stratified for age groups. Each individual bar represents the mean increase in bone mineral density with 95% confidence interval. The age distribution in transwomen is: 18–20 ($n = 36$), 21–29 ($n = 88$), 30–49 ($n = 83$), and ≥50 ($n = 24$) years; in transmen: 18–20 ($n = 56$), 21–29 ($n = 87$), 30–49 ($n = 46$), and ≥50 ($n = 10$) years. Differences between these age groups were analyzed using linear regression analyses, with percentage change in BMD as outcome variable and age groups as categorical dependent variable. $^{*}p \leq 0.001$; $^{**}0.001 < p \leq 0.01$; $^{***}0.01 < p \leq 0.05$. LS = lumbar spine; TH = total hip; FN = femoral neck; BMD = bone mineral density.

The larger increase in BMD in transmen of postmenopausal age compared with the other age groups may be the result of decreased bone resorption due to higher levels of estradiol after aromatization of testosterone to estradiol, as serum estradiol levels were low at baseline and largely increased during CHT. These findings may lead to the hypothesis that the increase in bone mineral density in older transmen is the result of aromatization of testosterone to estradiol and therefore an increase in estradiol levels, instead of direct effects of testosterone. This is in line with Finkelstein and colleagues,[24] who demonstrated that effects of hypogonadism on bone in men are mainly due to estrogen deficiency and not to testosterone deficiency. In addition, the results are compatible with findings in older natal men, whose BMD is better correlated with bioavailable estradiol levels than with other sex steroid measures.[25] However, it is not known whether these findings can be extrapolated to transmen. The larger increase in LS BMD in transwomen aged 18 to 20 years compared with transwomen aged 30 to 49 years could be explained by the fact that the youngest group has not reached the peak bone mass yet and therefore the increase in BMD is larger.

In both transwomen and transmen, the TH BMD increase attenuated after adjustment for change in body weight,

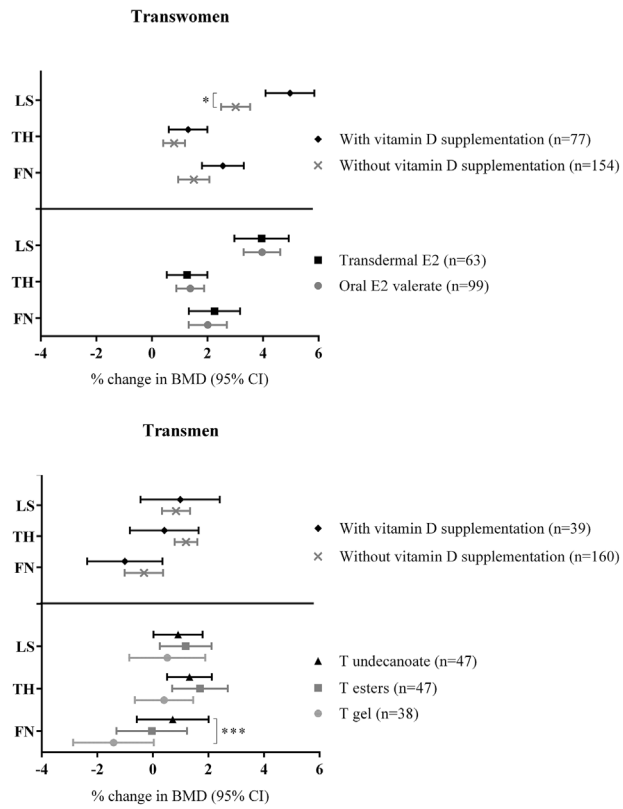

Fig. 4. Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen, stratified for vitamin D supplementation or administration routes, respectively. Linear regression analyses with change in BMD as outcome variable and vitamin D supplementation or administration route as independent variable were performed. Only persons using the same dose and administration route during the entire year were included for analyses. $^{*}p \leq 0.001$; $^{***}0.01 < p \leq 0.05$. LS = lumbar spine; TH = total hip; FN = femoral neck; BMD = bone mineral density; T = testosterone.

indicating that the increase in hip BMD is partly mediated by an increase in body weight. No differences in BMD change were observed between administration routes of estradiol or testosterone, except a small difference in FN BMD change between testosterone gel and testosterone undecanoate. However, the groups of testosterone administration routes were small and the analysis might not have enough power to detect small differences. No differences were observed in mean estradiol levels between transdermal estradiol or estradiol valerate. Testosterone esters gave higher testosterone levels compared with testosterone gel and testosterone undecanoate. However, testosterone ester therapy results in highly fluctuating serum testosterone levels, and because blood determination was independent of the last injection of testosterone esters, no representative testosterone levels were obtained from persons using testosterone esters. The current using dose of testosterone esters is thought to be similar to the dose of testosterone gel and testosterone undecanoate.[26]

Previously, small prospective studies were performed to investigate the change in BMD in transgender persons after 1 year of CHT. For transwomen, our results are comparable to most other studies, as an increase in LS BMD was found in transwomen treated with anti-androgens and estrogens[18,27,28] or treated with estrogens and gonadotropin-releasing hormone agonists.[13,29] One prospective study did not find a change in BMD in 12 transwomen.[30] Although the same results were found in most other studies, our results cannot be generalized to all parts of the world, as the included transwomen used cyproterone acetate and this is not approved for use in the United States.

We found different results for the change in BMD in transmen than described in literature. Although previous prospective studies did not find a change in LS BMD after 1 year of CHT,[14,19,27,28,30] we found a small increase in the total group and even a larger increase in the postmenopausal subgroup. This difference might be because of the small sample sizes of other studies and consequently lack of power to allow these studies to detect any differences. In addition, a subgroup analysis of the change in BMD in postmenopausal transmen has not been described before, as transmen included in previously mentioned studies were 18 to 47 years,[19] 16 to 39 years,[28] and 20 to 46 years[30] of age.

This study is a large multicenter prospective study, including transwomen and transmen with a wide range of age. All trans persons were treated according to a defined treatment protocol, and measurements were performed at baseline and during follow-up. Only a small percentage of participants were lost to follow-up (8.5%). There are also some limitations to our study. First, because of the multicenter aspect of this study, the BMD of participants was measured using different DXA devices. To compare the baseline values, Oslo and Florence were excluded for the present analyses and only Ghent and Amsterdam were included. Each individual had a baseline and follow-up BMD measurements on the same DXA device, and no differences were observed between Ghent and Amsterdam. Between the study centers, different laboratory assays were used, and within one study center, the assay was adjusted when more accurate assays became available. Conversion formulas within one center allowed for comparison of the data. However, because no conversion formulas between the centers were available, the analysis had to be stratified by center. The associations found within both centers were similar, which allows for higher generalizability of the results. Second, the study was performed during regular patient care. Data about smoking habits, alcohol use, medication use, and comorbidities were self-reported and were collected during their outpatient clinic visits. It is possible that some medication use or comorbidities were not reported, including the use of additional testosterone or estradiol preparations next to the prescribed sex hormones. Third, no data about physical exercise or calcium intake was available. Persons with low baseline BMD were advised about factors of positive influence on BMD, including exercise, calcium intake, and vitamin D supplements. Therefore, we cannot prove that the increase in BMD is solely a result of CHT. Because of ethical and practical reasons, it is not possible to add a placebo group to this study. Fourth, because this study is only performed in trans persons without a control group, we cannot rule out that passing time is partly an explanation for the change in BMD. However, because most persons had already reached the age of peak bone mass achievement at the time of inclusion in the study, the natural course of BMD

is to decrease over time. Lastly, for the present analyses, we only assessed BMD using a DXA scan, which does not provide any information about bone geometry. Further research is needed into whether changes in bone geometry can be found after CHT. However, in regular patient care, BMD is also assessed using a DXA scan; therefore, this study may be valuable for clinical practice.

One study reported a lower BMD in transwomen at the start of CHT compared with age-matched control men.[31] Hormonal treatment may influence the achievement of higher BMD on short term. A healthier lifestyle including more exercise and vitamin D exposure may also contribute to this change in BMD. Therefore, with regard to the clinical practice, monitoring bone quality in trans persons is relevant.

In conclusion, an increase in BMD in both transwomen and transmen after 1 year of CHT was found. For further research, it is desirable to investigate alterations in BMD after long-term CHT, to monitor bone turnover markers, and to add other imaging modalities to gain more insight into the actual changes in bone metabolism due to sex steroid therapy.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Study design: ADF, TS, GT, and MdH. Study conduct: GT and MdH. Data collection: CMW, MCV, MK, NMN, CJMdB, ADF, TS, GT, and MdH. Data analysis: CMW and MCV. Data interpretation: CMW and MCV. Drafting manuscript: CMW. Revising manuscript content: CMW, MCV, MK, NMN, CJMdB, RTdJ, PL, GT, and MdH. Approving final version of manuscript: CMW, MCV, MK, NMN, CJMdB, ACH, RTdJ, PL, ADF, TS, GT, and MdH. MdH takes responsibility for the integrity of the data analysis.

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