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Wiepjes, C.M.

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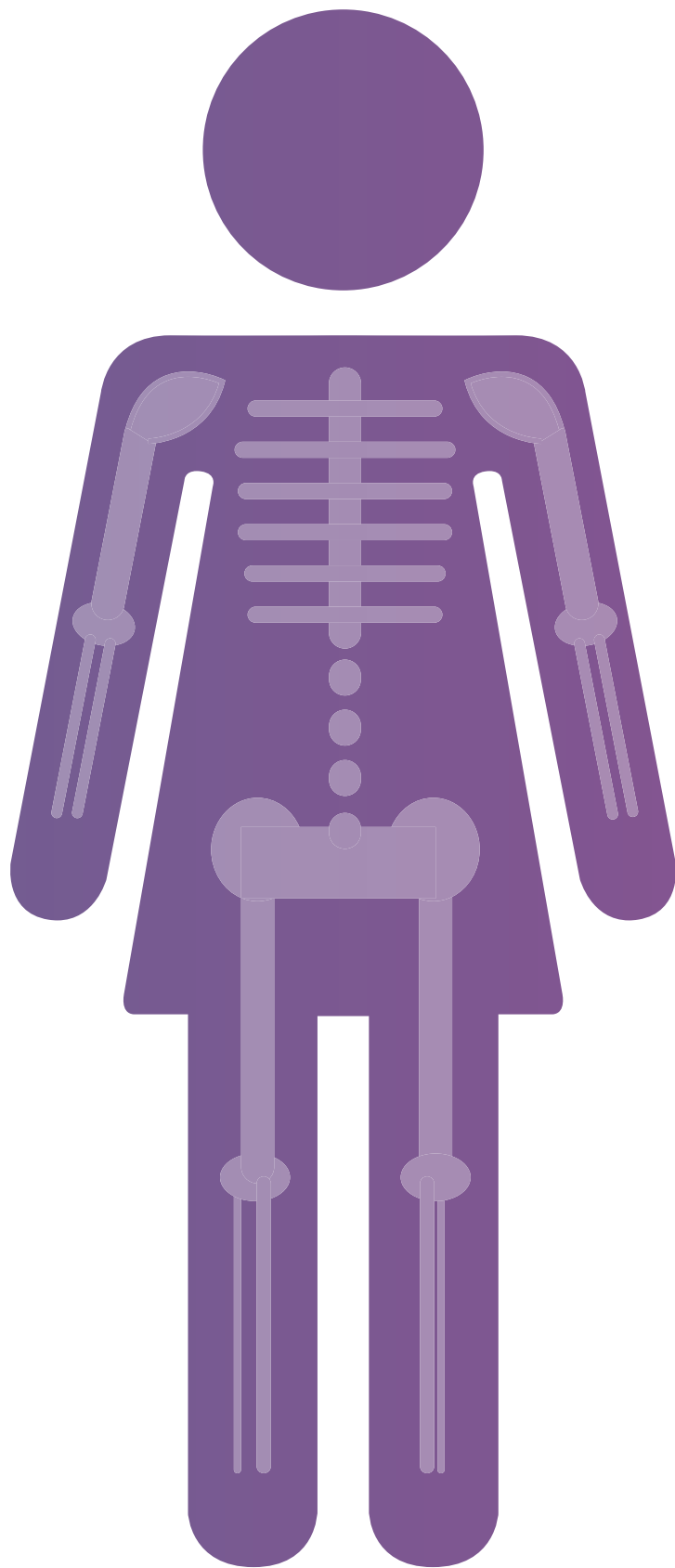
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Chapter 7

Bone safety during the first ten years of
gender-affirming hormonal treatment
in transwomen and transmen

CM Wiepjes, RT de Jongh, CJM de Blok, MC Vlot, P Lips, JWR Twisk,
M den Heijer

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Abstract

Concerns about the effects of gender-affirming hormonal treatment (HT) on bone mineral density (BMD) in transgender people exist, particularly regarding the decrease in estrogen concentrations in transmen. Although it is known that HT is safe for BMD on short term, long-term follow-up studies are lacking. Therefore this study aimed to investigate the change in BMD during the first ten years of HT, in order to determine whether HT is safe and if assessing BMD during HT is necessary. A follow-up study was performed in adult trans people receiving HT at the VU University medical center Amsterdam between 1998 and 2016. People were included if they were HT naïve and had a dual-energy X-ray absorptiometry (DXA) scan at the start of HT. Follow-up DXA scans performed after two, five, and/or ten years of HT were used for analyses. The course of BMD of the lumbar spine during the first ten years of HT was analyzed using multilevel analyses. 711 transwomen (median age 35 years, IQR 26-46) and 543 transmen (median age 25 years, IQR 21-34) were included. Prior to the start of HT, 21.9% of transwomen and 4.3% of transmen had low BMD for age (Z-score < -2.0). In transwomen lumbar spine BMD did not change (+0.006, 95%CI -0.005; +0.017), but lumbar spine Z-score increased with +0.22 (95%CI +0.12; +0.32) after ten years of HT. Also in transmen lumbar spine BMD did not change (+0.008, 95%CI -0.004; +0.019), but lumbar spine Z-score increased with +0.34 (95%CI +0.23; +0.45) after ten years of HT. This study showed that HT does not have negative effects on BMD, indicating that regularly assessing BMD during HT is not necessary. However, a high percentage of low BMD was found prior to HT, especially in transwomen. Therefore, evaluation of BMD before start of HT may be considered.

Introduction

Sex hormones influence bone acquisition and metabolism. Men develop wider bones and greater cortical bone size than women due to periosteal apposition.^(1,2) In women, the decline in estrogen during menopause leads to an increase in bone resorption⁽³⁾ and a decrease in bone mineral density (BMD).⁽⁴⁾ A higher trabecular BMD was found in women with androgen excess⁽⁵⁾, indicating that testosterone also influences BMD in women. In men, it was found that testosterone deficiency following orchiectomy was associated with accelerated bone loss.⁽⁶⁾ However, more recent studies indicated that the effects of hypogonadism on bone in men are mainly due to estrogen instead of testosterone deficiency.^(7,8) In both hypogonadal men and women, treatment with sex hormones increases BMD.^(9,10)

Gender-affirming hormonal treatment (HT) in trans people influences bone metabolism. After one year of HT the BMD increases in transwomen (male-to-female transgender people)⁽¹¹⁻¹⁹⁾, while in transmen (female-to-male transgender people) a maintenance^(12,17,19-21) or increase⁽¹⁶⁾ in BMD is described. More specifically, a larger increase in BMD was found in postmenopausal transmen with estrogen deficiency prior to HT compared with premenopausal transmen with normal estradiol concentrations. This suggests that the increase in BMD in transmen is mainly caused by the aromatization of testosterone into estradiol and therefore increasing the estradiol concentrations, instead of the direct effects of testosterone.⁽¹⁶⁾

The long-term effects of HT on BMD have been investigated using small-sample (n<50) cross-sectional case-control studies, with contradictory results. In transwomen compared with control men, higher⁽²²⁾, similar⁽²³⁾, and lower⁽²⁴⁾ BMD was found after 17, 12, and eight years of HT, respectively. Also, in transmen compared with control women contradictory results are published: one study found no difference in BMD after ten years of HT⁽²⁵⁾, while another study found higher whole body Z-scores after 7.6 years of HT.⁽²³⁾ Even prior to HT differences are observed: transwomen had lower BMD compared with control men^(12,26), while transmen had higher⁽¹²⁾ or similar⁽²⁵⁾ BMD than control women, indicating that the difference in BMD after long-term HT might already be present at baseline.

The transgender population who seek for help is steeply growing⁽²⁷⁾, which leads to an increasing demand for knowledge about the safety of HT, including bone health. While multiple studies investigated BMD after a longer period of HT, no studies have investigated the course of BMD during long-term HT. Therefore, our study aimed to describe BMD prior to the start of HT in a large population of transwomen and transmen, to investigate if BMD changes during the first ten years of HT, and as a result, to generate information about whether it is necessary to assess bone health in trans people during regular patient care. Furthermore, we studied whether different age groups and sex hormone concentrations were associated with a change in BMD during HT.

Materials and Methods

Design and population

This study is part of the retrospective Amsterdam Cohort of Gender Dysphoria (ACOG) study, including all 6,793 people who once presented themselves at the gender identity clinic of the VU University medical center (VUmc), Amsterdam, the Netherlands, between 1972 and 2016. Study design is described previously.⁽²⁷⁾ Briefly, data was collected by reviewing medical records. Age, medical history, medication use, type and dose of HT, prior HT use, gender-affirming surgery, smoking habits (in cigarettes per day), body weight (in kilogram), body height (in centimeter), laboratory test results, and dual-energy X-ray absorptiometry (DXA) results were retrieved. HT consisted of oral or transdermal estrogens and, usually until gonadectomy, anti-androgens in transwomen. Transmen were treated with oral, transdermal, or intramuscular testosterone. After at least one to 1.5 years of HT, surgery could be performed. For the current study, only people of 18 years and older with a baseline DXA scan (range one year before to four months after start with HT) who started with HT after 1998 were included, as BMD measurements were available since then. People who had undergone gonadectomy (orchiectomy in transwomen or oophorectomy in transmen) or used gender-affirming hormones prior to HT were excluded. We calculated that with 711 included transwomen and 543 included transmen (Figure 1), a mean difference in lumbar spine BMD of 0.021 g/cm² and 0.022 g/cm² could be detected, respectively, with a power of 80% and an alpha of 0.05. Our local ethics committee reviewed the study and determined that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study, and necessity for informed consent was waived due to the retrospective design.

Bone mineral density

BMD measurements were performed during regular patient care before the start of HT and were usually repeated every two to five years during HT. BMD of the lumbar spine (LS), total hip (TH), and femoral neck (FN) were measured using a DXA Hologic Delphi, which was updated in July 2004 and replaced by a Hologic Discovery A in February 2011. Phantom calibration allowed for comparison of BMD values with a difference of <1.0% between the densitometers. Coefficients of variation were <1.0% for both densitometers and least significant change was 0.022 g/cm². Absolute BMD values (g/cm²) were obtained and were converted into T-scores (standard deviation (SD) difference between the person's BMD and the BMD of a young-adult reference population of the same birth-sex and ethnicity) and Z-scores (SD difference between the person's BMD and an age-matched BMD of the same birth-sex and ethnicity), based on the National Health and Nutrition Examination Survey (NHANES). In addition, T-scores and Z-scores on baseline were also calculated using the identified sex as reference population. Osteoporosis was defined as a T-score below -2.5, and a Z-score below -2.0 was classified as low bone density for age.⁽²⁸⁾

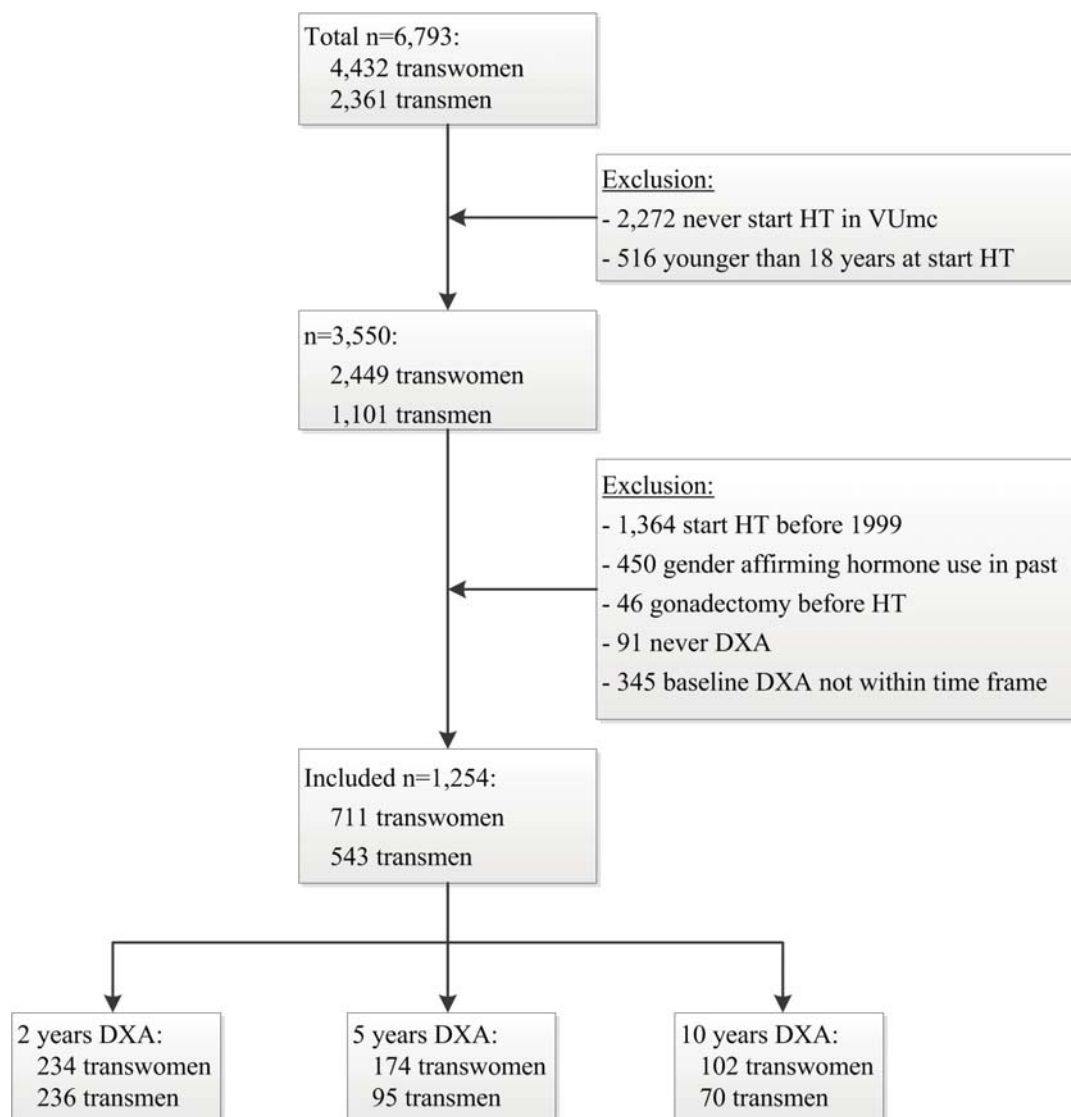


Figure 1.

Flowchart of inclusion of study population. DXA not within time frame: more than one year before start of gender-affirming hormonal treatment or more than 4 months after start of HT. The reported percentages are the percentages of transwomen or transmen with a DXA scan at that time point compared with the total included transwomen or transmen, respectively. Abbreviations: n=number, HT=gender-affirming hormonal treatment, VUmc=VU University medical center, DXA=dual-energy X-ray absorptiometry

Biochemical assessments

Blood samples were obtained during regular patient care before the start of HT, after three months of HT, and thereafter usually every one to two years. Assays with higher quality were implemented when available, and conversion formulas were generated to allow for comparison of the old and new values.

Estradiol was measured using a radioimmunoassay (Diasorin, Saluggia, Italy) with a lower limit of quantitation (LOQ) of 18 pmol/L and an inter-assay coefficient of variation (CV) of 10% until January 2010, where after a competitive immunoassay

(Delfia, Wallac, Turku, Finland) was used until July 2014 (LOQ 20 pmol/L, inter-assay CV 10%). The formula $\text{Delfia} = 1.267 * \text{Diasorin} - 28.87$ was used for conversion. After July 2014, LC-MS/MS (VUmc, Amsterdam, the Netherlands) was used (LOQ 20 pmol/L, inter-assay CV 7%). For conversion, the formula $\text{LC-MS/MS} = 1.60 * \text{Delfia} - 29$ was used.

Testosterone was measured using a radioimmunoassay (RIA; Coat-A-Count, Siemens, USA) until January 2013 (LOQ 1 nmol/L, inter-assay CV 7-20%). Thereafter, a competitive immunoassay (Architect, Abbott, USA) was used (LOQ 0.1 nmol/L, inter-assay CV 6-10%). For conversion, two formulas were used: $\text{Architect} = 1.1 * \text{RIA} + 0.2$ for testosterone concentrations <8 nmol/L; $\text{Architect} = 1.34 * \text{RIA} - 1.65$ for testosterone concentrations >8 nmol/L.

Luteinizing hormone (LH) was measured using an immunometric assay (Delfia, Wallac, Turku, Finland) until June 2011 (LOQ 0.5 U/l, inter-assay CV <7%). Thereafter, an immunometric assay (Architect, Abbott, Abbott Park, IL, USA) was used (LOQ 2 U/L, inter-assay CV <4-6%). For conversion, the formula $\text{Architect} = 0.91 * \text{Delfia} - 0.01$ was used.

Statistical analyses

Baseline characteristics are reported as mean (\pm SD), median (interquartile range, IQR), or percentages. Independent t-tests (or Wilcoxon rank sum tests in case of non-normal distribution) or chi square tests were used to analyze the differences in baseline characteristics between people with at least one follow-up measurement and people without a follow-up measurement.

Linear multilevel analyses with measurements clustered within people were performed to analyze the course of absolute BMD values and Z-scores during the first ten years of HT. Time was analyzed as a categorical variable with set time points at baseline (range: one year before to four months after baseline), after two years of HT (range: after one to three years of HT), after five years of HT (range: after three to 7.5 years of HT), or after ten years of HT (range: after 7.5 to twelve years of HT) using dummy variables. Next, stratified analyses were performed to investigate the change in BMD over time for different groups of age (<30 years, 30-40 years, and \geq 40 years), sex hormone concentrations during HT, and baseline BMD. The mean estradiol, testosterone, and LH concentrations during HT were calculated by averaging the results from the laboratory measurements after one, two, five, and ten years of HT (mean number of measurements 2.5, SD 0.8). As no linear associations existed between change in BMD and hormone concentrations, estradiol concentrations were divided into tertiles, LH concentrations into suppressed (<1 U/L) or not suppressed (>1 U/L), and testosterone concentrations into suppressed (<2 nmol/L) or not suppressed (>2 nmol/L) for transwomen, and tertiles for transmen. Baseline BMD was divided into tertiles. Last, the multilevel analyses were repeated by adding age, estradiol, testosterone, and LH concentrations as interactions with time to the model.

For all analyses, STATA Statistical Software (StataCorp, College Station, TX, USA) version 13.1 was used.

Results

Study participants

The flowchart of the inclusion of participants is shown in Figure 1. In total, 711 transwomen and 543 transmen with a baseline LS DXA were included for analyses, of which 440 transwomen and 358 transmen also had at least one follow-up DXA. No differences were found in age, ethnicity, BMD and Z-score of the LS, estradiol, testosterone, LH, and 25 (OH)D concentrations at baseline between people with and without follow-up BMD measurements (data not shown). The baseline characteristics of the study population are shown in Table 1.

Lumbar spine

Transwomen on estradiol based treatment

Transwomen had a mean LS Z-score of $-0.93 (\pm 1.32)$ before start of HT. At baseline, 14.2% of the transwomen were classified as having osteoporosis (T-score ≤ -2.5), and 21.9% had low bone density (Z-score < -2.0) for age.

After ten years of HT, LS BMD was not different from baseline ($+0.006 \text{ g/cm}^2$, 95%CI $-0.005 ; +0.017 \text{ g/cm}^2$), but LS Z-score was $+0.22$ (95%CI $+0.12 ; +0.32$) higher compared with baseline (Figure 2). As shown in Figure 3, no differences in change in LS BMD were observed between different age groups. In transwomen in the highest estradiol tertile (mean 443 pmol/L), an increase in LS BMD was observed ($+0.044 \text{ g/cm}^2$, 95%CI $+0.025 ; +0.063 \text{ g/cm}^2$), while it decreased in those in the lowest tertile (mean 118 pmol/L , -0.026 g/cm^2 , 95%CI $-0.044 ; -0.009 \text{ g/cm}^2$). There was no difference in change in LS BMD between suppressed versus not suppressed testosterone and LH. Transwomen in the lowest baseline BMD tertile (mean 0.828 g/cm^2) increased with $+0.026$ (95%CI $+0.004 ; +0.048$), while no change was observed in those in the middle (mean 0.972 g/cm^2 , change -0.014 , 95%CI $-0.031 ; +0.003$) or highest tertile (mean 1.129 g/cm^2 , change $+0.006$, 95%CI $-0.010 ; +0.022$).

In the multivariable analysis (Table 2), no differences in change in LS BMD were found between age groups, testosterone concentrations, and LH concentrations. Higher estradiol tertiles were associated with a larger increase in LS BMD than lower estradiol tertiles.

Table 1. Baseline characteristics of the study population

	Transwomen		Transmen	
<u>Baseline characteristics</u>				
Age, yr	35 (26 – 46)		25 (21 – 34)	
Ethnicity (% Caucasian)	97.2		95.2	
BMI, kg/m ²	23.7 (4.3)		25.6 (5.7)	
Smoking (% yes)	34.9		39.5	
<u>Bone mineral density</u>	<i>Male reference</i>	<i>Female reference</i>	<i>Male reference</i>	<i>Female reference</i>
Lumbar spine				
- Absolute BMD, g/cm ²	0.976 (±0.140)		1.030 (±0.127)	
- T-score	-1.07 (±1.27)	-0.67 (±1.27)	-0.61 (±1.14)	-0.20 (±1.14)
- Z-score	-0.93 (±1.32)	-0.31 (±1.39)	-0.54 (±1.15)	+0.01 (±1.14)
Total hip				
- Absolute BMD, g/cm ²	0.928 (±0.136)		0.948 (±0.118)	
- T-score	-0.72 (±0.89)	-0.13 (±1.09)	-0.61 (±0.75)	+0.01 (±0.92)
- Z-score	-0.58 (±0.92)	+0.07 (±1.16)	-0.55 (±0.76)	+0.07 ± (0.93)
Femoral neck				
- Absolute BMD, g/cm ²	0.789 (±0.129)		0.838 (±0.118)	
- T-score	-1.06 (±0.93)	-0.56 (±1.14)	-0.72 (±0.83)	-0.14 (±1.02)
- Z-score	-0.73 (±0.94)	-0.25 (±1.16)	-0.59 (±0.85)	-0.05 (±1.02)
Osteoporosis, %	14.2	5.8	5.2	2.4
Low bone density, %	21.9	9.4	10.3	4.3
<u>Laboratory measurements *</u>	Baseline	During HT	Baseline	During HT
Estradiol, pmol/L	95 (68 – 124)	235 (160 – 338)	185 (59 – 390)	159 (113 – 220)
Testosterone, nmol/L	20 (16 – 25)	1.1 (0.7 – 1.3)	1.3 (1.2 – 1.7)	26 (18 – 38)
LH, U/L	3.4 (2.3 – 4.6)	1.2 (0.1 – 6.2)	4.2 (2.4 – 7.1)	3.3 (0.7 – 8.8)
25(OH)D, nmol/L	42 (26 – 58)	53 (35 – 72)	50 (30 – 73)	57 (40 – 78)
Calcium, mmol/L	2.36 (0.08)	2.32 (0.08)	2.34 (0.08)	2.36 (0.08)
Creatinine, µmol/L	76 (11)	72 (11)	66 (10)	78 (11)
AF, U/L	71 (19)	67 (23)	70 (22)	78 (21)
SHBG, nmol/L	35 (26 – 46)	43 (29 – 59)	51 (31 – 81)	28 (20 – 36)

Data are shown as median with interquartile range, mean with standard deviation, or percentages. * Concentrations were not known for the entire population. Available percentage at baseline: estradiol, testosterone, and LH (75-85%), 25(OH)D, calcium, and creatinine (45-70%), AF and SHBG (15-25%). During HT: estradiol, testosterone, LH, creatinine (>90%), calcium and AF (75-90%), 25(OH)D and SHBG (50-60%). Abbreviations: yr = year, BMD = bone mineral density, LH = luteinizing hormone, 25(OH)D = 25-hydroxy vitamin D, AF = alkaline phosphatase, SHBG = sex-hormone binding globulin

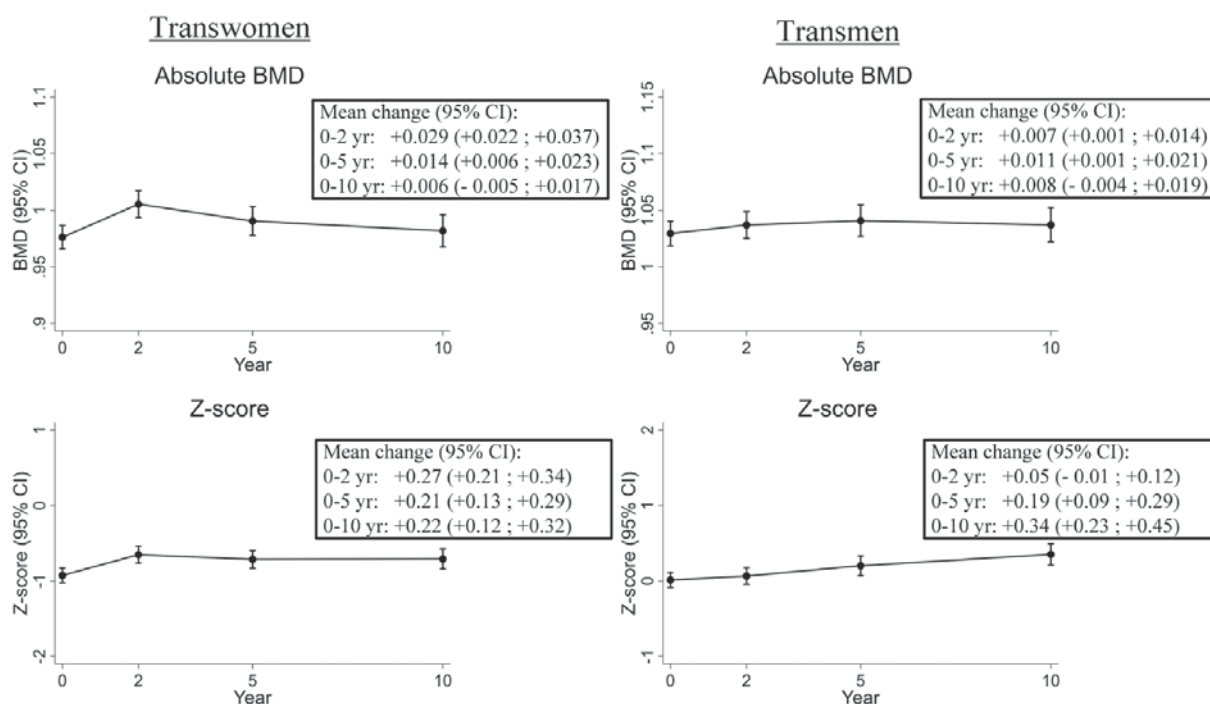


Figure 2.

Change in absolute BMD and Z-score in transwomen and transmen during the first ten years of gender-affirming hormonal treatment. Abbreviations: BMD = bone mineral density, 95% CI = 95% confidence interval, yr = year

Transmen on testosterone based treatment

The baseline LS Z-score was +0.01(±1.14). Osteoporosis (T-score ≤-2.5) was found in 2.4%, and low bone density for age (Z-score <-2.0) in 4.3% at baseline.

LS BMD after ten years of HT was similar to baseline (+0.008 g/cm², 95%CI -0.004 ; +0.019 g/cm²), but LS Z-score increased with +0.34 (95%CI +0.23 ; +0.45) from baseline to ten years (Figure 2). In the oldest age group (≥40 years) LS BMD increased (+0.054 g/cm², 95%CI +0.032 ; +0.076 g/cm²), while it did not change in the younger two age groups. Baseline estradiol concentrations were lower in transmen ≥40 years (median 104 pmol/L, IQR 20-386) than younger transmen (median 197 pmol/L, IQR 77-390). No change in LS BMD was found in transmen who were categorized in the two lowest estradiol tertiles (means 95 and 160 pmol/L, respectively), but it increased in those who were categorized in the highest estradiol tertile (mean 323 pmol/L, Figure 3). Testosterone concentrations were not associated with change in LS BMD. An increase in LS BMD was found in transmen with suppressed LH concentrations (<1 U/L), while no change was observed in those with higher LH concentrations (Figure 3). Transmen in the lowest baseline BMD tertile (mean 0.896 g/cm²) increased in BMD (+0.030, 95%CI +0.010 ; +0.051), while it did not change in the middle (mean 1.021 g/cm², change +0.004, 95%CI -0.018 ; +0.026) or highest (mean 1.172 g/cm², change -0.005, 95%CI -0.020 ; +0.010) tertile.

In the multivariable analysis (Table 2), estradiol concentrations were no longer associated with the change in LS BMD. Testosterone was not associated with change in LS BMD. Older transmen had a larger increase in LS BMD compared with younger transmen. Transmen with lower LH concentrations had a larger increase in LS BMD compared to those with higher LH concentrations.

Table 2. Adjusted differences of the change in lumbar spine bone mineral density between baseline and ten years.

	Transwomen	Transmen
	Difference in change (95% CI), g/cm ²	Difference in change (95% CI), g/cm ²
Age		
30-40 years vs. <30 years	+0.008 (-0.020 ; +0.037)	-0.007 (-0.033 ; +0.018)
>40 years vs. <30 years	-0.004 (-0.031 ; +0.024)	+0.052 (+0.023 ; +0.081)
>40 years vs. 30-40 years	-0.012 (-0.036 ; +0.013)	+0.059 (+0.030 ; +0.088)
Estradiol concentration		
Second tertile vs. first tertile	+0.033 (+0.006 ; +0.059)	+0.009 (-0.017 ; +0.034)
Third tertile vs. first tertile	+0.076 (+0.050 ; +0.103)	+0.025 (-0.005 ; +0.055)
Third tertile vs. second tertile	+0.044 (+0.018 ; +0.070)	+0.017 (-0.014 ; +0.047)
LH concentration		
Suppressed vs. not suppressed	-0.008 (-0.033 ; +0.017)	+0.043 (+0.013 ; +0.073)
Testosterone concentration		
Suppressed vs. not suppressed	-0.011 (-0.034 ; +0.012)	
Second tertile vs. first tertile		+0.013 (-0.016 ; +0.041)
Third tertile vs. first tertile		-0.002 (-0.030 ; +0.025)
Third tertile vs. second tertile		-0.015 (-0.043 ; +0.012)

Age is defined as age at start of HT. Number of transwomen per group: <30 years n=265, 30-40 years n=166, ≥40 years n=280; number of transmen per group: <30 years n=352, 30-40 years n=110, ≥40 years n=81. Estradiol, testosterone, and LH concentrations were assessed during HT. In transwomen, the mean and range estradiol concentrations were 118 (20 – 182) pmol/L for the 1st tertile (n=229), 238 (182 – 298) pmol/L for the 2nd tertile (n=229), and 443 (229-1411) pmol/L for the 3rd tertile (n=228). Testosterone concentration was divided into suppressed (<2 nmol/L, n=565) and not suppressed (>2 nmol/L, n=144), and LH concentration was divided into suppressed (<1 U/L, n=330) and not suppressed (>1 U/L, n=353). In transmen, the mean and range estradiol concentrations were 95 (20 – 131) pmol/L for the 1st tertile (n=179), 160 (132 – 192) pmol/L for the 2nd tertile (n=178), and 323 (192 – 1251) pmol/L for the 3rd tertile (n=178). The mean and range testosterone concentrations were 15 (1 – 21) nmol/L for the 1st tertile (n=179), 26 (21 – 33) nmol/L for the 2nd tertile (n=178), and 57 (33 – 213) nmol/L for the 3rd tertile (n=178). LH concentration was divided into suppressed (<1 U/L, n=150) or not suppressed (>1 U/L, n=384). Abbreviations: 95%CI = 95% confidence interval, vs = versus.

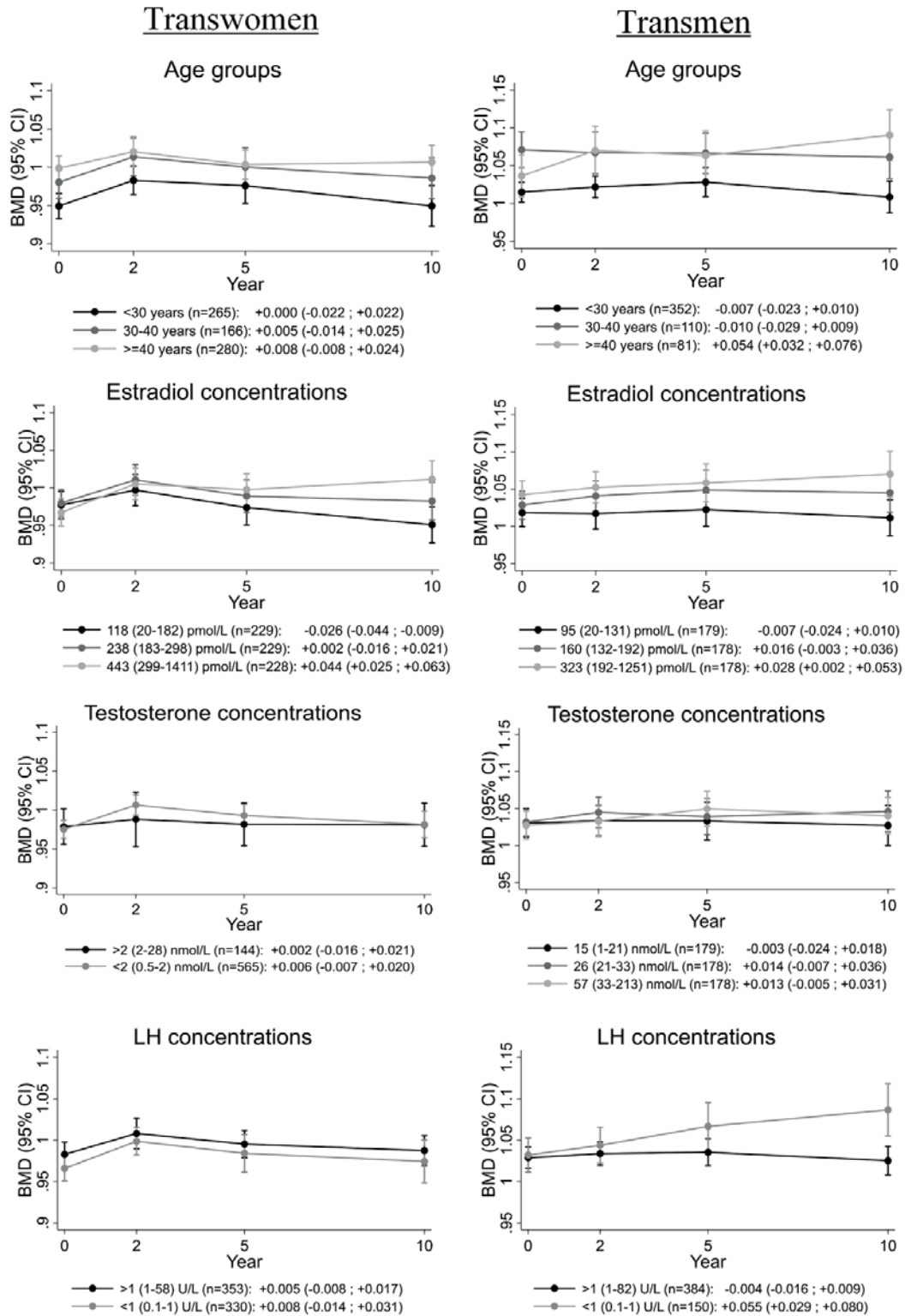


Figure 3.

Change in BMD during the first ten years of gender-affirming hormonal treatment in transwomen and transmen, stratified for age groups, concentrations of estradiol, testosterone, and LH. For each group, the mean change with 95% confidence interval is reported below the graphs. Age is defined as age at start of HT. Estradiol, testosterone, and LH concentrations were assessed during HT and are shown as mean (range). Abbreviations: BMD = bone mineral density, 95% CI = 95% confidence interval, LH=luteinizing hormone

Total hip and femoral neck

Only a small number of BMD measurements of TH and FN were performed after five years (n=24) and ten years (n=4) of HT. In the overall analyses, no changes in TH BMD (-0.034 g/cm², 95%CI -0.070 ; +0.002 g/cm²) and FN BMD (+0.057 g/cm², 95%CI -0.036 ; +0.047 g/cm²) were found in transwomen. Also in transmen, no changes in TH BMD (-0.024 g/cm², 95%CI -0.075 ; +0.026 g/cm²) and FN BMD (-0.033 g/cm², 95%CI -0.092 ; +0.026 g/cm²) were found. No subgroup analyses could be performed due to the limited number of people in each of the subgroups.

Discussion

In this study, we found that transwomen had a low mean LS Z-score before the start of HT, while this was not found in transmen. During the first ten years of HT, no change in LS BMD was found, while the LS Z-score increased in both transwomen and transmen. Higher estradiol concentrations were associated with an increase in LS BMD in transwomen, while in transmen lower LH concentrations were associated with an increase in LS BMD.

A low bone density in transwomen before the start of HT was earlier described by van Caenegem et al. ⁽²⁶⁾, who found that transwomen had lower 25(OH)D concentrations and lower muscle mass than control men, possibly due to less activities because of social isolation. Although we did not have a control group, we did find that transwomen had lower 25(OH)D concentrations at baseline than transmen.

As BMD is influenced by time, it was also analyzed as Z-score in order to compare the results with the general age-matched population. For the current study, the Z-score was calculated using the BMD of the sex assigned at birth, because the majority of the population started hormonal treatment after puberty and therefore already reached the age of peak bone mass that belongs to the sex assigned at birth. The natural course of BMD over time is to decrease after the peak bone mass, while we found that LS BMD did not change during the first ten years of HT. In addition, the LS Z-score increased, which may indicate that HT does not negatively influence BMD.

In transwomen, no differences in change in LS BMD between age groups were present, while in transmen LS BMD increased more in the oldest age group (≥40 years) compared with younger age groups. This finding was also described in a prospective one year follow-up study.⁽¹⁶⁾ In the current study, we found that the increase continued after one year. An explanation for the larger increase in LS BMD in this age group may be that these people were perimenopausal or postmenopausal with low estrogen concentrations prior to HT. Treatment with testosterone increased both testosterone and estradiol concentrations in these transmen (due to aromatization of testosterone to estradiol), while in the younger transmen only testosterone concentrations increased as

they were not estrogen deficient at baseline. This may indicate that the effects of HT on BMD in transmen were mainly due to indirect effects of estradiol instead of direct effects of testosterone. This is also in line with earlier studies who reported that BMD was better correlated with estradiol concentrations than testosterone concentrations.^(7,29) Moreover, when we adjusted this analysis for change in estradiol concentrations (mean estradiol concentration during HT minus estradiol concentration at baseline), no differences between age groups remained present (data not shown). This indicates that the difference in change in BMD between age groups is (partially) explained by a difference in change in estradiol concentrations. In transwomen with higher estradiol concentrations during HT an increase in LS BMD was found, while low estradiol concentrations were associated with a decrease in LS BMD. Testosterone concentrations were suppressed in the majority of the population and were not associated with the change in LS BMD. LH concentrations were not associated with change in LS BMD, but were also suppressed by the use of cyproterone acetate and is therefore not indicative of adequate estrogen substitution. In transmen, estradiol and testosterone concentrations were not associated with change in LS BMD. In transmen with low LH concentrations the LS BMD increased, while it did not change in those with higher LH concentrations. These results indicate that in transmen, LH concentrations may be used to evaluate the adequacy of testosterone dosing for bone health, while in transwomen estradiol concentrations may be used to evaluate the adequacy of estradiol dosing. In addition, as LS BMD decreased in transwomen with low estradiol concentrations, therapy compliance should be stimulated.

Our study was performed in the largest gender identity clinic of the Netherlands, including a large population of transwomen and transmen with a wide age range. Previous studies investigated the long-term effects of HT on BMD in cross-sectional studies, without baseline differences taken into account. In this study, long-term follow-up analyses were performed, also looking at the influence of age and sex hormone concentrations. However, there are also some limitations. First, as this study is a retrospective study, data were collected during regular patient care. Clinical data, such as smoking habits, alcohol intake, and body weight, were usually only assessed at baseline but not structurally during medical check-ups and could not be reliably analyzed. Also, data about calcium intake, vitamin D supplementation, or physical activity were not available, which could also influence BMD. It might be possible that trans people gain a healthier lifestyle during HT, for example more exercising, quit smoking, and using vitamin D supplementation. As a control group was lacking, the change in BMD cannot only be contributed to the use of HT. Although this study is retrospective, LS BMD measurements and sex hormone concentrations were regularly assessed as this was part of the treatment protocol. For this study, people were included for analyses if they had a DXA scan prior to the start of HT. No differences in baseline characteristics were observed between people with and without follow-up LS BMD measurements, which

decreases the risk of selection bias. Second, differences in change in BMD for different treatment regimens were not analyzed. As trans people often change the type of HT they use, it could not be analyzed whether the change in BMD differs for people who use transdermal, oral, or intramuscular estrogen or testosterone. In addition, the majority of the population underwent gonadectomy during the first ten years of HT ⁽²⁷⁾, so the influence of surgery on change in BMD could not be analyzed. Third, in this study only the LS BMD was analyzed in subgroup analyses, as not enough data were available for subgroup analyses of the TH or FN BMD. However, the overall analyses showed the same pattern as the LS analysis, so it is not expected that it would give different conclusions. Fourth, the type of densitometer changed during the study period, while most ideally all people would be scanned by the same densitometer. However, during replacement of the densitometer, phantom calibration was performed and a difference of less than one percent in absolute BMD values between the densitometers was found. This indicates that the change in densitometer did not affect our result. Last, only the change in BMD was assessed and no fracture data was available. As HT does not seem to have negative effects on BMD, it is not expected that fracture risk will increase because of HT.

In clinical practice, concerns about the safety of HT on bone health are present, probably because earlier studies found low BMD after long-term HT in transwomen compared with control men, and because of fear that HT decreases estradiol concentrations in transmen, which could have negative effects on BMD. The current results may indicate that HT does not have negative effects on bone health in trans people, because no change in LS BMD and an increase in LS Z-score was found. However, as prior to HT a high percentage of low bone density was found in transwomen, bone health should be an important topic, especially if therapy noncompliance is suspected. The results of this study support the statements of the Endocrine Society Guideline ⁽³⁰⁾ that clinicians should assess BMD only when risk factors for osteoporosis exists, and especially in those who stop HT after gonadectomy. For transwomen, testing BMD at baseline should be considered based on the high prevalence of low bone density at baseline, while this does not seem necessary for transmen. Regularly assessing BMD during HT without indication does not seem necessary based on the current results. However, the effect of HT on fracture risk is not known and is topic for further research.

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