Bone health in transgender people

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2020

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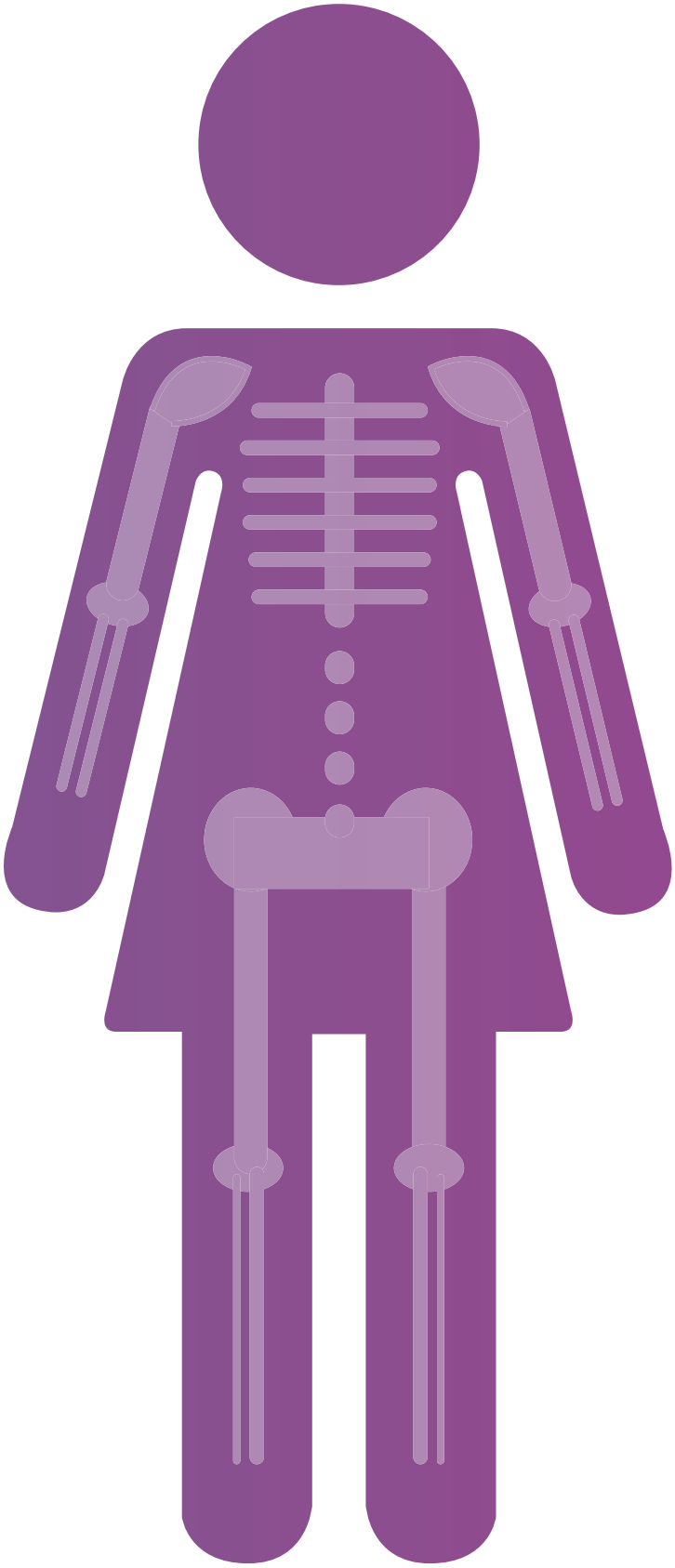

Chapter 8

Bone geometry and trabecular bone score in transgender people before and after short- and long-term hormonal treatment

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Bone. 2019;127:280-286

Abstract

Background: Gender-affirming hormonal treatment (HT) in adult transgender people influences bone mineral density (BMD). Besides BMD, bone geometry and trabecular bone score are associated with fracture risk. However, it is not known whether bone geometry and TBS changes during HT.
Purpose: To investigate the bone geometry and TBS in adult transgender people at different time points, up to 25 years, of HT.
Methods: A total of 535 trans women and 473 trans men were included, who were divided into three groups at time of their DXA: 20-29 years, 30-39 years, and 40-59 years. Subsequently, each group was divided into different HT durations: baseline, or after 5, 15, or 25 years of HT. Hip structure analysis was performed to measure subperiosteal width, endocortical diameter, average cortical thickness, and section modulus. TBS was calculated based on lumbar spine DXA images.
Results: In trans women in all age groups and in young trans men, no differences were observed in periosteal width, endocortical diameter, average cortical thickness, and section modulus for different durations of HT. In trans men aged 40-59 years, subperiosteal width, endocortical diameter, and section modulus were slightly higher in the groups who were using HT compared to the (peri- or postmenopausal) baseline group. In younger trans women, TBS tended to be higher in those using HT compared to the baseline groups, and in older trans women TBS was higher in those using HT for 25 years versus baseline (+0.04, 95%CI +0.00; +0.08). In younger trans men, TBS tended to be lower in those who used HT compared to the baseline groups, and in older trans men TBS was lower in those using 5 years HT versus baseline (-0.05, 95%CI -0.08; -0.01).
Conclusion: No differences in cortical bone geometry parameters were found during different HT-durations. TBS increased in trans women and decreased in trans men, indicating that estrogens have positive effects on TBS. These data may be helpful in determining what sex reference values for calculating T-scores and Z-scores in adult transgender people should be used.

Introduction

Sex steroids are important determinants of bone health, not only of bone maintenance but also of bone development. Prior to puberty, the bone geometry of boys and girls is similar, while differences in bone size and bone geometry occur during puberty.[1,2] Testosterone stimulates periosteal bone growth, leading to wider bones in men than in women.[3] This might be due to either direct effects of testosterone on bone via androgen receptor (AR) activation [4], or via indirect effects of testosterone on muscle mass. Testosterone increases muscle mass, resulting in increased mechanical loading of the bones which stimulates periosteal growth. Estrogen is known to stimulate endocortical growth, which narrows the medullary cavity.[2]

Transgender people often receive treatment with sex steroids: trans women (male sex assigned at birth, female gender identity) receive estrogens, with or without anti-androgens, while trans men (female sex assigned at birth, male gender identity) receive testosterone.[5] Although gender-affirming hormonal treatment (HT) in transgender people can influence areal bone mineral density (BMD) [6-8], data regarding changes in bone geometry during HT are scarce, especially when HT is started after peak bone mass is obtained.

In clinical practice, dual-energy X-ray absorptiometry (DXA) is used to assess areal BMD. However, these scans do not distinguish between cortical and trabecular bone, and do not provide insight whether bone geometry changes. To analyze the different structural properties of the bone, hip structure analysis (HSA) software was developed.[9,10] HSA uses distribution of the pixel bone mass across various regions in the hip to estimate, for example, periosteal width and endocortical diameter. The hip consists mainly of cortical bone, whereas trabecular bone is primarily present in vertebrae. Trabecular bone score (TBS) can be measured from lumbar spine DXA scans [11], by analyzing the variations in gray-level of the pixels. BMD assessed by DXA only provides information about bone quantity, whereas TBS is associated with the connectivity and number of trabeculae and the space in-between them. Different TBS values can be obtained in people with similar BMD. A high TBS indicates a dense microarchitecture, while a low TBS can indicate poor microarchitecture and wide spaces between trabeculae. TBS is known to predict fracture risk independently of BMD.[12] Earlier studies found that TBS is higher in non-trans (cis) women than in cis men [13] and that TBS decreases in cis women during menopause [14-16], indicating that estrogen is positively associated with trabecular bone.

Bone geometry and TBS (changes) are associated with fracture risk.[12] In transgender people using HT, changes in BMD are found with DXA. However, it is not known whether these differences in BMD measured by DXA are a result of changes in bone geometry or actual changes in BMD. In the present study, we aimed to investigate whether bone geometry and TBS changes in transgender people using HT, in order to

get more insight into the mechanistic effects of HT on bone. To study this, we used the HSA and TBS software in adult transgender people at different time points during HT, and in different age groups, as age also affects bone geometry. In addition, we compared these values with reference values of the general male and female population reported in literature, to provide data that can be helpful in determining which sex reference values for calculating T-scores and Z-scores should be used in adult transgender people undergoing a DXA.

Methods

Study design and population

This study is part of the Amsterdam Cohort of Gender dysphoria (ACOG) study [17], a retrospective study that includes all 6,793 people who once visited the gender identity clinic of the Amsterdam University Medical Centers, the Netherlands, between 1972 and 2016. Clinical data, including start date of HT, type of HT, age, body mass index (BMI, kilograms per square meter), smoking habits, medication use, and comorbidities were retrieved from the medical files. HT consisted in trans women of estrogens and, usually until gonadectomy, anti-androgens. The prescribed estrogens were ethinyl estradiol (50-150 μg daily), 17-beta oestradiol implants (20-40 mg per 3 months) or patches (50-150 μg twice a week), conjugated oestrogens (0.625 to 2.5 mg daily), oral oestradiol valerate (2-4 mg daily), or oestradiol gel (0.75 to 1.5 mg daily). Cyproterone acetate (50-100 mg daily) was most often used as anti-androgen until orchiectomy. Sometimes it was given in low dosages (10-25 mg daily) after orchiectomy if body hair growth persisted. Trans men were treated with testosterone gel (25-50 mg daily), intramuscular testosterone esters (250 mg every 2-3 weeks), or intramuscular or oral testosterone undecanoate (1000 mg per 12-14 weeks, or 40-240 mg daily, respectively). Lynestrenol (5-15 mg daily) was prescribed if menstrual bleeding persisted. After at least 1 to 1.5 years of HT, transgender people were eligible to undergo gonadectomy (orchiectomy in trans women and oophorectomy in trans men). During clinical care, DXA scans were performed regularly, usually at start of HT and every 5 years. For the current study, we only included people who started HT at age 18 years or older, and who had a DXA scan performed after February 2011, because the HSA software was only available since then. The latest DXA was used for analysis if more than one DXA was performed in a person, so every person is only included once. Non-Caucasian people were excluded, as ethnicity affects BMD and the majority of the population was Caucasian (93%). This led to a total study population of 535 trans women and 473 trans men. As age affects bone geometry and TBS, the included trans women and trans men were divided into 3 age groups at time of the DXA scan: 20-29 years, 30-39 years, and 40-59 years of age. Thereafter, each group was divided into different durations of HT: baseline, after 5 (range 3 to 10) years

of HT, after 15 (range 10 to 20) years of HT, and after 25 (range 20 to 37) years of HT. Due to the design of the study, no DXA scans were available in trans women and trans men aged 20-29 years after 15 and 25 years of HT, and in trans women and trans men aged 30-39 years after 25 years of HT. The local Medical Ethics Committee of the VU University Medical Center, Amsterdam, the Netherlands reviewed this study and determined that the law Medical Research Involving Human Subjects Act (WMO) did not apply to this study. As a result, necessity for informed consent was waived due to the retrospective design and the absence of interventions.

Bone mineral density

A Hologic Discovery A (Hologic Inc., Bedford, MA, USA) was used to measure BMD of the lumbar spine (LS), total hip (TH), and femoral neck (FN). All DXA measurements were performed between February 2011 and February 2017. Daily quality controls were performed using phantoms and the coefficient of variation was <1.0%. BMD values were extracted as absolute values (in g/cm^2) and were thereafter converted into T-scores by using the female reference data of the National Health and Nutrition Examination Survey (NHANES).

Hip structure analysis

APEX software version 4.0, HSA option, was applied to the standard hip DXA images to obtain structural geometrical data. Three regions of interest can be measured with HSA: narrow neck, intertrochanteric region, and femoral shaft. The narrow neck is the narrowest place of the femoral neck, the intertrochanteric region is the line where the femoral neck and the shaft axes cross, and the femoral shaft region is 1.5 times the width of the femoral neck below the cross of the femoral neck and shaft axes. The narrow neck region and intertrochanteric region contains both cortical and trabecular bone, while the femoral shaft region is thought to consist mainly of cortical bone.[18] Multiple variables are generated by the HSA software, which has been described previously.[18] For the current study, the subperiosteal width, the endocortical diameter, the average cortical thickness, and the section modulus of the narrow neck, intertrochanteric region, and femoral shaft were used. The subperiosteal width represents the outer diameter of the bone, which is the distance between the edges of the bone mineral content profile. The endocortical diameter is the estimate of the inside diameter of the cortex. The average cortical thickness is the difference between the subperiosteal and endocortical diameter divided by 2. The section modulus is an indicator of bending strength.

Trabecular bone score

TBS iNsight software version 3.0.1 (Medimaps Group) was used to calculate TBS. This technique evaluates variations in the gray-level of the pixels in the DXA lumbar spine image, thereby providing an index of trabecular microarchitecture. TBS was retrospectively calculated for L1 to L4 and then averaged. As TBS can be influenced by the amount of soft tissue, only people with a BMI between 15 and 37 kg/m^2 at time of the DXA scan were analyzed.

Statistical analyses

The characteristics of the study groups are shown as mean with standard deviation for normally distributed data, median with interquartile range for non-normally distributed data, and percentages for dichotomous data. Age at time of the DXA, age at time of the start of HT, the duration of HT, BMI, T-scores of the lumbar spine, total hip, and femoral neck were normally distributed. The estradiol and testosterone concentrations were non-normally distributed data. Smoking was analyzed as dichotomous variable (never smoker versus [former] smoker), just as medication use or comorbidity with possible influence on bone (yes versus no). To investigate whether these characteristics differed between the HT-duration groups within an age group, one-way ANOVA's were performed for the normally distributed variables, Kruskal-Wallis tests for the non-normally distributed variables, and chi-square tests for the dichotomous variables.

The bone variables subperiosteal width, endocortical diameter, average cortical thickness, section modulus, and trabecular bone score were all normally distributed. Differences in all these bone variables among the HT-duration groups per age group were analyzed using linear regression analyses, with bone parameters as continuous outcome variables and HT-duration group as determinant. The mean difference with 95% confidence intervals between these groups are reported. Sensitivity analyses were performed by repeating the linear regression analyses after excluding people with medication use (diuretics, corticosteroids, anti-epileptics, antidepressants, anti-convulsives, or bisphosphonates) or comorbidity (eating disorder, thyroid disease, diabetes mellitus, gastro-intestinal disease, alcohol abuse, malignancy, or Cushing's disease) with possible influence on bone.

Age- and sex-specific reference values of the narrow neck HSA were based on reports in literature [19] and were added to the graphs. Data was analyzed using STATA Statistical Software (Statacorp, College Station, Texas, USA, version 15.1). P-values below 0.050 were considered statistically significant.

Results

In Table 1 and 2, the number of people per group is shown, including age at time of the DXA, age at start of HT, duration of HT, BMI, T-scores of the LS, TH, and FN, smoking, bone-influencing medication, bone-influencing comorbidity, estradiol, and testosterone, separately for trans women and trans men. The BMI, smoking, bone-influencing medication, bone-influencing comorbidity, and T-scores of the LS, TH, and FN were not significantly different within the age groups. In trans women, estradiol and testosterone concentrations were higher, respectively lower, during HT than at baseline, but did not differ between the HT-duration groups. In trans men, estradiol and testosterone concentrations were lower, respectively higher, during HT than at baseline, but did not differ between the HT-duration groups.

Table 1. Characteristics of trans women

	20-29 years		30-39 years			40-59 years			
	Start HT *n=130*	5 yrs HT *n=16*	Start HT *n=63*	5 yrs HT *n=23*	15 yrs HT *n=25*	Start HT *n=93*	5 yrs HT *n=78*	15 yrs HT *n=67*	25 yrs HT *n=39*
Age DXA, yrs	24.7 (2.8)	26.8 (1.9)	34.5 (3.1)	35.6 (2.5)	36.2 (2.7)	50.0 (5.6)	50.3 (5.1)	50.2 (4.9)	50.6 (5.6)
Age start HT, yrs	24.6 (2.8)	21.1 (2.2)	34.5 (3.1)	29.8 (2.7)	22.2 (3.1)	49.9 (5.6)	43.7 (5.3)	34.8 (5.9)	25.5 (4.7)
Duration HT, yrs	0.0 (0.1)	5.7 (1.4)	0.0 (0.1)	5.8 (1.8)	14.0 (3.2)	0.1 (0.1)	6.5 (1.9)	15.4 (28)	25.1 (4.0)
BMI, kg/m^2	23.2 (4.6)	24.0 (4.0)	24.0 (4.1)	24.4 (5.3)	22.8 (5.2)	25.6 (4.9)	26.6 (4.1)	27.3 (5.6)	26.6 (7.5)
Smoking, %yes	33	25	35	59	52	31	43	41	53
Medication, %yes	16	13	17	35	28	37	28	36	31
Comorbidity, %yes	5	6	6	13	4	14	13	14	5
T-scores									
- LS	-0.9 (1.3)	-0.4 (1.5)	-0.8 (1.0)	-0.7 (1.2)	-0.7 (1.5)	-0.5 (1.3)	-0.3 (1.7)	-0.3 (1.3)	-0.5 (1.4)
- TH	-0.1 (1.0)	0.0 (1.3)	-0.2 (1.1)	-0.4 (1.1)	-0.5 (0.9)	0.0 (1.1)	-0.0 (1.2)	0.0 (0.9)	-0.2 (1.0)
- FN	-0.4 (1.1)	-0.2 (1.6)	-0.6 (1.2)	-0.5 (1.1)	-0.7 (0.9)	-0.7 (1.1)	-0.6 (1.1)	-0.6 (1.0)	-0.7 (1.2)
Laboratory values									
- Estradiol, pmol/L	107 (83–131)	313 (178–514)	103 (83–123)	220 (158–382)	272 (120–330)	99 (80–123)	245 (113–405)	205 (83–349)	194 (54–457)
- Testosterone, nmol/L	20 (16–25)	0.9 (0.6–1.2)	18 (14–23)	1.3 (0.8–1.3)	0.9 (0.5–1.3)	19 (14–25)	1.2 (0.6–1.3)	0.9 (0.5–1.3)	0.9 (0.5–1.1)

Age during the DXA scan, age at start of hormonal treatment (HT), duration of HT, body mass index (BMI), (former) smokers, bone-influencing medication, bone-influencing comorbidity, T-scores of the lumbar spine (LS), total hip (TH), and femoral neck (FN), and estradiol and testosterone concentrations are shown separately for different age groups (20-29 years, 30-39 years, and 40-59 years), and for different durations of HT (0 years, 5 years, 15 years, and 25 years). Data are shown as means with standard deviations, percentages, or median with interquartile range.

Table 2. Characteristics of trans men

	20-29 years		30-39 years			40-59 years			
	Start HT *n=174*	5 yrs HT *n=11*	Start HT *n=40*	5 yrs HT *n=29*	15 yrs HT *n=25*	Start HT *n=35*	5 yrs HT *n=38*	15 yrs HT *n=49*	25 yrs HT *n=72*
Age DXA, yrs	23.3 (2.7)	25.9 (2.8)	34.0 (2.5)	35.3 (3.4)	36.8 (3.0)	47.5 (4.4)	48.1 (6.0)	49.6 (6.2)	50.0 (4.8)
Age start HT, yrs	23.3 (2.7)	20.4 (2.5)	33.9 (2.5)	28.0 (3.6)	22.5 (2.7)	47.4 (4.4)	41.6 (6.7)	35.4 (6.9)	23.9 (4.3)
Duration HT, yrs	0.0 (0.1)	5.5 (1.9)	0.1 (0.2)	7.3 (1.8)	14.3 (2.5)	0.1 (0.1)	6.6 (1.6)	14.3 (2.6)	26.1 (3.8)
BMI, kg/m^2	25.4 (5.8)	24.0 (4.0)	28.9 (6.2)	27.4 (7.3)	27.2 (4.6)	25.6 (5.8)	27.0 (4.9)	25.4 (4.0)	25.1 (3.4)
Smoking, %yes	41	50	62	46	57	29	47	51	60
Medication, %yes	22	36	28	31	28	37	34	29	25
Comorbidity, %yes	6	0	15	10	0	14	24	18	13
T-scores									
- LS	-0.9 (1.3)	-0.4 (1.5)	0.2 (1.2)	0.1 (1.5)	-0.1 (1.2)	-0.2 (1.4)	0.1 (1.5)	0.2 (1.4)	-0.1 (1.3)
- TH	-0.1 (1.0)	0.0 (1.3)	0.3 (0.9)	0.3 (1.3)	0.3 (0.8)	-0.0 (0.9)	0.2 (1.1)	-0.0 (0.9)	-0.1 (1.0)
- FN	-0.4 (1.1)	-0.2 (1.6)	-0.0 (1.1)	-0.2 (1.3)	-0.0 (0.9)	-0.6 (1.0)	-0.3 (1.1)	-0.6 (1.0)	-0.7 (1.1)
Laboratory values									
- Estradiol, pmol/L	190 (64–373)	123 (95–186)	267 (78–558)	111 (78–168)	97 (57–137)	174 (29–537)	93 (72–150)	80 (49–131)	65 (36–109)
- Testosterone, nmol/L	1.4 (1.2–1.7)	20 (14–31)	1.4 (1.1–1.9)	18 (11–31)	21 (14–33)	1.3 (0.8–1.4)	22 (14–39)	20 (12–33)	18 (9–25)

Age during the DXA scan, age at start of hormonal treatment (HT), duration of HT, body mass index (BMI), (former) smokers, bone-influencing medication, bone-influencing comorbidity, T-scores of the lumbar spine (LS), total hip (TH), and femoral neck (FN), and estradiol and testosterone concentrations are shown separately for different age groups (20-29 years, 30-39 years, and 40-59 years), and for different durations of HT (0 years, 5 years, 15 years, and 25 years). Data are shown as means with standard deviations, percentages, or median with interquartile range.

Table 3. Mean differences (with 95% confidence intervals) between different durations of HT for different age groups, accompanying Figure 1 and Figure 2.

	Trans women			Trans men		
	5 vs 0 years	15 vs 0 years	25 vs 0 years	5 vs 0 years	15 vs 0 years	25 vs 0 years
20-29 years						
SW	-0.05 (-0.17; +0.07)	-	-	-0.14 (-0.34; +0.07)	-	-
ED	-0.07 (-0.19; +0.06)	-	-	-0.12 (-0.34; +0.10)	-	-
ACT	+0.01 (-0.01; +0.02)	-	-	-0.01 (-0.03; +0.01)	-	-
SM	+0.02 (-0.18; +0.21)	-	-	-0.08 (-0.27; +0.11)	-	-
TBS	+0.03 (-0.01; +0.07)	-	-	-0.04 (-0.09; +0.00)	-	-
30-39 years						
SW	-0.10 (-0.22; +0.02)	-0.09 (-0.21; +0.02)	-	-0.10 (-0.27; +0.08)	+0.09 (-0.10; +0.26)	-
ED	-0.11 (-0.24; +0.03)	-0.10 (-0.23; +0.03)	-	-0.09 (-0.28; +0.10)	+0.09 (-0.11; +0.29)	-
ACT	-0.00 (-0.02; +0.01)	-0.00 (-0.02; +0.02)	-	-0.00 (-0.02; +0.02)	-0.00 (-0.02; +0.02)	-
SM	+0.01 (-0.14; +0.17)	-0.01 (-0.17; +0.14)	-	-0.02 (-0.19; +0.16)	+0.04 (-0.14; +0.22)	-
TBS	+0.00 (-0.04; +0.04)	-0.02 (-0.06; +0.03)	-	-0.03 (-0.08; +0.02)	-0.03 (-0.09; +0.02)	-
40-59 years						
SW	+0.04 (-0.05; +0.12)	-0.04 (-0.13; +0.05)	-0.06 (-0.17; +0.04)	+0.17 (+0.02; +0.32)	+0.09 (-0.05; +0.23)	+0.14 (+0.01; +0.27)
ED	+0.03 (-0.06; +0.12)	-0.05 (-0.14; +0.05)	-0.07 (-0.18; +0.05)	+0.17 (+0.00; +0.33)	+0.10 (-0.06; +0.25)	+0.16 (+0.01; +0.30)
ACT	+0.00 (-0.01; +0.01)	+0.00 (-0.01; +0.01)	+0.00 (-0.01; +0.01)	+0.00 (-0.01; +0.02)	-0.00 (-0.02; +0.01)	-0.01 (-0.02; +0.00)
SM	+0.07 (-0.04; +0.18)	+0.01 (-0.11; +0.12)	-0.05 (-0.18; +0.08)	+0.16 (+0.01; +0.31)	+0.01 (-0.13; +0.15)	-0.00 (-0.13; +0.13)
TBS	+0.00 (-0.03; +0.03)	+0.01 (-0.02; +0.04)	+0.04 (+0.00; +0.08)	-0.05 (-0.09; -0.01)	-0.03 (-0.07; +0.01)	-0.03 (-0.06; +0.01)

Abbreviations: SW=subperiosteal width, ED=endocortical diameter, ACT=average cortical thickness, SM=section modulus, TBS=trabecular bone score

In Figure 1 and Table 3, the differences in subperiosteal width, endocortical diameter, average cortical thickness, and section modulus of the narrow neck among different hormone-duration groups are shown. Trans women had a larger subperiosteal width, endocortical diameter, and section modulus than trans men, while cortical thickness was lower in trans women than in trans men. In trans women in all age groups and in young trans men, no differences were observed in periosteal width, endocortical diameter, average cortical thickness, and section modulus for different durations of HT. In trans men aged 40-59 years, subperiosteal width, endocortical diameter, and section modulus were slightly higher in the groups who were using HT compared to the (peri- or postmenopausal) baseline group. These same patterns in trans women and trans men were also observed in the intertrochanteric region (supplementary Figure 1) and femoral shaft (supplementary Figure 2).

When comparing the narrow neck data with reference values reported in literature, trans women had mean subperiosteal widths, endocortical diameters, and section modulus closely related to the male reference values. The mean subperiosteal widths and endocortical diameters in trans men were in between the male and female reference values, whereas the section modulus in trans men was more related to the female reference values.

As shown in Figure 2 and Table 3, in younger trans women TBS tended to be higher in those using HT compared to the baseline groups, and in trans women in the oldest age group TBS was higher in those using HT for 25 years versus baseline (+0.04, 95%CI +0.00; +0.08). In younger trans men, TBS tended to be lower in those who used HT compared to the baseline groups, and in older trans men TBS was lower in those using 5 years HT versus baseline (-0.05, 95%CI -0.08; -0.01).

Repeating the analysis after excluding people with bone-influencing medication or bone-influencing comorbidity (n=174 trans women and 155 trans men), no changes in effect sizes were observed.

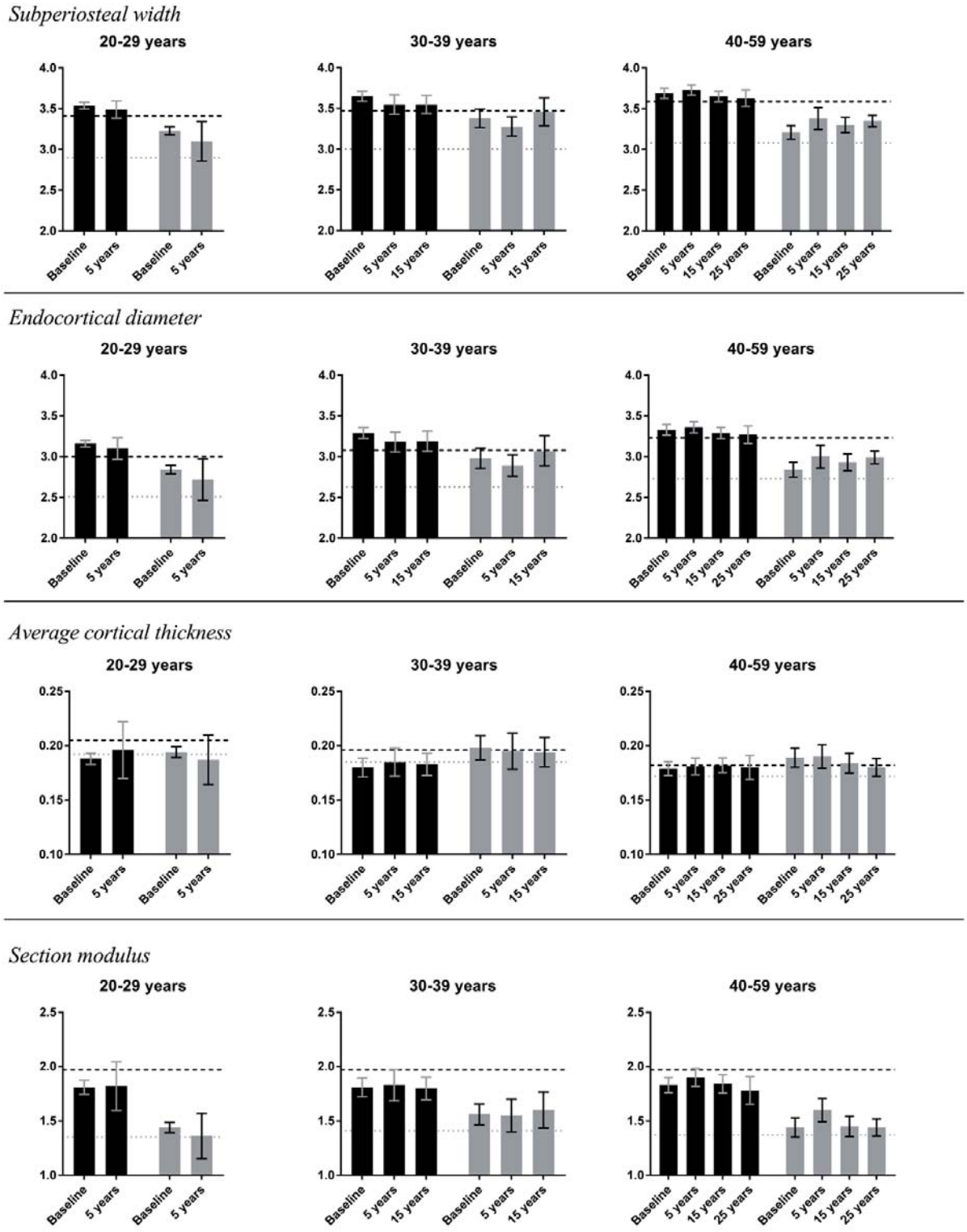


Figure 1.
Hip structure analyses of the narrow neck. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the y-axis the periosteal width, endocortical diameter, average cortical thickness, and section modulus in centimeters is shown for the narrow neck. On the x-axis, the different durations of HT are shown. The black horizontal lines are the reference values for cis men as reported in literature, while the grey horizontal lines are the reference values for cis women as reported in literature.[19]

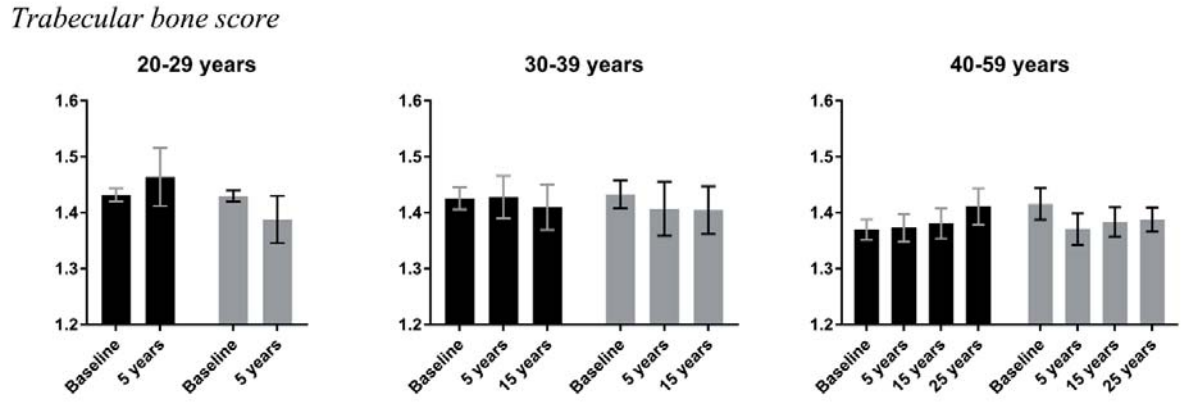


Figure 2.
Trabecular bone score. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the y-axis the trabecular bone score is shown. On the x-axis, the different durations of HT are shown.

Discussion

In this study, we aimed to study if bone geometry and TBS changes due to HT in adult transgender people. Hip structure analysis and trabecular bone score were measured in 3 age groups of trans women and trans men (20-29 years, 30-39 years, 40-59 years) using HT for different durations (baseline, 5 years, 15 years, 25 years). In trans women in all age groups and in young trans men, no differences were observed in periosteal width, endocortical diameter, average cortical thickness, and section modulus for different durations of HT. In trans men aged 40-59 years, subperiosteal width, endocortical diameter, and section modulus were slightly higher in the groups who were using HT compared to the (peri- or postmenopausal) baseline group. However, differences in trabecular bone score were observed. In younger trans women, TBS was (tending to be) higher in those using HT compared to the baseline groups, whereas in younger trans men, TBS was (tending to be) lower in those who used HT compared to the baseline groups.

In contrary to what we expected, we did not find any differences in periosteal and endocortical diameter in both trans women and trans men using HT for different durations. Several possible explanations can be given. Firstly, using the HSA software might not be the most accurate method to analyze bone geometry. DXA densitometers have a good precision for measuring BMD, but they are not designed to measure bone geometry. Even small changes in rotation of the femur can influence the geometry measurements. However, the scanner technologists were trained to scan the hip for measuring BMD adequately according to the manufacturer's instructions. It is expected that all participants were scanned in the same standardized way. This decreases the chance of differences between groups resulting from positioning errors. In addition,

previous studies compared the results obtained with HSA with quantitative computed tomography (qCT). One study found a high correlation of r=0.95 between HSA and a high-resolution qCT for periosteal width.[20] Another study described that in their study population the average value of the periosteal width was similar for HSA and qCT.[21] A second explanation for why we did not find any differences might be that most trans people already achieved the age of peak bone mass before the start of HT. It might be that differences in bone geometry only occur when treatment is started during puberty. This is also in line with the finding that in men with aromatase deficiency, estrogens given during puberty are still able to act at cortical level [22], whereas it seems less effective when given after puberty.[23] It is therefore interesting to investigate the bone geometry in trans people who started treatment during adolescence, by using gonadotropin-releasing hormones to suppress puberty with subsequently use of HT. Another explanation might be that differences in characteristics, such as physical activity, between groups influences the results, despite non-Caucasian people were excluded, and BMI and smoking was similar among the groups.

In trans women in our study, no differences in endocortical and periosteal diameters for different durations of HT in each age group were found. This is in line with earlier peripheral qCT studies performed in trans women. One study found no change in endocortical and periosteal circumference during the first 2 years of HT.[6] A smaller periosteal circumference in trans women compared with cis men was found before start of HT, while endocortical circumference was similar, leading to a smaller cortical thickness.[24] However, this is in contrast to our results, as we found that the periosteal width in trans women was similar to the male reference values reported in literature. One study after long-term HT found that trans women had a smaller periosteal circumference and similar endocortical circumference than cis men.[25] The reported point estimates were however quite similar to the point estimates reported in their baseline study, which confirms the finding that no changes in periosteal and endocortical circumference occurred during HT.

Also in trans men, no difference in periosteal and endocortical diameters were found for different durations of HT in different age groups. This is in agreement with earlier studies performed in trans men investigating the circumferences using peripheral qCT.[7,26] A 1-year follow-up study found no changes in periosteal and endocortical circumference in trans men.[7] After 10 years of HT, one study found no differences in periosteal and endocortical circumferences of the tibia compared with cis women [26], and only a slightly larger periosteal and endocortical circumferences of the radius. In addition, the authors report that the length of HT duration was not associated with these circumferences, which is in line with our results.

No earlier studies in transgender people have been performed analyzing the TBS. However, earlier studies found that cis women had higher TBS than cis men [13] and that TBS decreases in cis women during menopause.[14-16] These findings indicate that

estrogen is positively associated with TBS. Therefore, it was expected that TBS would increase in trans women as estrogen concentrations increase, whereas a decrease in TBS would be observed as estrogen concentrations decrease in trans men. Cut-off values for TBS are reported in literature. A TBS lower than 1.200 indicates poor microarchitecture, a TBS between 1.200 and 1.300 is considered to be partially degraded microarchitecture, and a TBS above 1.300 indicates normal microarchitecture.[12] Although TBS decreases in trans men, the mean values during HT are still above 1.300 and do not show a linear decrease with increasing duration of HT, indicating a still normal microarchitecture during HT.

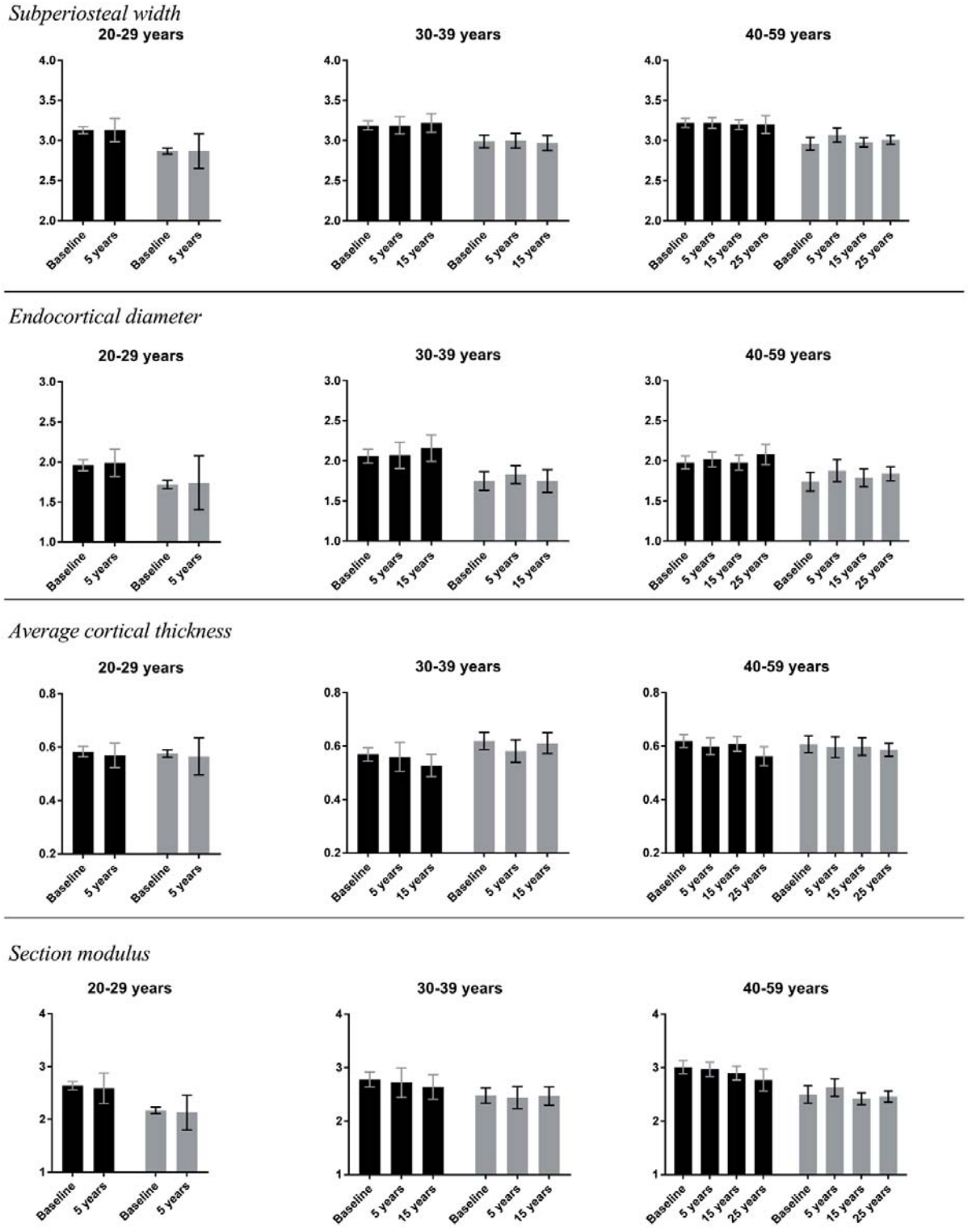
In clinical practice, DXA is the most commonly used technique to evaluate bone health. However, DXA is a 2-dimensional scan using two X-ray beams with different energy levels, to measure BMD (in g/cm^2) calculated as bone mineral content (in grams) divided by the bone area (in cm^2). In order to measure BMD more accurately, mass should be divided by volume and not area. Depth of the bones is however not measured by DXA. Taller people with larger bones will therefore have a higher areal BMD than people with smaller bones, even if volumetric BMD is similar. As earlier mentioned, men have larger bones than women and therefore also a higher areal BMD. Different reference values exist for men and women to evaluate the BMD and calculate T-scores and Z-scores. However, as some studies found a similar fracture risk among men and women with the same absolute BMD, there is discussion about whether or not sex-specific reference values should be used for cis men and women, or to use female reference ranges for everybody. In most hospitals in the Netherlands, female reference values are used for everybody. No guidelines exist about what reference values should be used for trans people if a center uses sex-specific reference values. Usually, the reference values of the sex-assigned at birth are used, because most trans people had already achieved their peak bone mass before the start of HT. In the most ideal situation, BMD of the trans population should be compared with those of cis men and women with a similar fracture risk, to determine what reference values should be used. Unfortunately, these data are not available. Based on the current results that bone geometry of the hip in trans women are more related to male reference values, and in trans men are in between male and female reference ranges or more related to female reference ranges, and did not change during HT, the birth-assigned sex reference values should be used. However, the change in TBS might indicate that the reference values of the identified sex should be used during HT. In addition, in an earlier study performed, both trans women and trans men had a mean BMD at baseline more closely related to female reference ranges than male reference [27], which can indicate that female reference ranges should also be used for transgender people. Therefore, data about which reference values should be used is not conclusive and more research is needed.

This study is the first study examining the bone geometry and TBS in transgender people using HT for different durations. We included a large population of trans women

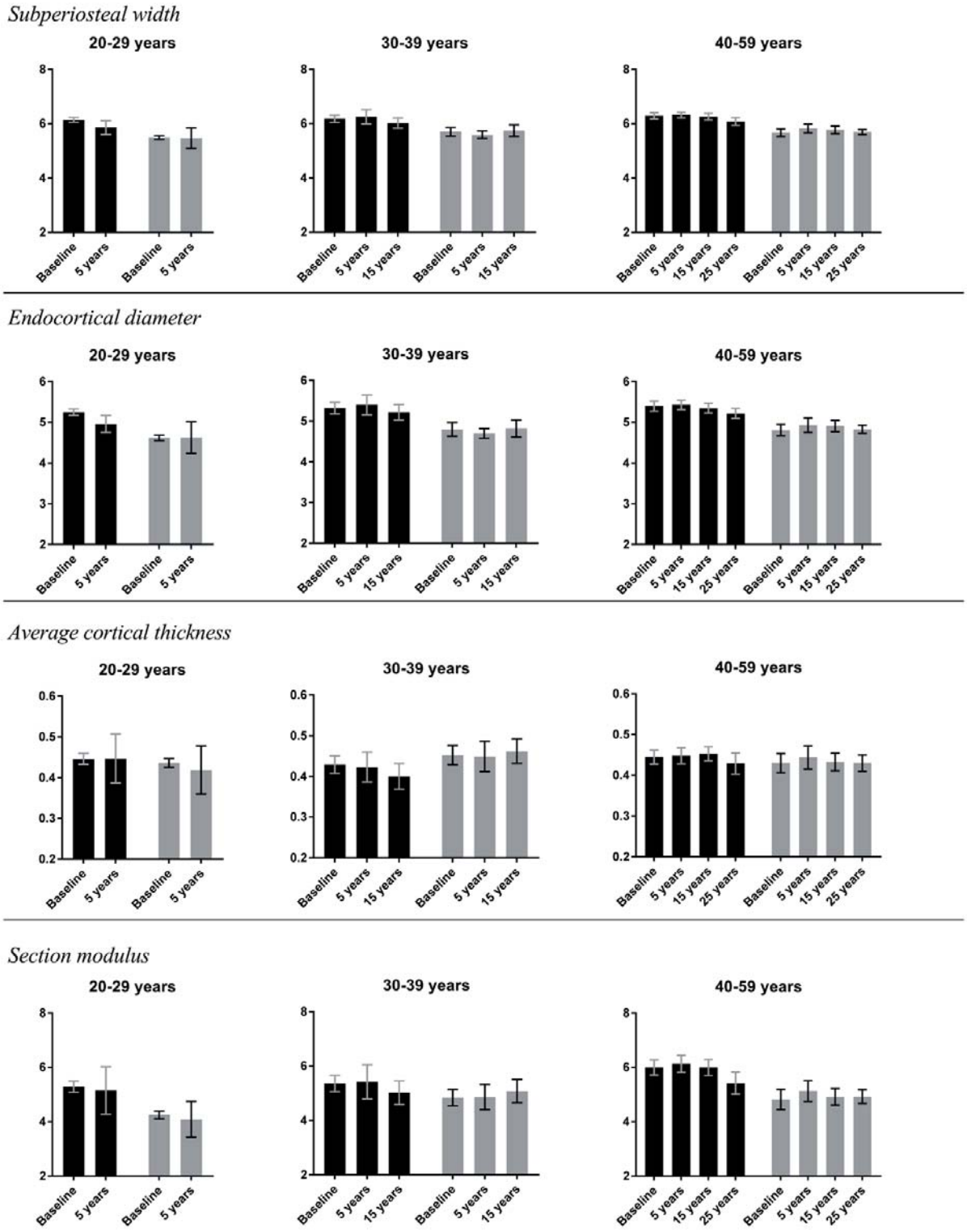
and trans men using HT for different durations, either before HT, short-term use of HT, and long-term use of HT. In addition, we included trans people with a wide age range. All measurements were performed using the same DXA scanner. However, there are also some limitations. Firstly, we only had one measurement for each person and no follow-up measurements. Secondly, a control group was lacking. Therefore, male and female reference values reported in literature were used for comparison. Thirdly, although we included a large population of trans women and trans men, we divided them into separate age groups and HT duration groups. Therefore, some groups were small resulting in large confidence intervals. Lastly, it was not possible to study relationships of concentrations of estradiol and testosterone with bone geometry and TBS, because the groups were too small.

Conclusion

We found no differences in cortical bone geometry parameters in transgender people using different durations of HT. TBS showed higher values in trans women using HT compared to trans women without use of HT, and lower values in trans men compared with trans men without use of HT. In trans women, the cortical bone geometry values were more related to male reference values, while in trans men the values were more related to the female reference values. These data may be helpful in determining what sex reference values for calculating T-scores and Z-scores in adult transgender people should be used.



Supplementary Figure 1.
Bone geometry parameters of the femoral shaft. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the y-axis the periosteal width, endocortical diameter, average cortical thickness, and section modulus in centimeters is shown for the femoral shaft. On the x-axis, the different durations of HT are shown.



Supplementary Figure 2.
Bone geometry parameters of the intertrochanteric region. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the y-axis the periosteal width, endocortical diameter, average cortical thickness, and section modulus in centimeters is shown for the intertrochanteric region. On the x-axis, the different durations of HT are shown.

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