Bone health in transgender people

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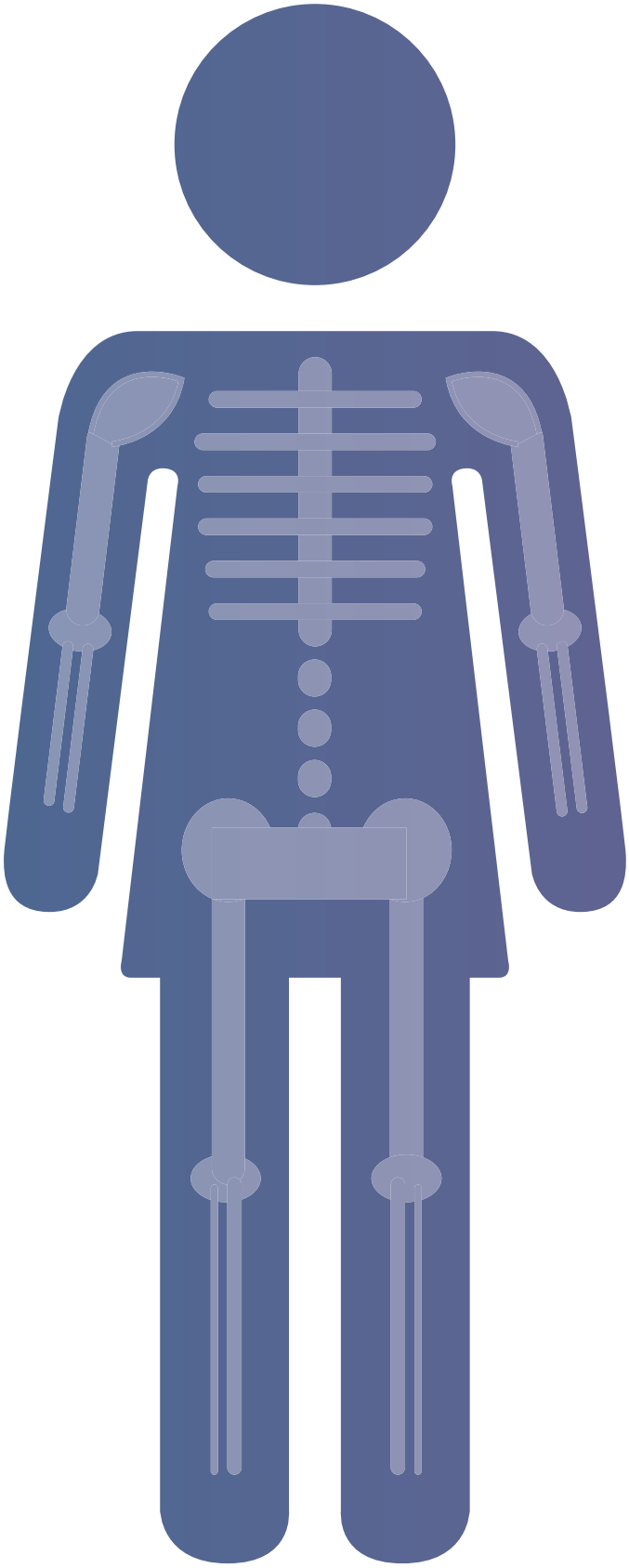

Chapter 5

Changes of vitamin D-binding protein, and total, bioavailable, and free 25-hydroxyvitamin D in transgender people

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Abstract

Context: Total 25-hydroxyvitamin D [25(OH)D] is mainly bound to vitamin D-binding protein (DBP). Bioavailable 25(OH)D consists of albumin-bound and free 25(OH)D and is available for metabolic processes. As sex steroids influence DBP, hormonal treatment (HT) in transgender people might affect DBP and consequently the available 25(OH)D. Total 25(OH)D might therefore not well represent bioavailable and free 25(OH)D.
Objective: To investigate the effects of HT on DBP, and total, bioavailable, and free 25(OH)D, and to assess whether total 25(OH)D well represents bioavailable and free 25(OH)D.
Design: A prospective study
Setting: A university hospital
Participants: 29 transwomen and 30 transmen
Intervention: Estradiol and cyproterone acetate in transwomen, and testosterone in transmen
Main Outcome Measures: DBP, total 25(OH)D, free 25(OH)D, and albumin were measured at baseline and after three months of HT, and deseasonalized total 25(OH)D and bioavailable 25(OH)D were calculated.
Results: DBP changed with +5% (95%CI -0; +10%, p=0.06) in transwomen and with -3% (95%CI -9; +3%, p=0.34) in transmen. No significant changes were found in total 25(OH)D, free, and bioavailable 25(OH)D concentrations. Total 25(OH)D was well correlated with bioavailable (R^2 0.75) and free (R^2 0.76) 25(OH)D.
Conclusions: DBP tended to increase in transwomen, but did not change in transmen. HT did not influence free 25(OH)D, total 25(OH)D, and bioavailable 25(OH)D concentrations in transwomen and transmen. As total 25(OH)D represents bioavailable and free 25(OH)D well, HT in transgender people does not interfere with the assessment of vitamin D status.

Introduction

Vitamin D status is usually assessed by measuring the total serum 25-hydroxyvitamin D (25(OH)D) concentration. In the circulation, more than 99% of total 25(OH)D is bound to serum proteins, mainly to vitamin D-binding protein (DBP) and to albumin. [1] In contrast to DBP-bound 25(OH)D, albumin-bound 25(OH)D is available for metabolic processes, and forms, together with the free circulating 25(OH)D, the bioavailable 25(OH)D.

Previous studies have shown that sex steroids can influence vitamin D metabolism, particularly DBP concentrations. Women have higher DBP concentrations than men.[2] During pregnancy [3,4] and the use of oral contraceptives [5], higher DBP concentrations were observed, while after menopause lower DBP concentrations were found.[6] In hypogonadal men, treatment with testosterone decreases DBP concentrations.[7]

Transgender people often receive treatment with gender-affirming hormonal treatment (HT) to induce physical changes, which consists of estrogen in transwomen (birth-assigned males, female identity) and testosterone in transmen (birth-assigned females, male identity).[8] This HT might affect the DBP concentration and consequently the bioavailable and free circulating 25(OH)D concentration. Measured total 25(OH)D might therefore not well represent the bioavailable and free circulating 25(OH)D in transgender people receiving HT. This may hamper the assessment of vitamin D status and vitamin D deficiency and its potential harmful effects on bone and muscle.

The aim of this study was to investigate the first three-month effects of estrogen and testosterone treatment on DBP, and total, bioavailable, and free serum 25(OH)D concentrations in transwomen and transmen, respectively, and to assess whether total 25(OH)D measurements well represent bioavailable and free 25(OH)D concentrations in transgender people.

Materials and Methods

Design and population

This study is part of the European Network for Investigation of Gender Incongruence (ENIGI) study, which is a multicenter prospective observational study performed in Amsterdam, Florence, Ghent, and Oslo. Study design and data collection have been described previously.[9,10] In short, people could be included if they had a confirmed diagnosis of gender dysphoria, if they were about to start with HT, if they had never used gender-affirming hormones before, and if they spoke the native language. Clinical data, as well as blood samples, were prospectively collected before and during HT. Blood samples were stored in the freezer for later analyses. For the present study, people were included in Amsterdam between June 2012 and May 2016. To exclude menopausal- or

age-related changes in vitamin D metabolism, the age range was restricted from 18 to 50 years, and to premenopausal status in transmen. Other exclusion criteria were the use of other medication with possible influence on vitamin D metabolism (oral contraceptives, finasteride, breast growth promoting agents, thyroid medication, spironolactone), renal insufficiency, and the use of vitamin D supplements in the first six months of HT. In addition, people who did not have enough stored serum were excluded for the present analyses. As in earlier studies increases in DBP of 25-50% using oral anticonceptives [5,11] and 100% during pregnancy [4,11] were found, we hypothesized that DBP would increase with 25% in transwomen. Based on reported means and SDs of the change in DBP in other populations [4,12], an alpha of 0.05, and a power of 90%, sample size calculation yielded 20 people per group. This was increased to 30 people per group to adjust for dropout. As more people were eligible according to the inclusion and exclusion criteria, these 30 people per group were randomly selected.

All transgender people received HT according to the Standards of Care Guidelines of the World Professional Association for Transgender Health (WPATH). [8] Transwomen were treated with cyproterone acetate (50 mg daily) combined with oral estradiol valerate (4 mg daily) or an estradiol patch (100 mg/24h twice a week). Transmen received intramuscular testosterone esters (250 mg every 2 to 3 weeks) or testosterone gel (50 mg daily). All people visited the gender identity clinic at baseline and after three months of HT. Medical history and medication use were reported. Physical examination included body height (in meters) and body weight (in kilograms) in indoor clothing without shoes.

The overall study protocol for the ENIGI study was approved by the Medical Ethical Review Board of the Ghent University Hospital, Belgium, and in the other centers approval for participation was obtained of the local medical ethical review boards. The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from all people according to the institutional guidelines.

Biochemical assessment

At baseline and after three months of HT, blood samples were obtained in the morning and immediately centrifuged and kept frozen at -80°C until analysis, including total 25(OH)D, DBP, free 25(OH)D, and albumin. Baseline and three-month samples of the same person were analyzed in the same run to exclude inter-assay variation. All analyses were carried out at the Endocrine Laboratory of the Amsterdam UMC, the Netherlands.

Total serum 25(OH)D was measured using a liquid chromatography-tandem mass spectrometry device (LC-MS/MS, Waters Acquity UPLC and Waters Quattro Premier XE MS/MS) with an intra-assay coefficient of variation (CV) of <9%, an inter-assay CV of ≤8%, and a lower limit of quantitation (LLOQ) of 4 nmol/L[4]. DBP was measured using a polyclonal ELISA (Immundiagnostik AG) with an intra- and inter-

assay CV of <13% and a LLOQ of 2.2 ng/mL. The free 25(OH)D was measured using the EIA of Diasource (intra-assay CV <13% and LLOQ of 3.3 pg/mL). Albumin was measured using Bromocresol purper method (Cobas, Roche Diagnostics).

Estradiol was measured using a competitive immunoassay (Delfia, PerkinElmer, Turku, Finland) with an inter-assay CV of 10-13% and LLOQ of 20 pmol/L until July 2014. After July 2014, estradiol was measured using a LC-MS/MS (inter-assay CV 7%, LLOQ 20 pmol/L). To make the estradiol concentrations measured with both methods comparable, the concentrations obtained with Delfia were converted to the concentrations obtained with the LC-MS/MS, using the formula LC-MS/MS=1.60×Delfia–29 generated by using Passing-Bablock regression for the method comparison. Testosterone was measured using a competitive immunoassay (Architect, Abbott, Abbott Park, IL, USA) with an intra-assay CV of 4-7%, an inter-assay CV of 6-10%, and a LLOQ of 0.1 nmol/L[13]. LH was measured using an immunometric assay (Architect, Abbott, Abbott Park, IL, USA) with an intra-assay CV of <5%, an inter-assay CV of <6%, and a LLOQ of 2 U/L.

Statistical analysis

Baseline characteristics of the study population are presented as mean with standard deviation (SD) or median with interquartile range (IQR). All analyses were performed separately for transwomen and transmen.

To adjust for seasonal variation in circulating total 25(OH)D concentrations, the measured serum total 25(OH)D concentrations were deseasonalized as described by Elstgeest et al.[14] A cosine model was fitted to the measurements at baseline and three months follow-up separately in transwomen and transmen. Bioavailable, albumin-bound, and free 25(OH)D concentrations were calculated using formulas by Bikle et al.[1] and Vermeulen et al.[15] Deseasonalized total 25(OH)D concentrations were used for calculation of the free 25(OH)D concentrations.

Differences between values at baseline and three months were tested using paired-samples *t*-test for normally distributed values, and Wilcoxon signed rank test for skewed variables. In transwomen, differences between transdermal and oral estradiol use were analyzed using linear regression analyses.

Linear regression analyses were performed and Pearson's correlation coefficients were calculated between measured and calculated free 25(OH)D, measured free and total 25(OH)D concentrations, and bioavailable and total 25(OH)D concentrations. In addition, linear regression analyses were performed between change in DBP and changes in estradiol and testosterone concentrations.

For all analyses, STATA Statistical Software (StataCorp, College Station, TX, USA) version 15.1 was used.

Results

One 24-year-old transwoman had an extremely high baseline DBP concentration of 1172 µg/mL, which was 385 µg/mL after three months of HT. Because measured free 25(OH)D and total 25(OH)D concentrations were quite similar at baseline and after 3 months of HT (free 25(OH)D: 10.9 pmol/L at baseline, 10.8 pmol/L after three months of HT; total 25(OH)D: 43 nmol/L at baseline, 37 nmol/L after three months of HT), we suppose that the high baseline DBP is a measurement error. Therefore this person was excluded for further analyses. In total, 29 transwomen (median age 26 years, IQR 22 – 35 years) and 30 transmen (median age 22 years, IQR 21 – 29 years) were included for analyses. The characteristics are presented in Table 1. At baseline, 12 transwomen (41%) had a total 25(OH)D concentration between 25 and 50 nmol/L, and 8 (28%) below 25 nmol/L. Also 14 (47%) and 6 (20%) transmen had a total 25(OH)D concentration between 25 and 50 or below 25 nmol/L, respectively. Altogether, 69% of transwomen and 67% of transmen were vitamin D deficient (serum 25(OH)D <50 nmol/L).

Table 1. Baseline and three-month values in transwomen and transmen

	Transwomen (n=29)			Transmen (n=30)		
	Baseline	**3 months**	**p-value**	**Baseline**	**3 months**	**p-value**
Age, yr	26 (22 – 35)			22 (21 – 29)		
Weight, kg	72.4 (67.7 – 80.5)	72.0 (68.6 – 86.1)	0.16	68.4 (59.1 – 85.1)	73.3 (62.8 – 88.8)	<0.01
BMI, kg/m^2	22.1 (20.5 – 26.3)	22.1 (20.8 – 26.7)	0.13	23.2 (21.3 – 29.0)	25.1 (21.6 – 30.0)	<0.01
ALT, IU/L	21 (17 – 26)	21 (14 – 25)	0.16	16 (13 – 23)	20 (13 – 23)	0.42
AST, IU/L	20 (18 – 23)	18 (15 – 21)	<0.01	21 (18 – 23)	21 (19 – 26)	0.16
GGT, IU/L	19 (14 – 34)	19 (15 – 25)	0.55	14 (10 – 19)	16 (12 – 20)	0.01
Creatinine, µmol/L	77 ± 10	72 ± 10	<0.01	67 ± 10	74 ± 10	<0.01
Albumin, g/L	48.5 ± 2.4	46.4 ± 2.6	<0.01	45.8 ± 2.4	45.9 ± 2.8	0.64
Testosterone, nmol/L	21.0 (17.0 – 28.0)	0.6 (0.5 – 0.8)	<0.01	1.3 (1.0 – 1.6)	28.5 (19.0 – 34.0)	<0.01
Estradiol, pmol/L	99 (79 – 113)	228 (158 – 337)	<0.01	358 (214 – 632)	186 (156 – 269)	0.01
LH, IU/L	3.2 (2.5 – 4.6)	0.1 (0.1 – 0.1)	<0.01	5.7 (2.7 – 7.7)	3.9 (0.9 – 6.1)	0.13

Data are shown as median (inter quartile range) or mean with standard deviation. Abbreviations: yr=year, kg=kilogram, BMI=body mass index, kg/m^2=kilogram per square meter, ALT=alanine amino transferase, IU/L=international units per liter, AST=aspartate amino transferase, GGT=gamma-glutamyl transferase, LH=luteinizing hormone

In transwomen, DBP tended to increase with +5% (95%CI -0; +10%, p=0.06) and measured free 25(OH)D changed with -2% (95%CI -12; +9%, p=0.75). Total 25(OH)D concentrations increased, but after seasonal adjustment no change was observed. Deseasonalized free, bioavailable, and albumin-bound 25(OH)D concentrations did not significantly change during the first three months of HT (Table 2).

Transwomen using transdermal estradiol tended to have a larger increase in DBP than transwomen using oral estradiol (difference +29 μg/mL, 95%CI -5; +63 μg/mL), although not statistically significant, while no differences were found in deseasonalized 25(OH)D (difference +0.1 nmol/L, 95%CI -7.7; +7.9 nmol/L), measured free 25(OH)D (difference +0.5 pmol/L, 95%CI -1.5; +2.5 pmol/L), and bioavailable 25(OH)D (difference +0.2 nmol/L, 95%CI -0.6; +1.0 nmol/L) concentrations.

In transmen, no significant changes were found in DBP (-3%, 95%CI -9; +3%, p=0.34), measured free 25(OH)D (+9%, 95%CI -13; +32%, p=0.41), deseasonalized total 25(OH)D, bioavailable 25(OH)D, and albumin-bound 25(OH)D concentrations during the first three months of HT (Table 2).

Table 2. Changes in different 25-hydroxyvitamin D fractions, for transwomen and transmen

	Transwomen (n=29)			Transmen (n=30)		
	Baseline	**3 months**	**Difference (95%CI)**	**Baseline**	**3 months**	**Difference (95%CI)**
Total 25(OH)D, nmol/L	40.5 ± 19.4	32.8 ± 16.3	-7.7 (-13.5; -1.9), p=0.01	44.2 ± 22.0	42.2 ± 23.1	-2.0 (-9.5; +5.5), p=0.59
Deseasonalized total 25(OH)D, nmol/L	37.7 ± 16.4	37.6 ± 13.6	-0.1 (-3.9; +3.7), p=0.94	43.1 ± 21.2	45.3 ± 20.5	+2.2 (-3.9; +8.3), p=0.46
Measured free 25(OH)D, pmol/L	7.7 (6.2–11.2)	7.5 (6.3–9.1)	-0.6 (-1.6; +0.3), p=0.18	8.6 (6.4–11.0)	8.1 (6.3–12.2)	+0.1 (-1.5; +1.7), p=0.89
Deseasonalized measured free 25(OH)D, pmol/L	8.1 ± 2.9	8.2 ± 1.9	+0.1 (-0.7; +1.0), p=0.74	9.1 ± 4.2	9.7 ± 4.0	+0.7 (-0.7; +2.1), p=0.32
Calculated free 25(OH)D, pmol/L	7.9 ± 3.5	7.5 ± 3.1	-0.4 (-1.3; +0.5), p=0.41	8.8 ± 4.2	9.5 ± 3.9	+0.7 (-0.5; +1.9), p=0.24
Albumin-bound 25(OH)D, nmol/L	3.3 ± 1.5	3.0 ± 1.2	-0.3 (-0.7; +0.1), p=0.09	3.5 ± 1.7	3.8 ± 1.7	+0.3 (-0.2; +0.8), p=0.19
Bioavailable 25(OH)D, nmol/L	3.3 ± 1.5	3.0 ± 1.2	-0.3 (-0.7; +0.1), p=0.09	3.5 ± 1.7	3.8 ± 1.7	+0.3 (-0.2; +0.8), p=0.19
DBP, μg/mL	333 (311–371) 337 ± 52	327 (311–394) 351 ± 56	+14 (-3; +31), p=0.11	336 (313–360) 344 ± 47	321 (293–359) 329 ± 41	-15 (-38; +8), p=0.20

Data are shown as median (inter quartile range) and/or mean with standard deviation. Abbreviations: 25(OH)D=25-hydroxy-vitamin D, DBP=vitamin D binding protein

An increase in estradiol concentrations tended to be associated with an increase in DBP concentrations (per 100 pmol/L: +3 µg/mL, 95%CI -1 ; +7 µg/mL), while an increase in testosterone levels tended to be associated with a decrease in DBP (per 10 nmol/L: -3 µg/mL, 95%CI -7 ; +1 µg/mL).

As shown in Figure 1, total 25(OH)D concentration was well correlated with bioavailable 25(OH)D (R^2 0.75) and free 25(OH)D (R^2 0.76). Measured and calculated free 25(OH)D concentrations were correlated (R^2 0.69). Stratification of the correlation analyses for baseline and three months measurements, and for transwomen and transmen, did not change the numbers of the beta's, Pearson's correlation coefficients, and R^2 with more than 6% (data not shown).

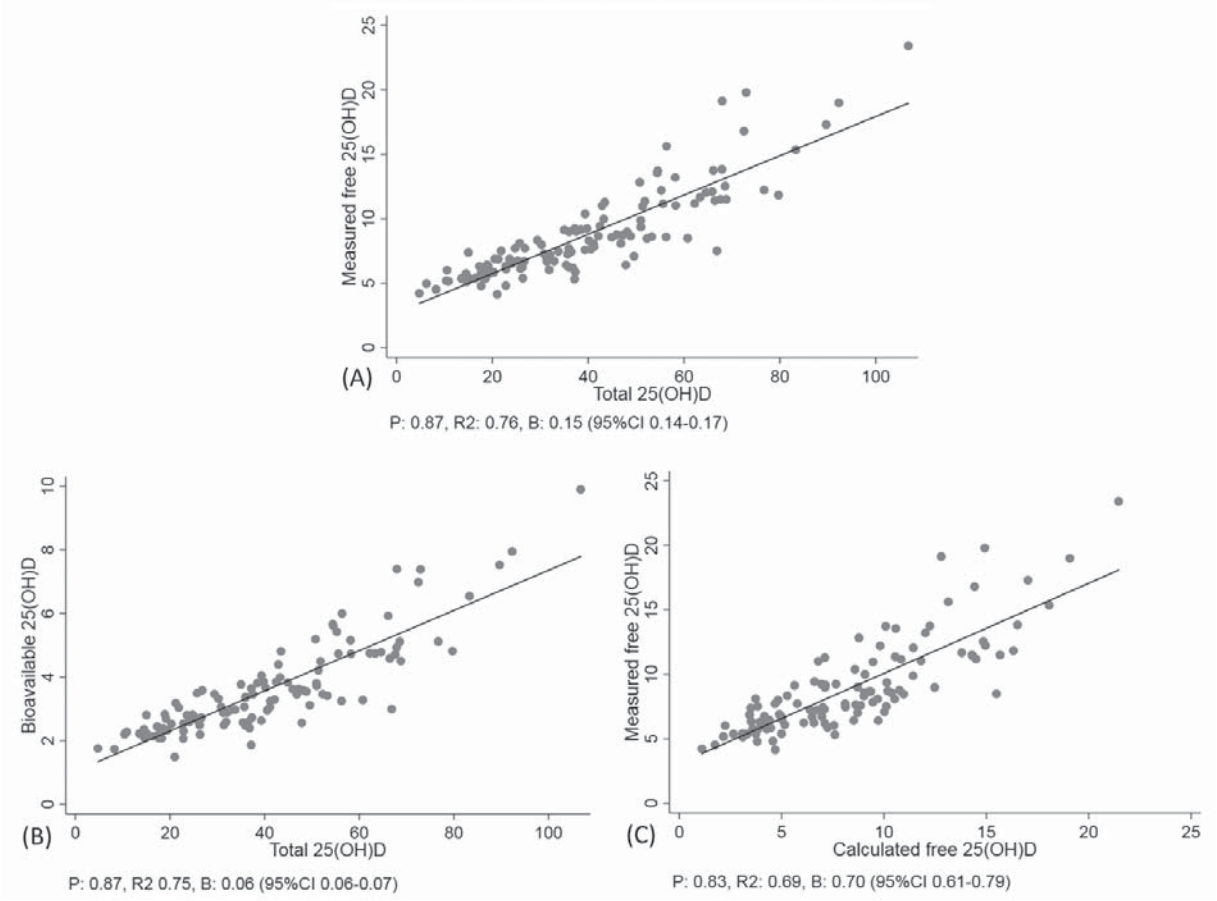

Figure 1.
Correlations between concentrations of (A) total 25(OH)D and measured free 25(OH)D, (B) total 25(OH)D and bioavailable 25(OH)D, and (C) measured and calculated free 25(OH)D. Abbreviations: P = Pearson correlation coefficient, R2 = R squared, B = beta

Discussion

In this study, we aimed to investigate whether DBP, total 25(OH)D, free 25(OH)D, and bioavailable 25(OH)D concentrations would change during HT in transgender people. We found that the percentage of people with vitamin D deficiency at baseline was high. HT was effective in both transwomen and transmen, as reflected by an increase in estradiol concentrations and decreases in testosterone and creatinine concentrations in transwomen, and a decrease in estradiol concentrations and increases in testosterone and creatinine concentrations in transmen. DBP tended to increase in transwomen, but did not change in transmen. No statistically significant changes were observed for either free 25(OH)D, deseasonalized total 25(OH)D, and bioavailable 25(OH)D concentrations for both transwomen and transmen. Total 25(OHD concentrations were well correlated with free and bioavailable 25(OH)D concentrations, and measured and calculated free 25(OH)D concentrations were also well correlated.

Although the changes in both transwomen and transmen were not statistically significant, they are pointing towards the direction that was expected and hypothesized. In transwomen, mean DBP concentrations were slightly higher after three months of HT, while free 25(OH)D and bioavailable 25(OH)D concentrations were lower. Opposite results were observed in transmen: DBP concentrations were lower after three months of HT, whereas free 25(OH)D and bioavailable 25(OH)D concentrations were slightly higher. In addition, the correlation with change in estradiol and testosterone concentrations pointed towards the same direction: an increase in estradiol concentration tended to be associated with an increase in DBP concentrations, while an increase in testosterone levels tended to be associated with a decrease in DBP. This is in line with earlier studies, which also reported a correlation between estradiol concentrations and change in DBP.[6]

Earlier studies reported that DBP changes under influence of estrogen. For example, it increased with 25-50% during the use of oral anticonceptives [5,11] and with 100% during pregnancy.[3,4,11] We hypothesized that DBP would increase with 25% in transwomen using HT. However, we observed that DBP increased with only 5%, but this change was not statistically significant. A possible explanation for the smaller increase in DBP is that, although the decrease in testosterone was large, the increase in estradiol concentrations was smaller. In our study, median estradiol concentrations increased from 99 pmol/L at baseline to 228 pmol/L at three months, while in pregnancy estradiol concentration can increase to concentrations over 10,000 pmol/L.[16] Oral contraceptives contain the potent synthetic estrogen ethinylestradiol, which may induce a larger increase in DBP. In addition, all participants used cyproterone acetate, which might also influence DBP metabolism. As DBP is produced by the liver, the route of administration of estrogen could influence DBP change.[17] However, in contrary to what we expected, we found that transwomen using transdermal estradiol tended to

have a larger increase in DBP than transwomen using oral estradiol. We do not have a clear explanation for this finding and further studies are needed to explore this.

In postmenopausal women (with low estradiol concentrations), DBP concentrations are 10% lower than in premenopausal women.[6] Contrary to studies in rats, where an increase in DBP concentrations was found after exposure to androgens [18], in hypogonadal men treated with testosterone, DBP concentrations decreased with 8% [7], while both testosterone and estradiol concentrations increased. Therefore, it was expected that DBP concentrations would decrease in transmen, because of decreasing estradiol concentrations and increasing testosterone concentrations. In our study, we found that DBP concentrations decreased with only 3% and this was not statistically significant. The estradiol concentrations decreased more in transmen than after menopause (transmen: 358 to 186 pmol/L, menopause: 195 to 48 pmol/L [6], although the percentage change was less. The increases in testosterone concentrations are relatively larger than the decreases in estradiol concentrations. Because testosterone administration also leads to aromatization of testosterone into estradiol, the estradiol concentrations did not decrease to a low level. It might therefore be that the estradiol concentrations were still above a certain threshold in transmen, preventing a substantial decrease in DBP.

Vitamin D deficiency was very common in our study population, with 68% of the participants having a serum 25(OH)D concentration <50 nmol/L. This may be caused by lack of sun exposure, as trans people may go outside less often and not expose themselves as they are not happy with their body.

The literature finding that PTH better correlates with bioavailable 25(OH)D concentrations than with total 25(OH)D concentrations indicates that bioavailable 25(OH)D may provide a better assessment of vitamin D deficiency.[19] However, as measurements of free and bioavailable 25(OH)D concentrations are not widely available, these are usually calculated. It is not known whether total 25(OH)D reflects the measured free and bioavailable 25(OH)D concentrations in trans people. In this study, we found that total 25(OH)D concentrations were well correlated with measured free 25(OH)D concentrations. In addition, the correlation between bioavailable 25(OH)D and total 25(OH)D concentrations was similar to that between measured free 25(OH)D and total 25(OH)D concentrations. The finding that total 25(OH)D was well correlated with free and bioavailable 25(OH)D, also during HT, indicates that total 25(OH)D concentrations can be used in transgender people using HT to assess vitamin D status.

This study is a prospective study including transwomen and transmen. It has several strengths. Measurements were performed before and during treatment with estradiol and testosterone, respectively. Inclusion and exclusion criteria were applied to exclude age- or menopause- related changes in vitamin D metabolism, and people with diseases or medication with possible influence on vitamin D metabolism were

excluded. All vitamin D measurements were analyzed in one run. However, this study also has some limitations. First, no PTH concentrations were available, which could be informative as well. A high correlation between the free 25(OH)D concentration and serum PTH could indicate the clinical importance of free 25(OH)D. Second, a control group was lacking, which was not possible because of ethical reasons. Third, the sample size might be too small. Although a sample size calculation was performed before the study, the changes in DBP were smaller than expected. Last, as only the changes during the first three months were evaluated, the long-term effects are not known and clinical endpoints were not measured.

In conclusion, HT did not substantially influence DBP, free 25(OH)D, total 25(OH)D, and bioavailable 25(OH)D concentrations in transwomen and transmen, as the observed changes were small and not statistically significant. In addition, total 25(OH)D concentrations seem to reflect free and bioavailable 25(OH)D concentrations well. Therefore diagnostics of the commonly occurring vitamin D deficiency in trans people does not seem to be hampered by hormonal treatment.

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