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# Access to gender-affirming hormones during adolescence and mental health outcomes among transgender adults

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## Abstract

### Objective

To examine associations between recalled access to gender-affirming hormones (GAH) during adolescence and mental health outcomes among transgender adults in the U.S.

### Methods

We conducted a secondary analysis of the 2015 U.S. Transgender Survey, a cross-sectional non-probability sample of 27,715 transgender adults in the U.S. Using multivariable logistic regression adjusting for potential confounders, we examined associations between access to GAH during early adolescence (age 14–15), late adolescence (age 16–17), or adulthood (age ≥18) and adult mental health outcomes, with participants who desired but never accessed GAH as the reference group.

### Results

21,598 participants (77.9%) reported ever desiring GAH. Of these, 8,860 (41.0%) never accessed GAH, 119 (0.6%) accessed GAH in early adolescence, 362 (1.7%) accessed GAH in late adolescence, and 12,257 (56.8%) accessed GAH in adulthood. After adjusting for potential confounders, accessing GAH during early adolescence (aOR = 0.4, 95% CI = 0.2–0.6, p < .0001), late adolescence (aOR = 0.5, 95% CI = 0.4–0.7, p < .0001), or adulthood (aOR = 0.8, 95% CI = 0.7–0.8, p < .0001) was associated with lower odds of past-year suicidal ideation when compared to desiring but never accessing GAH. In post hoc analyses, access to GAH during adolescence (ages 14–17) was associated with lower odds of past-year suicidal ideation (aOR = 0.7, 95% CI = 0.6–0.9, p = .0007) when compared to accessing GAH during adulthood.

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**Competing interests:** I have read the journal's policy and the authors of this manuscript have the following competing interests: Dr. Turban reports receiving textbook royalties from Springer Nature and Dr. Keuroghlian reports receiving textbook royalties from McGraw Hill. Dr. Turban has received expert witness payments from the ACLU.

## Conclusion

Access to GAH during adolescence and adulthood is associated with favorable mental health outcomes compared to desiring but not accessing GAH.

## Introduction

A recent representative sample of adolescents in the United States (U.S.) found that 1.8% identified as transgender [1]. Unfortunately, these young people face a range of mental health disparities, including elevated rates of anxiety, depression, and suicide attempts [2]. Suicide attempt prevalence among transgender young adults has been estimated to be as high as 40% [3]. These disparities are generally thought to be due to two processes: gender minority stress and dysphoria related to one's body developing in ways that are incongruent with one's gender identity (i.e., a person's psychological sense of their own gender) [2].

Gender minority stress refers to the ways in which society's mistreatment of transgender people results in worse mental and physical health outcomes. This includes distal factors (gender-related discrimination, gender-related rejection, gender-related victimization, and non-affirmation of gender identity), as well as subsequent proximal factors (internalized transphobia, negative expectations, and concealment) [4]. Creating safe and affirming social environments for transgender adolescents is thus considered paramount in preventing adverse mental health outcomes [5].

In addition to creating safe and affirming environments, care for transgender people often involves the provision of gender-affirming medical interventions to alleviate the psychological distress related to one's body developing in ways that do not align with one's gender identity [6, 7]. This may include pubertal suppression for younger adolescents and gender-affirming hormones (GAH, e.g., estrogen and testosterone) from adolescence onward to induce physical changes that match the person's gender identity [6–8]. Some adolescents may undergo gender-affirming surgery to reduce psychological distress [9, 10]. Of note, past Endocrine Society guidelines recommended that GAH not be considered until an adolescent reaches age 16 [11]. More recent guidelines state that initiation of GAH can be considered as early as age 14, to allow transgender adolescents to undergo puberty at ages more comparable to their peers, and to reduce the risk of delayed bone development due to prolonged pubertal suppression [7]. In this article, we therefore consider two age groups of adolescents who initiated GAH: those who started GAH during late adolescence (i.e., between their $16^{th}$ and $18^{th}$ birthdays) and those who started GAH during early adolescence (i.e., between their $14^{th}$ and $16^{th}$ birthdays).

To date, there have been six longitudinal cohort studies examining the impact of GAH initiation during adolescence on mental health [12–17]. These studies have generally found improvement in mental health following adolescent GAH initiation, including decreases in internalizing psychopathology, improved general wellbeing, and decreased suicidality. Of note, these studies did not include a comparison group of adolescents who did not access GAH. Furthermore, these studies did not examine separately those who initiated GAH during early or late adolescence, nor did they compare initiation of GAH during adolescence with initiation of GAH during adulthood.

The impact of GAH initiated in adolescence on the mental health of transgender adults is of particular policy relevance today, as several U.S. states have introduced legislation to limit access to GAH for transgender adolescents, despite opposition from major medical organizations including The American Medical Association, The American Academy of Pediatrics,

The American Psychiatric Association, The American Academy of Child & Adolescent Psychiatry, The Endocrine Society, The Pediatric Endocrine Society, and others [18]. This is an area of active policy debate where additional quantitative data are needed to guide policy decisions. Parents of transgender youth have been particularly concerned about these restrictive legislative efforts, with a parent in one recent qualitative sudy noting, "this could mean death for my child" [19].

The current study uses the largest survey of transgender people conducted to date to examine associations between recalled access to GAH during early adolescence (ages 14–15), late adolescence (ages 16–17), or adulthood (age ≥18), and adult mental health outcomes including measures of suicidality. It is the first study of GAH initiation during adolescence that includes a comparison group of those who desired but never accessed GAH. It is also the first to compare access to GAH during adolescence with access to GAH during adulthood. Given the large sample size, we were able to adjust for a wide range of potential confounding variables known to be associated with mental health outcomes for transgender people. We hypothesized that access to GAH during both early and late adolescence would be associated with more favorable mental health outcomes reported in adulthood, when compared to desiring but never accessing GAH.

## Methods

### Study population

The 2015 U.S. Transgender Survey (USTS) is the largest existing dataset of transgender people to date [3]. The cross-sectional non-probability survey was conducted between August and September of 2015. Transgender adults ages 18 years or older were recruited in collaboration with over 400 community organizations and completed measures online. The final survey had 27,715 participants from all 50 U.S. states, as well as Washington D.C., Puerto Rico, and U.S. territories abroad. Because not all transgender people necessarily desire GAH, we restricted the current study to participants who reported ever desiring GAH for gender affirmation, as this is a more clinically relevant group. This was assessed by choosing "hormone therapy/HRT (an acronym for 'Hormone Replacement Therapy')" in response to the question, "Have you ever wanted any of the health care listed below for your gender identity or gender transition? (Mark all that apply)." Options included "counseling/therapy," "hormone treatment/HRT," "puberty blocking hormones (usually used by youth ages 9–16)," and "none of the above." This resulted in inclusion of 21,598 participants.

### Ethical considerations

The protocol for the USTS was approved by the University of California Los Angeles Institutional Review Board. The protocol for the current study was reviewed by The Fenway Institute Institutional Review Board. All participants provided informed consent for study participation.

### Age of initiation of GAH

Participants were divided into four categories. The first group, "wanted but never accessed GAH" (No GAH), reported never accessing GAH despite desiring these medications. The second group consisted of participants who reported they first accessed GAH during early adolescence, defined as the period between their 14$^{th}$ and 16$^{th}$ birthdays (GAH 14–15), which corresponds to the age group most recently added to the Endocrine Society Guidelines [7]. The third group consisted of participants who reported they first accessed GAH during late

adolescence, defined as the period between their 16th and 18th birthdays (GAH 16–17), corresponding to the narrower age group in the prior, 2009 Endocrine Society Guidelines [11]. The fourth group consisted of participants who reported they first accessed GAH after their 18th birthday (GAH $\geq$ 18).

## Outcomes

Severe psychological distress in the month prior to the survey was defined as a score $\geq$ 13 on the Kessler-6 Psychological Distress Scale [20]. Binge drinking in the month prior to the survey was defined as drinking 5 or more standard alcoholic drinks on a single occasion, a threshold for use in research with transgender adults that has been discussed in prior reports [21]. Lifetime illicit drug use (excluding marijuana) was also assessed as a binary "yes" or "no" self-report outcome. Measures of suicidality were examined, including suicidal ideation during the year prior to the survey, suicidal ideation with plan during the prior year, suicide attempt during the prior year, and suicide attempt requiring hospitalization during the prior year [8]. All suicidality measures were binary outcome variables in which participants reported "yes" or "no."

## Demographic and other potential confounding variables

Demographic and other potential confounding variables that are known to be associated with adverse mental health outcomes among transgender people were collected for participants and included age at time of survey completion (U.S. census categories), gender identity, sex assigned at birth, sexual orientation, race/ethnicity (U.S. census categories), level of family support for gender identity (unsupportive, neutral, supportive, or not asked because participant had not disclosed being transgender to their family) [22], relationship status, level of education, employment status, household income, having ever received pubertal suppression (e.g., treatment with gonadotropin-releasing hormone agonists) [8], having ever been exposed to gender identity conversion efforts [23], and having experienced any harassment based on gender identity in K-12 (verbal, physical, or sexual) [5].

## Statistical analyses

All statistical analyses were performed with SAS 9.4. The data in the analytic sample had minimal missing data for both exposure and outcome variables. Each control variable had under 8% missing data within all comparison groups. Therefore no imputation was performed, since listwise deletion with missingness as high as 10% can be acceptable under particular assumptions of missingness [24].

Analyses were performed for the three age groups of participants who accessed GAH and participants who desired but never accessed GAH, on demographic variables listed above. Variables were analyzed with Rao-Scott $\chi^2$ tests. Logistic regression tests were used to identify demographics and other potential confounding variables associated with each outcome.

Multivariable logistic regression was then performed, comparing mental health outcomes for participants who reported access to GAH during early adolescence, late adolescence, or adulthood with those for participants who desired but never accessed GAH. Models were fit to test associations with mental health outcomes, after adjusting for demographic and potential confounding variables that were found to be associated with each outcome. All hypothesis tests were 2-sided. The percentage decrease in adjusted odds for the outcomes was calculated from the model coefficients for each age group.

In order to account for multiple comparisons, a modified Bonferroni correction was applied for the approximately 50 comparisons performed. A significance threshold of 0.001 (.05/50) was used for our analyses.

After all aforementioned analyses were completed, we identified further analyses of interest that were not included in the original study design, and therefore not included in the Bonferroni correction. In these post hoc analyses, we compared access to GAH during adolescence (ages 14–17) to access during adulthood (ages ≥18), and access to GAH during early adolescence (ages 14–15) to access during late adolescence (ages 16–17).

## Results

### Demographic differences & potential confounding variables

In total, 21,598 participants (77.9%) reported ever desiring GAH. Of these, 8,860 (41.0%) never accessed GAH, 119 (0.6%) reported access to GAH in early adolescence, 362 (1.7%) reported access to GAH in late adolescence, and 12,257 (56.8%) reported access to GAH in adulthood. Significant differences were found based on age at time of study participation, gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, relationship status, level of education, employment status, household income, having ever received pubertal suppression, having ever been exposed to gender identity conversion efforts, and having experienced verbal, physical, or sexual harassment based on gender identity in K-12 (Table 1).

### GAH during early adolescence

The median age of participants who reported accessing GAH during early adolescence was 21.0 (IQR 18.0–35.0). After adjusting for demographic and potential confounding variables, recalled access to GAH during early adolescence was associated with lower odds of past-month severe psychological distress (aOR = 0.3, 95% CI = 0.2–0.4, $p < .0001$) and past-year suicidal ideation (aOR = 0.4, 95% CI = 0.2–0.6, $p < .001$) when compared to desiring GAH but never accessed them. For participants who recalled GAH access in early adolescence, these results represent a 222% decrease in adjusted odds for past-month severe psychological distress and a 135% decrease for past-year suicidal ideation. We detected no difference for other mental health variables measured (Table 2).

### GAH during late adolescence

The median age of participants who reported accessing GAH during late adolescence was 19.0 (IQR 18.0–22.0). After adjusting for demographic and potential confounding variables, recalled access to GAH during late adolescence was associated with lower odds of past-month severe psychological distress (aOR = 0.3, 95% CI = 0.3–0.4, $p < .0001$) and past-year suicidal ideation (aOR = 0.5, 95% CI = 0.4–0.7, $p < .0001$) when compared to desiring GAH but never accessing them. These results represent a 153% decrease in the adjusted odds for past-month severe psychological distress and a 62% decrease for past-year suicide ideation. We detected no difference for other mental health variables measured (Table 2).

### GAH during adulthood

The median age of participants who reported accessing GAH during adulthood was 31.0 (IQR 25.0–45.0). After adjusting for demographic and potential confounding variables, participants who recalled access to GAH during adulthood had lower odds of past-month severe psychological distress (aOR = 0.6, 95% CI = 0.5–0.6, $p < .0001$) and past-year suicidal ideation

**Table 1. Sample demographics.**

| Total N = 21,598 | | No GAH | GAH 14–15 | GAH 16–17 | GAH ≥ 18 | |
|---|---|---|---|---|---|---|
| | | n = 8860 | n = 119 | n = 362 | n = 12257 | |
| | | n (%) | n (%) | n (%) | n (%) | p |
| Age (Census) | | | | | | <0.001 |
| | 18–24 | 5315 (60.0) | 75 (63.03) | 297 (82.04) | 2856 (23.30) | |
| | 25–44 | 2653 (29.9) | 23 (19.33) | 54 (14.92) | 6285 (51.28) | |
| | 45–64 | 753 (8.5) | 19 (15.97) | 11 (3.04) | 2660 (21.70) | |
| | 65+ | 139 (1.57) | 2 (1.68) | 0 (0.00) | 456 (3.72) | |
| Gender Identity | | | | | | <0.001 |
| | Trans man / male | 02620 (29.57) | 00048 (40.34) | 00214 (59.12) | 04713 (38.45) | |
| | Trans woman / female | 02324 (26.23) | 00054 (45.38) | 00109 (30.11) | 06340 (51.73) | |
| | AFAB GQ/NB | 02829 (31.93) | 00013 (10.92) | 00035 (9.67) | 00834 (6.80) | |
| | AMAB GQ/NB | 00766 (8.65) | 00004 (3.36) | 00004 (1.10) | 00330 (2.69) | |
| | Other | 00321 (3.62) | 00000 (0.00) | 00000 (0.00) | 00040 (0.33) | |
| Sex Assigned at Birth | | | | | | <0.001 |
| | Female | 05475 (61.79) | 00061 (51.26) | 00249 (68.78) | 05561 (45.37) | |
| | Male | 03385 (38.21) | 00058 (48.74) | 00113 (31.22) | 06696 (54.63) | |
| Sexual Orientation | | | | | | <0.001 |
| | Asexual | 01220 (13.77) | 00006 (5.04) | 00022 (6.08) | 00771 (06.29) | |
| | Bisexual | 01391 (15.70) | 00007 (5.88) | 00056 (15.47) | 01900 (15.50) | |
| | Gay/Lesbian/Same Gender Loving | 01337 (15.09) | 00022 (18.49) | 00064 (17.68) | 02535 (20.68) | |
| | Heterosexual/Straight | 00743 (8.39) | 00031 (26.05) | 00071 (19.61) | 02019 (16.47) | |
| | Pansexual | 01875 (21.16) | 00021 (17.65) | 00066 (18.23) | 01877 (15.31) | |
| | Queer | 01573 (17.75) | 00019 (15.97) | 00058 (16.02) | 02525 (20.60) | |
| | Other | 00721 (08.14) | 00013 (10.92) | 00025 (6.91) | 00630 (5.14) | |
| Race / Ethnicity | | | | | | <0.001 |
| | Alaska Native/American Indian | 00105 (1.19) | 00002 (1.68) | 00003 (0.83) | 00149 (1.22) | |
| | Asian/Native Hawaiian/Pacific Islander | 00273 (3.08) | 00008 (6.72) | 00010 (2.76) | 00292 (2.38) | |
| | Biracial/Multiracial | 00475 (5.36) | 00009 (7.56) | 00027 (7.46) | 00571 (4.66) | |
| | Black/African American | 00210 (2.37) | 00011 (9.24) | 00016 (4.42) | 00378 (3.08) | |
| | Latin/Hispanic | 00499 (5.63) | 00008 (6.72) | 00025 (6.91) | 00572 (4.67) | |
| | White/Middle Eastern/North African | 07298 (82.37) | 00081 (68.07) | 00281 (77.62) | 10295 (83.99) | |
| Family Support of Gender Identity | | | | | | <0.001 |
| | Not Asked (Not Out to Family as Transgender) | 03067 (34.64) | 00003 (2.52) | 00015 (4.14) | 00901 (7.36) | |
| | Neutral | 01564 (17.66) | 00012 (10.08) | 00032 (8.84) | 01980 (16.16) | |
| | Supportive | 02904 (32.80) | 00091 (76.47) | 00291 (80.39) | 07321 (59.77) | |

(*Continued*)

**Table 1.** (Continued)

| Total N = 21,598 | | No GAH | GAH 14–15 | GAH 16–17 | GAH ≥ 18 | |
|---|---|---|---|---|---|---|
| | | n = 8860 | n = 119 | n = 362 | n = 12257 | |
| | | n (%) | n (%) | n (%) | n (%) | p |
| | Unsupportive | 01319 (14.90) | 00013 (10.92) | 00024 (6.63) | 02047 (16.71) | |
| | Missing | 6 (0.07) | 0 (0.00) | 0 (00.00) | 8 (0.08) | |
| Relationship Status | | | | | | <**0.001** |
| | Partnered | 04028 (46.90) | 00049 (43.36) | 00135 (38.03) | 06257 (52.99) | |
| | Unpartnered | 04560 (53.10) | 00064 (56.64) | 00220 (61.97) | 05551 (47.01) | |
| | Other | 272 (3.07) | 6 (5.04) | 7 (1.93) | 449 (3.66) | |
| Education | | | | | | <**0.001** |
| | Bachelor's degree or higher | 02219 (25.05) | 00023 (19.33) | 00048 (13.26) | 05911 (48.23) | |
| | Some college (no degree)/Associate's | 04555 (51.41) | 00061 (51.26) | 00171 (47.24) | 05199 (42.42) | |
| | High school grad (including GED) | 01617 (18.25) | 00023 (19.33) | 00099 (27.35) | 00975 (7.95) | |
| | Less than high school | 00469 (5.29) | 00012 (10.08) | 00044 (12.15) | 00172 (1.40) | |
| Employment Status | | | | | | <**0.001** |
| | Employed | 05213 (59.10) | 00060 (50.85) | 00189 (52.50) | 08788 (72.01) | |
| | Out of the labor force | 02038 (23.10) | 00039 (33.05) | 00108 (30.00) | 02283 (18.71) | |
| | Unemployed | 01570 (17.80) | 00019 (16.10) | 00063 (17.50) | 01133 (9.28) | |
| | Excluded (status unclear) | 4 (0.05) | 0 (0) | 2 (00.55) | 2 (0.02) | |
| | Missing | 35 (0.40) | 1 (0.48) | 0 (0) | 51 (0.42) | |
| Household Income | | | | | | <**0.001** |
| | $1 to $9,999 | 01163 (14.75) | 00016 (14.81) | 00041 (12.65) | 01160 (10.10) | |
| | $10,000 to $24,999 | 01714 (21.73) | 00013 (12.04) | 00053 (16.36) | 02252 (19.62) | |
| | $100,000 or more | 01136 (14.40) | 00023 (21.30) | 00079 (24.38) | 02064 (17.98) | |
| | $25,000 to $49,999 | 01717 (21.77) | 00028 (25.93) | 00059 (18.21) | 02652 (23.10) | |
| | $50,000 to $100,000 | 01772 (22.47) | 00024 (22.22) | 00071 (21.91) | 03035 (26.44) | |
| | No income | 00385 (4.88) | 00004 (3.70) | 00021 (6.48) | 00317 (2.76) | |
| | Excluded | 275 (3.10) | 7 (5.88) | 11 (3.04) | 313 (2.55) | |
| | Missing | 698 (7.88) | 4 (3.36) | 27 (7.46) | 464 (3.79) | |
| Ever Received Pubertal Suppression | | | | | | <**0.001** |
| | Yes | 00031 (0.36) | 00041 (34.45) | 00044 (12.15) | 00221 (01.80) | |
| | No | 08659 (99.64) | 00078 (65.55) | 00318 (87.85) | 12036 (98.20) | |
| | Missing | 00170 (1.92)) | 0 (0.00)) | 0 (0.00)) | 0 (0.00)) | |
| Ever Experienced Gender Identity Conversion Efforts | | | | | | <**0.001** |

(*Continued*)

**Table 1.** (Continued)

| Total N = 21,598 | | No GAH | GAH 14–15 | GAH 16–17 | GAH ≥ 18 | |
|---|---|---|---|---|---|---|
| | | n = 8860 | n = 119 | n = 362 | n = 12257 | |
| | | n (%) | n (%) | n (%) | n (%) | p |
| | Yes | 00998 (11.28) | 00031 (26.27) | 00092 (25.48) | 02208 (18.03) | |
| | No | 07852 (88.72) | 00087 (73.73) | 00269 (74.52) | 10037 (81.97) | |
| | Missing | 10 (0.11) | 1 (0.84) | 1 (0.28) | 12 (0.10) | |
| K-12 Harassment | | | | | | **<0.001** |
| | Verbal, physical or sexual | 2026 (22.9) | 80 (67.2) | 226 (62.4) | 2612 (21.3) | |
| | None | 6834 (77.1) | 39 (32.8) | 136 (37.6) | 9645 (78.7) | |

Descriptive statistics for transgender adults in the U.S. who ever desired gender-affirming hormones (GAH) for their gender identity or gender transition, comparing those who never accessed this treatment (No GAH), those who accessed GAH between their 14th and 16th birthdays (GAH 14–15), those who accessed GAH after their 16th birthday and before their 18th birthday (GAH 16–18) and those who accessed GAH after their18th birthday (GAH ≥ 18).

Abbreviations: AFAB (assigned female at birth), AMAB (assigned male at birth), GQ/NB (gender queer or non-binary).

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(aOR = 0.8, 95% CI = 0.7–0.8, p < .0001) when compared to those who desired GAH but never accessed them. Access to GAH during adulthood was associated with an 81% decrease in adjusted odds of past-month severe psychological distress and a 21% decrease in past-year suicidal ideation. Access to GAH during adulthood was also associated with greater odds of past-month binge drinking (aOR = 1.2, 95% CI = 1.1–1.3, p < .0001) and lifetime illicit drug use (aOR = 1.7, 95% CI = 1.6–1.8, p < .0001) when compared to desiring but never accessing GAH. Results indicated an adjusted odds increase of 20% for past-month binge drinking and 70% increase for lifetime illicit drug use. We detected no difference for other mental health variables measured (Table 2).

## Raw frequencies of outcome variables

Raw frequencies for outcome variables are shown in Table 3.

## Post hoc analyses

**GAH during adolescence vs. GAH during adulthood.** After adjusting for demographic and potential confounding variables, access to GAH during adolescence (ages 14–17) was associated with lower odds of past-month severe psychological distress (aOR = 0.6, 95% CI = 0.5–0.8, p < .0001), past-year suicidal ideation (aOR = 0.7, 95% CI = 0.6–0.9, p = .0007), past-month binge drinking (aOR = 0.7, 95% CI = 0.5–0.9, p = .001), and lifetime illicit drug use (aOR = 0.7, 95% CI = 0.5–0.8, p = .0003) when compared to access to GAH during adulthood. We detected no difference for other mental health variables measured (Table 4).

**Access to GAH during early vs. late adolescence.** After adjusting for demographic and potential confounding variables, we detected no difference in odds of any mental health variables measured when comparing access to GAH during early adolescence with access to GAH during late adolescence (Table 4).

**Lifetime but no past year suicidality.** Due to the cross-sectional nature of the study, it was possible that we detected an association between favorable mental health outcomes and access to GAH because people with better mental health were more likely to be able to access GAH. Given that baseline mental health status could confound associations between access to GAH and mental health outcomes, in post hoc analyses we examined two outcome measures

**Table 2. Outcomes for participants who accessed gender-affirming hormones (estrogen or testosterone).**

| | Participants who Accessed GAH | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N = 12,598 | | | | | | | | | | | |
| | Accessed GAH at Age 14 or 15 | | | | Accessed GAH at Age 16 or 17 | | | | Accessed GAH at Age ≥ 18 | | | |
| | n = 119 | | | | n = 362 | | | | n = 12257 | | | |
| | OR (95% CI) | p | aOR (95% CI) | p | OR (95% CI) | p | aOR (95% CI) | p | OR (95% CI) | p | aOR (95% CI) | p |
| **Suicidality (Past 12 months)** | | | | | | | | | | | | |
| Past-year suicidal ideation [a] | 0.5 (0.3–0.7) | .0001 | 0.4 (0.2–0.6) | < **.0001** | 1.0 (0.8–1.2) | .73 | 0.5 (0.4–0.7) | < **.0001** | 0.5 (0.5–0.6) | < .0001 | 0.8 (0.7–0.8) | < **.0001** |
| Past-year suicidal ideation with plan [b] | 1.3 (0.8–2.4) | .31 | 0.8 (0.4–1.6) | .58 | 1.1 (0.9–1.5) | .41 | 0.9 (0.7–1.2) | .49 | 0.8 (0.8–0.9) | < .0001 | 0.9 (0.8–1.0) | .09 |
| Past-year suicide attempt [c] | 1.0 (0.5–2.2) | .99 | 0.4 (0.2–1.1) | .08 | 1.4 (1.0–2.0) | .04 | 0.9 (0.6–1.4) | .79 | 0.8 (0.8–0.9) | .002 | 1.0 (0.9–1.1) | .89 |
| Past-year suicide attempt requiring inpatient hospitalization [d] | -- | -- | -- | -- | 2.2 (1.2–4.0) | .01 | 2.2 (1.2–4.2) | .01 | 1.4 (1.1–1.7) | .002 | 1.2 (0.9–1.5) | .26 |
| **Mental Health & Substance Use** | | | | | | | | | | | | |
| Past-month severe psychological distress (K6 ≥ 13) [c] | 0.5 (0.3–0.7) | .0004 | 0.3 (0.2–0.4) | < **.0001** | 0.6 (0.5–0.8) | < .0001 | 0.3 (0.3–0.4) | < **.0001** | 0.4 (0.3–0.4) | < .0001 | 0.6 (0.5–0.6) | < **.0001** |
| Past-month binge drinking [e] | 1.6 (1.1–2.3) | .02 | 1.6 (1.0–2.4) | .04 | 0.8 (0.6–1.1) | .17 | 0.9 (0.6–1.1) | .27 | 1.2 (1.1–1.2) | < .0001 | 1.2 (1.1–1.3) | < **.0001** |
| Lifetime illicit drug use [f] | 1.8 (1.2–2.6) | .003 | 1.5 (1.0–2.2) | .08 | 1.2 (1.0–1.6) | .08 | 1.3 (1.0–1.6) | .07 | 2.1 (1.9–2.2) | < .0001 | 1.7 (1.6–1.8) | < **.0001** |

Mental health outcomes of transgender adults who recalled access to gender-affirming hormones (GAH) during various age groups. Reference group for all analyses is participants who desired GAH but did not access them. All models adjusted for age, partnership status, employment status, K-12 harassment, and having experienced gender identity conversion efforts.

Abbreviations: OR (odds ratio), aOR (adjusted odds ratio), 95% CI (95% confidence interval).

[a] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, and total household income.

[b] Model also adjusted for sexual orientation, race/ethnicity, educational attainment, and total household income.

[c] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, total household income, and having received pubertal suppression.

[d] Model also adjusted for family support of gender identity. Only one participant in the GAH < 16 group endorsed a past–year suicide attempt requiring inpatient hospitalization, precluding calculation of an aOR for this outcome.

[e] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, family support of gender identity, educational attainment, and total household income.

[f] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, and educational attainment.

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relevant to this question of temporality: lifetime but no past-year suicidal ideation, and lifetime but no past-year suicide attempt. We found that access to GAH in adulthood was associated with greater odds of lifetime but no past-year suicidal ideation (aOR = 1.4, 95% CI = 1.3–1.5, p < .0001) when compared to desiring but not accessing GAH (Table 5). The association of access to GAH during late adolescence with lifetime but no past year suicidal ideation (aOR = 1.4, 95% CI = 1.1–1.8, p = .005) was no longer significant after Bonferroni correction, though some have noted that Bonferroni adjustment may be overly conservative, suggesting that this finding may be considered significant [25].

## Discussion

In this large national cross-sectional non-probability study, transgender people who accessed GAH during early adolescence, late adolescence, or adulthood had better mental health

**Table 3. Raw outcome frequencies of mental health outcomes.**

| Total N = 21,598 | No GAH | GAH 14–15 | GAH 16–17 | GAH ≥ 18 |
|---|---|---|---|---|
| | n = 8860 | n = 119 | n = 362 | n = 12257 |
| | n (%) | n (%) | n (%) | n (%) |
| **Suicidality (Past 12 months)** | | | | |
| Past-year suicidal ideation | 5144 (58.1) | 48 (40.3) | 40 (33.6) | 5237 (42.7) |
| Past-year suicidal ideation with plan | 2731 (30.8) | 29 (24.3) | 39 (32.8) | 02537 (20.7) |
| Past-year suicide attempt | 853 (9.6) | 8 (6.7) | 40 (33.6) | 756 (6.2) |
| Past-year suicide attempt requiring inpatient hospitalization | 220 (2.5) | 1 (0.8) | 40 (33.6) | 247 (2.0) |
| **Mental Health & Substance Use** | | | | |
| Past-month severe psychological distress (K6 ≥ 13) | 4545 (51.3) | 40 (33.6) | 145 (40.1) | 3419 (27.9) |
| Past-month binge drinking | 2083 (23.5) | 39 (32.8) | 74 (20.4) | 3214 (26.2) |
| Lifetime illicit drug use | 1918 (21.6) | 40 (33.6) | 93 (25.7) | 4455 (36.3) |

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outcomes when compared to those who desired but were unable to access GAH. Given the substantial mental health disparities faced by transgender people, these results are of particular importance [26].

For each time period of GAH initiation examined (early adolescence, late adolescence, and adulthood), access to GAH was associated with lower odds of past-year suicidal ideation and past-month severe psychological distress. When we compared participants who accessed GAH during adolescence (ages 14–17) with those who accessed GAH during adulthood (18+),

**Table 4. Outcomes for participants who accessed gender-affirming hormones (estrogen or testosterone).**

| | Accessed GAH at Age 14–17 (compared to GAH access at age ≥ 18) n = 481 | | | | Accessed GAH at Age 14 or 15 (compared to GAH access at age 16 or 17) n = 119 | | | |
|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | p | aOR (95% CI) | p | OR (95% CI) | p | aOR (95% CI) | p |
| **Suicidality (Past 12 months)** | | | | | | | | |
| Past-year suicidal ideation [a] | 1.5 (1.3–1.8) | < .0001 | 0.7 (0.6–0.9) | **.0007** | 0.5 (0.3–0.8) | .002 | 0.7 (0.4–1.2) | .16 |
| Past-year suicidal ideation with plan [b] | 1.4 (1.1–1.8) | .009 | 1.1 (0.8–1.5) | .51 | 1.2 (0.6–2.3) | .58 | 1.0 (0.5–1.9) | .88 |
| Past-year suicide attempt [c] | 1.6 (1.2–2.2) | .003 | 1.0 (0.7–1.4) | .82 | 0.7 (0.3–1.6) | .40 | 0.4 (0.1–1.3) | .12 |
| Past-year suicide attempt requiring inpatient hospitalization [d] | 1.3 (0.7–2.3) | .35 | 1.7 (0.9–3.2) | .08 | 0.2 (0.0–1.6) | .13 | 0.2 (0.0–2.1) | .19 |
| **Mental Health & Substance Use** | | | | | | | | |
| Past-month severe psychological distress (K6 ≥ 13) [c] | 1.7 (1.4–2.0) | < .0001 | 0.6 (0.5–0.8) | **< .0001** | 0.8 (0.5–1.2) | .26 | 0.7 (0.4–1.3) | .30 |
| Past–month binge drinking [e] | 0.9 (0.7–1.1) | .17 | 0.7 (0.5–0.9) | **.001** | 1.9 (1.2–3.0) | .006 | 2.0 (1.2–3.5) | .01 |
| Lifetime illicit drug use [f] | 0.7 (0.5–0.8) | < .001 | 0.7 (0.5–0.8) | **.0003** | 1.4 (0.9–2.3) | .10 | 1.0 (0.6–1.7) | .98 |

All models adjusted for age, partnership status, employment status, K-12 harassment, and having experienced gender identity conversion efforts.

Abbreviations: OR (odds ratio), aOR (adjusted odds ratio), 95% CI (95% confidence interval).

[a] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, and total household income.

[b] Model also adjusted for sexual orientation, race/ethnicity, educational attainment, and total household income.

[c] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, total household income, and having received pubertal suppression.

[d] Model also adjusted for family support of gender identity.

[e] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, family support of gender identity, educational attainment, and total household income.

[f] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, and educational attainment.

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**Table 5. Lifetime but no past-year suicide ideation and attempts for participants who accessed gender-affirming hormones (estrogen or testosterone).**

| | Participants who Accessed GAH | | | | | |
|---|---|---|---|---|---|---|
| | N = 12,598 | | | | | |
| | Accessed GAH at Age 14 or 15 | | Accessed GAH at Age 16 or 17 | | Accessed GAH at Age ≥ 18 | |
| | n = 119 | | n = 362 | | n = 12,257 | |
| | aOR (95% CI) | p | aOR (95% CI) | p | aOR (95% CI) | p |
| Lifetime suicidal ideation and no past-year ideation [a] | 1.3 (0.8–2.0) | .28 | 1.4 (1.1–1.8) | .005 | 1.4 (1.3–1.5) | < **.0001** |
| Lifetime suicide attempt and no past-year attempt [b] | 0.8 (0.5–1.2) | .24 | 0.7 (0.6–1.0) | .03 | 1.0 (0.9–1.1) | .67 |

Mental health outcomes of transgender adults who recalled access to gender-affirming hormones (GAH) during various age groups. Reference group for all analyses is participants who desired GAH but did not access them. Both models adjusted for age, partnership status, employment status, K-12 harassment, and having experienced gender identity conversion efforts.

[a] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, and total household income.

[b] Model also adjusted for gender identity, sex assigned at birth, sexual orientation, race/ethnicity, family support of gender identity, educational attainment, total household income, and having received pubertal suppression.

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participants who accessed GAH earlier had better mental health outcomes, including lower odds of past-year suicidal ideation, past-month severe psychological distress, past-month binge drinking, and lifetime illicit drug use. These results argue against waiting until adulthood to offer GAH to transgender adolescents and suggest that doing so may put patients at greater mental health risk.

The current study has a few advantages over past published studies in this area. While past studies have not included a comparison group of people who did not access GAH and were also underpowered to adjust for potential cofounders, this large sample size enabled comparison of participants who reported access to GAH to those who desired but did not access GAH, while adjusting for a wide range of potential confounding variables known to be associated with mental health outcomes for transgender people.

One unexpected finding was that participants who initiated GAH during adulthood, compared to those who desired but never accessed GAH, had greater odds of past-month binge drinking and lifetime illicit substance use. Transgender people often become more socially engaged following the increased confidence that results from gender affirmation, which may partly explain these results [27]. Given the high prevalence of substance use disorders in this population, clinicians ought to routinely screen for substance use disorders among transgender people, and researchers ought to focus on development of culturally responsive substance use disorder prevention and treatment interventions with transgender communities [27].

Notably, even participants who recalled access to GAH had high rates of past-year suicidal ideation. Though access to GAH during adolescence appears to be related to more favorable mental health outcomes, transgender people face a range of other psychosocial stressors that contribute to chronic minority stress, including but not limited to employment discrimination, lack of safe access to public facilities, and physical violence [4]. Future epidemiological and interventional research is needed to understand and address chronic minority stress among transgender people who access GAH as well as those who do not. For transgender adolescents, creating safe and affirming school environments appears to be of particular importance [28], in addition to providing gender-affirming medical care, as well as psychological, legal and surgical gender affirmation as needed [6].

This study also suggests that a large proportion of transgender people desire but never access GAH. Though prevalence in a non-probability sample should be interpreted with

caution, 41% of those who desired GAH in this study reported that they were unable to access them. Barriers to accessing prescribed GAH, in addition to leaving many without treatment, may also drive use of non-prescribed GAH, which is highly prevalent and associated with stigmatizing healthcare policies [29]. Future studies ought to examine if non-prescribed GAH use, when compared to prescribed GAH, is linked to worse mental health outcomes or adverse physical health outcomes (e.g., blood clot risk from estradiol use without standard medical monitoring).

### Strengths and limitations

Strengths of this study include its large sample size and broad geographic representation within the U.S. The large sample size enabled adjustment for a wide range of potential confounding variables. Limitations include its non-probability cross-sectional design, which reduces generalizability and limits determination of causality. It is possible that people with better mental health status at baseline are more likely to be able to access GAH, thus confounding associations between GAH access and adult mental health outcomes measured: we therefore examined lifetime but no past-year suicidal ideation as an outcome, with results suggesting a lack of reverse causation due to such confounding. Nonetheless, this method is imperfect for investigating mental health changes following GAH, and future longitudinal studies are needed. Longitudinal waitlist control studies would be of particular value. Though a randomized controlled trial would help determine causality, many have noted that such a trial design is unethical in this context [2]. Age of GAH initiation reported by participants at time of data collection is vulnerable to recall bias. It is possible that participants in older age cohorts (45–65; 65+) were more vulnerable to recall bias; in our clinical experience, however, starting GAH is a major event in one's life, making it less susceptible to recall bias than more routine events [30]. It was unexpected that the median age at time of survey completion for participants who recalled accessing GAH in early adolescence was older than for those in the late adolescence group, which may be indicative of recall bias. Of note, though it is often presumed that GAH were not offered to adolescents in the U.S. until the past three decades, recent historical analyses have pointed out that adolescents have been receiving GAH as early as the 1970s [31]. The 2015 USTS sample is younger, with fewer racial minorities, fewer heterosexual participants, and higher educational attainment when compared with probability samples of TGD people in the U.S [32]. Because all participants identified as non-cisgender, those who initiated GAH and subsequently identified as cisgender would not necessarily be represented in this study; existing literature, however, suggests that this is a rare occurrence [2, 33].

## Conclusion

This study found that transgender people who accessed GAH during early or late adolescence had a lower odds of past-month suicidal ideation and past-month severe psychological distress in adulthood, when compared to those who desired but did not access GAH, after adjusting for a range of potential confounding variables. The findings support updated 2017 recomendations from The Endocrine Society [7] and WPATH [6] that these medical interventions be made available for transgender adolescents. The results also provide additional evidence to suggest that legislation restricting transgender adolescents' access to gender-affirming medical care would result in adverse mental health outcomes [18].

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## Author Contributions

**Conceptualization:** Jack L. Turban, Dana King, Sari L. Reisner, Alex S. Keuroghlian.

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# Pubertal Suppression for Transgender Youth and Risk of Suicidal Ideation

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abstract

**BACKGROUND AND OBJECTIVES:** Gonadotropin-releasing hormone analogues are commonly prescribed to suppress endogenous puberty for transgender adolescents. There are limited data regarding the mental health benefits of this treatment. Our objective for this study was to examine associations between access to pubertal suppression during adolescence and adult mental health outcomes.

**METHODS:** Using a cross-sectional survey of 20 619 transgender adults aged 18 to 36 years, we examined self-reported history of pubertal suppression during adolescence. Using multivariable logistic regression, we examined associations between access to pubertal suppression and adult mental health outcomes, including multiple measures of suicidality.

**RESULTS:** Of the sample, 16.9% reported that they ever wanted pubertal suppression as part of their gender-related care. Their mean age was 23.4 years, and 45.2% were assigned male sex at birth. Of them, 2.5% received pubertal suppression. After adjustment for demographic variables and level of family support for gender identity, those who received treatment with pubertal suppression, when compared with those who wanted pubertal suppression but did not receive it, had lower odds of lifetime suicidal ideation (adjusted odds ratio = 0.3; 95% confidence interval = 0.2–0.6).

**CONCLUSIONS:** This is the first study in which associations between access to pubertal suppression and suicidality are examined. There is a significant inverse association between treatment with pubertal suppression during adolescence and lifetime suicidal ideation among transgender adults who ever wanted this treatment. These results align with past literature, suggesting that pubertal suppression for transgender adolescents who want this treatment is associated with favorable mental health outcomes.

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Dr Turban conceptualized and designed the study, drafted the initial manuscript, and incorporated all revisions and comments; Ms King conducted statistical analyses and reviewed and revised the manuscript for important intellectual content, with a focus on statistical aspects of the manuscript; Dr Carswell assisted in the design of the study and in interpretation of the data analyses and critically reviewed and revised the manuscript for important intellectual content, with a focus on relevant clinical endocrinology; Dr Keuroghlian supervised and contributed to the conceptualization and design of the study and the design of the statistical analyses and reviewed and revised the manuscript for important intellectual content as it relates to mental health considerations for transgender people; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**WHAT'S KNOWN ON THIS SUBJECT:** Gonadotropin-releasing hormone analogues are commonly used to suppress endogenous puberty for transgender adolescents. Small studies have revealed that pubertal suppression results in favorable mental health outcomes. No studies to date have examined associations between pubertal suppression and suicidality.

**WHAT THIS STUDY ADDS:** In this study, using the largest survey of transgender adults to date, we show that access to pubertal suppression during adolescence is associated with lower odds of lifetime suicidal ideation among transgender young adults.

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Pl. Trial Ex. 215

According to the Centers for Disease Control and Prevention's Youth Risk Behavior Surveillance System, ~1.8% of adolescents in the United States identify as transgender.[1] These youth suffer mental health disparities that include higher rates of internalizing psychopathology (ie, anxiety and depression) and suicidality, theorized to be due to a combination of dysphoria toward their bodies and minority stress.[2–5] In a large study of transgender adults in the United States, 40% endorsed a lifetime suicide attempt.[6]

Over the past 2 decades, protocols have been developed to provide transgender adolescents with gender-affirming medical interventions that align their bodies with their gender identities. Most prominent among these are the Endocrine Society guidelines[7] and the World Professional Association for Transgender Health (WPATH) Standards of Care.[8] Both sets of guidelines recommend that transgender adolescents be offered gonadotropin-releasing hormone analogues (GnRHas), colloquially referred to as "puberty blockers," once they reach Tanner 2 of puberty. These medications are provided as subcutaneous implants or are administered as either 1- or 3-month depot injections. GnRHa therapy effectively halts the production of gonadal sex steroids (testosterone and estrogen) by persistently activating and thereby desensitizing the gonadotropin-releasing hormone receptor, which in turn leads to suppression of luteinizing hormone and follicle-stimulating hormone release from the anterior pituitary gland.[9] This process inhibits endogenous puberty for the duration of GnRHa use. Once further pubertal development is delayed, youth are able to explore gender identities without the pressure of dysphoria associated with gender-incongruent physical development.[10] GnRHa therapy is unique among gender-affirming medical interventions in that the resultant pubertal suppression is fully reversible, with the resumption of endogenous puberty after their discontinuation.[7,8]

Since the publication of the WPATH Standards of Care and the Endocrine Society guidelines, the use of pubertal suppression for transgender youth has become more common in the United States[9] There are limited data, however, regarding the mental health outcomes of pubertal suppression. To date, there have been 2 published studies in which the effects of this treatment on the mental health of transgender youth were examined. In the first study, the authors assessed changes in mental health among 55 Dutch adolescents who received pubertal suppression.[11] This study, which notably lacked a control group, revealed that internalizing psychopathology improved after treatment with pubertal suppression. In the second study, researchers followed a group of 201 adolescents with gender dysphoria and found that those who received pubertal suppression in addition to psychological support ($n$ = 101) had superior global functioning, measured by the Children's Global Assessment Scale, when compared with those who received psychological support alone ($n$ = 100).[12]

In the current study, we use the largest survey of transgender people to date, a community-recruited sample of transgender adults in the United States, to conduct the first-ever investigation into associations between pubertal suppression and suicidality.

Transgender youth present to clinicians with a range of concerns. Some have minimal body dysphoria and do not desire pubertal suppression, whereas others report significant dysphoria around the physical changes related to puberty. Because not all transgender and gender-diverse youth desire medical interventions, we examined only those youth who desired pubertal suppression because these are the young people who would present to care and for whom clinicians would need to decide about whether to initiate pubertal suppression. We specifically examined measures of past-year suicidality, lifetime suicidality, past-month severe psychological distress, past-month binge drinking, and lifetime illicit drug use. We hypothesized that among those who wanted pubertal suppression, those who received it would have superior mental health outcomes when compared with those who wanted but did not receive it.

## METHODS

### Study Design and Data Source

The 2015 US Transgender Survey (USTS) was conducted over a 1-month period in 2015 by the National Center for Transgender Equality (NCTE). It is, to our knowledge, the largest existing data set of transgender adults and includes data regarding demographics, past gender-affirming medical treatment, family support, and mental health outcomes. Participants were recruited through community outreach in collaboration with >400 lesbian, gay, bisexual, and transgender organizations and were provided with a Web address to complete the survey online. Details regarding outreach efforts are further described in the NCTE report on the survey.[6] The USTS protocol was approved by the University of California, Los Angeles Institutional Review Board. For the purposes of the current study, data were obtained via a data-sharing agreement with the NCTE, and the current protocol was reviewed by The Fenway Institute

Institutional Review Board and determined to not comprise human subjects research.

### Study Population

The USTS data set contains responses from 27 715 US transgender adults, with respondents from all 50 states, the District of Columbia, American Samoa, Guam, Puerto Rico, and US military bases overseas. Given that pubertal suppression for transgender youth was not available in the United States until 1998,[4] only participants who were 17 or younger in 1998 would have had health care access to GnRHa for pubertal suppression. We thus restricted the analysis to participants who were 36 or younger at the time of the survey, resulting in a sample of 20 619 participants. Data were further restricted to those who selected "puberty blocking hormones (usually used by youth ages 9–16)" in response to the question "Have you ever wanted any of the health care listed below for your gender identity or gender transition? (Mark all that apply)." Response options for this question were "counseling/therapy," "hormone treatment/HRT," "puberty blocking hormones (usually used by youth ages 9–16)," or "none of the above." This resulted in a sample of 3494 individuals between the ages of 18 and 36 who ever wanted pubertal suppression as part of their gender-affirming medical care.

### Exposures

Exposure to pubertal suppression was defined as selecting "puberty blocking hormones (usually used by youth ages 9–16)" in response to the question "Have you ever had any of the health care listed below for your gender identity or gender transition? (Mark all that apply)." Response options for this question were "counseling/therapy," "hormone treatment/HRT," "puberty blocking hormones (usually used by youth ages 9–16)," and "none of the above." Participants who reported having pubertal suppression were also asked, "At what age did you begin taking Puberty Blocking Hormones?" Those who reported beginning treatment after age 17 were excluded to only include participants who likely had pubertal suppression during active endogenous puberty. The vast majority of adolescents would have reached Tanner 5, the final stage of puberty, by age 17.[13,14]

### Outcomes

Comparing those who received pubertal suppression with those who did not, we examined past-month severe psychological distress (defined as a score of ≥13 on the Kessler Psychological Distress Scale [K6], a cutoff previously validated among US adults[15]), past-month binge drinking (operationalized as drinking ≥5 standard alcoholic beverages during 1 occasion; the rationale for this threshold when studying alcohol use among transgender people has been discussed previously[16]), lifetime illicit drug use (not including marijuana), past-year suicidal ideation, past-year suicidal ideation with a plan, past-year suicide attempts, past-year suicide attempts resulting in inpatient care, lifetime suicidal ideation, and lifetime suicide attempts.

### Control Variables

Demographic variables collected included age, age of social transition, age of initiation of gender-affirming hormone therapy, current gender identity, sex assigned at birth, sexual orientation, race, education level, employment status, relationship status, total household income at the time of data collection in 2015, family support for gender identity, and current hormone treatment.

### Statistical Analysis

Data were analyzed by using SPSS software version 25 (IBM SPSS Statistics, IBM Corporation, Armonk, NY). Descriptive statistics were conducted and are presented as frequency (percentage) or mean (SD). Analysis of variance and $\chi^2$ tests were used to assess significance by age, gender identity, sex assigned at birth, race, education level, employment status, relationship status, total household income, family support for gender identity, and current hormone treatment between those who received pubertal suppression and those who did not. We used univariate logistic regression to examine associations between receiving pubertal suppression and each mental health outcome, as well as between age and both ever wanting and receiving pubertal suppression. $P < .05$ defined statistical significance. Multivariable logistic regression models were adjusted for using the demographic variables associated with each outcome at the level of $P \leq .20$. Because all outcomes were associated with level of family support, sexual orientation, education level, employment status, and total household income, all models were adjusted for these variables. Lifetime suicide attempts were associated with gender identity, and this model was therefore additionally adjusted for this variable. Past-month severe psychological distress and past-year suicidal ideation were additionally associated with age, gender identity, and relationship status, and therefore models were adjusted for these variables as well. Race was found to be associated with lifetime suicidal ideation and lifetime suicide attempts; therefore models were therefore additionally adjusted for race.

## RESULTS

Of the 20 619 survey respondents 18 to 36 years of age, 3494 (16.9%) reported that they had ever wanted pubertal suppression. Of those who wanted pubertal suppression, only 89 (2.5%) had

received this treatment. The following variables were found to be associated with those who wanted and received pubertal suppression compared with those who wanted pubertal suppression but did not receive it: younger age, age of social transition, age of initiation of hormone therapy, feminine gender identity, male sex assigned at birth, heterosexual sexual orientation, higher total household income, and greater family support of gender identity (Table 1).

In univariate analyses, when comparing those who received pubertal suppression with those who did not, receiving pubertal suppression was associated with decreased odds of past-year suicidal ideation, lifetime suicidal ideation, and past-month severe psychological distress (Table 2). After controlling for demographic variables from Table 1, pubertal suppression was associated with decreased odds of lifetime suicidal ideation. Raw

**TABLE 1** Sample Demographics

| | | Have You Ever Had [Pubertal Suppression] for Your Gender Identity or Gender Transition? | | | |
|---|---|---|---|---|---|
| | All ($N$ = 3494) | Yes ($n$ = 89; 2.5%) | No ($n$ = 3405; 97.5%) | F | $P$ |
| $n$ (%) | $n$ (%) | $n$ (%) | | | |
| Age | 23.4 (5.0) | 21.7 (4.7) | 23.4 (5.0) | 10.3 | .001* |
| Age of social transition | 20.0 (5.5) | 15.2 (4.5) | 20.1 (5.5) | 67.5 | <.001* |
| Age began hormone therapy | 22.1 (4.5) | 15.7 (2.4) | 22.5 (4.3) | 217.4 | <.001* |
| Gender identity | | | | 25.5[a] | <.001* |
| Woman | | 23 (25.8) | 617 (18.2) | | |
| Man | | 19 (21.3) | 383 (11.3) | | |
| Transgender woman | | 25 (28.1) | 720 (21.3) | | |
| Transgender man | | 16 (18.0) | 795 (23.5) | | |
| Nonbinary or genderqueer | | 6 (6.7) | 866 (25.6) | | |
| Sex assigned at birth | | | | 4.4[a] | .04* |
| Female | | 39 (43.8) | 1874 (55.0) | | |
| Male | | 50 (56.2) | 1531 (45.0) | | |
| Sexual orientation | | | | 36.5[a] | <.001* |
| Heterosexual or straight | | 27 (30.3) | 350 (10.3) | | |
| Asexual | | 9 (10.1) | 437 (12.8) | | |
| Pansexual or queer | | 36 (40.4) | 1784 (52.4) | | |
| Gay or lesbian | | 12 (13.5) | 539 (15.8) | | |
| Not listed | | 5 (5.6) | 295 (8.7) | | |
| Race, $n$ (%) | | | | 3.5[a] | .06 |
| Racial minority | | 28 (31.5) | 782 (23.0) | | |
| Not racial minority (white or European American) | | 61 (68.5) | 2623 (77.0) | | |
| Education level | | | | 2.9[a] | .41 |
| Less than high school | | 9 (10.1) | 220 (6.5) | | |
| High school graduate or GED | | 20 (22.5) | 683 (20.1) | | |
| Some college or associate degree | | 39 (43.8) | 1729 (50.8) | | |
| Bachelor's degree or higher | | 21 (23.6) | 773 (22.7) | | |
| Employment status | | | | 0.6[a] | .45 |
| Employed | | 51 (79.7) | 1976 (75.6) | | |
| Unemployed | | 13 (20.3) | 638 (24.4) | | |
| Relationship status | | | | 0.5[a] | .47 |
| Partnered | | 35 (40.2) | 1447 (44.1) | | |
| Unpartnered | | 52 (59.8) | 1834 (55.9) | | |
| Total household income, $ | | | | 21.9[a] | <.001* |
| <25 000 | | 21 (26.3) | 1153 (38.3) | | |
| 25 000–49 999 | | 13 (16.3) | 652 (21.7) | | |
| 50 000–99 000 | | 14 (17.5) | 630 (20.9) | | |
| >100 000 | | 32 (40.0) | 574 (19.1) | | |
| Family support for gender identity | | | | | |
| Supportive | | 71 (81.6) | 1551 (55.8) | 24.3[a] | <.001* |
| Neutral | | 11 (12.6) | 573 (20.6) | | |
| Unsupportive | | 5 (5.7) | 658 (23.7) | | |
| Current hormone treatment | | 87 (97.8) | 1617 (96.3) | 0.5[a] | .48 |

Descriptive statistics for transgender adults in the United States who ever wanted pubertal suppression for their gender identity or gender transition when comparing those who received this treatment with those who did not receive this treatment (total $N$ = 3494). Percentages were calculated from the total of nonmissing values.

*Indicates statistical significance.

[a] $\chi^2$.

**TABLE 2** Mental Health Outcomes Among Those Who Received Pubertal Suppression

| | Univariate Analyses | | Multivariable Analyses | |
|---|---|---|---|---|
| | OR (95% CI) | *P* | aOR (95% CI) | *P* |
| Suicidality, past 12 mo | | | | |
| Ideation | 0.6 (0.4–0.8) | .006* | 0.6 (0.3–1.1) | 0.09 |
| Ideation with plan | 0.9 (0.5–1.6) | .73 | | |
| Ideation with plan and attempt | 1.2 (0.6–2.3) | .64 | | |
| Attempt resulting in inpatient care | 2.8 (0.8–9.4) | .09 | | |
| Suicidality, lifetime | | | | |
| Ideation | 0.3 (0.2–0.5) | <.001* | 0.3 (0.2–0.6) | 0.001* |
| Attempts | 0.7 (0.4–1.0) | .08 | | |
| Mental health and substance use | | | | |
| Past-month severe psychological distress, K6 ≥13 | 0.5 (0.3–0.8) | .001* | 0.8 (0.4–1.4) | 0.38 |
| Past-month binge drinking | 0.3 (0.8–2.0) | .29 | | |
| Lifetime illicit drug use | 1.1 (0.7–1.8) | .67 | | |

Univariate and multivariable analyses of mental health outcomes among transgender adults in the United States who ever wanted pubertal suppression when comparing those who received this treatment with those who did not. Multivariable logistic regression models were adjusted for using the demographic variables associated with each outcome at the level of $P \leq .20$. Because all outcomes were associated with family support, sexual orientation, education level, employment status, and total household income, all models were adjusted for these variables. Lifetime suicide attempts were associated with gender identity, and this model was additionally adjusted for this variable. Past-month severe psychological distress and past-year suicidal ideation were additionally associated with age, gender identity, and relationship status, and thus these models were adjusted for these variables as well. Race was found to be associated with lifetime suicidal ideation and lifetime suicide attempts, and thus these models were additionally adjusted for race. Models for psychological distress and past-year suicidal ideation were also adjusted for age, gender identity, and relationship status. aOR, adjusted odds ratio.

[a] Indicates statistical significance.

frequency outcomes are presented in Table 3.

To examine associations between age, ever wanting, and ever receiving pubertal suppression, we divided participants into 2 age groups with the cutoff point at the median, 18 to 22 and 23 to 36, in light of the skewed distribution of age.[17] The younger age group had increased odds both of ever wanting pubertal suppression (odds ratio [OR] = 1.4, $P < .001$, 95% confidence interval [CI]: 1.3–3.5) and of receiving pubertal suppression (OR = 2.1, $P = .001$, 95% CI: 1.4–3.4).

Among those who had ever received pubertal suppression, 60% reported traveling <25 miles for gender-affirming health care, 29% traveled between 25 and 100 miles, and 11% traveled >100 miles.

**TABLE 3** Raw Frequencies of Outcome Variables

| | Have You Ever Had [Pubertal Suppression] for Your Gender Identity or Gender Transition? | |
|---|---|---|
| | Yes (*n* = 89; 2.5%) | No (*n* = 3405; 97.5%) |
| | *n* (%) | *n* (%) |
| Suicidality (past 12 mo) | | |
| Ideation | 45 (50.6) | 2204 (64.8) |
| Ideation with plan | 25 (55.6) | 1281 (58.2) |
| Ideation with plan and attempt | 11 (24.4) | 473 (21.5) |
| Attempt resulting in inpatient care | 5 (45.5) | 108 (22.8) |
| Suicidality (lifetime) | | |
| Ideation | 67 (75.3) | 3062 (90.2) |
| Attempts | 37 (41.6) | 1738 (51.2) |
| Mental health and substance use | | |
| Past-month severe psychological distress (K6 ≥13) | 32 (37.2) | 1847 (55.1) |
| Past-month binge drinking | 26 (29.2) | 825 (24.3) |
| Lifetime illicit drug use | 24 (27.3) | 850 (25.3) |

Raw frequencies of mental health outcomes among transgender adults in the United States who ever wanted pubertal suppression. Percentages were calculated from the total of nonmissing values.

## DISCUSSION

This study is the first in which the association between access to pubertal suppression and measures of suicidality is examined. Treatment with pubertal suppression among those who wanted it was associated with lower odds of lifetime suicidal ideation when compared with those who wanted pubertal suppression but did not receive it. Suicidality is of particular concern for this population because the estimated lifetime prevalence of suicide attempts among transgender people is as high as 40%.[6] Approximately 9 of 10 transgender adults who wanted pubertal suppression but did not receive it endorsed lifetime suicidal ideation in the current study (Table 3). Access to pubertal suppression was associated with male sex assignment at birth, heterosexual sexual orientation, higher total household income, and higher level of family support for gender identity.

Results from this study suggest that the majority of transgender adults in the United States who have wanted pubertal suppression did not receive it. Of surveyed transgender adults in

the current study, 16.9% reported ever desiring pubertal suppression as part of their gender-related care; however, only 2.5% of these respondents indicated they had in fact received this wanted treatment. This was the case even for the youngest survey respondents, who were 18 years old at the time of data collection in 2015. Only 4.7% of 18-year-olds who wanted the treatment reported receiving it.

Although rates both of desiring and of receiving pubertal suppression were higher among younger respondents, results from the current study indicate that still only 29.2% of the youngest participants in the study (ie, those who were 18 years of age in the year 2015) reported ever desiring pubertal suppression as part of gender-related care. No individuals <18 years of age were captured by this data set; future research should investigate the rate of desiring pubertal suppression among younger populations. Some respondents may have simply never been aware of the possibility of puberty suppression while still within the range of developmentally suitable candidates for receiving this treatment, or they may have believed that they were not suitable candidates. This finding may also reflect the diversity of experience among transgender and gender-diverse people, highlighting that not all will want every type of gender-affirming intervention.[7,8] Future research is needed to understand why younger participants reported desiring pubertal suppression at higher rates; we hypothesize that this is likely due in part to recent increased public awareness about and access to gender-affirming interventions.[5]

Access to pubertal suppression was associated with a greater total household income. Without insurance, the annual cost of GnRHa therapy ranges from $4000 to $25 000.[18] Among adolescents treated with pubertal suppression at the Boston Children's Hospital Gender Management Service before 2012, <20% obtained insurance coverage.[19] More recently, insurance coverage for these medications has increased: a study from 2 academic medical centers in 2015 revealed that insurance covered the cost of GnRHa therapy in 72% of cases.[18] This is 1 potential explanation for why younger age was found to be associated with accessing pubertal suppression in the current study (Table 1). It is also plausible that those who receive pubertal suppression experience more improvement in mental health, which in turn may contribute to greater socioeconomic advancement.[20] This study's cross-sectional design limits further interpretation.

Participants who endorsed a heterosexual sexual orientation were more likely to have received pubertal suppression. This is in line with past research revealing that nonheterosexual transgender people are less likely to access gender-affirming surgical interventions.[21] Some clinicians may be biased against administering pubertal suppression to patients whose sexual orientation identities do not align with society's heteronormative assumptions.[21] In the current study, nonbinary and genderqueer respondents were also less likely to have accessed pubertal suppression, suggesting that clinicians may additionally be uncomfortable with delivering this treatment to patients whose gender identities defy more traditional binary categorization. Of note, because research on gender-affirming hormonal interventions for adolescents has been focused on transgender youth with binary gender identities,[11] some clinicians have reservations about prescribing pubertal suppression interventions to nonbinary youth in the event of a potentially prolonged state of low sex-steroid milieu.

Family support was also associated with receiving pubertal suppression among those who wanted this treatment. This finding is unsurprising given that most states require parental consent for adolescents to receive pubertal suppression.[22] Past studies have revealed that family support of gender identity is associated with favorable mental health outcomes.[6] Of note, treatment with pubertal suppression in the current study was associated with lower odds of lifetime suicidal ideation, even after adjustment for family support (Table 2).

We did not detect a difference in the odds of lifetime or past-year suicide attempts or attempts resulting in hospitalization. It is possible that we were underpowered to detect these differences given that suicide attempt items were less frequently endorsed than suicidal ideation items (Table 3). Given this study's retrospective self-report survey design, we were unable to capture information regarding completed suicides, which may have also reduced the number of suicide attempts we were able to account for. Given that suicidal ideation alone is a known predictor of future suicide attempts and deaths from suicide, the current results warrant particular concern.[23]

This study adds to the existing literature[11,12] on the relationship of pubertal suppression to favorable mental health outcomes. The theoretical basis for these improved mental health outcomes is that pubertal suppression prevents irreversible, gender-noncongruent changes that result from endogenous puberty (eg, bone structure, voice changes, breast development, and body hair growth) and that may cause significant distress among transgender youth. Pubertal suppression allows these adolescents more time to decide if they wish to either induce exogenous gender-congruent puberty or allow

endogenous puberty to progress.[7,8] Some have also theorized that gender-affirming medical care may have mental health benefits that are separate from its physical effects because it provides implied affirmation of gender identity from clinicians, which may in turn buffer against minority stress.[24]

Strengths of this study include its large sample size and representation of a broad geographic area of the United States. It is the first study in which associations between pubertal suppression for transgender youth and suicidality are examined. Limitations include the study's cross-sectional design, which does not allow for determination of causation. Longitudinal clinical trials are needed to better understand the efficacy of pubertal suppression. Because the 2015 USTS data do not contain the relevant variables, we were unable to examine associations between access to pubertal suppression and degree of body dysphoria in this study. Notably, past studies have revealed that body image difficulties persist through pubertal suppression and remit only after administration of gender-affirming hormone therapy with estrogen or testosterone.[11] It is also limited by its nonprobability sample design. Future researchers should work toward the collection of population-based survey data that include variables related to gender-affirming medical interventions. Of note, because pubertal suppression for transgender youth is a relatively recent intervention, some participants might not have known that these interventions existed and thus would not have reported ever wanting them. Had these individuals known about pubertal suppression, it is possible that they might have desired it. Because we do not have data on whether individuals who did not desire pubertal suppression would have wanted it had they known about it, we restricted our analysis to those who reported ever desiring pubertal suppression. Reverse causation cannot be ruled out: it is plausible that those without suicidal ideation had better mental health when seeking care and thus were more likely to be considered eligible for pubertal suppression. The Endocrine Society guidelines for pubertal suppression eligibility recommend that other mental health concerns be "reasonably well controlled."[7] Because this study includes only adults who identify as transgender, it does not include outcomes for people who may have initiated pubertal suppression and subsequently no longer identify as transgender. Notably, however, a recent study from the Netherlands of 812 adolescents with gender dysphoria revealed that only 1.9% of adolescents who initiated pubertal suppression discontinued this treatment without proceeding to gender-affirming hormone therapy with estrogen or testosterone.[25]

## CONCLUSIONS

Among transgender adults in the United States who have wanted pubertal suppression, access to this treatment is associated with lower odds of lifetime suicidal ideation. This study strengthens recommendations by the Endocrine Society and WPATH for this treatment to be made available for transgender adolescents who want it.

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## ABBREVIATIONS

CI: confidence interval
GnRHa: gonadotropin-releasing hormone analogue
K6: Kessler Psychological Distress Scale
NCTE: National Center for Transgender Equality
OR: odds ratio
USTS: US Transgender Survey
WPATH: World Professional Association for Transgender Health

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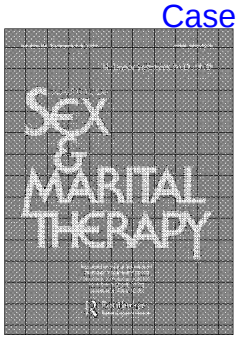

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# Surgical Satisfaction, Quality of Life, and Their Association After Gender-Affirming Surgery: A Follow-up Study

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**ABSTRACT**

We assessed the outcomes of gender-affirming surgery (GAS, or sex-reassignment surgery) 4 to 6 years after first clinical contact, and the associations between postoperative (dis)satisfaction and quality of life (QoL). Our multicenter, cross-sectional follow-up study involved persons diagnosed with gender dysphoria (*DSM-IV-TR*) who applied for medical interventions from 2007 until 2009. Of 546 eligible persons, 201 (37%) responded, of whom 136 had undergone GAS (genital, chest, facial, vocal cord and/or thyroid cartilage surgery). Main outcome measures were procedure performed, self-reported complications, and satisfaction with surgical outcomes (standardized questionnaires), QoL (Satisfaction With Life Scale, Subjective Happiness Scale, Cantril Ladder), gender dysphoria (Utrecht Gender Dysphoria Scale), and psychological symptoms (Symptom Checklist-90). Postoperative satisfaction was 94% to 100%, depending on the type of surgery performed. Eight (6%) of the participants reported dissatisfaction and/or regret, which was associated with preoperative psychological symptoms or self-reported surgical complications ($OR = 6.07$). Satisfied respondents' QoL scores were similar to reference values; dissatisfied or regretful respondents' scores were lower. Therefore, dissatisfaction after GAS may be viewed as indicator of unfavorable psychological and QoL outcomes.

## Introduction

Gender dysphoria refers to the phenomenon where people experience psychological distress resulting from the discrepancy between their sex assigned at birth and gender identity. Within this group, one generally distinguishes assigned males with female gender identity (trans women) and assigned females with male gender identity (trans men). Yet, it is increasingly acknowledged that gender is less binary than this distinction reflects. People desiring the body characteristics of their experienced gender may apply for medical treatments. In addition to cross-sex hormone therapy, gender-affirming surgery (GAS)—formerly known as sex-reassignment surgery (SRS)—aims to surgically align physical characteristics with one's identity. These surgical interventions target sex-specific physical characteristics such as genitals (e.g., vaginoplasty), chest (e.g., mastectomy), face, gonads, voice, and Adam's apple.

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In the past decades, (surgical) care for people diagnosed with gender dysphoria is increasingly provided in specialized, interdisciplinary health-care facilities following the Standards of Care (Coleman et al., 2012). A beneficial consequence of the increase in specialized centers is the opportunity to perform prospective follow-up studies on the effectiveness of surgery.

On the measures of this outcome research, Kuiper and Cohen-Kettenis (1988) stated, "In our opinion an evaluation of SRS can be made only on the basis of subjective data, because SRS is intended to solve a problem that cannot be determined objectively" (p. 441). Quality of life (QoL) reflects one's perceived well-being and functioning, and often includes physical, psychological, and social parameters. Ultimately, knowledge of treatment outcomes may result in a reciprocal relation between quality of care and QoL (van den Bos & Triemstra, 1999). Individuals diagnosed with gender dysphoria report a lower QoL compared to the general population, although this difference was not always statistically significant or found in both sexes (de Vries et al., 2014; Motmans, Meier, Ponnet, & T'Sjoen, 2012; Newfield, Hart, Dibble, & Kohler, 2006). In their 2010 review, Murad and colleagues (2010) conclude, albeit based on low-quality evidence, that cross-sex hormone therapy improves various domains of QoL of people diagnosed with gender dysphoria. For various GAS procedures, QoL improvement is reported as well. Although many studies have methodological limitations, trans women rate their QoL after vaginoplasty higher than before surgery (Horbach et al., 2015). Similarly, QoL is also higher in trans women who have undergone facial feminization surgery, compared to trans women without surgery (Ainsworth & Spiegel, 2010). Regarding trans men, Newfield and colleagues (2006) report higher scores in multiple QoL domains after mastectomy when compared to trans men who did not receive this intervention. All these studies indicate the positive impact on QoL of having received surgery aligning physique and identity. In an explorative Belgian study, however, no significant differences in QoL were found between the groups who did and did not undergo GAS procedures (Motmans et al., 2012). The authors explain that the majority of the no-GAS group was receiving psychological counseling and planning surgical procedures in the (near) future. A less positive picture comes from a study by Kuhn et al. (2009); compared to matched controls, transgender people report a lower QoL 15 years after GAS, mostly relating to social and sexual domains. Despite the positive effects of surgery, the average QoL of people who received GAS may thus still be lower than the general population.

Dissatisfaction and/or regret regarding the outcomes of GAS may be a source of impaired postoperative QoL. In the context of gender-affirming medical treatments, Pfäfflin (1993) distinguishes between minor and major regret. Major regret (the wish to detransition) is rare and associated with psychological morbidity and poor social support (Gijs & Brewaeys, 2007). Minor regret is considered as disappointment and can overlap with dissatisfaction. In general, the literature gives high percentages of satisfaction with GAS procedures: breast augmentation 87% to 100% (De Cuypere et al., 2005; Smith, van Goozen, Kuiper, & Cohen-Kettenis, 2005; Weigert, Frison, Sessiecq, Al Mutairi, & Casoli, 2013), vaginoplasty 83% to 100% (De Cuypere et al., 2005; Horbach et al., 2015; Lawrence, 2003; Rehman, Lazer, Benet, Schaefer, & Melman, 1999; Smith et al., 2005), subcutaneous mastectomy 92% to 100% (De Cuypere et al., 2005; Nelson, Whallett, & McGregor, 2009; Smith et al., 2005), and phalloplasty 100% (De Cuypere et al., 2005). Clinical evaluation studies show positive appraisal of both genital functionality and aesthetics after surgery, whereas sexual outcomes were found to be less positive (Bouman et al., 2016; Buncamper et al., 2015). In a cohort of 232 operated trans women, Lawrence (2003) examined the effect of three categories of characteristics on (dis)satisfaction with genital GAS, namely personal characteristics (e.g., age), therapy characteristics (e.g., psychotherapy provided), and psychosocial characteristics. The factor contributing most to satisfaction with GAS was the self-reported physical and functional result of surgery. Experienced complications were associated with less positive evaluation of the postoperative result (Lawrence, 2006).

Overall, the current evidence suggests that postoperative QoL of people surgically treated for gender dysphoria may be influenced by feelings of (minor) regret and dissatisfaction with GAS. Although individuals generally report high satisfaction rates with their GAS, little is known on dissatisfaction/regret and its impact on psychological and QoL outcomes. We believe that gaining insight into the influence of experienced complications and disappointing results after GAS (including genital, chest, facial, and voice/thyroid cartilage surgery) on these measures will help improve preoperative information and

identify and support people at risk for disappointing outcomes. More insight into the technical and psychological results of GAS, and their interrelationship of these aspects may improve our understanding of the overall outcomes of these treatments, and improve the effectiveness of care.

### Aims

Therefore, the objectives of the current study are to:

- describe satisfaction with and complications after any of the aforementioned GAS procedures in a cohort of people surgically treated for gender dysphoria five years after first contact with a gender clinic;
- explore the frequency and reported reasons of unsatisfactory and/or regretted outcomes;
- assess the association of dissatisfaction and/or (minor) regret with preoperative psychological symptoms, life satisfaction, and expectations regarding GAS, as well as with experienced complications at follow-up;
- assess differences in postoperative QoL, gender dysphoria, and psychological symptoms amongst satisfied and dissatisfied participants, and compare their scores to reference values.

## Method

### Procedure and participants

This follow-up study was conducted within the European Network on the Investigation of Gender Incongruence (ENIGI). All individuals diagnosed with gender dysphoria applying for gender-affirming interventions in Amsterdam (the Netherlands), Ghent (Belgium), Hamburg (Germany), and Oslo (Norway) filled out a battery of questionnaires during their diagnostic procedure (Kreukels et al., 2012).

Between July 2013 and February 2014, the cohort that had their first contact with the clinics in Amsterdam, Ghent, and Hamburg during 2007, 2008, and 2009 was invited to fill out an online survey. Because the Oslo clinic did not get ethical approval to contact Norwegian applicants again, the current article includes only three clinics. Institutional review board approval was obtained in the participating centers. Based on sex assigned at birth and treatments received, participants were automatically routed throughout the survey. Information on surgical procedures was retrieved from medical records or the responsible psychologist. More information on the procedures of the current study can be found in van de Grift et al. (2017). Finishing the survey typically took around 60 minutes.

In total, 546 people were invited to participate. At the end of the recruitment period, 201 (37%) people filled out the survey (Figure 1). Twenty-nine did not receive medical interventions and 36 received hormonal therapy. The remaining 136 (67%), who had received both hormonal therapy and GAS (genital, chest, facial, vocal cord, and/or thyroid cartilage surgery), were included for analysis. The majority of trans women had undergone a vaginoplasty, and some also received mamma augmentation. Other feminizing procedures included thyroid cartilage reduction, facial feminization surgery, and vocal cord surgery. The vast majority of trans men had undergone mastectomy and/or uterus extirpation and ovariectomy, and a minority received penis construction (phalloplasty or metoidioplasty).

The background characteristics of the study population are displayed in Table 1. Except for education level, none of the descriptive measures differed significantly between responders and nonresponders. Applicants with lower education were underrepresented in the present sample (12%) in comparison to the group of individuals who did not participate (21%).

### Main outcome measures

#### Baseline information

Demographic and diagnostic information were collected at admission (Kreukels et al., 2012). At the end of the diagnostic phase, the diagnostic criteria of gender identity disorder (currently referred to as *gender*

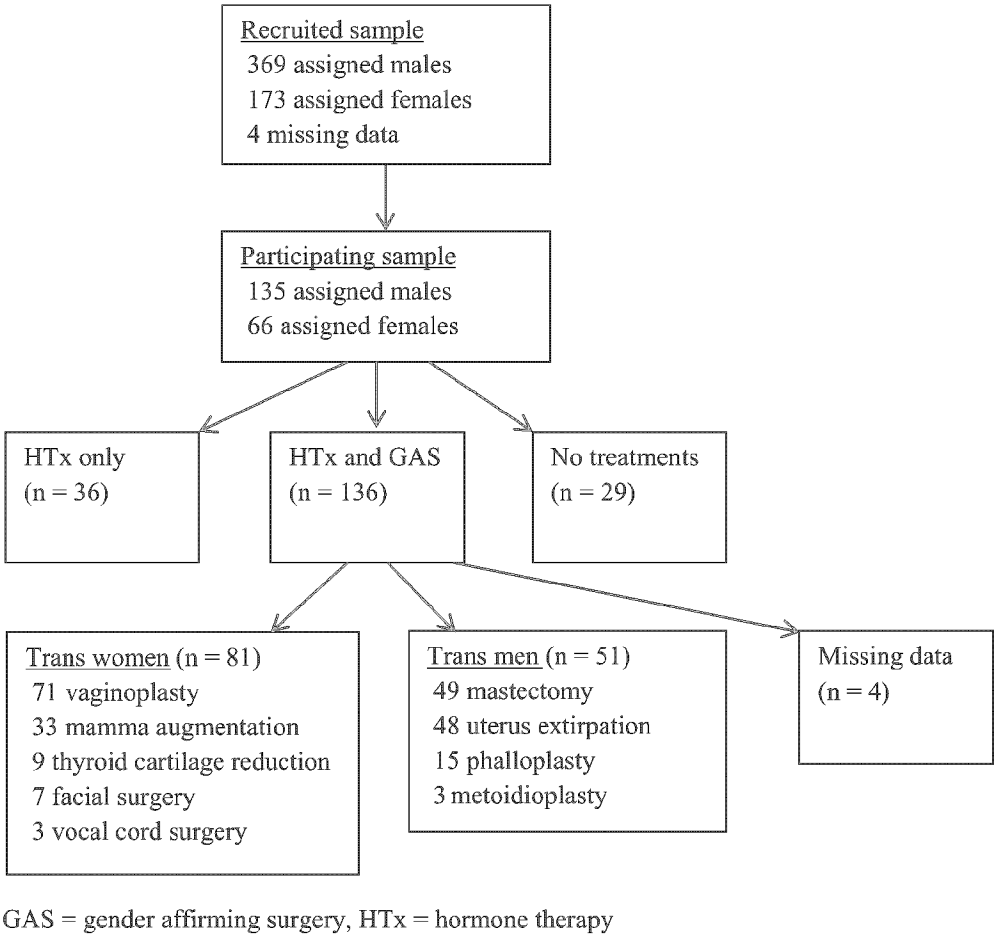


Figure 1. Flowchart of study participants.

*dysphoria* in the *DSM-5*) (American Psychiatric Association, 2013) were scored by the clinician on a self-constructed form based on the *DSM-IV-TR* criteria (American Psychiatric Association, 2000; Paap et al., 2011). With regard to the medical treatment, participants were asked if they were aware of the risks of treatments (*yes/no*) and how sure they were of receiving medical treatments (*sure/pretty sure/unsure*; both self-constructed). Also, people were asked to rate overall feelings with regard to their life (*good/fair/not good*) (Bradburn, 1969).

### *Treatment evaluation*

At follow-up, transition status and the wish to undergo additional GAS procedures were surveyed via questionnaires constructed by the researchers. For each surgical procedure received, participants were surveyed on experienced complications (*yes/no*) and to specify each complication. Satisfaction with the outcome of GAS procedures was assessed on a 5-point Likert scale (*very dissatisfied* to *very satisfied*). For each surgical procedure received, participants were asked whether they regretted having undergone the procedure and to comment on this. For each country, language-specific versions of all measures were used.

### *Gender dysphoria and psychological symptoms*

*Utrecht Gender Dysphoria Scale (UGDS).* This 12-item scale assesses the level of experienced gender dysphoria. Respondents rated each item on a 5-point Likert scale. The sum score ranges from 12 (minimal dysphoria) to 60 (maximal dysphoria) (Cohen-Kettenis & van Goozen, 1997). At follow-up, participants received the UGDS version of the gender they currently lived in.

Table 1. Sample characteristics and baseline scores of the study participants ($n = 136$).

| | *n (%)* |
|---|---|
| Sex | |
| Trans woman | 81 (61) |
| Trans man | 51 (39) |
| Age (*M*, range)* | 36.3 (17–63) |
| Education* | |
| Lower | 16 (12) |
| Intermediate | 56 (42) |
| Higher | 63 (47) |
| Expecting risks of gender-affirming medical treatments*^ | |
| Yes | 76 (64) |
| No | 43 (36) |
| Sure of gender-affirming medical treatments*# | |
| Sure | 100 (80) |
| Pretty sure | 22 (18) |
| Unsure | 3 (2) |
| SCL-90, GSI (*M*, SD)* | .48 (.44) |
| Overall feelings about life*$ | |
| Good | 41 (33) |
| Fair | 55 (44) |
| Not good | 29 (23) |
| Social transition | |
| Yes | 134 (99) |
| No transition/retransition to assigned gender | 1 (1) |
| Future medical treatments& | |
| Yes | 51 (38) |
| Unsure | 27 (20) |
| No | 55 (41) |
| Clinic | |
| Amsterdam, The Netherlands | 60 (46) |
| Ghent, Belgium | 50 (38) |
| Hamburg, Germany | 22 (17) |

*Note*. SCL-90, GSI = Symptoms Checklist-90, Global Severity Index. Sums of percentages may not equal 100 due to rounding of decimals.
*baseline data. ^"Do you expect risks related to your gender-affirming medical treatments?" #"How sure are you with regard to undergoing gender-affirming medical treatments?" $"How do you feel when considering your life as a whole?" &"Are there any additional medical treatments you wish to receive?"

*Symptom Checklist 90-R (SCL-90).* The SCL-90 is a 90-item self-report questionnaire assessing psychological symptoms on a 5-point Likert scale, ranging from zero (*no symptoms*) to four (*severe symptoms*). The Global Severity Index (GSI) is the average of all items and indicates overall experienced psychological symptoms (Derogatis, 1992). The measure was administered both at baseline and at follow-up.

### Quality of life

*Satisfaction With Life Scale (SWLS).* In this scale, satisfaction with life is measured through the agreement with five statements on a 7-point Likert scale. The sum score represents an overall measure of satisfaction—from low (5), to neutral (20), and high (35) (Diener, Emmons, Larsen, & Griffin, 1985).

*Subjective Happiness Scale (SHS).* This scale assesses the level of experienced happiness. The participant reports the agreement with four statements regarding happiness on a 7-point Likert scale. Higher scores represent a higher degree of happiness (Lyubomirsky & Lepper, 1999).

*Cantril Ladder (CL).* In this one-item self-anchoring scale, the participant rates how well one overall feels at that moment, ranging from 0 (*extremely bad*) to 10 (*extremely good*) (Cantril, 1965).

### Statistical analysis

Sex, education level, attitudes toward surgery, feelings about life, transition status, and planned GAS were reported as frequencies. Means and standard deviations were calculated for age and baseline SCL-90 GSI score. For all surgical procedures, the number of recipients and percentage per assigned sex were calculated, as well as self-reported complications and satisfaction. For participants indicating dissatisfaction and/or regret, the reported complications and comments were reviewed manually. Individuals who answered "dissatisfied" or "very dissatisfied" were considered dissatisfied, and individuals answering "yes" to the question about regretting a specific surgery were considered to have a (minor) regret. Associations of the following factors with GAS dissatisfaction/regret (*yes*/*no*) were calculated via odds ratios or chi-square calculations: "aware of risks of medical therapy," "sure of medical therapy," "overall feelings about life," and "experienced complications" and "planning additional surgery," or via correlation: baseline SCL-90 GSI. The relationship between dissatisfaction and QoL was assessed by comparing the dissatisfied and satisfied participants with regard to UGDS, follow-up SCL-90 GSI, SWLS, SHS, and CL scores. Because the test assumptions of normality were violated in the group with negative outcomes, nonparametric Mann-Whitney U tests were performed to compare the satisfied and dissatisfied groups. Values of the satisfied group were compared with control samples (nonclinical and general population samples including cisgender males and females) (Arrindell & Ettema, 2005; Arrindell, Heeskink, & Feij, 1999; Kahneman & Deaton, 2010; Lyubomirsky & Lepper, 1999; Steensma, 2013) using one-sample *t* tests and Cohen's *d* values of effect size. All analyses were performed using SPSS 22.0.

## Results

### Surgical procedures, complications, and postoperative satisfaction

Table 2 displays GAS procedures, self-reported complications, and satisfaction rates. Overall, complications included both medical (e.g., thrombosis), functional (e.g., voiding problems), and aesthetic issues (e.g., tissue necrosis). For trans women, complications were reported after vaginoplasty, mamma augmentation, and vocal cord surgery. The satisfaction with feminizing surgeries was 96% to 100%, except for a single person receiving vocal cord surgery who was not satisfied. For trans men, complication rates were highest for penis construction and mastectomy procedures. Satisfaction with the surgeries ranged from 94% (mastectomy) to 100% (penis construction), although some procedures were provided to only a few participants.

Table 2. Gender-affirming surgery received and self-reported complications and satisfaction.

| | | Medical Record Data* | Self-Reported Data^ | | |
|---|---|---|---|---|---|
| | | Received *n (%)* | Complications *n (%)* | Satisfied *n (%)* | Missing Data& *n (%)* |
| Feminizing surgery | Vaginoplasty | 71 (88) | 21 (38) | 53 (96) | 16 (23) |
| | Mamma augmentation | 33 (41) | 6 (22) | 25 (96) | 7 (21) |
| | Thyroid cartilage reduction | 9 (11) | 0 (0) | 6 (100) | 3 (33) |
| | Facial surgery | 7 (9) | 0 (0) | 7 (100) | 0 (0) |
| | Vocal cord surgery | 3 (4) | 1 (100) | 0 (0) | 2 (67) |
| Masculinizing surgery | Mastectomy | 49 (96) | 19 (53) | 34 (94) | 13 (27) |
| | Uterus extirpation | 48 (94) | 5 (14) | 34 (97) | 13 (27) |
| | Phalloplasty | 15 (29) | 4 (44) | 9 (100) | 6 (40) |
| | Metoidioplasty | 3 (6) | 2 (100) | 2 (100) | 1 (33) |

*Note*. *Data on the frequency of surgical procedures were collected from medical records. Percentages are calculated per sex: 81 trans women and 51 trans men; ^Data on complications and satisfaction were participant-reported. As some participants did not fill out some measures, percentages are calculated over the available data per procedure; &Numbers apply to participants who had received a surgical procedure but did not answer the questions on postoperative complications and/or satisfaction.

Table 3. Respondents reporting dissatisfaction after gender-affirming surgery.

| | Reported | Characteristics (incl. surgical procedures) | Complications | Reasons for Dissatisfaction |
|---|---|---|---|---|
| 1. | Dissatisfaction | 46 y/o trans woman breast augmentation; vaginoplasty; vocal cord surgery | Labia correction; no effect of vocal cord surgery | Vocal cord surgery had poor outcome |
| 2. | Minor regret | 42 y/o trans woman vaginoplasty | None | My body does not meet the feminine ideal |
| 3. | Dissatisfaction | 47 y/o trans woman vaginoplasty | None | Unspecified, dissatisfied with hormone therapy as well |
| 4. | Minor regret & dissatisfaction | 19 y/o trans man mastectomy; uterus extirpation | Hematoma; skin numbness of the chest; abdominal adhesions | Recurring abdominal pains, dependence on exogenous hormones |
| 5. | Dissatisfaction | 20 y/o trans woman breast augmentation; vaginoplasty | Capsule formation; vaginal fistula | Dissatisfied with the result of the breast augmentation |
| 6. | Dissatisfaction | 20 y/o trans man mastectomy | Infection; excessive scar tissue | Dissatisfied with the cosmetic outcome of the mastectomy |
| 7. | Dissatisfaction | 35 y/o trans woman vaginoplasty | Pain | Experiences technically poor outcome (chronic pain and poor cosmetic outcome) |
| 8. | Dissatisfaction | 19 y/o trans man mastectomy; uterus extirpation | Skin surplus | Dissatisfied with the cosmetic outcome of the mastectomy |

Dissatisfaction: "How do you feel about [surgical procedure] you have received?" Regret: "Do you regret your [surgical procedure] treatment?" See method section.

### *Respondents reporting postoperative dissatisfaction*

None of the respondents reported major regret. Eight respondents reported minor regrets (disappointment) or/and dissatisfaction with the outcomes of surgery (Table 3). The group included five trans women and three trans men who represented all three clinics. Three participants reported dissatisfaction after vaginoplasty, two after mastectomy, one after vocal cord surgery, one after uterus extirpation, and one after breast augmentation. One person (no. 3) was more generally dissatisfied; she was also dissatisfied with the hormonal treatment. Two participants reported dissatisfaction related to long-term complications, mostly pain (no. 4 and no. 7). The remaining five reported dissatisfaction with other outcomes, both functional (no. 1: no effect of vocal cord surgery) and aesthetic (nos. 2, 5, 6, and 8).

Reporting dissatisfaction and/or regret at follow-up was associated with less positive feelings about life, $\chi^2(2) = 7.47$, $p = .02$, and a higher SCL-90 GSI score at baseline, $r(127) = .24$, $p = .006$, and with reporting complications at follow-up ($OR = 6.07$, 95% CI: 1.18–31.38). Awareness of the risks and being sure about medical interventions at baseline were not significantly associated with dissatisfaction at follow-up, nor were the wishes for obtaining additional medical interventions at follow-up.

### *Relationship between postoperative satisfaction and quality of life*

Table 4 shows the mean QoL, gender dysphoria, and psychological symptom scores of the groups reporting dissatisfaction/regret and satisfaction with the outcomes of GAS, as well as control values retrieved from the literature. Satisfied participants had lower SCL-90 scores compared to the dissatisfied group; a trend toward a difference was seen for gender dysphoria (the UGDS score of the dissatisfied group was higher, but still low compared to the levels at baseline). For the other measures, a significant difference was seen for the Subjective Happiness Scale, with the satisfied group reporting a higher level of happiness; a trend in the same direction was seen for the Cantril Ladder.

When comparing the satisfied group with the normative control samples, significantly more psychological symptoms (SCL-90; $d = .22$) and lower satisfaction with life (SWLS; $d = .21$) were reported in our study sample. No significant differences were found for the levels of gender dysphoria, subjective happiness, and overall feelings about life when compared to control males and females, and to adolescents and young adults after GAS (de Vries et al., 2014).

**Table 4.** (Dis)satisfaction with surgery; differences in gender dysphoria, psychological symptoms, and quality of life, and the relationship with reference values.

| | Dissatisfied $n = 8$ *M (SD)* | Satisfied $n = 127$ *M (SD)* | Reference *M* | Test statistics, Satisfied vs. | |
|---|---|---|---|---|---|
| | | | | Dissatisfied | Reference |
| Gender Dysphoria | | | | | |
| UGDS | 19.0 (6.2) | 15.9 (4.3) | 15.8[1] | $U = 303, p = .08$ | $t = .13, p = .90$ |
| Psychological Symptoms | | | | | |
| SCL-90 (GSI) | .808 (.73) | .410 (.47) | .31[2] | **$U = 300, p = .05$** | **$t = 2.42, p = .02$** |
| Satisfaction With Life | | | | | |
| SWLS | 20.1 (7.6) | 24.2 (7.0) | 26.2[3] | $U = 294, p = .16$ | **$t = -3.19, p = .002$** |
| SHS | 3.57 (1.6) | 4.87 (1.4) | 4.89[4] | **$U = 233, p = .04$** | $t = -.17, p = .86$ |
| CL | 5.29 (2.7) | 7.07 (2.2) | 6.76[5] | $U = 267, p = .08$ | $t = 1.60, p = .11$ |

*Note*. One person had missing data on this measure, n = 135. CL = Cantril Ladder; SCL-90, GSI = Symptom Checklist-90, Global Severity Index; SHS = Subjective Happiness Scale; SWLS = Satisfaction With Life Scale; UGDS = Utrecht Gender Dysphoria Scale. Reference data includes data from both male and female cisgender controls, reported in the literature. Significant differences in bold.
[1]Steensma, 2013 (sample of 219 nonclinical heterosexual males and females). [2]Arrindell & Ettema, 2005 (general population sample of 2,368 males and females). [3]Arrindell, Heesink, & Feij, 1999 (general population of 1,700 young males and females). [4]Lyubomirsky & Lepper, 1999 (college sample of 551 males and females). [5]Kahneman & Deaton, 2010 (general population of 450,000 males and females).

## Discussion

Gender-affirming surgeries form an important part of medical treatment of gender dysphoria. In our study, participants reported high surgical satisfaction rates despite considerable numbers of postoperative complications. From the literature it is clear that complication rates differ per GAS procedure and depend on how data are being collected. Most studies point out the substantial risks of these surgeries (Horbach et al., 2015), whereas data collection via surveys obtained similarly high self-reported complication rates (Lawrence, 2006). The high number of satisfied respondents found in the present study is comparable to earlier studies (Bouman et al., 2016; Buncamper et al., 2015; De Cuypere et al., 2005; Horbach et al., 2015; Lawrence, 2003; Lawrence, 2006; Nelson, Whallett, & McGregor, 2009; Rehman et al., 1999; Smith et al., 2005; Weigert et al., 2013) and emphasizes the effectiveness of gender-affirming procedures. Yet, most treatment evaluation studies have collected data in a clinical setting, whereas the present study reports on a cohort that was surveyed in their home environment with limited dependence on clinicians (although the participants were invited through the clinics).

With regard to regret, similar to other studies (De Cuypere et al., 2005; Lawrence, 2006; Smith et al., 2005), only a few study participants reported feelings of regret, which was exclusively related to disappointment and not to the wish to detransition. Amongst the eight people who reported dissatisfaction or/and regret with GAS, both genders and most surgical procedures were represented.

Unlike many previous evaluation studies, we have looked into the background of this (dis)satisfaction. Smith and colleagues (2005) did not find an association between dissatisfaction with surgery and sex assigned at birth. Lawrence (2006), who only studied trans women, reported that self-reported complications and less favorable physical functioning (mostly pain) were negatively associated with satisfaction with GAS. Comments of our dissatisfied group showed that dissatisfaction was related to general treatment dissatisfaction, long-term complications, and disappointing (functional/aesthetic) outcomes. With respect to the last group, the surgical outcomes were mostly regarded as disappointing in relation to the feminizing or masculinizing aim (e.g., limited change in voice pitch after vocal cord surgery, or skin surplus after mastectomy that kept the chest's appearance feminine). Some reasons of dissatisfaction could be considered the direct result of postoperative complications, resulting in a six-fold increased risk of dissatisfaction amongst people who reported complications. The influence of perceived surgical outcomes on satisfaction has been described earlier (Landén, Wålinder, Hambert, & Lundström, 1998; Lawrence, 2003; Lawrence, 2006; Peyrot & Rubin, 2009). However, not all dissatisfied respondents reported complications with surgery, indicating that psychological characteristics may also have played a role.

Psychological symptoms and life dissatisfaction at baseline were associated with treatment dissatisfaction at follow-up. These findings emphasize the importance of paying attention to psychological mechanisms in addition to technical outcomes. Some may relate to psychological characteristics already present

before medical transition. Participants experiencing more psychological problems at clinical entry (along with and/or resulting from gender dysphoria) seem to differ from those with fewer psychological problems with regard to treatment evaluation. Earlier studies pointed out that pretreatment psychological functioning influences evaluation of the outcomes of medical transition (de Vries et al., 2014; Smith et al., 2005). Psychological symptoms, mostly depression, are associated with impaired treatment outcomes (Mayou et al., 2000), but also with disappointment (Saisto, Salmela-Aro, Nurmi, & Halmesmaki, 2001) and poor coping behavior (Kelly, Tyrka, Price, & Carpenter, 2008), albeit these studies did not include surgical samples. In the light of our findings, this could imply that individuals with more psychological symptoms at baseline may be at risk for poorer experienced outcomes of GAS. Although technical outcomes are important, psychological characteristics can influence how individuals cope once disappointing situations occur. Interestingly, participants were most satisfied with the procedure that had the highest prevalence of reported complications: penis construction. One explanation may be that these participants received more or better preoperative attention than was the case in other types of surgery. Their motivations and expectations concerning surgery were not only discussed with the responsible surgeon, but frequently also with a psychologist-sexologist. Additionally, only highly motivated people probably choose to undergo a procedure with a high risk of complications, after which disappointment may be more difficult to admit.

The relationship between (dis)satisfaction and QoL measures shows that treatment satisfaction can be viewed as an indicator for other experienced outcomes. Compared to the satisfied group, participants reporting dissatisfaction with any of their GAS procedures scored less favorably on psychological symptoms and QoL measures; they also displayed a nonsignificant trend toward less favorable gender dysphoria scores. Our study is the first assessing this relationship between treatment satisfaction, psychological well-being, and QoL in a large prospectively followed cohort. Lawrence (2006) has described the associations between postoperative satisfaction and improvement in QoL, but did not objectify the latter by using standardized measures. The dissatisfied participants no longer scored in the clinical gender dysphoria range at follow-up (Steensma, 2013), and no statistically significant differences were found with the satisfied group. Among dissatisfied respondents, less favorable scores on psychological symptoms and life satisfaction may relate to the higher number of self-reported complications (e.g., chronic pain), suggesting lower physical well-being.

Compared to other studies reporting on values of operated and norm groups (de Vries et al., 2014; Motmans et al., 2012; Newfield et al., 2006), our results show that satisfied respondents reported a relatively positive QoL. This confirms that medical transition alleviates feelings of gender dysphoria and improves life satisfaction to normative levels. This "normalization" of QoL most likely results from affirming one's gender identity by making physical characteristics more congruent to one's experienced gender and all its positive consequences on life. Remaining issues on some QoL measures could be explained by the fact that a number of people did not undergo all GAS desired procedures or by issues faced in their social life.

This study's main limitation was the sample representativeness. With a response rate of 37%, similar to the attrition rates of most follow-up studies (Gijs & Brewaeys, 2007), our study has probably suffered from a selection bias. Particularly individuals with lower education were underrepresented. In the future, this might be improved by decreasing the effort to participate, by reducing the length and complexity of the questionnaire, or finding alternatives for questionnaire evaluation only. Potentially, less satisfied individuals may have been underrepresented as well, although, the contrary cannot be ruled out either.

For surgeons it is important to know what type of "objective" results are considered desirable. In order to gain more insight into the relation between "objective" and "subjective" outcomes (Haid et al., 2016; Nordenstrom et al., 2010), future studies may collect both self-reported outcomes as well as objective measures (e.g., photography). Preferably, questionnaires should be used that are valid in this population and enable pre- and postintervention comparisons. The UGDS, which we used to assess postoperative gender dysphoria, was not originally developed for use in this setting, possibly reducing its sensitivity. Also, our study objective was to follow up all people five years after clinical entry, irrespective of their treatment phase. Therefore not all respondents were in the same postsurgical phase, lumping individuals with different postoperative follow-up lengths into single groups. Lastly, it should be mentioned that

treatment satisfaction and quality of life are more dynamic concepts than we most likely have been able to detect. Overall feelings of well-being may change over time, partly in response to external events. Therefore, it would be valuable to survey the participants of the present study again after five more years to see how these indicators further develop.

### Conclusions

The results of our study suggest that satisfaction with gender-affirming surgery is related to a variety of factors; although dissatisfaction may not be very prevalent, it can be viewed as an indicator of more impaired outcomes. Predicting dissatisfaction with postoperative outcomes is difficult, but the present data suggest associations with preoperative psychological symptoms and life satisfaction, as well as with self-reported complications at follow-up. Satisfaction with the relatively complicated phalloplasty procedure, before which participants are mostly thoroughly counseled by both the surgeon and the psychologist, was high. This may suggest that a concerted effort by specialized clinicians of both specialties may improve experienced outcomes.

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# Psychological Functioning in Transgender Adolescents Before and After Gender-Affirmative Care Compared With Cisgender General Population Peers

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# Psychological Functioning in Transgender Adolescents Before and After Gender-Affirmative Care Compared With Cisgender General Population Peers

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A B S T R A C T

**Purpose:** Transgender adolescents are at risk for internalizing and externalizing problems, along with high suicidality rates, and poor peer relations. The present study compared transgender adolescents before and after gender-affirmative care with a sample of nonclinical age-equivalent cisgender adolescents from the general population on psychological well-being and aimed to investigate the possible effect of transgender care involving puberty suppression.
**Methods:** In this cross-sectional study, emotional and behavioral problems were assessed by the Youth Self-Report in a sample of 272 adolescents referred to a specialized gender identity clinic who did not yet receive any affirmative medical treatment and compared with 178 transgender adolescents receiving affirmative care consisting of puberty suppression and compared with 651 Dutch high school cisgender adolescents from the general population.
**Results:** Before medical treatment, clinic-referred adolescents showed more internalizing problems and reported increased self-harm/suicidality and poorer peer relations compared with their age-equivalent peers. Transgender adolescents receiving puberty suppression had fewer emotional and behavioral problems than the group that had just been referred to transgender care and had similar or fewer problems than their same-age cisgender peers on the Youth Self-Report domains.
**Conclusions:** Transgender adolescents show poorer psychological well-being before treatment but show similar or better psychological functioning compared with cisgender peers from the general population after the start of specialized transgender care involving puberty suppression.


**IMPLICATIONS AND CONTRIBUTION**

This study found increased behavioral and emotional problems among adolescents referred to a specialized gender identity clinic compared with their cisgender peers from the general population. After the start of gender-affirming treatment, the transgender adolescents showed similar or better psychological functioning compared with their cisgender peers from the general population.

In recent years, a sharp increase has been seen in media attention, clinical referrals, and number of publications on adolescents with gender dysphoria (GD), the DSM-5 term used to describe the incongruence between one's birth-assigned gender and the experienced gender [1,2]. A number of these studies report on psychological functioning and show that feelings of GD are frequently associated with psychological difficulties [3].

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Adolescents referred to specialized gender identity clinics have prevalence rates of depression ranging from 12% to 58% and for anxiety 16% to 24% [3–8]. In these studies, histories of suicidal thoughts and self-harming behaviors were reported by 34%–51% and 12%–39% of youth, respectively, in the various studies [3–8]. In addition, comparison studies of transgender youth with lesbian, gay, and bisexual adolescents revealed comparable rates of psychological difficulties [9]. Several studies have used the standardized self-report and parental measures of the Youth Self-Report (YSR) and the Child Behavior Checklist [10,11] and found more behavioral and emotional problems in transgender youth compared with the normative samples of these measures [12,13]. In general, comparisons made with normative samples drawn from the general population show similar findings, with a predominance of internalizing problems over externalizing problems (for an overview, see [14]). Summarizing the YSR and Child Behavior Checklist results, transgender adolescents show psychological problems comparable to clinical norm populations, with some cross-national variation in levels of psychological problems between North America and Europe [15,16].

A framework for understanding GD and the associated mental health disparities is offered by the minority stress model that posits that sexual minorities experience chronic stressors related to the stigmatization of their identities [17,18]. Psychological functioning is better when there is more acceptance of GD by the youth and their environment, including better peer relations [12,16]. In addition, other more general risk factors might be related, and other models of explanations have been proposed [14]. In addition, the onset of puberty and the developing body might endorse an intensification of psychological distress [19].

Transgender care for adolescents with GD is often offered in a step-wise model. During the first phase, the nature of the adolescent's gender identity and general psychosocial functioning are explored, and medical interventions are not yet provided [19]. During the second phase, adolescents with GD receive puberty suppression by means of reversible gonadotropin-releasing hormone analogs to "create time" to enable further exploration of the decision for gender-affirming treatments without the accompanying distress caused by the physical changes of puberty [19]. Thereafter, gender-affirming hormones (GAHs) can be provided, androgens in assigned girls at birth and estrogens in assigned boys at birth to induce the development of secondary sex characteristics of the experienced gender [19–21]. The present article will refer to assigned boys or girls when assigned gender at birth is boy or girl, respectively, which may be incongruent from the experienced gender in the group of adolescents with GD.

The first follow-up studies evaluating the use of puberty suppression in relation to psychological well-being in adolescents with GD come from the Netherlands and showed that behavioral and emotional problems and depressive symptoms decreased and general functioning significantly improved during treatment [22,23]. A study from the United Kingdom showed that psychological support and puberty suppression were associated with an improved global psychosocial functioning in adolescents with GD with a combination of psychological support and puberty suppression, attributing to a greater improvement than psychological support only [24]. These psychological evaluation studies were performed using self-reported psychosocial functioning (internalizing and externalizing problems, suicidality, and peer relations) in comparison with normative standardization samples. The YSR normative sample was recruited over 20 years ago, and a more recent recruited sample from the general population is lacking [11]. The present study is the first to compare transgender adolescents receiving gender-affirmative treatment by means of puberty suppression with recently collected nonclinical cisgender peers from the general population, exploring psychological functioning and the role of specialized transgender care.

## Methods

### *Participants and procedure*

The samples in this study consisted of consecutive referrals to the Center of Expertise on Gender Dysphoria of the VU University Medical Center (VUmc) in Amsterdam, the Netherlands, between 2012 and 2015, and a control group of cisgender adolescents recruited in 2015 in the general population. During this period, 504 adolescents were seen in our gender identity service. Fifty-three participants did not complete the assessment process and did, therefore, not participate in this study. The reason for dropout was failure to complete the questionnaire or alternation of symptoms of GD. Of the adolescents diagnosed with GD, 179 were about to start GAH treatment. One participant did not complete the questionnaire and was thus excluded.

Therefore, in this cross-sectional study, the three groups that were compared consisted of (1) adolescents who just started the assessment process (n = 272; mean age = 14.5 years; 116 assigned boys at birth and 156 assigned girls at birth), (2) adolescents diagnosed with GD who were on puberty suppression and about to start GAH treatment (n = 178; mean age = 16.8 years; 68 assigned boys at birth and 110 assigned girls at birth), and (3) cisgender adolescents recruited from the general population (n = 651; mean age = 15.4 years; 346 assigned boys at birth and 305 assigned girls at birth). Adolescents who just started the diagnostic procedure were assessed during their first sessions at the VUmc. Adolescents diagnosed with GD were assessed before the start of GAH. During both assessments, parents and children completed several questionnaires [20].

Data from the comparison group of cisgender adolescents from the general population were recruited by means of the help of different secondary schools in different provinces in the Netherlands. After consent of the parents, the adolescents completed a paper-pencil survey during regular class times.

### *Measures*

Key demographic variables that were collected included the adolescents' birth-assigned gender, age, ethnicity, level of education, and parent's marital status. The demographic characteristics of the three groups are shown in Table 1.

The Dutch version of the YSR was used to assess internalizing and externalizing problem behavior, self-harm/suicidality, and poor peer relations [11]. The YSR consists of a total of 118 items, rated on a 0- to 2-point scale: "never," "sometimes," or "often," asking adolescents about their emotional and behavioral problems during the previous 6 months. The YSR is well established with regard to reliability and validity and has acceptable reliability and adequate criterion and construct validity [11]. The YSR has one item specifically pertaining to GD: "wish to be of the opposite sex" (Item 110). In line with previous studies, this item was scored as 0 to avoid increased associations with psychological challenges and GD [25]. For internalizing and

**Table 1**
General characteristics for transgender adolescents and the general population sample

| Variable | General population (n = 651) | Transgender at referral (n = 272) | Transgender using puberty suppression (n = 178) |
|---|---|---|---|
| Age (in years) | | | |
| Mean (SD) | 15.39 (1.36) | 14.47 (2.18) | 16.75 (1.24) |
| Ethnicity, n (%) | | | |
| Dutch | 580 (89.1) | 185 (68) | 131 (73.6) |
| Non-Dutch | 67 (10.3) | 30 (11) | 16 (9) |
| Unknown | 4 (.6) | 57 (21) | 31 (17.4) |
| Level of education, n (%) | | | |
| VMBO | 99 (15.2) | 203 (74.3) | 126 (70.6) |
| HAVO | 274 (42.1) | 29 (10.8) | 29 (16.4) |
| VWO | 278 (42.7) | 40 (14.9) | 23 (13) |
| Parent's marital status, n (%) | | | |
| Both parents | 520 (79.9) | 153 (56.3) | 103 (57.9) |
| Other | 129 (19.8) | 116 (42.6) | 74 (41.6) |
| Unknown | 2 (.3) | 3 (1.1) | 1 (.6) |

HAVO = higher general continued education; SD = standard deviation; VMBO = prevocational education; VWO = preparatory scholarly education.

externalizing problems, mean scale scores and clinical range percentages (>90th percentile in nonreferred samples) were calculated. To assess peer relations, and following the procedure as done in previous studies [25], a Peer Relations scale was created from three YSR items: "I don't get along with other kids" (Item 25), "I get teased a lot" (Item 38), and "I am not liked by other kids" (Item 48). Self-harm/suicidality was examined by two YSR items, namely, "I deliberately try to hurt or kill myself" (Item 18) and "I think about killing myself" (Item 91) as metrics of suicidality.

### *Analyses*

First, multivariate general linear modeling (GLM) analysis was used to analyze between-group differences for internalizing, externalizing, suicidality, and peer relations together. Second, a multivariate GLM analysis with assigned gender at birth and a gender by group interaction as additional predictors was used to identify possible gender differences. These analyses were followed by univariate GLM analyses with Bonferroni correction to correct for multiple comparisons. Third, multivariate GLM analyses with group and assigned gender at birth as predictors and age, ethnicity, level of education, and parent's marital status as covariates were performed. Fourth, Cohen's *d* was used to measure the effect sizes between the groups [26]. Finally, clinical range percentages were calculated for internalizing and externalizing.

## Results

### *Mean scores for internalizing, externalizing, suicidality, and peer relations*

Table 2 shows the mean scores for internalizing, externalizing, suicidality, and peer relations per sample. On average, the scores of the transgender adolescents who have just been referred on internalizing, suicidality, and peer relations were higher than the scores of the transgender adolescents using puberty suppression and the cisgender comparison group, respectively. A multivariate GLM analysis with group as a fixed factor and the internalizing, externalizing, suicidality, and poor peer relations as the dependent measures showed an overall difference using Pillai's trace ($F = 707.61$, $df = 4$; $p < .001$). Subsequent analyses for the internalizing, externalizing, suicidality, and poor peer relations indicated that groups differed from each other on internalizing, suicidality, and poor peer relations (all three univariate $p$ values $< .001$) but not on externalizing ($p = .709$).

### *Post hoc analyses*

Post hoc analyses showed that transgender adolescents who just have been referred had significantly higher scores on internalizing, suicidality, and peer relations compared with the cisgender comparison group and transgender adolescents using puberty suppression. In addition, the transgender adolescents using puberty suppression scored significantly lower on internalizing problems but higher on peer relations compared with the comparison group. No differences were found between adolescents using puberty suppression and the comparison group on self-harm/suicidality (Table 2 provides all effect sizes).

### *Gender differences*

When we added assigned gender at birth as a predictor, we confirmed the main effect of group ($F = 686.47$, $df = 4$; $p < .001$), and the previously mentioned univariate group effects for internalizing, suicidality, and peer relations were also confirmed (all $p < .001$). In addition, we found a main effect for gender ($F = 14.22$, $df = 4$; $p < .001$) and a group by gender interaction effect ($F = 9.52$, $df = 8$; $p < .001$). Subsequent univariate analysis found an effect for gender and an interaction effect on internalizing and peer relations. Within-group post hoc *t* tests revealed that the interaction arose on internalizing because in the cisgender comparison group, assigned girls at birth had higher mean scores than assigned boys at birth, whereas in both the transgender groups, no differences were found in internalizing scores between assigned girls and assigned boys at birth. On the peer relations, the interaction arose because in both transgender groups, assigned boys at birth had higher scores, whereas in the cisgender comparison group, assigned girls at birth had higher scores. Table 3 provides mean scores by assigned gender at birth. In addition, as for the demographic variables age, ethnicity, level of education, and parent's marital status statistical group differences were found, all analyses were repeated with these variables as covariates and showed similar findings.

**Table 2**
Mean scores on the Youth Self-Report for internalizing, externalizing, peer relations, and suicidality problems for transgender adolescents and the general population sample

| Measures[c] | General population (n = 651) | | Transgender at referral (n = 272) | | Transgender using puberty suppression (n = 178) | | Statistical analysis[a] | | Effect sizes Cohen's *d*[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | F[d] | *p* values | GP versus T0[e] | GP versus T1[e] | T0 versus T1[e] |
| Internalizing | 9.71 | 7.73 | 11.67 | 8.38 | 7.76 | 6.68 | 14.16 | **<.001** | −.24 | .30 | .52 |
| Externalizing | 10.25 | 6.10 | 10.19 | 6.33 | 9.82 | 5.79 | .34 | .709 | .01 | .07 | .06 |
| Peer relations | .41 | .81 | 1.08 | 1.31 | .70 | 1.06 | 12.58 | **<.001** | −.62 | −.31 | .32 |
| Suicidality | .19 | .60 | .41 | .78 | .17 | .52 | 44.26 | **<.001** | −.32 | .04 | .36 |

SD = standard deviation.
[a] Additional post hoc analyses comparing the transgender group at referral, the transgender group using puberty blockers, and the general population sample, demonstrated that on internalizing, peer relations, and suicidality, the adolescents at referral had significantly higher scores than the adolescents using suppression and the adolescents from the general population. In addition, the adolescents using puberty suppression scored significantly lower on internalizing but significantly higher on peer relations compared with the general population sample.
[b] Effect sizes Cohen's *d*: .80 or higher is a large effect size, .50–.79 a medium effect size, .20–.49 small, and effect sizes <.20 are negligible [26].
[c] Internalizing problems = disturbances of emotions (e.g., depression, anxiety; absolute range: 0–62); externalizing problems = behavioral excess or disturbances of conduct (e.g., aggression, hyperactivity; absolute range: 0–64); peer relations = problems with relations with peers (absolute range: 0–6); suicidality = thinking about or attempting suicide (absolute range: 0–4) [11].
[d] *df* = 2.
[e] GP = sample of cisgender adolescents from the general population; T0 = sample of transgender adolescents referred to transgender affirmative care who did not receive any medical treatment; T1 = transgender adolescents receiving affirmative care consisting of puberty suppression.

Finally, four (internalizing, externalizing, poor peer relations, and self-harm/suicidality) between-group analyses for each assigned gender at birth were performed using Bonferroni correction. These analyses showed that of the four between group comparisons for assigned boys at birth at referral with cisgender boys, significant higher scores were found for internalizing ($d = -.66$), peer relations ($d = -.92$), and self-harm/suicidality ($d = -.63$) for the assigned boys who just started the assessment. Assigned girls at birth who just started the assessment only scored significantly higher than the cisgender girls on peer relations ($d = -.36$). The three other scales were not significantly different.

In the transgender adolescent sample using puberty suppression, the assigned boys at birth scored only higher on peer relations ($d = -.53$) but not on the three other scales compared with the cisgender boys. For the assigned girls at birth using puberty suppression compared with the cisgender girls, the scores on internalizing were found to be significantly lower ($d = .63$). No other significant differences were found.

Of the four scale comparisons for assigned boys at birth at referral with the assigned boys at birth using puberty suppression, significant lower scores were found for those using puberty suppression on internalizing ($d = .54$), peer relations ($d = .41$), and self-harm/suicidality ($d = .37$). For the comparisons between the assigned girls at referral with the assigned girls using puberty suppression, significant lower scores were found for those using puberty suppression on internalizing ($d = .50$) and self-harm/suicidality ($d = .35$).

### *Clinical range percentages*

Of the transgender adolescents just referred to the clinic, 31.3% had clinical range scores for internalizing problems (assigned boys at birth: 35.3% and assigned girls at birth: 28.2%), and 17.3% (assigned boys at birth: 6.0% and assigned girls at birth: 25.6%) had those for externalizing compared with 22.9% (assigned boys at birth: 13.0% and assigned girls at birth: 34.1%) and 13.8% (assigned boys at birth: 11.3% and assigned girls at birth: 16.7%) of the cisgender comparison sample. For the transgender adolescents using puberty suppression, the percentages were 16.3% for internalizing (assigned boys at birth: 16.2% and assigned girls at birth: 16.4%) and 14.0% for externalizing (assigned boys at birth: 8.8% and assigned girls at birth:17.3%).

**Table 3**
Mean scores on the Youth Self-Report by gender assigned at birth for internalizing, externalizing, peer relations, and suicidality for transgender adolescents and the general population sample

| Measures[a] | General population | | | | | Transgender at referral | | | | | Transgender using puberty suppression | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Assigned boys (n = 346) | | Assigned girls (n = 305) | | Effect sizes Cohen's *d*[b] | Assigned boys (n = 116) | | Assigned girls (n = 156) | | Effect sizes Cohen's *d*[b] | Assigned boys (n = 68) | | Assigned girls (n = 110) | | Effect sizes Cohen's *d*[b] |
| | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| Internalizing | 7.21 | 5.89 | 12.54 | 8.55 | −.73 | 11.74 | 7.74 | 11.62 | 8.84 | .01 | 7.79 | 6.76 | 7.74 | 6.66 | .01 |
| Externalizing | 10.90 | 5.91 | 9.50 | 6.24 | .23 | 9.69 | 5.52 | 10.56 | 6.86 | −.14 | 10.32 | 6.26 | 9.51 | 5.31 | .14 |
| Peer relations | .38 | .77 | .45 | .85 | −.09 | 1.45 | 1.46 | .81 | 1.11 | .49 | .91 | 1.18 | .57 | .95 | .32 |
| Suicidality | .12 | .44 | .27 | .73 | −.25 | .39 | .73 | .42 | .81 | −.04 | .16 | .48 | .18 | .54 | −.04 |

SD = standard deviation.
[a] Internalizing problems = disturbances of emotions (e.g., depression, anxiety; absolute range: 0–62); externalizing problems = behavioral excess or disturbances of conduct (e.g., aggression, hyperactivity; absolute range: 0–64); peer relations = problems with relations with peers (absolute range: 0–6); suicidality = thinking about or attempting suicide (absolute range: 0–4) [11].
[b] Within group effect size differences; Cohen's *d*: .80 or higher is a large effect size, .50–.79 a medium effect size, .20–.49 small, and <.20 is negligible [26].

*Endorsement of self-harm/suicidality*

In the sample of transgender adolescents at referral, 74 (27.2%) endorsed the metric of suicidality. In the sample of transgender adolescents using puberty suppression, this was n = 22 (12.4%). In the cisgender comparison group, the percentage was 11.9% (n = 77).

## Discussion

Our study revealed that adolescents referred for gender-affirmative care have increased behavioral and emotional problems, especially internalizing problems, reported increased self-harm/suicidality, and poorer peer relations compared with cisgender adolescents from the general population. This finding, including the clinical range percentage for internalizing problems, is in line with the current literature that in general, transgender adolescents are at risk for mental health problems [3–8]. However, our study also showed that transgender adolescents receiving gender-affirmative care involving puberty suppressing treatment not only have less emotional and behavior problems than transgender adolescents who have just been referred to gender-affirmative care but also reported similar rates of mental health problems as their nonclinical cisgender peers on internalizing problems (with a lower clinical range percentage) and self-harm/suicidality but not on peer relation problems. This second finding of less internalizing problems and self-harm/suicidality is also in line with previous follow-up studies on transgender adolescents [22,23], providing further evidence that transgender adolescents could benefit from gender-affirmative care.

With regard to gender differences, we found that in both the transgender samples, assigned boys at birth scored higher on internalizing than assigned girls at birth, which is contrary to general population adolescents' mean scores but in line with previous findings [12]. For externalizing, and also in contrast with general population mean scores, assigned girls at birth who have just been referred but not assigned girls at birth on puberty suppression scored somewhat higher than assigned boys at birth with GD. These findings are partly in line with the hypothesis that the sex-typical pattern of more internalizing problems in girls and more externalizing problems in boys in the cisgender population might be inversed in transgender people [12]. This hypothesis deserves more research.

A clinical implication of these findings is the need for worldwide availability of gender-affirmative care, including puberty suppression for transgender adolescents to alleviate mental health problems of transgender adolescents. It should be acknowledged that the care provided in the present study also involved the offering of appropriate mental health care. Thus, transgender care providers need to actively screen for mental health problems and offer this care. In addition, clinicians should receive special training to provide this care, for example, to become more experienced in disentangling psychological problems stemming from bullying related to GD or having other origins. Our study found that transgender adolescents using puberty suppression consider their peer relations better than adolescents at referral but still reported more challenges with peers than the cisgender adolescents. As it has been established in different studies that stigmatization and peer victimization seem to be common for transgender people [27], and psychological problems are correlated with peer support [28], clinicians should also take the importance of peer support during the transition into account.

Although the treatment with puberty suppression for adolescents with GD is now available in an increasing number of countries, the small amount of scientific evidence of the medical safety and efficacy and the psychological efficacy comes from a limited number of studies, mostly performed in the Netherlands [22]. It should, therefore, additionally be stressed that the gender-affirmative treatment described in the Dutch protocol is a highly protocolled treatment with regard to eligibility criteria and psychological support, including affirmative psychoeducation of GD for youth and parents or caregivers and the continued discussion of psychosexual development with themes such as school and friendships but also dating and romantic relationships [29]. This does imply that the findings of our study might not apply to all transgender adolescents, as, for example, in other health care systems, psychological support is incomparable to the psychological support received following the Dutch protocol [29]. More research is needed to see whether our findings of effective affirmative care involving puberty suppression improving the mental health of transgender adolescents is generalizable to other countries.

In addition, the results of this study should be seen in the light of three limitations. First, this study did not make use of a random nonclinical national probability sample. However, although the mean scores in this study of the general population comparison sample were consistent with the findings of the YSR standardization sample used in other studies in the Dutch population [11,22], the generalizability of our findings might not be corroborated. Second, although the YSR is a well-validated questionnaire for behavioral and emotional challenges [11], it cannot be equated with a diagnosis of any mental health condition made by clinical assessment. Third and most important, although those individuals with and without a GD diagnosis after assessment did not differ in internalizing, externalizing, peer relations, and suicidality scores at baseline in the group that has just been referred to the clinic, the cross-sectional design of this study with different participants in the groups before and after puberty suppression may potentially limit the results with participants being different on characteristics not measured and controlled for. The present study can, therefore, not provide evidence about the direct benefits of puberty suppression over time and long-term mental health outcomes. Conclusions about long-term benefits of puberty suppression should thus be made with extreme caution needing prospective long-term follow-up studies with a repeated measure design with individuals being followed over time to confirm the current findings.

Future studies should, therefore, not only investigate the benefit of gender-affirmative care in other health care settings together with a matched nonclinical general population sample but should also make comparisons to transgender adolescents receiving GAH treatment and gender-affirming surgery to investigate the impact of these treatments on long-term mental health. As this study did not ask specifically for the increasingly recognized nonbinary identities [30], future studies should also cover if nonbinary transgender adolescents might equally benefit from this type of gender-affirmative care. Despite the previously mentioned limitations, this first study comparing a group of transgender adolescents just referred for gender-affirmative care, a group of transgender adolescents receiving treatment with puberty suppression, and a group of cisgender adolescents

from the general population showed that when affirmative care involving puberty suppression is provided, transgender adolescents may have comparable mental health levels to their cisgender peers. This type of gender-affirmative care seems thus extremely important for this group.

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## Bones in balance

Vlot, M.C.

2020



Pl. Trial Ex. 215

# BONES IN BALANCE

## Use of bone turnover markers in clinical practice

Mariska Caroline Vlot

# Bones in balance

## Use of bone turnover markers in clinical practice

Mariska Caroline Vlot

This PhD thesis was embedded within Amsterdam Movement Sciences research institute, at the Department of Internal Medicine and Department of Clinical Chemistry, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands.

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# Bones in balance

## Use of bone turnover markers in clinical practice

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door

Mariska Caroline Vlot
geboren te Leiden

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*"Your bones are for life.*
*Look after them and they will carry you far."*
Susan Hampshire

# Table of Contents

# Part I

## INTRODUCTION

# Chapter 1

# General introduction

The overall objective of this thesis is to assess the use of different bone turnover markers in clinical practice and to gain insight in changes of bone turnover in various patient settings. **Chapter 1** of the first part of this thesis consists of a general introduction that provides more background on the anatomy and physiology of bone tissue. Next, bone turnover markers and their clinical utility are addressed. Subsequently, different bone cells and their function are explained in more detail. Last, assessment of bone health by dual-energy X-ray absorptiometry (DXA) is described, followed by several factors affecting bone homeostasis. This chapter ends with an outline of the thesis.

## Bone

During growth and also in adult life, bone is constantly broken down and (re)built, a process known as bone remodeling [1]. Bone remodeling resembles a coupled process that starts with bone resorption and is followed by bone formation [2–5] (see Figure 1). Osteoclasts, osteoblasts and osteocytes are the essential cells controlling this bone remodeling process, which will be explained in more detail in the next paragraphs. These cells produce or release several factors called bone turnover markers (BTMs), when bone is degraded or formed and these BTMs can be measured in blood or urine. As a result these BTMs can be used in clinical practice e.g. to assist in diagnosing (metabolic) bone disease.

The main function of bone is to provide structure and protection of the internal organs by resisting mechanical forces [6]. Furthermore, bone contains bone marrow of which stem cells originate and bone is a dynamic reservoir for calcium and phosphate metabolism. Next to this, bone has several endocrine functions e.g. glucose homeostasis and insulin sensitivity which are partially mediated by the matrix protein osteocalcin (OC) [7]. Lastly, bone acts as a buffer in acid-base imbalances.

Bone can be differentiated in either trabecular or cortical bone. Trabecular bone, also known as spongy or cancellous bone, is predominantly present in the lumbar spine and contains many trabeculae between which bone marrow is located. Cortical bone is found primarily in the shaft of long bones and forms the outer shell around cancellous bone at the end of joints and the vertebrae. Cortical, or compact bone, is mainly present in the hip and represents 80% of the total bone volume of the body [8–10]. Within bone tissue, all bone cells are embedded within a bone matrix, which provide bone its rigidity. The main components of this matrix are the organic type I collagen and anorganic components of which calcium hydroxyapatite is the most abundant. Osteoid, newly formed bone which is not yet mineralized, consists mainly of type I collagen which is composed of triple helix polypeptide chains [6]. This type of collagen is predominantly found in bone, but also in skin and tendons, albeit to a lesser extent [11]. The anorganic calcium hydroxyapatite provides bone its additional rigidity and strength, especially once mineralization of the osteoid is completed [6,10–12].

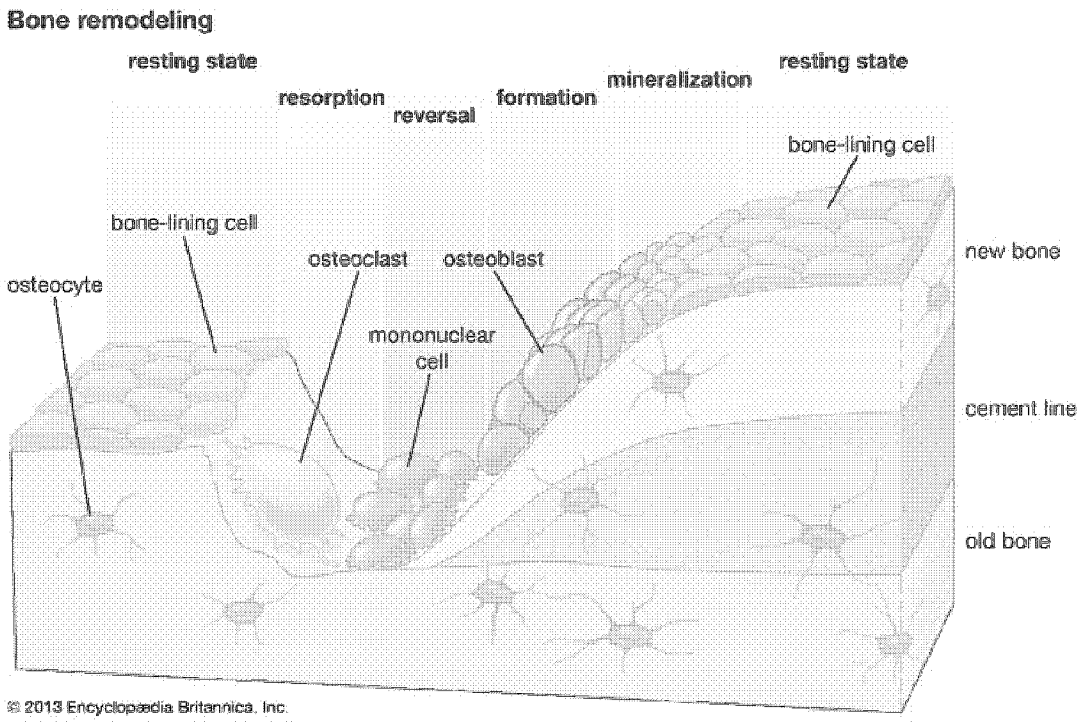


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**Figure 1.** Overview of bone remodeling

## Bone turnover markers and clinical utility

BTMs are considered indicators of bone remodeling as their release from bone into blood reflects the bone resorption and/or bone formation directly. Figure 2. shows an overview of several BTMs that are addressed in this thesis. Nowadays, the use of BTMs increased substantially as they can be measured more easily, as they are useful either to diagnose bone (related) disease, to follow its natural history, but also to monitor the effects of interventions [13–16]. However, it is challenging to choose the BTM with the best clinical utility in order to optimize patient care as many bone formation and bone resorption markers can be measured in one individual patient.

Throughout this thesis the concept "clinic utility" of BTMs is used. With clinical utility of a BTM we mean that a BTM is suitable either 1) to diagnose bone (related) disease, 2) to assist in making a prognosis of either improvement or deterioration of bone (related) disease, or 3) to define high risk patients e.g. patients with low bone mineral density (BMD) or high fracture risk. Based on these aspects, the clinician can provide medical advice to his/her patients in order to preserve the best possible bone health. This will result in an overall improved health outcome for the patient and also in a reduction of health care costs eventually. In this thesis, we will evaluate whether and when BTMs are of additional value in clinical practice based on this definition of clinical utility.

The best BTM for the clinician to use would be produced in bone tissue only, can be detected by a standardized laboratory assay at low costs with low variability and high diagnostic test accuracy, can be interpreted based on proper reference values and is associated with clinical

outcomes related to bone disease such as a decrease of BMD, and/or bone strength or increased fracture risk. As BTM concentrations are affected by several factors such as sex, age, kidney function, hepatic function, recent fracture, fasting state, circadian rhythm and assay variability, the clinician should be aware of possible pitfalls when selecting a BTM and interpreting its concentration. These possible pitfalls will be addressed throughout the next chapters of this thesis.

# Bone cells

## Osteoclast

Osteoclasts originate from the monocyte cell line and are therefore related to macrophage cells. By secreting acid substances and using lysosomal enzymes osteoclasts resorb bone matrix. BTMs carboxy-terminal telopeptide of type I collagen (CTX), cross–linked carboxy-terminal telopeptide of type I collagen (ICTP), amino-terminal telopeptide of type I collagen (NTX), deoxypyridinoline

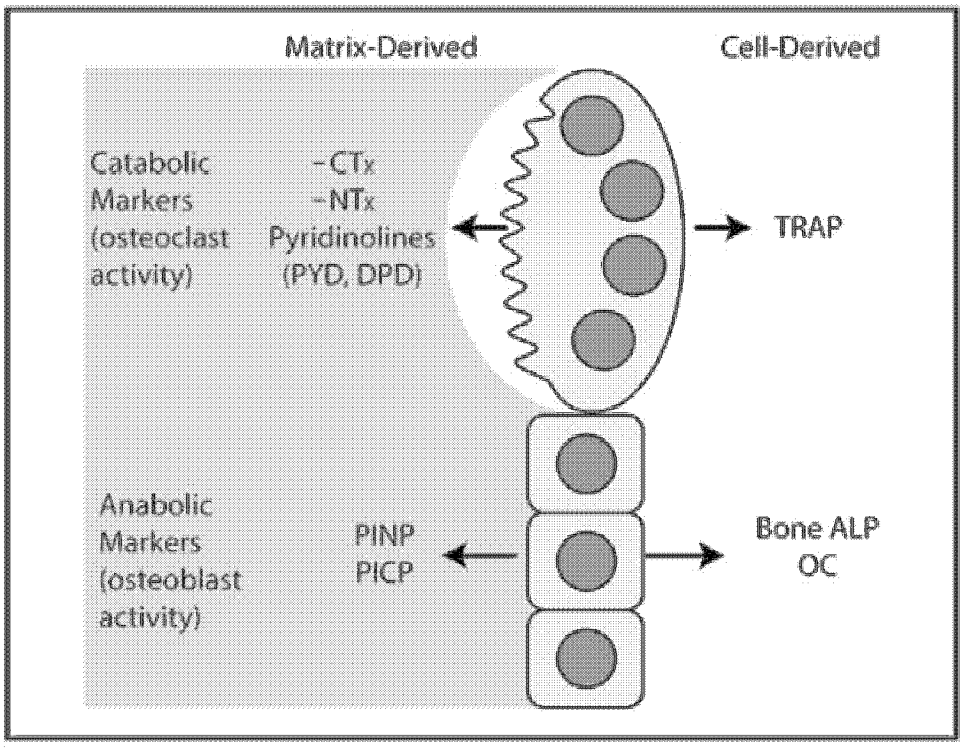


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**Figure 2.** Overview of bone turnover markers either released or produced during bone resorption or bone formation. **Legend**: CTx = carboxy-terminal telopeptide of type I collagen, NTx = amino-terminal telopeptide of type I collagen, PYD = pyridinoline, DPD = deoxypyridinoline , TRAP = TRAP5b = tartrate resistant alkaline phosphatase 5b, PINP = amino-terminal propeptide of type I procollagen, PICP = carboxy-terminal propeptide of type I procollagen, bone ALP = BALP = bone specific alkaline phosphatase, OC = osteocalcin. Resorption marker cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) is not displayed in Figure 2.

(DPD), and tartrate resistant alkaline phosphatase 5b (TRAP5b) are released during bone resorption. These markers are degradation products of type I collagen or represent enzymes that are present in the osteoclast and can be measured in blood and/or urine samples [11,17].

### Osteoblast

Osteoblasts are bone forming cells and originate from osteoprogenitor cells derived from stem cells. The osteoblasts are essential for synthesizing bone matrix by producing type I collagen and also to mineralize osteoid. Osteoblasts release the BTMs amino-terminal propeptide of type I procollagen (PINP), carboxy-terminal propeptide of type I procollagen (PICP), osteocalcin (OC) and bone specific alkaline phosphatase (BALP) during bone formation. In the process of mineralization of bone especially alkaline phosphatase (ALP) and OC play an important role [11]. Bone formation markers are peptides, proteins or enzymes that are derived from, or are present in the osteoblasts and can be measured in blood and some also in urine samples [11,17].

### Osteocyte

Osteocytes are older inactivated osteoblasts and lie embedded in the bone matrix. Osteocytes are connected to each other by small canaliculi, which allows them to affect bone turnover by producing fibroblast growth factor-23 (FGF23) and sclerostin. FGF23 affects bone metabolism by increasing the activity of the enzyme 24-hydroxylase in the kidney and the phosphate excretion by the kidney. In addition, the activity of the enzyme 1-alpha-hydroxylase and parathormone (PTH) concentration decrease, all resulting in a decrease of the concentration of active vitamin D [18,19]. Sclerostin inhibits the anabolic Wnt-signaling pathway, which results in decreased bone formation [20].

## Assessing bone health by DXA

Currently, BMD measurements by DXA are most commonly used to evaluate bone health in patient care. The two-dimensional DXA measurements display the BMD in grams of mineralized bone per square cm ($g/cm^2$) [21]. However, the DXA does not account for differences in shape and size of the bone which can result in either under- or overestimation of BMD, which is especially relevant in younger persons with growing bones and those who have not reached their peak bone mass yet [22]. To account for these changes in bone size a volumetric assessment of the BMD is required [23]. Also, a DXA scan does not discriminate between trabecular or cortical bone. Furthermore, only mineralized bone can be reliably measured by DXA and complete mineralization of newly formed bone can take up to 1-3 years to be completed [10]. As a result, DXA scans are especially useful to display longer term changes in BMD. This is in contrast to measurements of BTMs as these display the actual bone metabolism. Therefore BTMs can be valuable in early analysis of bone health in clinical patient care. In this thesis, we often relate changes in bone remodeling measured by BTMs to changes in BMD measured by DXA to assess both short and longer term changes in bone metabolism and bone health of our study participants.

Nowadays, DXA is predominantly used in the clinic to diagnose osteoporosis. Osteoporosis is characterized by a deterioration of bone structure and quality resulting in impaired bone strength and increased fracture risk. These fractures are associated with increased mortality, especially in the elderly [2,24] and also result in a great financial burden on the healthcare system. The prevalence of osteoporosis in Europe is estimated at 22 million mainly post-menopausal women and 5.5 million men [24]. Osteoporosis is diagnosed based on calculating T-scores according to WHO criteria [25]. T-scores are calculated by comparing the BMD of the patient to a reference BMD of young healthy women or men with the same ethnicity. Alternatively, in younger patients, a Z-score can be calculated, in which the BMD of the patient is compared to the BMD of a reference BMD of women or men with the same age and ethnicity. A T-score of -1.0 and above is considered normal, osteopenia is classified as a T-score between -1.0 and -2.5 and osteoporosis is present when a T-score of -2.5 or below is calculated. A Z-score of -2.0 or below is considered as osteoporotic [22,25]. Treatment of osteoporosis by antiresorptive therapy such as bisphosphonates results in suppression of bone turnover and therefore an increase in BMD and a reduction of fracture risk. Currently, BTMs do not play a key role in the diagnosis of osteoporosis, because the diagnostic predictive value of BTMs for fractures in the individual patient at baseline in osteoporosis treatment is low [26]. However, the International Osteoporosis Foundation (IOF) and also the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) guidelines do favor using PINP as formation marker and CTX as resorption marker as BTM of choice to monitor and to evaluate the effect of osteoporosis therapy [2,24,26,27].

## Factors affecting bone turnover and BMD

From childhood until adulthood, several factors are known to affect bone health such as insulin like growth factor (IGF-1), sex steroids, parathyroid hormone (PTH), cortisol, calcium, vitamin D and mechanical loading of the skeleton either by muscle tension and/or body weight [27,28]. Bone growth starts with endosteal growth, implying formation of new bone by osteoblasts at the medullary cavity of the bone, where estrogens play an important role. Later, also periosteal apposition of bone occurs, especially due to the effect of androgens. The longitudinal growth of bones is terminated after closure of the epiphyseal growth plates due to high concentrations of estrogen in both girls and boys towards the end of puberty. In boys aromatization of (a part of) testosterone results in estrogen and thereby exerts its anabolic effect on bone as well. After termination of longitudinal bone growth in puberty, bone mineralization continues and the peak bone mass is reached at an average age of 30 years [1]. In general, adult men have higher BMD than women [29]. During adulthood, bone resorption starts to exceed bone formation, resulting in a gradual decrease of BMD during aging in both men and women [1,10]. Bone loss occurs predominantly in the cortical bone, which is seen especially in post-menopausal women due to loss of estrogen which induces bone resorption [10,30–32]. In addition, the number of trabeculae in the trabecular bone decreases as well. These physiological changes are shown in Figure 3.

Besides physiological changes in bone metabolism, other factors also affect bone metabolism. Drugs affecting sex steroid concentrations (e.g. gender affirming hormone therapy in transgender

persons , aromatase inhibitors or gonadotropin-releasing hormone (GnRH) analogues) can result in changes in bone metabolism [33–35]. Furthermore, inflammatory factors such as interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) in case of inflammation or tumor derived factors such as tumor growth factor beta (TGF-β) in case of malignant disease can negatively affect bone metabolism [36–38]. These factors often result in an increase of bone resorption and therefore exert possible deleterious effects on BMD and bone strength. Consequently, treatment of these inflammatory or malignant diseases resulting in a decrease of inflammation or tumor derived factors may restore bone health in part.

## Objective of this thesis

The overall objective of this thesis is to assess the use of different bone turnover markers in clinical practice and to gain insight in changes in bone turnover in various patient settings. This thesis will provide additional knowledge of the clinical utility of several BTMs. As a result, this thesis consists of five parts in which the effects of sex steroids, inflammation and malignant disease on bone turnover and BTMs are assessed. An overview of the different parts is described below.

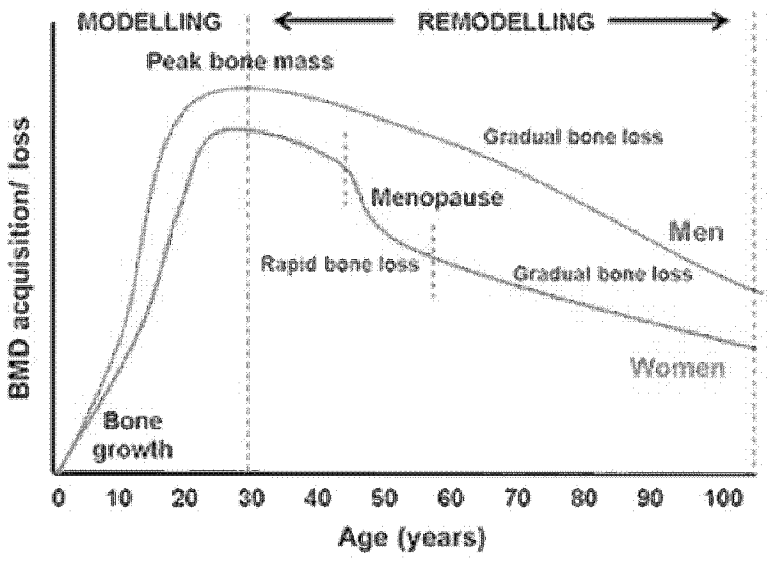


*Image reprinted according to Creative Commons Attribution 4.0. International Licence of Springer Nature, based on original article "Bone mineral density in people living with HIV; a narrative review of the literature", Kruger MJ and Nell TA, AIDS Res Ther 2017 Jul 26;14(1):35. No changes were made, only a legend was added.*

**Figure 3.** Bone development in males and females throughout life. **Legend**: BMD = bone mineral density.

# Outline

## Part I: Introduction

After the general introduction, **chapter 2** shows a review of the current literature regarding clinical utility of BTMs in various diseases. Also possible pitfalls resulting from either patient or assay characteristics are evaluated when interpreting BTM concentrations.

## Part II: Effects of sex steroids on bone health in transgender persons

**Chapters 3 to 5** all focus on changes in BTMs in transgender persons during treatment with gender-affirming hormonal therapy (HT). Gender dysphoria is defined as incongruence between a persons experienced gender and gender assigned at birth, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM V) [39]. Transgender persons are treated with HT consisting of testosterone in trans men (female-to-males) and with cyproteronacetate and estrogen in trans women (male-to-females) to achieve desired body changes. As a result, these studies are a model to evaluate the effects of sex steroids on bone metabolism in both adolescent and adult transgender persons. The first study, **chapter 3,** contains a study in adolescent transgender persons. As puberty is highly important for accumulation of bone mass, we studied effects of pubertal suppression and use of HT on bone turnover and volumetric bone mineral apparent density (BMAD) of both trans men and trans women. **Chapter 4** evaluates the changes in BMD in adult transgender persons after one year of HT. **Chapter 5** looks into more detail into the changes of BTMs and its relation to BMD changes in adult transgender persons after one year of HT.

## Part III: Effects of inflammation and auto-immune disease on bone turnover

In **chapter 6 and 7** the effects of inflammation on bone turnover caused by either viral inflammation or by auto-immune disease is studied. Human immunodeficiency virus (HIV) infection and also treatment with combination antiretroviral therapy (cART) are known to affect bone turnover. **Chapter 6** evaluates BTMs and BMD in adult men with primary HIV infection, either receiving cART or not. Next, vitamin D metabolism and BMD are known to be negatively affected in patients with the auto-immune disease multiple sclerosis (MS). **Chapter 7** looks into changes in vitamin D-FGF23 axis and bone turnover in patients with MS compared to healthy controls.

## Part IV: Possible role of FGF23 as tumor marker in malignant disease

In **chapter 8**, a pilot study is displayed, which evaluates whether FGF23 could be a promising biomarker in patients with prostate cancer.

## Part V: Discussion and summary

**Chapter 9** includes the most important findings of the thesis, both in English and Dutch. Furthermore, future perspectives are discussed.

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# Chapter 2

# Clinical utility of bone markers in various diseases

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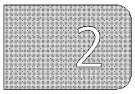
*Bone 2018 Sep;114:215-225.*




## Abstract

Measurements of bone markers (BMs) in peripheral blood or urine are a pivotal part of modern clinical medicine. In recent years the use of BMs increased substantially as they can be useful either to diagnose bone (related) disease and to follow its natural history, but also to monitor the effects of interventions. However, the use of BMs is still complicated mainly due to (pre)analytical variability of these substances, limited accessibility of assays, variable cut-off values in different countries and laboratories and heterogeneous results with regard to clinical implications of measuring BMs in several studies. This review will provide the clinician with a practical guide, based on current evidence, in which circumstances to test which bone markers for optimal diagnostic purposes, in order to improve patient care in different areas of bone diseases including Paget's disease, primary osteoporosis, tumor induced osteomalacia, hypophosphatemic rickets, van Buchem disease, chronic kidney disease, rheumatoid arthritis, neoplasma/multiple myeloma, type 2 diabetes mellitus and primary hyperparathyroidism. The clinician should consider fasting state, recent fractures, aging, menopausal status, concomitant liver and kidney disease when ordering and interpreting BM measurements as these factors might result in misleading BM concentrations. We found that BMs are clearly useful in the current diagnosis of tumor induced osteomalacia, van Buchem disease, Paget's disease and hypophosphatemic rickets. In addition, BMs are useful to monitor disease activity in chronic kidney disease, Paget's disease and are useful to monitor treatment adherence in osteoporosis.

# Introduction

Measurements of bone markers (BMs) in peripheral blood or urine are a pivotal part of modern clinical medicine. In recent years the use of BMs increased substantially as they can be useful either to diagnose bone (related) disease and to follow its natural history, but also to monitor the effects of interventions [1–5]. BMs are the only indicators of overall bone turnover and reflectors of the actual bone remodeling, a coupled process that consists of bone resorption which is followed by bone formation [6–8]. The release of BMs from bone into blood reflect the activity of bone formation and/or bone resorption. Key-players in this process are osteoblasts, osteoclasts and osteocytes. We used the term 'bone markers' as a broad term for those factors that are produced by these bone cells or are degradation products of bone. Vitamin D and its metabolites will be addressed very briefly, as this review focuses on true BMs only. The best bone marker of choice for the clinician to use would be produced in bone tissue only, can be detected by a standardized laboratory assay at low costs with low variability and high diagnostic test accuracy, can be interpreted based on proper reference values and is associated with clinical outcomes related to bone disease such as a decrease of bone mineral density (BMD), and/or bone strength or increased fracture risk.

Nevertheless, the use of BMs is still complicated mainly due to (pre)analytical variability of these substances, limited accessibility of assays, variable cut-off values according to the assay used in different countries and laboratories and heterogeneous results with regard to clinical implications of measuring BMs in several studies [8–16]. Therefore, it is challenging for clinicians to select the markers with best clinical utility in daily clinical practice to optimize patient care. Also, clinicians should be aware of several important issues when measuring or interpreting BMs. Some BMs display a circadian rhythm with concentrations generally showing their peak during nighttime and early morning and a nadir in the afternoon. This circadian rhythm is specifically seen in resorption markers such as carboxy-terminal telopeptide of type I collagen (CTX-I), amino-terminal telopeptide of type I collagen (NTX-I) and deoxypyridinoline (DPD) and to a lesser extent in formation markers such as amino-terminal propeptide of type I procollagen (PINP) and osteocalcin (OC) [6,7,11,17]. No circadian variation is seen in bone-related enzymes such as the resorption marker tartrate resistant alkaline phosphatase 5b (TRAP5b) or the formation marker bone specific alkaline phosphatase (BALP). Furthermore, food ingestion is critical with regard to CTX-I measurements as these concentrations drop after food intake resulting in the need of measuring CTX-I in fasting state [18]. CTX-I can undergo post-translational modifications which results in various circulating isomers. Beta CTX-I is measured in most laboratories. Most BMs are cleared by the kidney, with the exception of formation marker trimeric PINP and the enzymes BALP and TRAP5b. This implies that concentrations of CTX-I, NTX-I, resorption marker carboxy-terminal telopeptide of type I collagen (ICTP), OC and monomeric PINP increase when renal function decreases [6,18,19]. Also liver disease affects the hepatic clearance of PINP and PICP and concentrations of ALP and BALP [20]. Furthermore, it is of note that haemolysis result in decreased levels of OC [21] and repetitive freeze/thaw cycli should be avoided due to the instability of this protein [8]. It also has to be kept in mind that, depending on the bone and the surface of consolidation, BMs can be elevated especially within the first 6 months and up to or even more than 1 year after a fracture. In addition,

the biological age of a patients results into different patterns of BM e.g. high levels in children due to growth and higher levels in postmenopausal women compared to premenopausal women. To summarize, when measuring BMs the preferred timing is in the morning after an overnight fast, to limit effects of both circadian rhythm and effect of food on turnover as much as possible and when interpreting the BMs the age, sex, kidney function, liver function and previous fractures should be taken into account. In case of urinary BMs, the use of a second morning void after overnight fast has been advised, although this is often challenging or even inconvenient for patients. The BMs of choice for this review can be categorized in markers involved in bone resorption or bone formation. CTX-I, NTX-I, ICTP ,collagen cross-links pyridinoline (PYD), DPD and hydroxyproline (HOP) are released from the collagen matrix during its degradation by the osteoclasts. The enzymes TRAP5b and cathepsin K are expressed and released by osteoclasts. The osteoblasts produce the BMs bone specific alkaline phosphatase (BALP), OC, osteoblast derived osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL is not specific for bone, as it can also be produced in B lymphocytes. PINP and carboxy-terminal propeptide of type I procollagen (PICP) are released during formation of type I collagen. Finally, the osteocyte itself is the main source of other factors derived from bone namely fibroblast growth factor-23 (FGF23) and sclerostin, which inhibits the Wnt- signaling pathway, thereby resulting in decreased bone formation.

Overall, this review will provide an overview of these BMs with regard to their possible clinical use in a selected number of diseases. The order of the diseases is based on the clinical relevance of measuring BMs, ranging from having high to low additional value. Review articles and original studies related to ten diseases or conditions in which bone health is compromised were assessed. Mainly articles that were published between 2005 and 2016 were included in this review. The final aim of this review is to provide the clinician with a practical guide in which circumstances to test which bone markers for optimal diagnostic purposes, in order to improve patient care in different areas of bone (related) diseases, e.g. Paget's disease, osteoporosis, rheumatoid arthritis, malignant disease, diabetes mellitus and chronic kidney disease. Furthermore, an additional aim of this review is to make clinicians aware of possible pitfalls that can be encountered during measurements or interpretation of bone marker assays.

# Results

## Paget's disease

Paget's disease (PD) is a focal bone disorder that results from increased and disorganised bone remodelling in typically one (mono-ostotic disease) or in multiple (poly-ostotic disease) bones. Paget's disease is often treated using bisphosphonates, which results in improvement of bone pain. In addition, bisphosphonates reduce fracture risk after normalisation of bone turnover [22].

### Clinically useful markers in Paget's disease

BMs play a role in both diagnosing and follow-up of Paget's disease, as bone turnover increases during active disease [11]. In general, concentrations of resorption markers urinary NTX-I, HOP and RANKL, and formation markers ALP, BALP, PINP are highly elevated in active Paget's disease,

whereas resorption markers CTX-I, ICTP, uDPD, sclerostin [7,23], and formation markers OC and PICP are elevated to a lesser extent or show heterogeneous results [1,5–10]. In Paget's disease, bone turnover is very rapid and bone is resorbed before substantial beta-isomerization may develop. This results in mainly non-isomerized (i.e. alpha) CTX-I in the blood. Measurements of only beta CTX-I therefore lead to an underestimation of the response to treatment of Paget's disease [11,22,27]. BMs reach higher concentrations in patients with poly-ostotic disease compared to mono-ostotic Paget's disease [27]. The highly elevated BMs, especially formation markers ALP, BALP and PINP can be used next to radiographic imaging of the bones to confirm the diagnosis of Paget's disease and in asymptomatic patients the increased turnover can even be the first clinical feature of the disease. Treatment with bisphosphonates results into normalisation of BM concentrations, starting with a rapid decline in resorption markers first followed by a decrease in formation markers, starting already within the first two months of treatment [22,27]. When more bones are affected in Paget's disease, this will result in higher levels of BMs before starting treatment. As a result, a stronger decrease of BM levels will be seen in patients who are more severely affected compared to patients who have a single affected bone only. Also, if a more potent bisphosphonate is used, the decrease of BMs will be more pronounced [22,28]. Aim of the treatment is to reduce the BMs into the reference intervals or even the lower half of these intervals [22,23,26,29]. Return of bone pain reported by the patient and increasing concentrations of BMs indicate recurrence of disease activity [22,23].

### *(Bone) Alkaline phosphatase ((B)ALP)*

Total ALP is general available, cheap and shows low variability [7,11,23,27]. Many studies highlight ALP as the BM of choice in diagnosis and follow up of Paget's disease, as this BM is often highly increased and has a high sensitivity (approximately 70 to 100%) [11,21–24,27,29]. ALP concentrations decrease within 10 days to several weeks after starting bisphosphonate therapy and strongest suppression will occur within 3-6 months [23,26,30]. Measurements of total ALP every 1-2 years is advised after initial treatment with bisphosphonates [21]. Depending on the guidelines adhered to, treatment should be restarted if ALP concentrations increase 25% in comparison to post-treatment concentrations after an initial normalisation upon bisphosphonate treatment [26,27] or if ALP increases up to three times the upper reference range [21]. BALP has an even slightly higher sensitivity than total ALP (approximately 80-100%) [27]. Furthermore, BALP is the preferred markers to monitor disease activity as the least significant change (LSC) of 25% of BALP implies a flare of the disease [7,11,22,23,30] LSC displays the actual physiological change of a BM as it comprehends both the within subject coefficient of variation of the assay and the total analytical imprecision as well. If Paget's disease is suspected in presence of a normal total ALP, then measuring BALP is recommended as sometimes a normal concentration of ALP does not rule out Paget's disease completely as total ALP is not always elevated, especially in mono-ostotic disease [26,30,31]. However, as BALP is more expensive than total ALP and not widely available, this results in more frequent use of total ALP instead of BALP. In case of abnormal function of liver or biliary tract, measurement of total ALP is not possible, as it could consist of a hepatic instead of bone origin [22,25–27,31,32]. In these cases BALP or another BM should be used.

*Amino-terminal propeptide of type I procollagen (PINP)*

PINP can be used as alternative to BALP, since this BM has a high sensitivity as well (approximately 75 – 100% ) [27]. Besides total ALP and BALP, PINP is the preferred marker to monitor disease activity as a LSC of 35% of PINP implies a change in activity of the disease [7,11,22,23,30]. Especially in monostotic disease or in case of abnormal kidney function trimeric PINP is a good marker for follow up of disease activity [23]. In severe liver disease, PINP is not the BM of choice as this marker is cleared from the circulation predominantly via the liver [27]. Measurement of PINP is favoured compared to total ALP by some as this marker is showing the greatest changes during relapse, yet it is more expensive than total ALP [32,33].

*Amino-terminal telopeptide of type I collagen (NTX-I)*

Urinary NTX-I also has high sensitivity (approximately 95 – 100%) and can also be used in diagnosis or follow up of Paget's disease in case of normal total ALP or liver disease [7,11,23,26,27,30]. The review of Nofal et al. [27] showed a R of 0.52 between uCTX-I and uNTx-I in Paget's disease before any treatment at baseline. Urine NTX showed the highest correlation with bone scintigraphy in Paget's disease. Furthermore, in this review a study of Garnero et al. [34] was described in which uNTX showed greater decrease compared to uCTX in patients with Paget's who were treated with zoledronate. It should however be noted that urinary NTX-I shows high variability within patients and is more difficult to collect properly, which makes it a less valuable marker compared to measurements of (B)ALP and PINP [22,28,29].

### Conclusion bone markers in Paget's disease

(B)ALP and PINP are the best markers to assist in diagnosis and monitoring of the disease activity and the effect of treatment in Paget's disease. Especially due to the general availability, long half-life, low costs and small variation of the measurement, total ALP is most often used in Paget's disease. BALP and PINP are the markers of choice in case of high clinical suspicion of Page's disease and a normal total ALP and in case of abnormal function of liver or biliary tract.

## Primary osteoporosis

Most patients with osteoporosis have increased concentrations of both formation and resorption BMs due to increased bone turnover [7,9,24,35,36]. However, possible selection bias plays a role in evaluating literature, as there is high amount of variation between previously studied patient groups. Overall, BMs do not play a role in the diagnosis of osteoporosis currently, because the diagnostic predictive value of BMs for fractures in the individual patient at baseline in osteoporosis treatment is very low [9]. After the start of treatment with antiresorptive medication, BMs decrease within several days to 3 months and reach a plateau phase within 3 to 6 months [1,12,15,16,66,67]. Comparing baseline concentrations of BMs with follow-up values can therefore provide information about the amount of bone turnover reduction due to the therapy. BMs change already within months after start of therapy in contrast to BMD, which takes several months to years to change [6,37]. One study showed that BMs can be helpful to provide information regarding the compliance of patients

and also to identify possible non-responders to the prescribed anti-resorptive or osteoanabolic drugs [38]. Several other studies emphasize using BMs to evaluate treatment compliance [6,7,11,39].

## Clinically useful markers in osteoporosis

BMs might also be useful for fracture risk prediction, as several studies found an association between an early decrease in BMs due to anti-resorptive medication and subsequent lower fracture incidence, although not all studies were corrected for drug compliance of the patients [7,11,14,40]. Higher BALP was associated with higher fracture risk and a greater decrease in BALP due to therapy was associated with a greater decrease in the fracture risk [14,41]. Also, high concentrations of beta CTX-I, uPYD, DPYR, TRAP5b, BALP and OC were associated with an increased fracture risk [7,41]. A modest significant association was found between the risk of fracture and elevated PINP and beta CTX levels [12]. These studies all show that the change in bone turnover has an impact on bone strength given the association between the change in BMs and change in fracture risk. The clinical applicability of BMs as risk prediction tool for fractures however is still limited due to their large biologic variability and BMs are not yet recommended to be used for the prediction of an individual fracture risk [6]. Beta CTX-I, PINP, BALP, OC, DPD and TRAP5b decrease upon treatment with antiresorptive therapy and can therefore be used to evaluate adherence [7,9,11,24,35,36,42]. BM concentrations return to initial values after cessation of antiresorptive therapy. This may take several years in case of bisphosphonates use due to the prolonged presence of bisphosphonates in bone and may occur within weeks after cessation of denosumab therapy [6,15,40]. During osteoanabolic treatment, BMs increase within weeks and are markers for the therapy adherence [44]. BMs decrease to normal again within weeks after cessation of osteoanabolic therapy [15,44].

New therapies such as the PTH-related protein peptide analog abaloparatide results in a smaller increase of bone resorption and formation markers compared to teriparatide [45]. Other new therapies are based on sclerostin antibodies, e.g. romosozumab, results in a decrease of bone resorption markers and increase of bone formation markers towards the concentrations close to the re-treatment status already within 3 months of therapy, while these effects diminish after discontinuation of therapy [46].

Currently, the International Osteoporosis Foundation (IOF) and also the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) guidelines favor using PINP as formation marker and beta CTX-I as resorption marker as the standard markers to monitor and follow-up osteoporosis-therapy [7,9]. Treatment efficacy is displayed as a decrease of BMs towards the concentrations that fit the lower half of reference values of premenopausal women [9].

### *Carboxy-terminal telopeptide of type I collagen (CTX-I)*

Several studies showed that beta CTX-I concentrations are higher in osteoporotic patients compared to controls [9,24,35]. Beta CTX-I concentrations shows a reduction of 50-70% after 12 weeks of bisphosphonates treatment [11] and decrease already within days or weeks after the start of treatment with intravenous bisphosphonates [9]. A decrease of beta CTX-I below the premenopausal

reference interval is seen already within one month of denosumab therapy [7]. The selective estrogen receptor modulator (SERM) raloxifene resulted in an decrease of beta CTX-I within weeks of therapy [47]. After starting teriparatide, beta CTX-I concentrations increases on average 5-15% in the first month of therapy [6,9,11,14]. It has to be kept in mind that beta CTX-I is influenced by feeding status, so measurements should be performed in a fasting sample in the morning as beta CTX–I levels decrease directly after food intake and showing a persistent decrease up to 50% in the hours after having a meal [7,9,48].

*Amino-terminal propeptide of type I procollagen (PINP)*

PINP decreases following bisphosphonate therapy [7,11] and a decline of 45-60% is seen within three months of oral bisphosphonate treatment [39,49]. Teriparatide results in an increase of PINP already after 2 days of treatment [11,15,32], resulting in PINP being the most sensitive (approximately 94%) marker to evaluate this therapy [7,11,14,50]. Furthermore, during abaloparatide treatment, PINP increases as well [51]. The SERM raloxifene resulted in an decrease of PINP within weeks of therapy [47]. In general, PINP is less sensitive to variations during the day and after food intake. To assess therapy success PINP is the marker of choice to monitor osteoporosis therapy. It should be measured before start of therapy and repeated 3-6 months after start of therapy [36,48].

### Conclusion bone markers in osteoporosis

To conclude, the value of BMs to diagnose osteoporosis or predict fracture risk seems to be limited. Fasting beta CTX-I and PINP are currently the recommended BMs to be used in individual monitoring of treatment compliance

## Tumor induced osteomalacia

Tumor induced osteomalacia (TIO) is a rare disorder which is caused by mostly benign and sometimes malign mesenchymal tumors. It is estimated that 50% of these tumors are located in the bones themselves [52] and these tumors produce phosphatonins such as FGF23. Regarding FGF23, both intact and C-terminal fragment FGF23 assays are available. Intact FGF23 is considered as the biologically active form, however as the C-terminal FGF23 displays a less significant diurnal pattern, and requires less pre-analytic precautions this assay is favoured by clinicians [42,53,54]. Treatment of TIO consists of surgically removal of the tumor [52]. After successful treatment, FGF23 and OC concentrations decrease and hypophosphatemia normalizes [52,55,56].

### Clinically useful markers in tumor induced osteomalacia

Increased concentrations of ALP and OC are found in TIO patients [56–58]. The measurement of FGF23 is useful for diagnosis of TIO, revealing inappropriate high concentrations of FGF23 [52,55,57,59,60]. Nuclear imaging techniques and sometimes venous FGF23 sampling [52,57,59] are used to detect the exact location of the tumor which can be challenging due to fluctuating concentrations of plasma FGF23 [59]. Assessment of BMD and evaluation of serum phosphate and 1.25(OH)2 vitamin D are used frequently in follow-up of patients with TIO [58,60], if FGF23

measurements are not available. Furthermore, in the absence of an FGF23 assay, the clinician can use the increase of phosphate and need for calcitriol supplementation for follow-up.

### Conclusion bone markers in tumor induced osteomalacia

To conclude, measurement of FGF23 is the most direct BM to use in the diagnosis and follow up of TIO. Alternatively, the BM ALP combined with serum phosphate and, if available, 1.25(OH)2 vitamin D and 25(OH) vitamin D measurements can be used as follow up parameters for patients when FGF23 measurements are not available. Currently, there is no information about the role of other bone markers.

## Hypophosphatemic rickets

Hypophosphatemic rickets (HR) comprise several hereditary diseases such as X-linked hypophosphatemia (XLH) and autosomal dominant hypophosphatemic rickets (ADHR). FGF23 concentrations have not been related to fracture risk or fracture incidence so far in these patients [61,62]. The therapy of hypophosphatemic rickets consists of active vitamin D analogues and phosphate supplementation [63–66], but will probably change to anti-FGF23 antibodies in the near future which might change the therapeutic landscape of these diseases [64].

## Clinically useful markers in hypophosphatemic rickets

Generally, in all hypophosphatemic rickets, serum ALP concentrations are often increased, yet may return to normal concentrations in adulthood [64,67,68]. Recent studies looking into other bone turnover markers in these heterogeneous conditions are lacking. FGF23 measurements are useful for clinical diagnosis of both XLH and ADHR. Although not in all [55], high concentrations of FGF23 are found in almost two thirds of patients with X-linked hypophosphatemia. The increase of FGF23 range between upper normal and up to 20 times higher than upper normal value [68]. The increased FGF23 concentration is due to a mutation in the PHEX gene, yet the exact mechanism is still unclear [62,64,66–69]. In ADHR FGF23 concentrations fluctuate reflecting disease severity [62]. The daily dose of therapeutic phosphate supplementation is mainly based on the clinical complaints and growth of the patient of the patient and ALP and PTH concentrations as FGF23 assays might not be generally accessible up until now [63]. It is advised to measure ALP, calcium, phosphate, FGF23 if available, PTH and 1.25(OH)2 vitamin D and 25(OH) vitamin D in HR during follow up [67]. If available, next to the clinical complaints of the patient, FGF23 can be used for follow up during supplementation therapy as FGF23 concentrations decrease upon treatment.

### Conclusion bone markers in hypophosphatemic rickets

In the current clinic practice the BM ALP is most often used for follow up of hypophosphatemic rickets patients during treatment. However, if available, FGF23 is the key BM to use for diagnosis of X-linked hypophosphatemia or ADHR and is also appropriate to measure for follow-up. However, the accessibility and costs of the FGF23 assay are limiting its use in hypophosphatemic rickets patients currently.

## Van Buchem disease



Van Buchem disease is an extremely rare autosomal recessive disorder caused by a mutation of the SOST gene resulting in loss of function and low concentration of sclerostin [70]. Sclerostin is produced in osteocytes and normally inhibits the anabolic Wnt-signalling pathway in osteoblasts. Therefore, van Buchem disease is characterised by an increased BMD, hyperostosis and severe osteosclerosis of the skeleton [70–72]. Currently, no treatment is available.

### Clinically useful markers in van Buchem disease

Due to increased bone formation in this disorder, increased ALP, PINP and OC concentrations are found, in presence of low resorption markers (e.g. urinary NTX-I) [73,74]. Low, but detectable concentrations of serum sclerostin are a distinctive feature of van Buchem disease and are used to confirm the diagnosis next to genetic testing [75]. As there is currently no treatment available for this genetic disorder, the sclerostin concentrations will not change and therefore measurements of this marker are not required during follow-up.

### Conclusion bone markers in van Buchem disease

High concentrations of ALP, PINP, or OC can be measured to confirm increased bone formation activity in patients suspected of van Buchem disease based on positive family history and radiographic imaging of the skeleton. The BM sclerostin is crucial in diagnosing van Buchem disease in addition to genetic testing, as low to absent concentrations of this BM are found.

## Chronic kidney disease

A higher fracture incidence of the hip and vertebrae is seen in patients with a glomerular filtration rate (GFR) already below 60 ml/min per 1.73 $m^2$ compared to the general population [76]. This fracture risk increases with the severity of chronic kidney disease (CKD). The collagen derived BMs of bone resorption CTX-I and NTX-I are generally considered not to be useful to predict fractures in patients with chronic kidney disease, according to the KDIGO group of 2017 and several other studies [4,76–78]. In particular in end stage renal disease bone turnover is often low, which can by accompanied with decreased mineralisation and bone volume [79–81]. Reduced renal clearance limits the use of urinary and some serum BMs such as CTX-I and OC in chronic kidney disease patients [23,82,83]. In addition, CTX-I is cleared by dialysis, further limiting its use in patients treated by hemodialysis (HD) or hemofiltration [19].

### Clinically useful markers in chronic kidney disease

To evaluate bone disease, it has been suggested to use BALP or PINP to detect high or low bone turnover state in chronic kidney disease patients as these BMs have low biological variation and low inter- and intra assay coefficient of variation (CV) [19,78,84,85]. Currently, the intact molecule 1-84 PTH, ALP or BALP are used to monitor bone turnover in chronic kidney disease patients [4]. PTH is not a bone-derived BM, and also probably most widely used to estimate bone turnover in clinical nephrology practice, detailed description of its clinical utility lies beyond the scope of this review,

and was reviewed recently [19]. (B)ALP, trimeric (intact) PINP, but not monomeric PINP, and TRAP5b are reliable BMs to interpret as they are not affected by kidney or dialytic clearance in chronic kidney disease patients [23,78]. Lower GFR is furthermore associated with increased FGF23, sclerostin and RANKL/OPG concentrations [4,42,52,55,80,86–89].

High BALP, PINP and TRAP5b concentrations were associated with prevalent fractures in all CKD stages [4,90]. BALP and TRAP5b can be used as independent predictors of bone loss in HD and peritoneal dialysis (PD) patients, because they are unaffected by either renal and peritoneal clearance and have low biological variability [4,78,82,91]. With regard to prediction of fractures, studies show heterogeneous results for sclerostin [4,78].

Besides its association with prevalent fractures, two recent studies assessed the ability of biomarkers to discriminate between high and low bone turnover, using bone histomorphometry as gold standard [92,93]. Collectively these studies found that BALP, intact PINP, TRAP5b and CTX (despite its limitations as described above), using C-statistics, had reasonable discriminative ability to classify bone turnover as either high or low. Intact PTH performed equally well to identify high turnover only, while results for intact PTH to detect low turnover were mixed.

## Fibroblast Growth Factor 23 (FGF23)

FGF23 concentrations rise when glomerular filtration rate declines [52,54,55,81,86,88,94,95]. In end stage renal disease such as HD patients, FGF23 concentrations increase up to 1000-fold above normal upper limits [86]. Although FGF23 is associated with a range of clinical outcomes, including CKD progression, cardiovascular events and mortality, these aspects are beyond the scope of this review. With regard to bone disease, FGF23 is a promising marker to detect early chronic kidney disease patients who are at risk for development of bone mineral disorders [86] as FGF23 concentrations increase as one of the first markers in early chronic kidney disease [96]. FGF23 could therefore be used as independent predictor of the loss of bone in HD patients [4,78]. Regarding the FGF23, both intact and C-terminal FGF23 assays can be used to measure FGF23. The C-terminal assay, which detects both intact FGF23 and its C-terminal fragment, is often the assay of choice due to less significant diurnal pattern and also less pre-analytical precautions [97]. However, the intact FGF23 is thought to be the biologically active form [42,53,54]. In severe CKD recent studies showed that both assays can be used reliably [98,99].

## Conclusion bone markers in chronic kidney disease

To conclude, (B)ALP, trimeric PINP and TRAP5b are useful markers to monitor bone turnover in CKD patients, also during progression of their CKD. Importantly, urinary and other BMs that are cleared by the kidneys are not reliable to use in patients with chronic kidney disease. Furthermore, FGF23 is the most promising marker in chronic kidney disease to detect disorders of bone metabolism early and to be able to identify chronic kidney disease patients that are at risk for adverse outcomes such as left ventricular hypertrophy and eventually early mortality. More studies are needed to understand the exact role and the usefulness of this marker for the individual patient.

## Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disorder resulting in chronic systemic inflammation, pain and erosions of the joints, resulting in deformities and bone loss. In acute destructive RA an uncoupling of bone remodelling is seen [100–103]; elevate bone resorption and inhibited bone formation. In general, active RA patients have higher concentrations of CTX-I, TRAP5b, and PYD concentrations [101,103–105]. OC concentrations were significantly lower in women with RA compared to controls in another study [106,107]. Furthermore, higher RANKL concentrations are seen in acute RA, resulting in higher RANKL/OPG ratio than in healthy controls [101,108]. During high disease activity in RA, specifically in acute RA, a decrease of bone formation markers such as OC and BALP and an increase of bone resorption markers such as CTX-I, ICTP, urinary NTX-I, PYD and TRAP5b was observed [24,106,107,109]. The production of cytokines such as RANKL by B lymphocytes is increased in RA which stimulate bone resorption, also resulting in a higher incidence of generalised osteoporosis in these patients. Consequently, an increased risk for fractures is seen in RA patients compared to healthy controls [110].

### Clinically useful markers in rheumatoid arthritis

To predict radiological progression such as cartilage degradation or erosive joint destruction, the elevated concentrations of ICTP [104,106], PYD [109] and RANKL [106,111] were eligible markers. Heterogeneous results were found with regard to CTX-I and biomarkers in urine, also in chronic RA [122,125,127]. When comparing the effect of different RA treatment regimens on BM, bone formation markers will increase during treatment, while their baseline will be often decreased in acute RA. Methotrexate therapy resulted in a decrease of urinary NTX-I after 6 months of treatment [106]. Anti-TNF therapy e.g. infliximab resulted in an increased ratio between bone formation markers and resorption markers, mainly caused by a decrease of NTX-I, CTX-I, ICTP and RANKL [101,106,107,109], and an increase in osteocalcin [113]. Tocilizumab treatment, an anti- interleukine-6 (IL-6) receptor therapy, resulted in heterogeneous results with either an decrease of CTX-I, ICTP and CTX-I /OC ratio or an increase of OC, PINP and RANKL/OPG ratio [42,101,103,107,109,110]. Treatment with prednisone resulted in a decrease of CTX-I and ICTP, although clinically often bone resorption increases first for a short period of time whereas the ratio of RANKL/OPG remained stable after 24 months treatment [112]. A comparison between patients treated with and without bisphosphonates did not reveal significant differences of CTX-I or TRAP5b concentrations [110].

### Carboxy-terminal telopeptide of type I collagen (CTX)

High concentrations of CTX-I in early active RA are associated with a higher risk of articular damage [101] and disease activity [107,109,112]. Therefore, a high concentration of CTX-I is accepted as indication to start early and aggressive treatment with anti-rheumatic drugs [111]. Furthermore, CTX-I can be used as predictor with regard to long-term radiological progression based on data from a RA cohort with 11 years of follow-up [111] with high levels indicating more severe joint destruction.

### Carboxy-terminal telopeptide of type I collagen (ICTP)

ICTP is statistically significantly increased in acute and chronic RA patients compared to healthy controls, is a sensitive BM to detect increased local bone turnover in inflamed joint tissue and correlates with joint inflammation [112]. Furthermore, ICTP is especially suitable to evaluate bone erosions associated with RA and a sensitive marker to reflect periarticular resorption of bone in RA [101].

### Receptor activator of nuclear factor kappa-B ligand / osteoprotegerin (RANKL/OPG) ratio

A high RANKL/OPG ratio is independently associated with joint damage and mandates early and aggressive treatment, as the RANKL/OPG ratio reflects local bone loss surrounding the joints caused by inflammation [111]. RANKL concentrations were positively correlated to the inflammation marker C-reactive peptide (CRP) [101]. Higher levels of CTX-I and RANKL/OPG ratio in active RA patients who did not receive treatment yet are predictors of progression of RA and increasing joint damage [107,111]. Lastly, a decrease in RANKL concentration after starting anti-TNF therapy is a predictor of the response to anti-TNF therapy expressed as lower RA disease activity [101]. Studies regarding RANKL often display various results due to low sensitivity and variable reliability of the immunoassays used the measure RANKL [114–116].

### Amino-terminal propeptide of type I procollagen (PINP)

Almost all treatments of RA result into an increase of PINP [106,110]. However, an unchanged concentration of PINP after 1 year of treatment with infliximab was found [103] and after one year of denosumab therapy PINP concentrations decreased as expected [106]. Daily teriparatide resulted in an increase of PINP already after one month of therapy, which was more clear in RA patients compared to control patients with primary osteoporosis [110]. The increase in PINP between baseline and 3 months of teriparatide therapy was a strong predictor for BMD changes of the LS after 18 months of therapy [110].

### Conclusion bone markers in rheumatoid arthritis

The heterogeneity of the studies with regard to duration of the disease and different treatment protocols in RA limits a robust recommendation of using BMs as standard part of RA patient care. Measuring disease activity by joint-scores (DAS) or simple biochemical markers as ESR or CRP seem to be as valuable as the BMs on an individual level in daily clinical practice in evaluating RA activity and monitoring of radiographic progression of the disease. Nevertheless, CTX-I, ICTP and RANKL/OPG ratio seem to be the most useful markers to evaluate the disease activity in especially acute RA, and they also to predict radiological progression of disease activity of the joints.

## Neoplasms and multiple myeloma

Bone metastases are prevalent in about 1 - 7% of the lung cancer patients and 17 - 37% in breast cancer patients [117]. On average 90% of males who die from prostate carcinoma suffer from bone metastases [118]. Extensive bone metastases result in high concentrations of BMs. Therefore, BMs

may be useful to provide prognostic information in malignant disease [2] as elevated concentrations correlate with pathological fractures, pain, the number of bone metastases and overall survival [119]. However, the specificity and sensitivity of BMs are currently still too low to be used for diagnosing bone metastasis in malignant disease [2,13,120]. In general, several post-hoc studies on zoledronic acid in lung, prostate, breast cancer and multiple myeloma showed a clear association of decrease in NTX-I and BALP after zoledronic acid therapy and prediction of skeletal related events and better survival [121–124].

## Clinically useful markers in neoplasms and multiple myeloma

### *Lung*

With regard to bone metastases, higher concentrations of ICTP, urinary NTX-I, serum NTX-I, PYD, DPD, TRAP5b and (B)ALP were measured in patients with compared to those without bone metastasis [125–131]. Nevertheless, solely measurements of BMs is not recommended to diagnose metastatic bone disease [120,132]. Treatment with zoledronic acid in patients with bone metastases resulted in a decrease of CTX-I, NTX-I, TRAP5b and RANKL [121,128]. The markers NTX-I, ICTP and TRAP5b can be used in monitoring of lung cancer patients. ICTP, TRAP5b and PINP could be used furthermore in assessing the prognosis in patients with bone metastases [120,128,133]. OC has not been shown as a suitable prognostic tool [128].

### *Prostate*

Patients with bone metastases in prostate cancer have increased concentrations of CTX-I, serum NTX-I, ICTP, HOP, uPYD, DPD,TRAP5b, sclerostin, (B)ALP, PINP, OC and PICP compared to patients without metastases or controls [2,120,133–138]. However, BALP, PICP and CTX-I display a low diagnostic sensitivity (ranging from 53-65 %) in diagnosis of bone metastases [120]. In contrast, another study showed that BALP, ICTP and TRAP5b concentrations were predictors of bone metastasis [144]. RANKL concentrations were not different between controls and prostate carcinoma patients [122]. Longer survival was seen if NTX-I, ICTP and BALP decreased during treatment with zoledronic acid [136, 153]. In prostate cancer patients heterogeneous results were found with regard to use of NTX-I to monitor the treatment response and predict skeletal related events (SRE). In general, elevated concentrations of NTX-I, CTX-I, DPD and BALP were related to worse survival [133]. In contrast, lower baseline concentration of NTX-I, PINP, ALP and DPD were correlated to a better survival, which was independent of the use of bisphosphonates or prostate specific antigen (PSA) concentrations [140]. Of note, the combination of urinary NTX-I and BALP to predict survival has been advised [141,142] and serial measurements of CTX-I and PINP were useful predictors of survival [120]. A decrease of ICTP and BALP after zoledronic acid was associated with better prognosis [143]. An increased RANKL/OPG ratio and increased RANKL were independent predictors of recurrence of prostate carcinoma in patients who were treated by prostatectomy [144].

### *Breast*

CTX-I, NTX-I, DPD, TRAP5b, RANKL and BALP increased in patients with breast cancer and were higher in patients with multiple bone metastases compared to patients with solitary bone lesions

[2,119,145–147]. ICTP, DPD, TRAP5b and BALP concentrations were correlated to the extent of the bone lesions [2,148]. Urinary NTX-I, ICTP and PINP decreased after treatment with zoledronic acid and can be used to monitor and predict bone metastases [149]. In breast cancer patients NTX-I, ICTP and PINP are most useful in follow up of patients to detect osteolytic metastases.

*Multiple myeloma*

CTX-I, urinary NTX-I, ICTP, PYD, DPD,TRAP5b and sclerostin concentrations were higher in multiple myeloma patients than in healthy controls [2,16,145,150–154]. Predominantly urinary NTX-I, CTX-I, ICTP, TRAP5b, RANKL/OPG ratio and also BALP can be used to estimate the extent of bone involvement in multiple myeloma, but not BMs such as PYD or DPD [16,152]. Especially ICTP, DPD, TRAP5b and BALP [152] are associated with progression of bone involvement [2,152,154]. Treatment with bortezomib resulted in a decrease of sclerostin, which was followed later by a decrease of CTX-I and an increase of BALP [155]. In patients in remission from MM, CTX-I, sclerostin, RANKL and PINP did not differ between baseline and 6 months of intravenous treatment of bisphosphonates [156].

### Conclusion bone markers in neoplasms and multiple myeloma

In lung, prostate, breast cancer and multiple myeloma BMs are not suitable as a diagnostic tool to detect bone metastases. However, especially the markers NTX-I, CTX-I, ICTP, TRAP5b, the RANKL/OPG ratio and BALP seem to be promising markers to monitor bone metastases, next to conventional radiography and other diagnostic tests. The markers ICTP, NTX-I, CTX-I or BALP can be used as indicators of progression, prognosis and survival or mortality in lung, prostate and breast cancer as well as multiple myeloma patients. The role of TRAP5b is not clear yet. PINP is a suitable marker to indicate survival in prostate cancer and ICTP and the RANKL/OPG ratio might indicate survival in multiple myeloma patients. Overall, in malignant disease, a large number of studies show heterogeneous results for a number of BM. This can be explained partially by difficulties of choosing correct cut-off points of maximum values, which are mainly based on cut-offs used in osteoporosis management. In addition, decreased kidney function is often seen as co-morbidity in malignant disease, thereby increasing the concentrations of BMs which are cleared by the kidney such as CTX-I, NTX-I and ICTP. Nevertheless, BM assessments might provide useful complementary information in addition to imaging techniques or tumor marker measurements to monitor disease activity, progression of bone metastasis and even mortality risk, especially also after treatment with zoledronic acid. In summary, NTX-I, CTX-I, ICTP, TRAP5b and BALP seem to be the currently most useful BMs to detect bone metastases in various malignancies. However, their ability to predict skeletal related events and overall survival remains to be elucidated and therefore BMs are not advised to use in daily clinic in neoplasms or multiple myeloma management.

## Bone disease in type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is defined by increased blood glucose concentrations and insulin resistance. Higher glucose concentrations are thought to lower bone turnover [157] and inhibit osteoclastogenesis which is induced by RANKL [158]. Previous research has also shown that patients with T2DM have a decreased number, and possibly also reduced functioning, of osteoblasts which

results in decreased bone turnover in these patients [158,159]. On the other hand, several studies showed an increase of bone turnover, due to an increase of especially bone resorption [160–162]. The increase of bone turnover is thought to also result from chronic inflammation as a result of adiposity in many T2DM patients [163,164]. Most studies showed an increased fracture risk in patients with T2DM compared to healthy controls [158,165–169]. The increased fracture risk could be the consequence of paradoxically increased mineralisation of the bone matrix and altered bone architecture due to increased cortical porosity, which is accompanied by higher concentrations of BALP in T2DM [102,103,105,110], and probably also by increased fall risk [168]. Higher concentrations of CTX-I, urinary NTX-I, PINP, BALP, and sclerostin have been associated with an increased fracture risk as well in patients with T2DM [159,165,170,171].

### Clinically useful markers in type 2 diabetes mellitus

Bone markers (both formation and resorption markers) are frequently, but not generally decreased in T2DM patients [42,158,159,165,166,170–174]. Depending on the setting even the same BMs can be different in studies on T2DM patients (e.g. (B)ALP [170,172,174], or sclerostin [166,170,171,175]). The only marker that seems to be consistently decreased in T2DM is osteocalcin [158,159,165,166,170–172,174,176,177], which implies a possible future for adding this BM to long-term T2DM management.

#### *Osteocalcin (OC)*

Based on several cross-sectional studies, serum OC showed an inverse relationship with glycaemic control [169], fasting insulin concentrations [171,176,178] and HbA1C [166,168,171,176–178]. A further decrease of OC is associated with more vertebral fractures [170] and a decreased ratio of OC to BALP indicates higher fracture risk, independent of BMD [165]. Also, a positive association of OC with insulin sensitivity was found and it was suggested that OC stimulates insulin secretion, thereby emphasising the positive effect of OC on glucose metabolism as higher concentrations of OC were also associated with improved glucose tolerance [176,178]. The uncarboxylated form of OC is considered to be related to glucose control and general energy metabolism [168].

### Conclusion bone markers in T2DM

The great variation in study populations in T2DM studies limit the possibility to make general recommendations about the utility of BMs in these patients. With regard to fracture risk, there is insufficient evidence to use BMs to distinguish patients with a high fracture risk in T2DM, as many markers showed conflicting results. Therefore, BMs have no additional value in diabetes care yet, but especially OC is a promising BM for the future.

## Primary hyperparathyroidism

Primary hyperparathyroidism (PHPT) is characterized by high PTH concentrations, hypercalcaemia and might be accompanied by nephrolithiasis or bone pains [179]. Bone turnover generally normalizes within one year [180,181] and BMD improves after surgery and drug therapy with a need for supplementation of calcium and vitamin D as well.

### Clinically useful markers in primary hyperparathyroidism

High concentrations of BMs, both resorption and formation markers (e.g. CTX-I,TRAP5b, PINP, BALP), are observed in primary hyperparathyroidism due to the increased bone turnover [3,5,180,182–185]. In general, higher PTH concentrations are associated with higher BMs [3]. BMs decrease after adenoma extirpation and during treatment with drugs [3,5,190,191,179,180,184–189]. Although bone turnover is clearly affected by primary hyperparathyroidism, no single BM proves to be useful on top of measuring PTH itself, for diagnosing this disease or show additional benefit in clinical follow up of the patients during or after treatment.

### Conclusion bone markers in primary hyperparathyroidism

Despite the abnormalities seen in BMs during primary hyperparathyroidism and the recovery during treatment, BMs do not play an additional role in standard care during diagnosis or follow up of primary hyperparathyroidism, as the PTH measurement itself is widely available.

## Overall conclusion

The clinical utility of BMs in patient care is rather heterogeneous, despite widespread research regarding their use. By all means, adequate standardized sample collection and analysis are the cornerstones of correct interpretation of BMs. In addition, intra- and inter patient variability such as circadian rhythm, fasting state and current pre- and analytical conditions such as different assays used are still limiting factors in daily clinic. Furthermore, the clinician should consider recent fractures, aging, menopausal status, concomitant liver and kidney disease when ordering and interpreting BM measurements as these factors might result in misleading BM concentrations. Also, the knowledge of use of bone-specific or bone-related medications by the patient is of high impact in the interpretation of BM results. Fortunately, an increasing amount of high quality assays are widely available at clinical laboratories, which enables both a more accurate display of concentrations of BMs and a reduction of the costs of the BM analysis as well. Therefore, BMs will be easier to order and results will be quicker accessible to the physician. In conclusion, based on this review BMs are clearly useful in the diagnosis of tumor induced osteomalacia, van Buchem disease, Paget's disease and hypophosphatemic rickets. In addition, BMs are useful to monitor disease activity in chronic kidney disease, Paget's disease and are also useful to monitor treatment effects in osteoporosis. Currently, osteoporosis is one of the few diseases in which CTX-I and PINP are recommended to use to monitor adherence to therapy and therapy success so far [7,9]. A summary of the outcomes mentioned in this review are displayed in Table 1. So far, no single BM has proven to be particularly suitable for prediction of the fracture risk in individual patients. Finally, preferably longitudinal trials and more clinical studies are needed to establish accurate age and sex dependent reference ranges of BMs of the ten bone (related) diseases that were described in this review. As a result, the clinical utility of BMs in patient care will possibly be extended in the near future.

**Table 1.** Diseases and bone markers.

| Disease/BM<br>Suitable for D and/or FU | (B)ALP | OC | PINP | CTX-I | NTX-I | ICTP | TRAP5b | Sclerostin | FGF23 | RANKL |
|---|---|---|---|---|---|---|---|---|---|---|
| Paget's disease | D + FU | - | D + FU | - | | | | | | |
| Primary osteoporosis | FU<br>(only BALP) | | FU | FU | | | | | | |
| Tumor-induced osteomalacia | FU | | | | | | | | D | |
| Hypophosphatemic rickets | FU | | | | | | | | D | |
| van Buchem disease | | | | | | | | D | | |
| Chronic kidney disease | FU | - | FU (trimeric form) | - | - | | FU | | FU | |
| Rheumatoid arthritis | | | | - | | - | | | | - |
| Neoplasms/ multiple myeloma | | - | | | | | | | | |
| Type 2 diabetes mellitus | | | | | | | | | | |
| Primary hyperparathyroidism | | | | | | | | | | |

D = useful marker for diagnosis, FU = useful marker for follow-up, - = no useful marker, empty = no data or insufficient evidence, (B)ALP = (bone specific) alkaline phosphatase, OC = osteocalcin, PINP = amino-terminal propeptide of type I procollagen, CTX-I = carboxy-terminal telopeptide of type I collagen, NTX-I = amino-terminal telopeptide of type I collagen, ICTP = carboxy-terminal telopeptide of type I collagen, TRAP5b = tartrate resistant alkaline phosphatase 5b, FGF23 = fibroblast growth factor-23, RANKL = receptor activator of nuclear factor kappa-B ligand

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### Authors' role

Study design: MV, AH, Data collection: MV, Data analysis: MV, AH. Drafting manuscript: MV, AH. Revising manuscript content: MV, AH, MH, RTdeJ, MVervloet, WL, RdeJ, BOP and AH. Approving final version of manuscript: AH. AH takes responsibility for the integrity of the data analysis.

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# Part II

## EFFECTS OF SEX STEROIDS ON BONE HEALTH IN TRANSGENDER PERSONS

# Chapter 3

# Effect of pubertal suppression and cross-sex hormone therapy on bone turnover markers and bone mineral apparent density (BMAD) in transgender adolescents

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## Abstract

Puberty is highly important for the accumulation of bone mass. Bone turnover and bone mineral density (BMD) can be affected in transgender adolescents when puberty is suppressed by gonadotropin-releasing hormone analogues (GnRHa), followed by treatment with cross-sex hormone therapy (CSHT). We aimed to investigate the effect of GnRHa and CSHT on bone turnover markers (BTMs) and bone mineral apparent density (BMAD) in transgender adolescents. Gender dysphoria was diagnosed based on diagnostic criteria according to the DSM-IV (TR). Thirty four female-to-male persons (transmen) and 22 male-to-female persons (transwomen) were included. Patients were allocated to a young (bone age of <15 years in transwomen or <14 in transmen) or old group (bone age of ≥15 years in transwomen or ≥ 14 years in transmen). All were treated with GnRHa triptorelin and CSHT was added in incremental doses from the age of 16 years. Transmen received testosterone esters (Sustanon, MSD) and transwomen received 17-β estradiol. P1NP, osteocalcin, ICTP and BMD of lumbar spine (LS) and femoral neck (FN) were measured at three time points. In addition, BMAD and Z-scores were calculated. We found a decrease of P1NP and 1CTP during GnRHa treatment, indicating decreased bone turnover (young transmen 95% CI - 74 to - 50% , *p=0.02*, young transwomen 95% CI - 73 to - 43, *p=0.008*). The decrease in bone turnover upon GnRHa treatment was accompanied by an unchanged BMAD of FN and LS, whereas BMAD Z-scores of predominantly the LS decreased especially in the young transwomen. Twenty-four months after CSHT the BTMs P1NP and ICTP were even more decreased in all groups except for the old transmen. During CSHT BMAD increased and Z-scores returned towards normal, especially of the LS (young transwomen CI 95% 0.1 to 0.6, *p=0.01*, old transwomen 95% CI 0.3 to 0.8, *p=0.04*). To conclude, suppressing puberty by GnRHa leads to a decrease of BTMs in both transwomen and transmen transgender adolescents. The increase of BMAD and BMAD Z-scores predominantly in the LS as a result of treatment with CSHT is accompanied by decreasing BTM concentrations after 24 months of CSHT. Therefore, the added value of evaluating BTMs seems to be limited and DXA-scans remain important in follow-up of bone health of transgender adolescents.

# Introduction

Puberty is the most important period in life regarding the accumulation of bone mass. In general, about 85% - 90% of the total bone mass will have been acquired at the end of puberty [1]. Sex steroids reach high concentrations as puberty progresses and play a key role in the augmented bone growth and bone mass accumulation in adolescents. Consequently, the process of bone turnover, bone remodelling and bone mineral apposition increase during puberty as well [2]. In adolescents with gender dysphoria the pubertal development of secondary sex characteristics during puberty can cause psychological distress because this physical maturation belongs to their unwanted sex assigned at birth. When transgender adolescents are treated, the first step of the so-called gender affirming (GA) therapy is the administration of gonadotropin-releasing hormone analogues (GnRHa) to suppress puberty. The GnRHa treatment induces a hypogonadal state, resulting in a developmental arrest of the undesired secondary sex characteristics of the sex assigned at birth [3-5]. Bone metabolism is affected by the GnRHa treatment as well and as a result the BMD as measured by DXA-scan can decrease [6-7]. The second step of the GA therapy of transgender adolescents consists of gender affirming hormones also known as cross-sex hormone therapy (CSHT) from the age of 16 years. The purpose of CSHT is to induce the development of secondary sex characteristics of the desired sex. Until now the effects of CSHT on both BMD and bone turnover in transgender adolescents are not known [6;8;9].

Bone turnover markers (BTMs) can be used to display the actual bone metabolism in transgender adolescents. Several studies show that BTMs reach high concentrations during biological puberty [10-12]. To date, there is little data on the course of BTMs in relation to BMD during pubertal suppression and treatment with CSHT in transgender adolescents. When GA therapy affects the bone quality during puberty this might have an impact on the bone quality in later adult life, especially with regard to a possible lower BMD and the risk of osteoporosis and fractures. Hence, studies are needed to assess both the immediate and the long-term effects of the GA therapy on bone metabolism in transgender adolescents.

The objective of this study is to investigate the course of three bone turnover markers in relation to bone mineral density, in transgender adolescents during gonadal suppression and during CSHT.

# Methods

## Subjects and treatment protocol

Adolescents diagnosed with gender dysphoria who were treated with GnRHa and CSHT were recruited at our clinic the Centre of Expertise on Gender dysphoria at the VU University Medical Centre, Amsterdam, the Netherlands. Gender dysphoria was diagnosed based on diagnostic criteria according to the DSM-IV (TR) [13]. This retrospective study was approved by the Ethical Committee of the VU University Medical Centre and data collection started only after the subjects and their parents or legal representatives provided written consent. Data for this study was collected at three moments in time: (1) D0: at start of GnRHa treatment to suppress puberty, (2) C0: at start of CSHT and (3) C24: at 24 months after C0.

Inclusion criteria of this study were: adolescents with diagnosed gender dysphoria, a serum BTM measurement of P1NP, osteocalcin or carboxy terminal cross linked telopeptide of type I collagen (ICTP) within 90 days before or after time point D0, C0 and C24, and/or a DXA-scan of the lumbar spine (LS) and/or femoral neck (FN) performed within 90 days before or after time point D0, C0 and C24. After applying these criteria to an eligible patient group of 85 transwomen (male-to-female persons) and 130 transmen (female-to-male persons) a cohort of 28 transwomen and 42 transmen were included in the study. The full treatment protocol and all clinical assessments were extensively described previously [6]. Briefly, the GA therapy of transgender adolescents starts with administration of GnRHa triptorelin (Decapeptyl – CR ®, Ferring) 3.75 mg subcutaneously every 4 weeks in order to suppress puberty of the sex assigned at birth (D0). In transmen triptorelin starts from Tanner B stage 2 or more and in transwomen when the testicle volume is at least 6-8 ml or when Tanner G is staged at 2 or 3. CSHT, the second phase of GA therapy, starts from the age of 16 year (C0) transmen receive testosterone esters (Sustanon ®, MSD), with an initial dose of 25 mg/m$^2$ body surface area IM every two weeks and doses are increased every six months until an adult maintenance dosage of 250 mg every 4 weeks is reached. The transwomen are treated with 17-β-estradiol orally, with an initial dose of 5 µg/kg daily with 6-monthly increments until an adult maintenance dosage of 2 mg daily.

All 28 transwomen and 42 transmen which were included in this study started the GA therapy between 2001 and 2011. The patients were categorised into a young and old pubertal group, based on their bone age. The young transmen had a bone age of <14 year and the old transmen had a bone age of ≥ 14 years. The young transwomen group had a bone age of <15 year and the old transwomen group ≥15 years. These groups were created to account for the difference between biological age and pubertal stages of the adolescents as the older patients already partially completed their puberty, resulting in higher bone mass accrual compared to younger patients. Groups were based on the median bone age of the groups and also because the peak height velocity ages were reached earlier and as a result the near-final height was reached at these respective bone ages. The bone age was measured by a X-ray of the left hand and was assessed using the method of Greulich and Pyle [14].

## Measurements

### General

Body weight and height were measured each visit (D0, C0 and C24). A wall-mounted Harpenden Stadiometer was used to measure the standing height and weight without shoes on. The stages of pubertal development were assessed according to Tanner by a paediatrician-endocrinologist each visit.

### Bone turnover markers

The formation markers P1NP and osteocalcin and the resorption marker ICTP were measured in non-fasting state. P1NP was measured using a RIA (Orion Diagnostica, Espoo, Finland) with an intra-assay coefficient of variation (CV) of 4-8% and inter-assay CV of 8%. The lower limit of quantitation (LOQ) was 5 µg/L. Osteocalcin was measured using an immunometric-assay (Biosource, Nivelles,

Belgium) with an intra-assay CV of <5%, inter-assay CV of 8-15% and LOQ of 0.4 nmol/L. ICTP was measured using a RIA (Orion Diagnostica, Espoo, Finland) with an intra-assay CV of 4-6%, inter-assay CV of 7% and LOQ of 1 µg/L.

### Bone densitometry (DXA-scan)

A DXA-scan (Hologic QDR 4500, Hologic Inc., Bedford, MA, USA) with a precision of <1% was used to measure BMD in g/cm$^2$ of the LS and FN of the non-dominant hip. The LS and FN were the anatomical sites of choice as reference values for BMD and BMAD of these regions in adolescents were studied before [15]. To correct for height and height gain the volumetric bone mineral apparent density (BMAD) in g/cm$^3$ for both LS and FN was calculated. BMAD Z-scores were calculated for sex assigned at birth using an UK reference population, due to the lack of consensus with regard to the use of either sex assigned at birth or desired sex reference values in transgender adolescents [15]. The lack of validated reference values of bone age needed to calculate the BMAD and Z-scores limits the use of bone age and therefore the chronological calendar age of the transgender adolescents was used. Furthermore, the reference values of L- M- and S-values of 17 year old biological males and females were used to calculate the BMAD for patients older than 17 year, due to the lack of reference values of adolescents exceeding the age of 17 years [15;16].

### Statistics

Stata/SE 13.0 software (StataCorp, LP) was used for calculations and statistical analysis. Normality was tested by normality plots and by Shapiro-Wilk tests. As described previously patients were categorised in different groups based on sex and bone age resulting in four groups: young transmen, old transmen, young transwomen and old transwomen. Further sub analyses were not possible due to the limited sample size. Wilcoxon signed rank tests were used to analyse the non-normal distributed data. All BTM results and BMAD results were standardized to the measurement performed at D0 which was set at 100 %. Subsequent measurements of BTMs and BMAD at C0 and C24 were expressed as the percentage of the measurement of D0. Changes in percentages of bone turnover markers and BMAD between D0, C0 and C24 were calculated as deltas (Δ) with corresponding 95% CI and *p*-values.

## Results

### Study population

Baseline subject characteristics are shown in Table 1. The categorisation into smaller groups of subjects did not result into different baseline characteristics compared to the total group. The inclusion criteria of BTM measurements implied that these measurements should be performed within 90 days before or after time point D0, C0 and C24. However, almost all samples for BTM measurements were drawn at the same day of start of GnRHa (D0) or CSHT (C0) with the exception of 3 transwomen (range of 11-16 days after D0) and 5 transmen (range of 3-32 days after D0). Likewise, only 1 transwoman had blood drawn for BTM measurements 5 days after C0.

**Table 1.** Baseline characteristics of transmen and transwomen adolescents receiving GnRHa and CSHT

| Total | | Transmen, n = 42 | | | Transwomen, n = 28 | |
|---|---|---|---|---|---|---|
| | D0 | C0 | C24 | D0 | C0 | C24 |
| Age, years, median (range) | 15.1 (11.7 - 18.6) | 16.3 (15.9 - 19.5) | 18.3 (17.9 - 21.5) | 13.5 (11.5 - 18.3) | 16.0 (14.0 - 18.9) | 18.0 (16.0 - 20.9) |
| Height, cm, median (range) | 164.2 (149.6 - 180.1) | 165.8 (152.6 - 181.2) | 168.6 (155.6 - 183) | 166.9 (153.9 - 185.7) | 176.3 (165.1 - 186.4) | 180.7 (167.4 - 195.0) |
| Weight, kg, median (range) | 57.1 (34 - 85) | 63.7 (44.5 - 84.5) | 68.9 (52.4 - 93.4) | 53.2 (38.5 - 74.2) | 61.5 (44.9 - 87.5) | 66.1 (49.4 - 94.8) |
| Bone age, years, median (range) | 15 (12 - 17) | 16 (12 - 17) | 17 (14 -17) | 13.5 (10 - 17) | 14 (13 - 17) | 16.75 (14.5 - 17) |
| Tanner stage breast, median (range) | 5 (2 - 5) | 5 (1 - 5) | 4 (1 - 5) | N.A. | N.A. | N.A. |
| Tanner stage genital, median (range) | N.A. | N.A. | N.A. | 3 (2-5) | 4 (2-5) | 4 (2-5) |
| Tanner stage pubic hair, median (range) | 5 (3 - 5) | 5 (3 - 5) | 6 (4 -5) | 3 (2 -5) | 4 (2-5) | (3-5) |

## Bone turnover markers

All BTM and BMAD results can be found as medians and ranges in Table 2, completed with a summary in Table 3.

### P1NP

At baseline, both young transmen and young transwomen showed higher concentrations of P1NP compared to the old transmen (*p=0.02)* and old transwomen (*p=0.03)* respectively. Old transwomen showed borderline higher concentrations of P1NP at baseline than old transmen (*p=0.05)*. During GnRHa a decrease of P1NP concentrations was seen in the young transmen, young transwomen

**Table 2.** BTM and BMAD results of transmen and transwomen adolescents at D0, C0 and C24.

| | D0 | C0 | C24 |
|---|---|---|---|
| **Transmen, young** | | | |
| P1NP median/range, n = 7 | 783 (516-1090) | 324 (194 - 402) | 186 (163 - 334) |
| OC median/range, n = 7 | 5 (2.2 - 11.7) | 6.8 (1.8 - 7.7) | 4.9 (4.2 - 7.8) |
| ICTP median/range, n = 7 | 24 (17 - 29.9) | 11 (7.8 - 12) | 12 (11 - 14) |
| BMAD HIP, n = 10 | 0.31 (0.26 - 0.36) | 0.30 (0.22 - 0.35) | 0.33 (0.23 - 0.37) |
| BMAD Z-score, n = 10 | - 0.01 (- 1.30 - 0.91) | - 0.37 (- 2.28 - 0.47) | - 0.37 (- 2.03 - 0.85) |
| BMAD LS, n = 11 | 0.23 (0.20 - 0.29) | 0.23 (0.19 - 0.28) | 0.25 (0.22 - 0.28) |
| BMAD LS Z-score,n = 11 | - 0.05 (- 0.78 - 2.94) | - 0.84 (- 2.2 - 0.87) | - 0.15 (- 1.38 - 0.94) |
| **Transmen, old** | | | |
| P1NP median/range, n = 15 | 110 (38-471) | 127 (61 - 321) | 101 (44 - 181) |
| OC median/range, n = 18 | 2.4 (0.4 - 4.6) | 3.9 (0.4 - 8.6) | 2.9 (0.8 - 5) |
| ICTP median/range, n = 15 | 7 (5.2 - 15) | 6.9 (4.6 - 14) | 8.2 (4.1 - 16) |
| BMAD HIP, n = 23 | 0.33 (0.25 - 0.39) | 0.30 (0.23 - 0.41) | 0.32 ( 0.23 - 0.41) |
| BMAD Z-score, n= 23 | 0.27 (- 1.39 - 1.32) | - 0.27 (- 1.91 - 1.29) | 0.02 (- 2.1 - 1.35) |
| BMAD LS,n = 23 | 0.26 (0.21 - 0.29) | 0.24 (0.20 - 0.28) | 0.25 (0.21 - 0.30) |
| BMAD LS Z-score, n = 23 | 0.27 (- 1.6 - 1.8) | - 0.29 (- 2.28 - 0.90) | - 0.06 (- 1.76 - 1.61) |
| **Transwomen, young** | | | |
| P1NP median/range, n = 9 | 935 (617 - 1348) | 363 (185 - 643) | 204 (137 - 314) |
| OC median/range , n = 11 | 4.8 (2.6 - 21.9) | 6.4 (0.7 - 12.8) | 5.4 (3.9 - 12.5) |
| ICTP median/range, n = 9 | 23 (15 - 34) | 13 (8.7 - 21) | 10 (8.5 - 13) |
| BMAD HIP, n = 16 | 0.29 (0.20 - 0.33) | 0.27 (0.20 - 0.33) | 0.27 (0.20 - 0.36) |
| BMAD Z-score, n = 16 | - 0.71 (- 3.35 - 0.37) | - 1.32 (- 3.39 - 0.21) | - 1.3 (- 3.51 - 0.92) |
| BMAD LS, n = 15 | 0.21 (0.17 - 0.25) | 0.20 (0.18 - 0.24) | 0.22 (0.19 - 0.27) |
| BMAD LS Z-score, n = 15 | - 0.2 (- 1.82 - 1.18) | - 1.52 (- 2.36 - 0.42) | - 1.10 (- 2.44 - 0.69) |
| **Transwomen, old** | | | |
| P1NP median/range, n = 6 | 191 (96 - 792) | 140 (111 - 467) | 119 (55 - 296) |
| OC median/range, n = 7 | 2.29 (0.8 - 11) | 2.2 (0.5 - 6.1) | 3.3 (1.8 - 6.8) |
| ICTP median/range, n = 5 | 12 (6.9 -21) | 7.4 (6.9 -13) | 6.8 (4.8 - 15) |
| BMAD HIP, n = 6 | 0.30 (0.26 - 0.36) | 0.30 (0.26 - 0.34) | 0.29 (0.24 - 0.38) |
| BMAD Z-score,n = 6 | - 0.44 (- 1.37 - 0.93) | - 0.36 (- 1.5 - 0.46) | - 0.56 (- 2.17 - 1.29) |
| BMAD LS, n = 5 | 0.22 (0.18 - 0.25) | 0.22 (0.19 - 0.24) | 0.23 (0.21 - 0.26) |
| BMAD LS Z-score, n = 5 | - 1.18 (- 1.78 - 1.09) | - 1.15 (- 2.21 - 0.08) | - 0.66 (- 1.66 - 0.54) |

**Table 3.** Overview of BTMs and BMAD and Z-scores of transmen and transwomen adolescents receiving GnRHa and CSHT, including legend.

| | | P1NP | | Osteocalcin | | ICTP | | BMAD hip | | Z score hip | | BMAD spine | | Zscore spine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D0-C0 | C0-C24 | D0-C0 | C0-C24 | D0-C0 | C0-C24 | D0-C0 | C0-C24 | D0-C0 | C0-C24 | D0-C0 | C0-C24 | D0-C0 | C0-C24 |
| Transmen | young | ↓↓ | ↓ | ↔ | ↔ | ↓↓ | ↑ | ↔ | ↑↑↑ | ↔ | ↑↑↑ | ↔ | ↑↑↑ | ↓↓↓ | ↑↑↑ |
| | old | ↔ | ↓↓↓ | ↑↑↑ | ↓↓ | ↔ | ↑ | ↓↓↓ | ↑↑↑ | ↓↓↓ | ↑↑ | ↓↓↓ | ↑↑↑ | ↓↓↓ | ↑↑↑ |
| Transwomen | young | ↓↓↓ | ↓↓↓ | ↔ | ↔ | ↓↓↓ | ↔ | ↓ | ↔ | ↓ | ↔ | ↔ | ↑↑↑ | ↓↓↓ | ↑↑ |
| | old | ↓ | ↓↓ | ↔ | ↔ | ↓ | ↔ | ↔ | ↔ | ↔ | ↔ | ↔ | ↑↑ | ↓ | ↑↑ |

↔ NS
↑/↓ trend ($p \leq 0.1$)
↑↑/↓↓ $p \leq 0.05$
↑↑↑/↓↓↓ $p \leq 0.01$

and old transwomen groups. P1NP concentrations decreased further in all but the young transmen group 24 months after CSHT (*Figure 1*).

## Osteocalcin

At baseline, both young transmen and young transwomen showed higher concentrations of osteocalcin compared to the old transmen (*p=0.02*) and old transwomen (*p=0.03*), respectively. No difference between transmen and transwomen was found. Suppression of puberty and CSHT treatment did not affect osteocalcin concentrations in most groups. Only in the old transmen group the osteocalcin concentration showed an increase after suppression of puberty and a decrease after 24 months of CSHT (*Figure 2*).



## ICTP

At baseline, young transmen showed higher concentrations of ICTP compared to the old transmen (*p=0.02*). No difference between young transwomen and old transwomen was found (*p=0.08*). transmen and transwomen did not differ regarding ICTP concentrations at baseline. During suppression of puberty a decrease of ICTP concentrations was seen in all groups except for the old

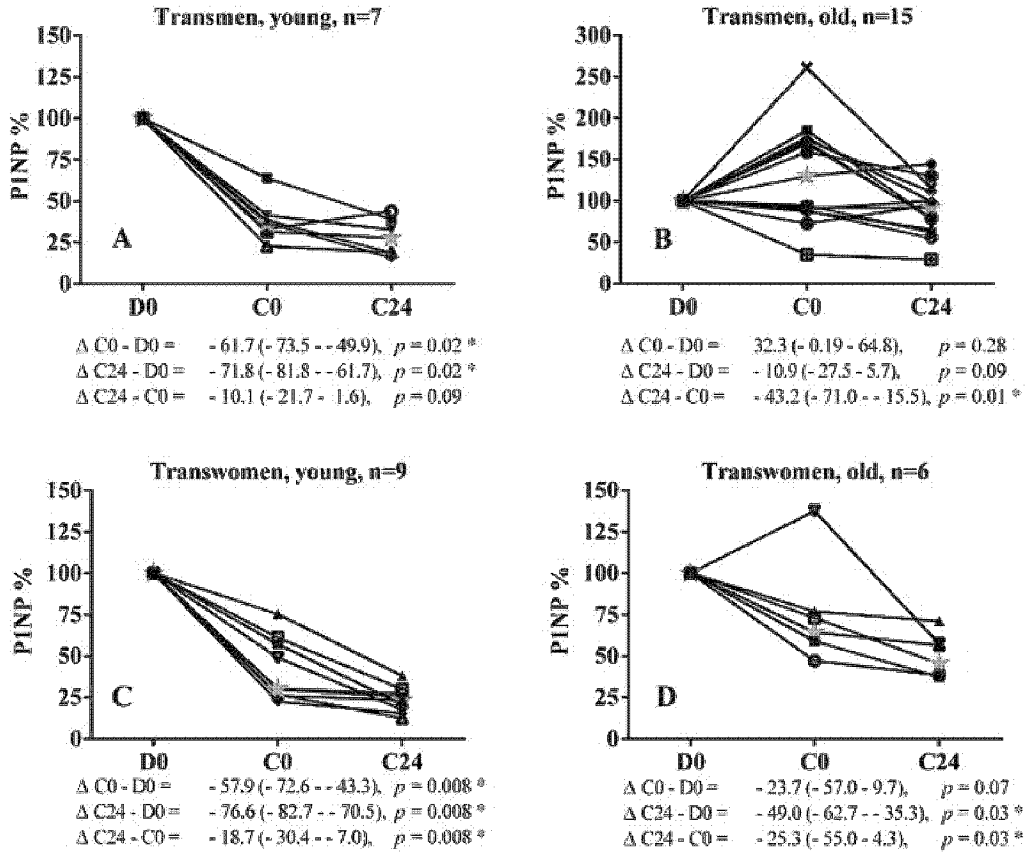



**Figure 1.** P1NP measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding *p*-value.

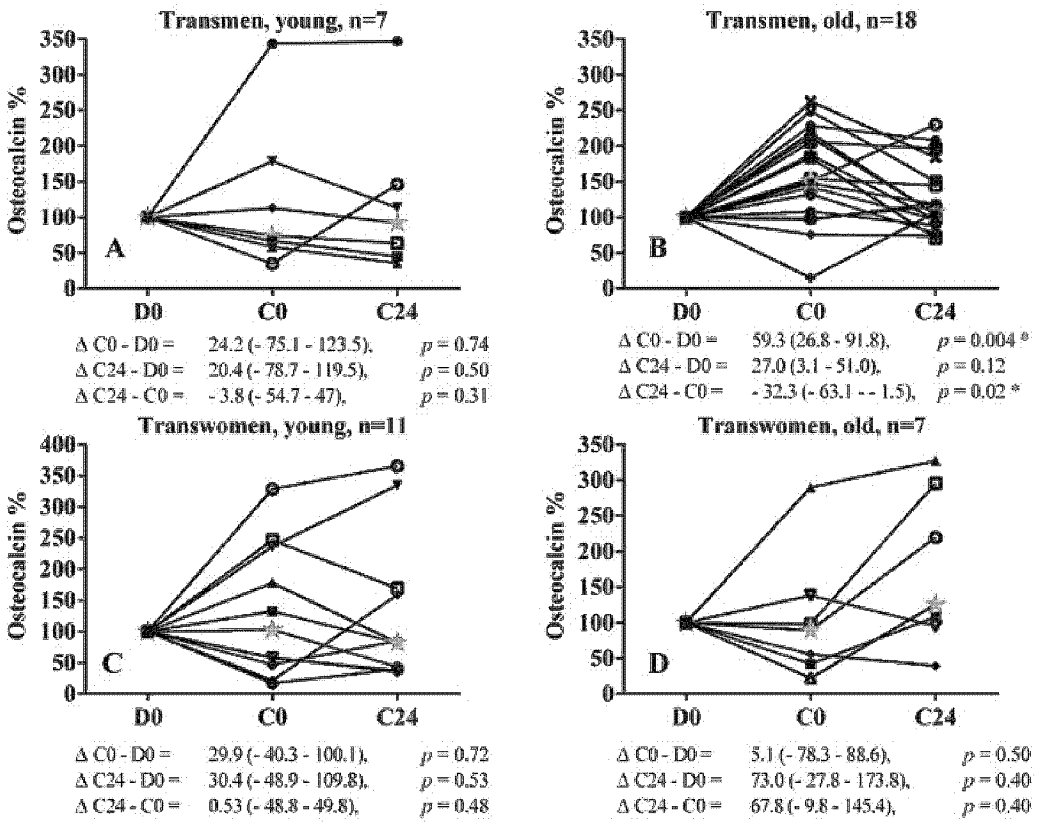



**Figure 2.** Osteocalcin measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding *p*-value.

transmen and transwomen. ICTP concentrations decreased especially in the young transmen group twenty four months after CSHT (*Figure 3*).

## BMAD and Z-scores

### FN

BMAD FN did not differ at baseline between young and old transmen (*p=0.7*). Also, no difference between young and old transwomen (*p=0.5*) was found. Furthermore, transmen and transwomen did not differ regarding their BMAD FN at baseline. During GnRHa therapy only the old transmen showed a decrease of the BMAD. In general, in both young and old transwomen the BMAD did not change after CSHT. In contrast, in young and old transmen an increase of BMAD after 24 months of CSHT was found *(Figure 4)*.

Regarding the FN BMAD Z-scores, both young and old transwomen groups showed a BMAD Z-score below zero at D0. In old transmen a decrease of the BMAD Z-score during GnRHa was seen (95% CI - 0.548 to - 0.147, *p=0.002)*. Young and old transmen showed an increase of the BMAD Z-score during CSHT *(*95% CI 0.276 to 0.639, *p=0.005* and 95% CI 0.038 to 0.470, *p=0.02)* respectively. In all transwomen the median BMAD Z-score was still below zero after 24 months of CSHT and the transwomen young group showed a lower BMAD-Z score compared to young transmen at C24 (*p=0.02*).

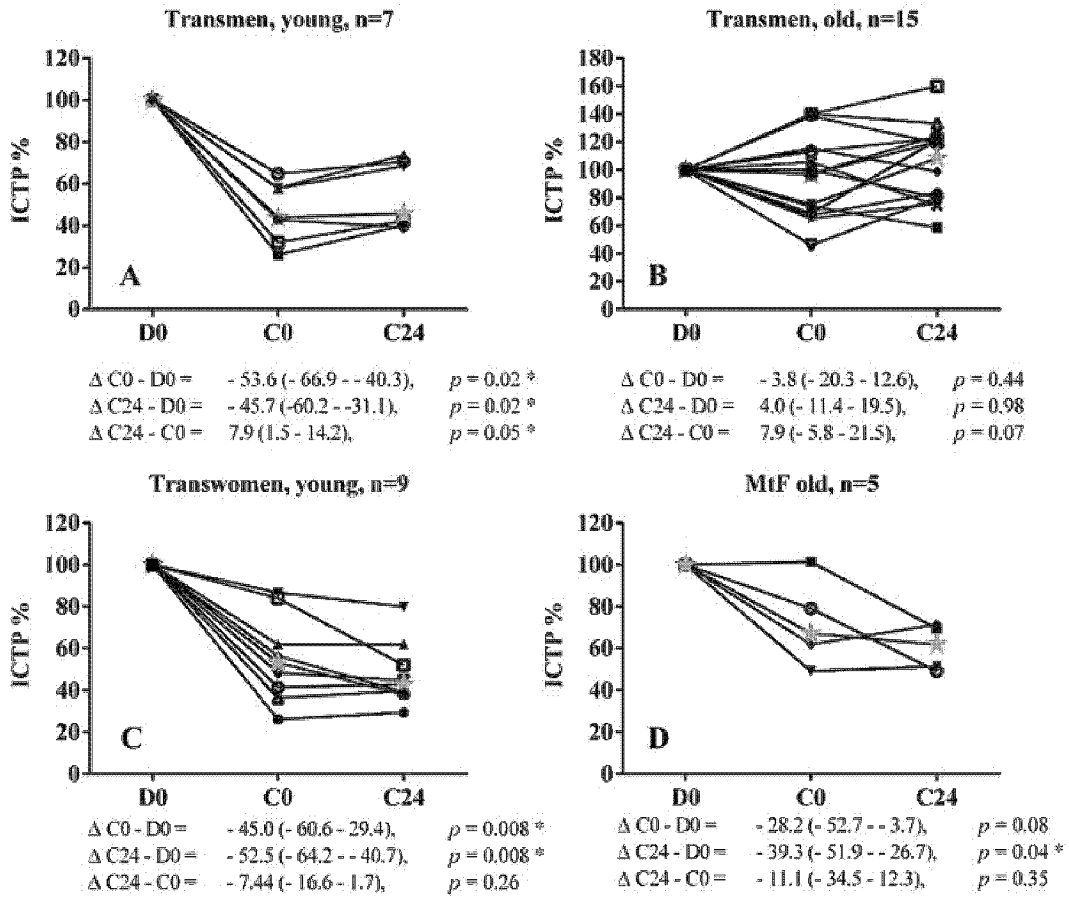



**Figure 3.** ICTP measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding *p*-value.

## LS

At baseline, the young transwomen had a lower BMAD LS than the young transmen (*p=0.003)*. At baseline, there was no difference between young and old transmen, young and old transwomen, or between old transmen and old transwomen. Suppression of puberty resulted in a decrease of BMAD of the old transmen. A substantial increase of BMAD in all groups was seen after 24 months of CSHT *(Figure 5)*.

Regarding the LS BMAD Z-scores, young transmen showed a lower Z-score compared to old transmen (*p=0.02)* at baseline. The median BMAD Z-score of both young and old transwomen was lower compared to the median of young and old transmen at baseline. In both transmen groups a decrease of the BMAD Z-score was seen after suppression of puberty (young transmen 95% CI - 1.304 to - 0.582, *p=0.003,* old transmen 95% CI - 0.973 to - 0.530, *p=<0.0001*) and the LS BMAD Z-score increased after 24 months of CSHT in both transmen groups (young transmen 95% CI 0.252 to 0.926, *p=0.008* and old transmen 95% CI 0.123 to 0.425, *p=0.001,* respectively). Only the BMAD Z-scores of the young transwomen decreased during GnRHa therapy (95% CI - 1.196 to - 0.678, *p=0.001)*. The LS BMAD Z-score of both transwomen groups increased after 24 months of CSHT (young transwomen 95% CI= 0.099 to 0.642, *p=0.01* and old transwomen 95% CI 0.316 to 0.753, *p=0.04*), but the BMAD

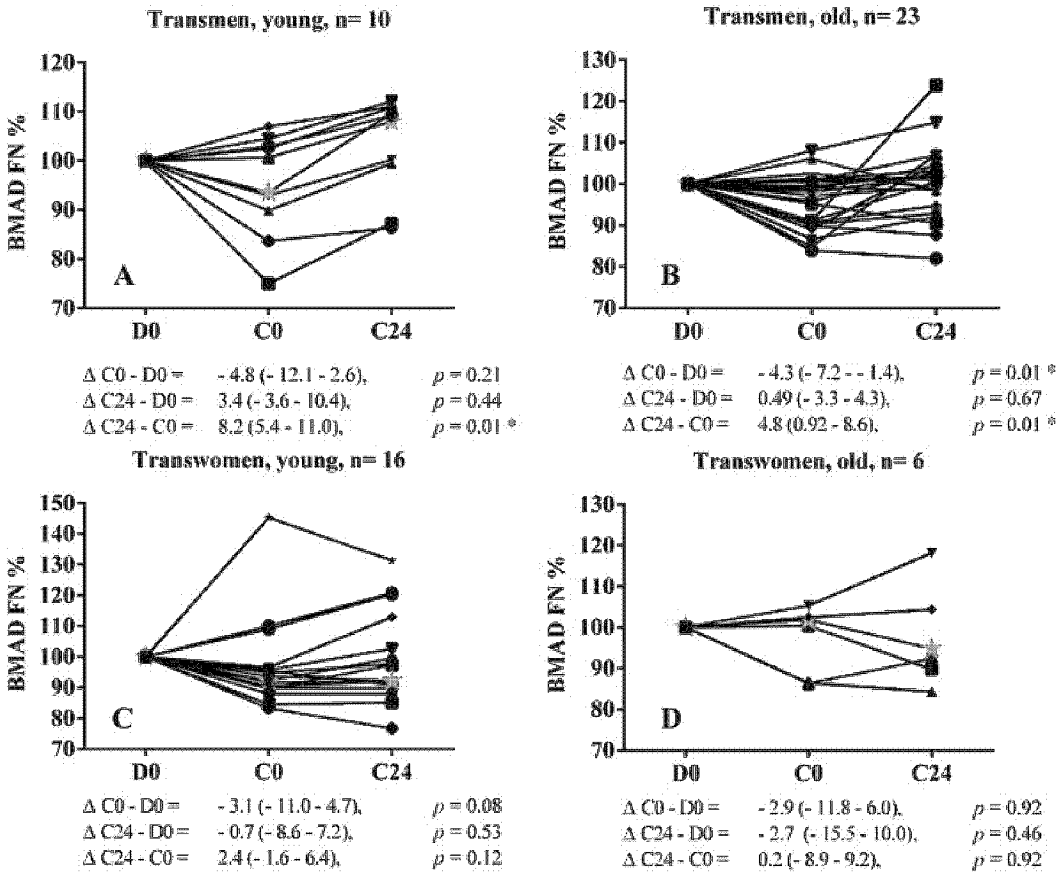



**Figure 4.** BMAD of the hip (FN) measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding *p*-value.

Z-score did not reach the zero level. In general, after 24 months of CSHT the median LS BMAD Z-score of the transwomen were still lower than the median BMAD Z-score of the transmen.

# Discussion

This study is the first to explore the effect of suppressing puberty and the administration of CSHT on bone turnover markers in transgender adolescents. We showed that suppression of puberty by GnRHa in young transmen and transwomen led to a decrease in the serum BTMs ICTP and P1NP. The decrease in bone turnover upon GnRHa treatment coincides with a decrease of BMAD Z-scores of predominantly the LS. During CSHT the BTMs further decreased in most adolescents, whereas the BMAD Z-scores improved, especially of the LS. However, pre-treatment Z-scores were not reached in most transgender adolescents after 24 months of treatment with CSHT.

## Effect of suppressed puberty on BTMs and BMAD in young patient groups

Previous studies describe a down regulation of bone turnover reflected by a decrease in BTMs in non-transgender adolescents using GnRHa [17;18]. As expected, our study showed that suppression of puberty by GnRHa resulted in a decrease of P1NP and ICTP in young transmen and transwomen

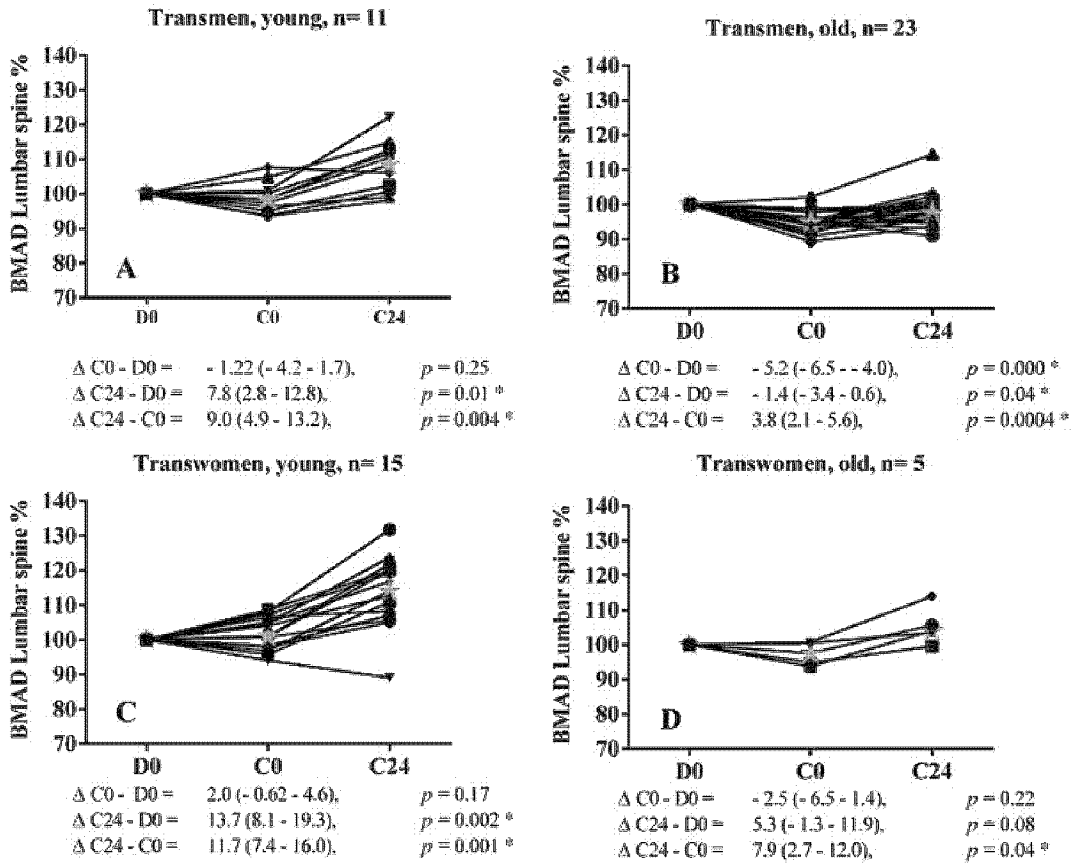



**Figure 5.** BMAD of the lumbar spine measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding *p*-value.

as well. The BTM osteocalcin did not show this decrease nor did it resemble the course of P1NP and ICTP. A previous study in middle-aged adult transwomen also described this aberrant pattern of osteocalcin [19]. The circadian rhythm of osteocalcin, with highest values in the morning, might be one of the causes as our samples were collected at various moments during the day [20;21].

Previous studies show an initial decrease of BMD in different patient groups using GnRHa followed by a normalisation or increase of the BMD [7;22;23]. In our study, the decrease in bone turnover upon GnRHa treatment only coincides with decreased BMAD Z-scores of predominantly the LS. BMAD itself remains stable in both transmen and transwomen, as previously shown [24]. The observed decrease in BMAD Z-scores in our study was expected. Normally, bone mass accumulates under the influence of sex steroids during puberty. However, in our study sex steroid deprivation due to GnRHa treatment results in stable bone mass, which implies a loss of Z-scores in transgender adolescents compared to their peers.

## Effect of CSHT on BTMs and BMAD in young patient groups

After the start of CSHT an increase of BMAD and BMAD Z-score of predominantly the LS is seen in both transmen and transwomen. Previous studies in adult transwomen showed an increase of

the BMD of the LS after one and two years of CSHT as well [19;25]. The difference between changes in LS and FN was seen earlier, also in normal puberty and might be due to several explanations [5;6;24]. First, trabecular bone is more biologically active than cortical bone and the estrogen receptor [ER]-α responds to different stimuli in trabecular versus cortical bone, the regulation of the number of osteoclast seems to be different in trabecular versus cortical bone and, lastly, estrogen seems to slow done bone resorption more in trabecular bone more than in cortical bone [26;27]. In other words, we found that estrogen affects the lumbar spine in particular, which consists predominantly of trabecular bone, whereas FN consists mainly of cortical bone. The delay in bone mass accrual during gonadal suppression was not annihilated after 24 months of CSHT as Z-scores were still lower despite the stimulating effect of CSHT. Indeed, in our previous study we reported a BMAD Z-score under pre-treatment level at the age of 22 in transgender patients after over 5 year of CSHT as well [6].

In contrast to the increasing BMAD and BMAD Z-scores, we observed a decrease of predominantly P1NP but also of ICTP in both transmen and transwomen after 24 months of CSHT. As we do not have serum BTM measurements between the start of CSHT and 2 years later, we can only speculate that an increase of BTMs took place within this 2 year period. After 2 years of CSHT most patients reached their adult maintenance dosage and therefore BTMs may already have decreased to levels corresponding with end of puberty. A decrease of bone turnover is described as well at the end of physiological puberty [10;28] and BTMs P1NP and CTX showed the highest levels mid puberty in comparison to early and late biological puberty [29]. Furthermore, several studies in adult transwomen showed a general decrease in bone turnover after CSHT as well [25;30]. The bone formation marker osteocalcin did not resemble the course of P1NP or ICTP after induction of puberty with CSHT. When comparing BTMs and BMAD Szulc et al. have shown that levels of bone turnover markers correlate more with linear growth of the bones during puberty than with the accrual of bone mass [20]. Alternatively, it can be postulated that the decrease of bone turnover we found in all our patient groups after 24 months of CSHT was linear and that increase of BMAD continues under influence of sex steroids, IGF-1 and alkaline phosphatase. These factors have been described to stimulate bone mineral accrual still in the post-pubertal years after the longitudinal growth of the bones has stopped and when bone turnover decreased already [20;28;31].

## Differences between young and old groups and between transmen and transwomen

Our study showed several differences between young and old groups and between transmen and transwomen. With regard to bone turnover, we found higher concentrations of P1NP, osteocalcin and ICTP in the young compared to old groups at baseline except for ICTP in the transwomen. This finding is in line with previous studies which showed high BTM concentrations in early and mid-puberty compared to BTM concentrations later in biological puberty [2;32]. As for LS BMAD we found that the transwomen had lower BMAD and median BMAD Z-scores than FtMs at baseline and during GnRHa and CSHT treatment. This difference between transwomen and transmen was shown in previous studies as well [6;33;34] The surprisingly lower median BMAD of the transwomen compared to reference values of biological boys in the study of Ward et al. [15] at baseline could be

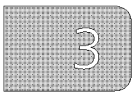
explained by the small number of subjects in this group in our study. Alternatively, lower 25OHD levels, reduced mechanical loading of their bones due to a less active lifestyle, less participation in sports and other physical activities of this group could have contributed to this phenomenon. Physical activity is important for periosteal bone formation and also for gain of bone mass and geometry of cortical bones especially during puberty [33;34]. Another possible explanation for the lower median BMAD in transwomen is the lower age of transwomen than transmen in our study. Girls with female sex assigned at birth reach their peak bone mass and thus higher BMAD earlier than boys [35;36]. This difference is also strengthened by the height gain between transmen and transwomen during GnRHa treatment with transwomen showing a more pronounced increase of height due to their earlier pubertal stage at D0 compared to transmen.

The LS BMAD showed more changes compared to the FN BMAD after both GnRHa and CSHT, especially in the transmen in our study, as shown previously [6]. Furthermore it was shown that in adult transwomen estrogen treatment resulted in an increase of BMD of both FN and LS[9;19] whereas in our transwomen groups bone mass predominantly accrued in the LS. This difference can be explained by i] BMD was measured after a longer period of CSHT treatment in the adult group; ii] the administration of higher dose of estrogen in adult transwomen or; iii] the use of anti-androgens instead of GnRHa in the present study. While FN BMAD increased in the transmen it did not in the transwomen. This could be explained by the difference in dosage scheme. The incremental and assumed physiological estrogen dosage in the transwomen group could still be too low to result in an adequate increase of BMAD of the FN in our study group of transwomen, whereas the administration of incremental testosterone dosage could be high enough to result in an increase of BMAD in the transmen in our study. Indeed, the cortical bone in the FN responds less to hormonal stimuli than the trabecular bone in the LS and therefore may require higher dosages [37]. Nevertheless, several studies in adult transwomen and transmen showed that long-term CSHT was able to preserve the BMD at adult age [38;39].

The strengths of this study are the use of a standardised treatment protocol, a standardized modification of BMD to BMAD and the use of the same BTM assays over time in all samples.

Furthermore, the vast majority of BTM measurements were performed at the exact same date of D0 and C0 time points respectively. These measurements therefore reflect the actual BTM changes in true GnRHa and cross-sex-hormone naïve patients. Furthermore, this is the first study in which the relationship between bone turnover and BMAD in transgender adolescents is studied. With regard to overlap of the study of Klink et al. [6] some of the study subjects (n=12) described in their study were part of the current study as well. However, overlap is minimal because the study by Klink et al. [6] focused on long-term effects on bone mass while this study focuses the short term effects on bone turnover and bone mass. In addition, the other study subjects included in this study were recruited at the same outpatient clinic with the same pediatricians conducting physical and study research. This contributed to a sufficient study population and standardized treatment of the patients. This study also has a number of limitations. First, the sample size of this retrospective study is small and lacks a control group and as a result the effects caused by normal growth during puberty on bone turnover and BMAD cannot be distinguished. Second, serial BTM measurements after the start of CSHT were not available as we presumed that these BTM levels were expected

to be high just after starting CSHT. Furthermore, standardized data on possible confounders such as physical activity, smoking, previous fractures and use of medication (especially calcium and colecalciferol) were not available. Lastly, BTMs were measured in a non-fasting state and at different times during the day and season.



In conclusion, suppressing puberty by GnRHa decreases bone turnover, as evidenced by a decrease of both P1NP and 1CTP in transgender adolescents. This decrease in bone turnover during GnRHa treatment coincides with decreased BMAD Z-scores of predominantly the LS in young transwomen. BTMs further decreased after 24 months of CSHT, except for the old transmen, whereas CSHT resulted in an increase BMAD Z-scores of especially the LS mainly in transwomen. The BMAD Z-scores did not reach baseline levels after 24 months of CSHT. Hence, the course of BTMs is not directly reflected in changes of BMAD and BMAD Z-scores in transgender adolescents. Therefore, based on this study, the added value of evaluating BTMs in transgender adolescents seems to be limited and requires further research. Meanwhile, DXA-scans remain important in follow-up of bone health of transgender adolescents.

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# Chapter 4

# Bone mineral density increases in trans persons after 1 year of hormonal treatment: a multicenter prospective observational study

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## 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 is to investigate in a prospective observational multicenter study the first-year effects of CHT on BMD in transgender persons. 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 was performed to measure lumbar spine (LS), total hip (TH), and femoral neck (FN) BMD before and after one year 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 one year 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 ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, p=0.001) compared with transmen <50 years (+0.68%, 95% CI 0.19 to 1.17%, p=0.007). In conclusion, BMD increased in transgender persons after one year 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.

# 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, transmen (female-to-male transgenders) are treated with testosterone. While a few studies have investigated the 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 of 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 (e.g. 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; Florence: Hologic Delphi A; Oslo: Lunar), 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 three months before or one

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 two 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 of 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 three 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 three months, and after 12 months of CHT, all after overnight fasting. In Amsterdam, estradiol was measured using a competitive immunoassay (Delfia, PerkinElmer, Finland) with an inter-assay coefficient of variation (CV) of 10-13% 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 inter-assay 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, USA) with an inter-assay CV of 7-20% and a LOQ of 1 nmol/L until January 2013. Thereafter, testosterone was measured using competitive immunoassay (Architect, Abbott, USA) with an inter-assay CV of 6-10% 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, Germany) until 19th March 2015. Thereafter, estradiol was measured using a E170 Modular (Gen III, Roche Diagnostics, Germany), with an inter-assay CV of 3.2% and a LOQ of 25 pg/mL (92 pmol/L). For conversion of estradiol values measured before 19th March 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 inter-assay CV of 2.6% and a LOQ of 10 ng/dL (0.4 nmol/L) for testosterone, and an inter-assay 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., Bedford, MA, USA). 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, L1–L4), 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 one 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 variable, respectively. In order 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 three 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, Texas, USA), version 13.1.

# Results

## General characteristics

The flowchart of the inclusion of participants in both centers is shown in Figure 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 to 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.

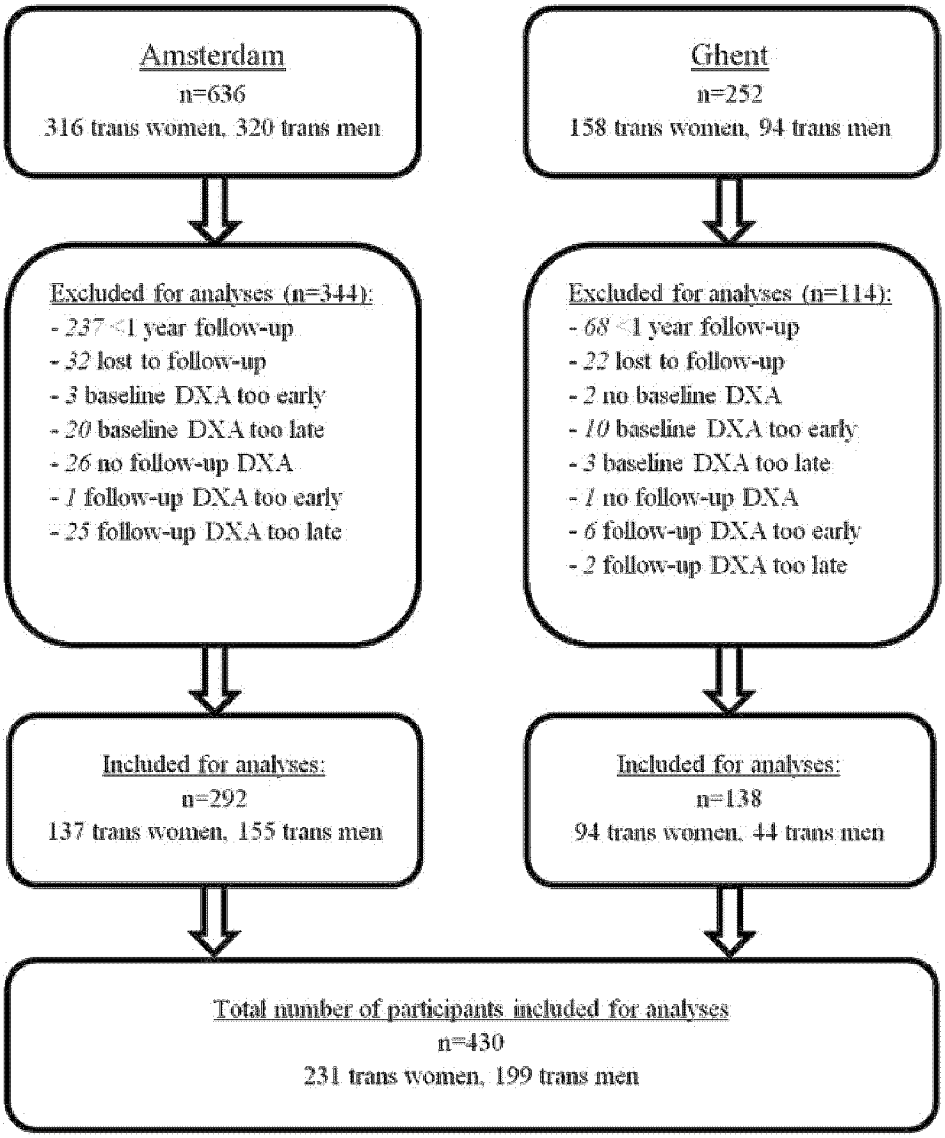


**Figure 1.** Flow chart of the inclusion of participants in the present study. Abbreviations: DXA=dual-energy X-ray absorptiometry.

**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 (% Caucasian) | 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) |

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



## 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, one 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, one 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 observed in FN BMD (-0.46%, 95% CI -1.07 to 0.16%, p=0.144) (Figure 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 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 Figure 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 18 to 20 year compared to transwomen of 30 to 49 years. In transmen, LS BMD increased more in persons ≥50 years (+4.32%, 95% CI 2.28 to 6.36%, p=0.001) compared to persons below 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 ≥50 years, compared to no increase in persons below 50 years (158 pmol/L to 194 pmol/L; +22%, 95% CI -2 to 53%, p=0.078).

**Table 2.** Baseline and one-year BMD in lumbar spine, total hip, and femoral neck for transwomen and transmen, stratified per center

| | Transwomen | | | |
|---|---|---|---|---|
| | **Amsterdam (n=137)** | **Ghent (n=94)** | **Total (n=231)** | **p-values** |
| Lumbar spine BMD | | | | |
| Baseline | 0.966 (0.138) | 0.983 (0.143) | 0.972 (0.140) | 0.362 |
| One-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 |
| One-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 |
| One-year | 0.814 (0.127) | 0.811 (0.135) | 0.813 (0.130) | 0.834 |
| | **Transmen** | | | |
| | **Amsterdam (n=155)** | **Ghent (n=44)** | **Total (n=199)** | **p-values** |
| Lumbar spine BMD | | | | |
| Baseline | 1.030 (0.124) | 1.022 (0.114) | 1.028 (0.121) | 0.705 |
| One-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 |
| One-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 |
| One-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.

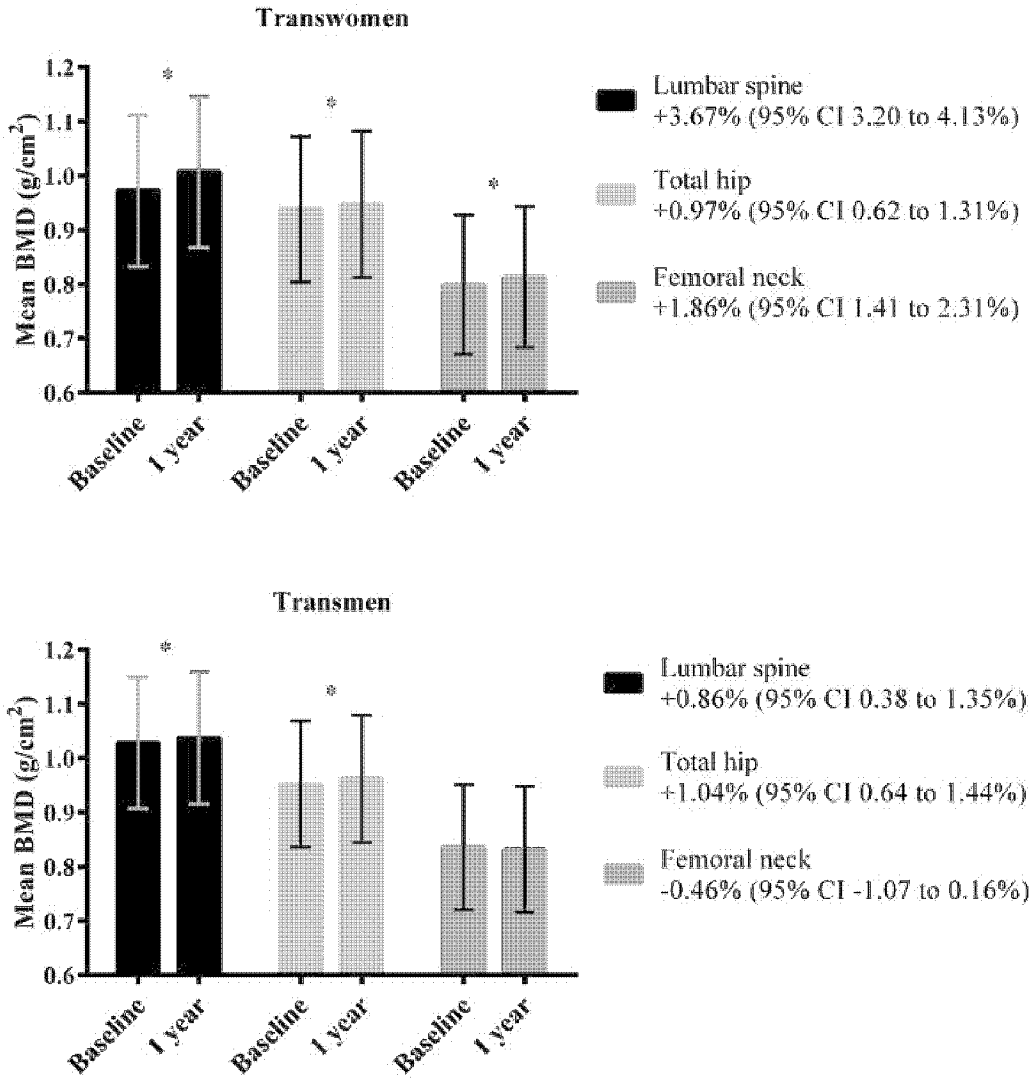


**Figure 2.** Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen after one year cross-sex hormonal treatment. Data represent mean baseline and mean one-year BMD with standard deviation. Percentage changes in BMD were calculated for every person 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), which are shown in the legends on the right. * p≤0.001. BMD=bone mineral density.

## The effect of vitamin D supplements on BMD change

As shown in Figure 4, LS and FN BMD increased more in transwomen who used vitamin D supplements compared to 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. As 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 (Figure 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 to testosterone gel (Figure 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), while 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), gastro-intestinal disease (n=8), malignancy (n=2)) did not change the effect sizes (data not shown).

## Discussion

This study showed that after one year 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.

The larger increase in BMD in transmen of postmenopausal age as compared to 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

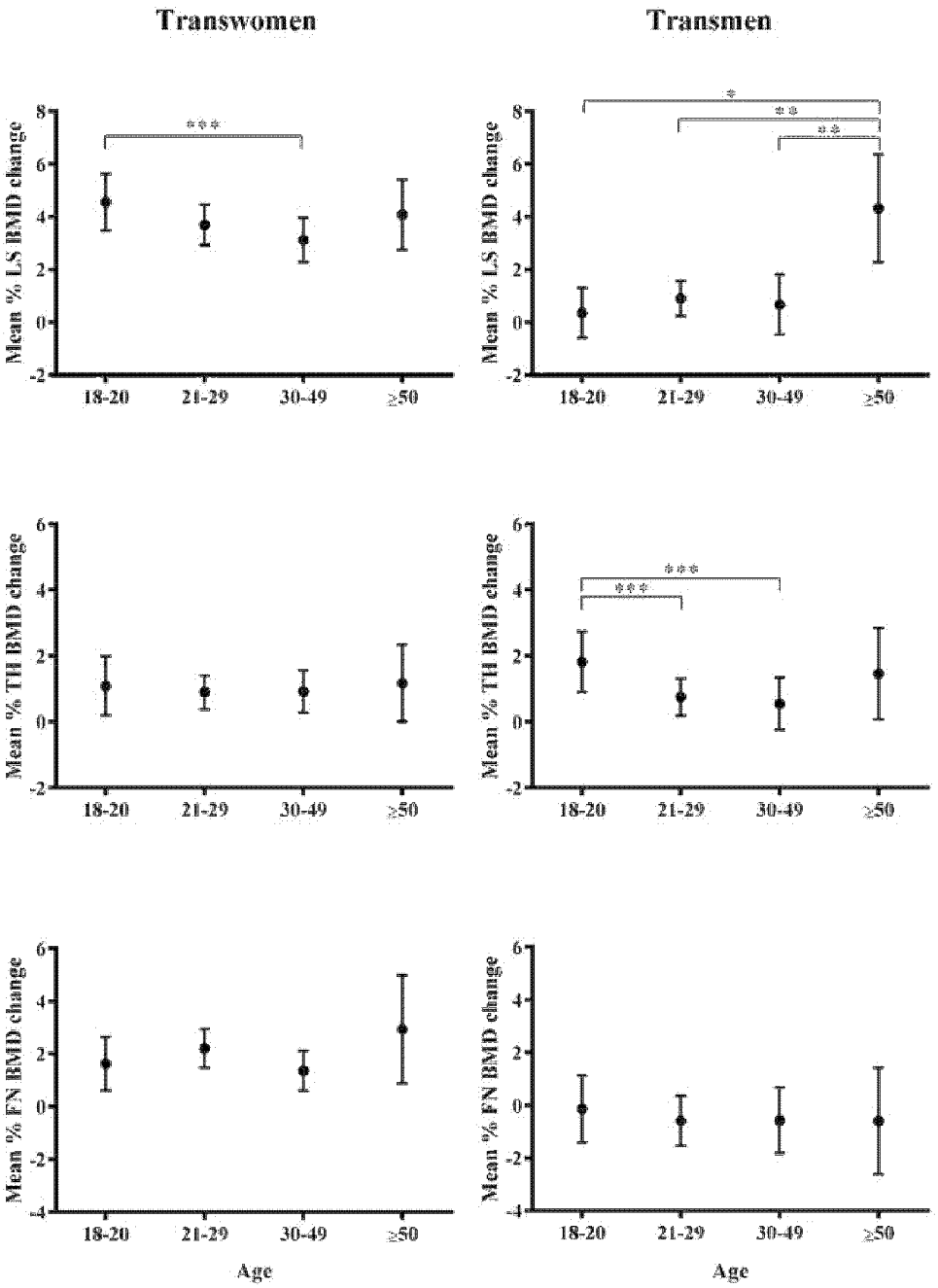


**Figure 3.** Change in bone mineral density of lumbar spine, total hip, and femoral neck, in transwomen and transmen, stratified for age groups. Each individual bar represent the mean increase in bone mineral density with 95% confidence interval. The age distribution in transwomen is: 18–20 (n=36), 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. Difference 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≤0.001; ** 0.001<p≤ 0.01; *** 0.01<p≤0.05. LS=lumbar spine; TH=total hip; FN=femoral neck; BMD=bone mineral density.

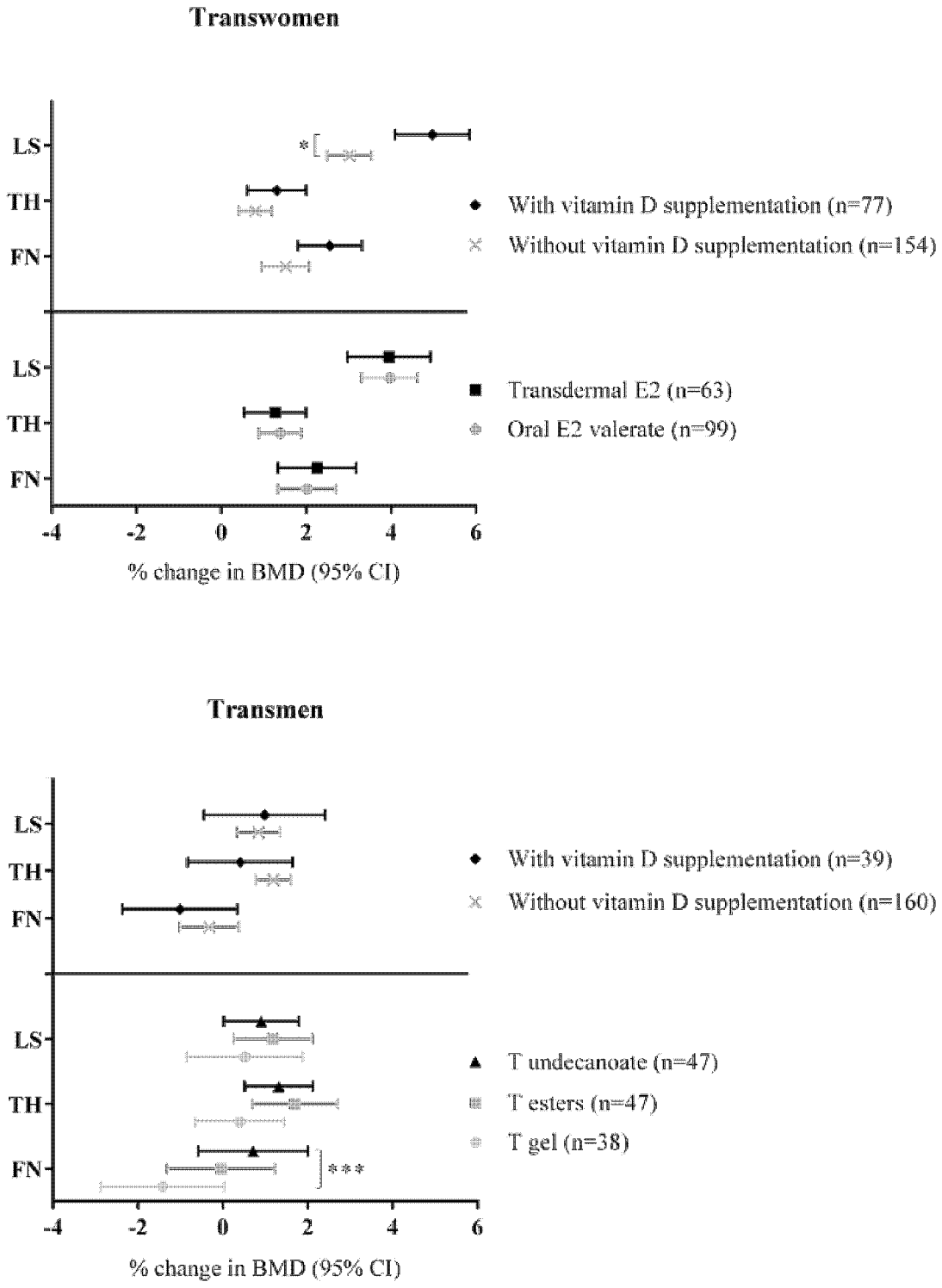


**Figure 4.** Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen, stratified for vitamin D supplementation or administration routes, respectively. Linear regression analyses with change in BMD as outcome variable and vitamin D supplementation 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. * $p \leq 0.001$; *** $0.01 < p \leq 0.05$. Abbreviations: LS=lumbar spine, TH=total hip, FN=femoral neck, BMD=bone mineral density, T=testosterone.

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 et al.[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 of 18 to 20 years compared to transwomen between 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, 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 to testosterone gel and testosterone undecanoate. However, testosterone ester therapy results in highly fluctuating serum testosterone levels and since 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 one year 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. While previous prospective studies did not find a change in LS BMD after one year 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 due to 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 between 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 was lost to follow-up (8.5%). There are also some limitations to our study. First, due to the multicenter aspect of this study, the BMD of participants was measured using different DXA devices. In order 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, as 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. Due to ethical and practical reasons, it is not possible to add a placebo group to this study. Fourth, as 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 one year 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 in order to gain more insight of the actual changes in bone metabolism due to sex steroid therapy.

## Disclosures

All authors state that they have no conflict of interest.

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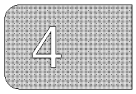
Authors' roles: Study design: AF, TS, GT, and MdH. Study conduct: GT and MdH. Data collection: CW, MV, MK, NN, CdB, AF, TS, GT, and MdH. Data analysis: CW and MV. Data interpretation: CW and MV. Drafting manuscript: CW. Revising manuscript content: CW, MV, MK, NN, CdB, RdJ, PL, GT, and MdH. Approving final version of manuscript: CW, MV, MK, NN, CdB, AH, RdJ, PL, AF, TS, GT, and MdH. MdH takes responsibility for the integrity of the data analysis.



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# Chapter 5

# Gender-affirming hormone treatment decreases bone turnover in trans women and older trans men

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## Abstract

Sex steroids play a key role in bone turnover and preserving BMD. Gender-affirming hormonal treatment (HT) in transgender people affects bone metabolism. Most studies looked into the effect of HT on changes in BMD, however this does not provide insights of changes in bone metabolism due to HT. This study investigated changes in bone turnover markers (BTMs), sclerostin and correlations with change in BMD in trans women and trans men during the first year of HT. Trans women received estradiol and anti-androgens, while trans men received testosterone. Sclerostin, P1NP, alkaline phosphatase (ALP), CTx, and BMD of total hip (TH), femoral neck (FN), and lumbar spine (LS) were evaluated at baseline and after 1 year of HT. 121 Trans women (median age 30 years, IQR 24-41) and 132 trans men (median age 24 years, IQR 21-33) were included. In trans women, ALP decreased with 19% (95%CI -21;-16), CTx with 11% (95%CI -18;-4) and sclerostin with 8% (95%CI -13;-4) after 1 year of HT. In contrast, in trans men P1NP, ALP, and sclerostin increased with 33% (95%CI 24;42), 16% (95%CI 12;20), and 15% (95%CI 10;20), respectively after 1 year of HT. No age differences were seen in trans women, whereas in trans men aged ≥50 years a decrease in all BTMs was found in contrast to the other age groups. These trans men had low estrogen concentration at start of HT, due to their postmenopausal state before start of HT, and estradiol concentrations increased during testosterone treatment. Changes in BTMs and BMD were weakly correlated (correlation coefficient all <0.30). To conclude, 1 year of HT resulted in decreased bone turnover in trans women and older trans men, while it increased in younger trans men. The decrease in bone resorption in the older trans men displays the importance of estrogen as key regulator of bone turnover.

# Introduction

Sex steroids are considered as pivotal regulators of bone metabolism. Estrogen inhibits the osteoclast function and thereby lowers bone resorption, resulting in a positive effect on BMD in both women and men [1–5]. Furthermore, it is well known that BMD decreases in postmenopausal women due to decreasing estrogen concentrations and consequently increased bone resorption by osteoclasts [6,7]. In men, estrogen is aromatized from testosterone and is also considered as the key sex steroid affecting bone homeostasis [8–10]. Previous research showed that bone metabolism and therefore BMD are affected by gender-affirming hormonal treatment (HT) in people diagnosed with gender dysphoria (GD) [5,11–18]. HT is used to accomplish desired body changes in transgender people. HT in trans men (female-to-male transgender people) consists of testosterone treatment, while trans women (male-to-female transgender people) receive a combination of anti-androgens and estrogens.



An increase in BMD after 1 to 10 years of treatment with HT in transgender people was described before [5,19]. BMD is evaluated by DXA scan, however these scans estimate the amount of mineralized bone only and therefore represent late changes in bone metabolism. In contrast, bone turnover markers (BTMs) represent the actual activity of the osteoblasts and osteoclasts. Consequently, measurements of BTMs display the balance between bone formation and bone resorption directly. Up until now, scarce data is available regarding specifically the effect of HT on bone turnover in transgender people [20–24], while no data on sclerostin is available yet. Clinically, increased bone turnover and lower BMD are risk factors for deterioration of bone quality resulting in possible osteopenia, osteoporosis, and even increased risk of fractures and associated co-morbidities and financial costs. As the transgender population receiving HT increases worldwide [25,26], more transgender people are possibly at risk for lower bone quality and associated problems regarding bone health. Therefore, the aim of this study is to investigate the change in bone turnover markers and to evaluate the correlations with changes in BMD in adult transgender people during their first year of HT.

This study will also look into possible age-related effects on bone turnover markers and BMD during HT by studying transgender people in various age groups. We hypothesize to find a decrease in bone turnover predominantly due to estrogen, as this sex steroid is known to inhibit osteoclast function and therefore exerts an anabolic effect on bone. This study will focus on bone formation markers P1NP and total alkaline phosphatase (ALP) and the bone resorption marker CTx. Furthermore, the glycoprotein sclerostin is studied, which is known to mediate an anti-anabolic effect on bone by promoting apoptosis of osteoblasts, stimulate RANKL production by osteocytes resulting in increased osteoclastogenesis, and inhibition of the activation of the Wnt/β-catenin pathway, all resulting in negative effects on BMD [27–32]. Sclerostin is mainly produced by osteocytes and can be used as a marker of bone metabolism as well.

# Methods

## Subjects and study protocol

This study is part of the European Network for Investigation of Gender Incongruence (ENIGI) study, which is a prospective multicenter observational study in Ghent (Belgium), Oslo (Norway), Florence (Italy), and Amsterdam (the Netherlands) [33,34]. The current study protocol was approved by the Ethical Committee of the Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands, and data was retrieved only after informed consent. Adults diagnosed with gender dysphoria based on the diagnostic criteria of the DSM-IV or DSM 5 [35,36] were recruited at the Center of Expertise on Gender Dysphoria of the Amsterdam University Medical Center, between June 2012 and April 2016. All transgender people included in this study were treated according to the Standards of Care Guidelines of the World Professional Association for Transgender Health (WPATH) [37]. Summarized, trans women were treated with anti-androgen treatment consisting of cyproterone acetate (50 to 100 mg daily, oral) accompanied by estrogen treatment consisting of either estradiol valerate (2 to 4 mg daily, oral) or estradiol patches (50 - 100 mcg/24 h twice a week, transdermal application). Trans men were treated with either testosterone gel (50 mg daily, dermal application), testosterone esters (250 mg every 2 to 3 weeks, i.m.), or testosterone undecanoate (1000 mg every 12 weeks, i.m.). Some trans men used lynestrenol for a short period of time if menses persisted while using testosterone.

People were not eligible to participate in the study if they had (I) insufficient knowledge of the native language, (II) were psychological vulnerable, (III) used HT earlier in life, or (IV) used other drug therapies which were not part of the standardized treatment protocol (e.g. spironolactone or gonadotropin-releasing hormone agonists). For the current analyses, people were excluded if they (I) did not completed 1 year of HT, (II) had no DXA scan at baseline and/or after 1 year of HT, (III) or had no blood drawn at baseline and after 1 year of HT. In addition, only people from the Amsterdam University Medical Center, Vrije Universiteit Amsterdam center were included, in order to exclude possible changes in BTM concentrations due to use of different BTM assays in the other medical centers.

## Measurements

### General

Participants visited the outpatient clinic every 3 months to evaluate their health and treatment effects. Body weight (kilograms) and height (centimeters) were measured without wearing shoes at baseline and follow-up. Blood samples were collected between 09:00 AM and 12:00 PM at baseline, after 3 months, and after 1 year of HT. Participants were instructed to draw blood in a fasting state.

## Bone turnover markers

### P1NP

The bone formation marker P1NP resembles osteoblast activity [38] and was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay coefficient of variation (CV) of <8% and lower limit of quantification (LOQ) of 5 µg/L.

### Alkaline phosphatase (ALP)

The bone formation marker ALP, also representing osteoblast activity [38] was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay CV of 2.5% and LOQ of 5 U/L.

### CTx

The bone resorption marker CTx displays osteoclast activity [38]. CTx was measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany), with an inter-assay CV of <6.5% and LOQ of 10 ng/L.



### Sclerostin

The osteocyte-derived glycoprotein sclerostin [38] was measured using an immunoassay (LiasonXL, Diasorin, Saluggia, Italy), with an inter-assay CV of 7.5% and LOQ of 2.2 pmol/L.

## Other measurements

### 25OHD

An LC-MS/MS method was used to measure 25OHD with an CV of 8% and LOQ of 4.0 nmol/L until 2015 [39]. Since then, another LC-MS/MS method was used [40]. Both methods resulted in comparable concentrations.

### Testosterone

A radio-immunofrequent assay (RIA) (Coat-A-Count, Siemens, USA; inter-assay CV of 7-20 %, LOQ of 1 nmol/L) was used to measure testosterone until January 2013. Since then, a competitive immunoassay was used (Architect, Abbott, USA; inter-assay CV of 6-10 %, LOQ of 0.1 nmol/L). The RIA based concentrations were converted to concentrations of the competitive immunoassay using the formulas Architect=1.1*RIA+0.20 (for testosterone concentrations <8 nmol/L) and Architect=1.34*RIA–1.65 (for testosterone concentrations >8 nmol/L) in order to evaluate and report comparable testosterone concentrations.

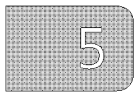
### Estradiol

A competitive immunoassay (Delfia, PerkinElmer, Finland; inter-assay CV of 10–13%, LOQ of 20 pmol/L) was used to measure estradiol until July 2014. Subsequently, an LC-MS/MS (Amsterdam University Medical Center, VUmc, Amsterdam, the Netherlands; inter-assay CV of <7%, LOQ of 20 pmol/L) was used. The Delfia concentrations were converted to the LC-MS/MS concentrations by using the formula LC-MS/MS=1.60*Delfia–29.

Creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyltransferase ($\gamma$GT) concentrations were all measured using an immunoassay (Cobas, Roche Diagnostics).

### DXA

DXA (Hologic Discovery A, Hologic Inc., Bedford, MA, USA) was used to measure BMD in g/cm2 of the total hip (TH) and femoral neck (FN) of the non-dominant hip and the lumbar spine (LS), measuring the first 4 lumbar vertebrae (L1–L4). The software was updated from version 13.3 to 13.5.3 in July 2015, which did not affect the results of the measurements. Baseline DXA was performed 3 months before to 1 month after start of HT. The follow-up DXA was performed between 10 and 14 months after start of HT.



### Statistics

For statistical analyses Stata/SE 15 (StataCorp, LP) was used. Median with corresponding interquartile range (IQR), percentages, or means with SD were used to describe baseline characteristics. The percentage change was calculated of all BTMs and BMD to evaluate differences between baseline and 1 year HT. As these changes were normally distributed, linear regression analyses were performed to evaluate mean changes in percent with corresponding 95% CI. Next, these percentage difference variables were adjusted for changes in BMI, alcohol and tobacco use, 25OHD, creatinine, AST, ALT, and -GT concentrations. Participants were stratified for both age and sex steroid concentrations, with the following age groups: 18 to 30 years, 30 to 50 years, and 50 years and older. By using these separate age groups, age related differences in BMD due to decreasing bone mass with increasing age after reaching peak bone mass is accounted for, as it is expected that bone mass decreases throughout time, as described before [5]. Linear regression was performed to evaluate possible differences between the separate age groups. Furthermore, participants were stratified into quartiles based on their mean estradiol and testosterone concentrations during HT, which were calculated by an average of the concentrations after 3 and 12 months of HT. This stratification was applied to detect possible differences between effect of either low or high sex steroid concentrations. Furthermore, a power analysis was performed. The analysis was applied to the study population of 121 trans women and 132 trans men in order to detect mean differences of both BMD and separate bone turnover markers with a power of 80% and alpha of 0.05. This resulted in detection of a mean difference of LS BMD of 0.021 g/cm2 in trans women and 0.022 g/cm 2 in trans men. Regarding bone turnover markers, in trans women a 10% change in CTx, 9% change in P1NP, 4% change in ALP, and 6% change in sclerostin could be detected. In trans men, a change of 10%, 13%, 6%, and 7% could be detected, respectively. Lastly, Pearson correlations were calculated between change in BTMs and BMD and are displayed with corresponding 95% CI.

## Results

### General

A total of 253 people were included in this study (Figure 1), which consisted of 121 trans women with median age of 30 (IQR 24 to 41) years and 132 trans men with median age of 24 (IQR 21 to 33) years. The baseline and follow-up characteristics are displayed in Table 1. In trans women, a median increase in estradiol of 129 pmol/L (IQR 56 to 232) implying a percentage change of estradiol of 128% (IQR 52 to 214) and a median decrease in testosterone of -18 nmol/L (IQR -22 to -14) with

a percentage decrease of -96% (IQR -97 to -94) was seen during the first year of HT. In trans men, a median increase in estradiol of 46 pmol/L (IQR -304 to 135) with a percentage change of 26% (IQR -63 to 198), which was accompanied by a median increase in testosterone of 27 nmol/L (IQR 20 to 38) and percentage increase of 2248% (IQR 1311 to 3338) was seen during the first year of HT.

In both groups, the BMI increased and tobacco use decreased during 1 year of HT (Table 1).



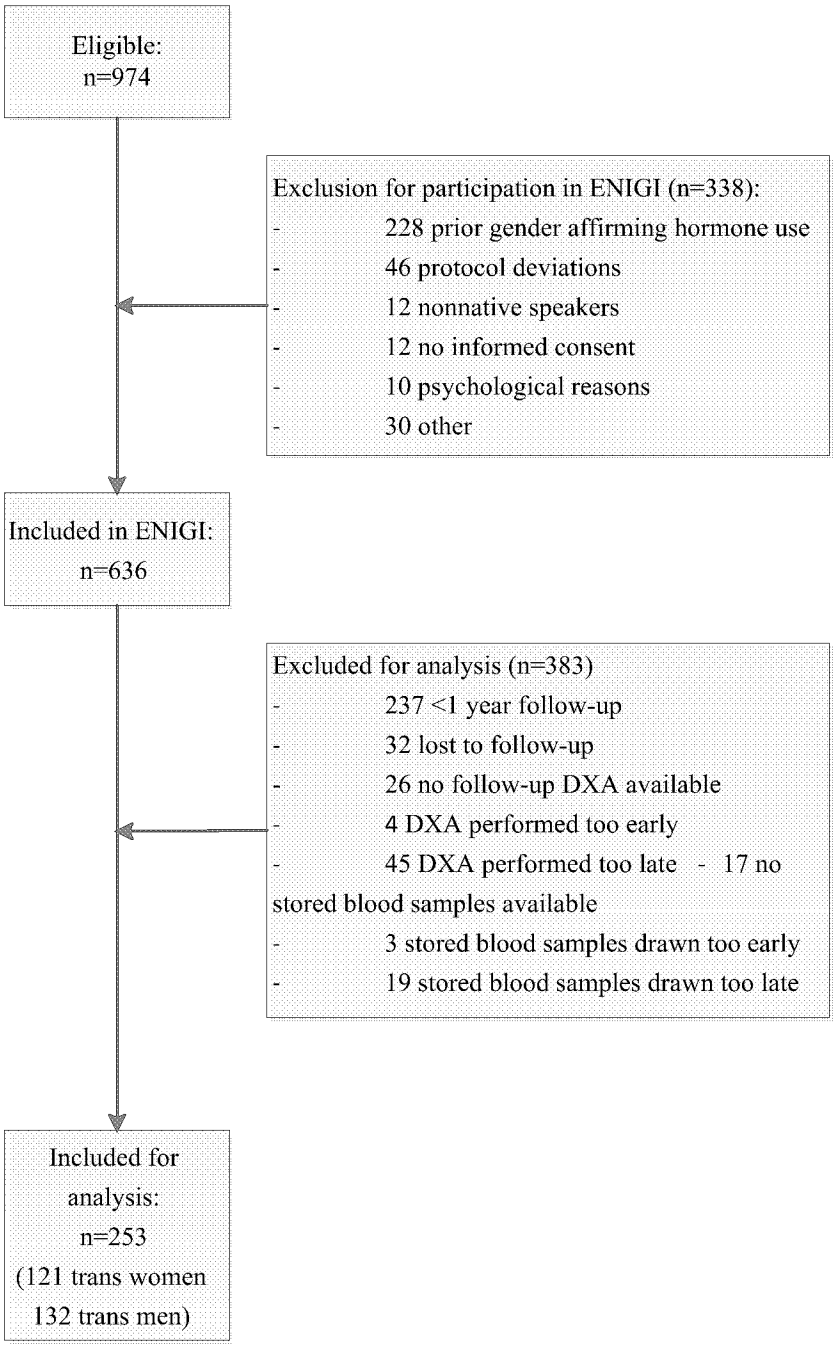



**Figure 1.** Flowchart of in- and exclusion of participants. ENIGI = European Network for Investigation of Gender Incongruence.

**Table 1.** Participant characteristics at baseline and after 1 year of HT

| | Trans women (n=121) | | Trans men (n=132) | |
|---|---|---|---|---|
| | **Baseline** | **1 year of HT** | **Baseline** | **1 year of HT** |
| General | | | | |
| Age, yr (median, IQR) | 30 (24 – 41) | | 24 (21 – 33) | |
| Ethnicity (% Caucasian) | 96.7 | | 91.7 | |
| BMI, kg/m$^2$ (median, IQR) | 22.9 (20.8 – 26.1) | 24.1 (21.9 – 26.3) | 24.5 (21.4 – 29.0) | 25.4 (22.5 – 29.2) |
| Tobacco use (% yes) | 24.0 | 14.0 | 29.2 | 20.0 |
| - Cigarettes/day (median, IQR) | 10 (5 – 10) | 6 (4 – 20) | 8 (4 – 15) | 7 (3 – 15) |
| Alcohol use (% yes) | 46.3 | 45.6 | 51.2 | 47.5 |
| - Units/week (median, IQR) | 2 (1 – 5) | 2 (2 – 4) | 2 (1 – 4) | 3 (2 – 5) |
| Biochemical results (median, IQR) | | | | |
| Estradiol, pmol/L | 105 (84 – 133) | 204 (137 – 328) | 187 (67 – 525) | 181 (132 – 261) |
| Testosterone, nmol/L | 19.0 (14.0 – 23.0) | 0.7 (0.5 – 1.0) | 1.3 (1.0 – 1.7) | 29 (20 – 39) |
| LH, U/L | 3.2 (2.3 – 4.3) | 0.1 (0.1 – 0.1) | 5.0 (2.7 – 6.9) | 1.5 (0.2 – 3.6) |
| 25OHD, nmol/L | 39 (25 – 57) | 60 (40 – 76) | 54 (30 – 77) | 57 (41 – 80) |
| Creatinine, µmol/L (mean ± SD) | 77 ± 10 | 73 ± 10 | 66 ± 10 | 79 ± 12 |
| AST, U/L | 24 (20 – 28) | 20 (17 – 23) | 21 (19 – 25) | 24 (20 – 28) |
| ALT, U/L | 22 (16 – 30) | 21 (15 – 27) | 17 (13 – 24) | 22 (17 – 29) |
| γGT, U/L | 20 (15 – 28) | 19 (15 – 26) | 15 (12 – 23) | 17 (12 – 26) |

HT = gender affirming hormonal treatment, IQR = interquartile range, LH = luteinizing hormone, AST = aspartate transaminase, ALT = alanine transaminase, γGT = gamma-glutamyltransferase

## Trans women

ALP, CTx, and sclerostin decreased with 19% (95%CI -21 to -16), 11% (95%CI -18 to -4), and 8% (95%CI -13 to -4), respectively, in the unadjusted model after 1 year of HT (Table 2). Adjusting the percentage changes in all BTMs for changes in BMI, smoking habits, alcohol use, 25OHD, creatinine, AST, ALT, and γGT concentrations did not affect the results (Table 2). No difference between the different age groups in change in BTMs were found (Figure 2). Sclerostin decreased in all but the lowest estradiol quartile (Figure 3).

## Trans men

P1NP, ALP, and sclerostin increased with 33% (95%CI 24 to 42), 16% (95%CI 12 to 20), and 15% (95%CI 10 to 20), respectively, after 1 year of HT (Table 2). Adjusting these percentage changes in BTMs for changes in BMI, smoking, alcohol use, creatinine, 25OHD, AST, ALT, and -GT did not affect the results (Table 2). More detailed analyses based on the earlier specified age groups revealed an opposite effect on bone turnover in the trans men aged ≥50 years after 1 year of HT compared to the younger trans men (Figure 2). In trans men aged ≥50 years, a decrease in P1NP of -19% (95%CI -35 to -4), CTx of -32% (95%CI -50 to -13), and sclerostin of -10% (95%CI -19 to -0) were found. Estradiol concentrations increased more in the trans men aged ≥50 years (median increase of 135 pmol/L, IQR 100 to 164) compared to trans men <50 years (median increase of 30 pmol/L, IQR -336 to 124). Different absolute

**Table 2.** Baseline and 1 year concentrations of bone turnover markers and BMD with corresponding percentage change (mean and 95%CI), for trans women and trans men separately.

| | Baseline | 1 yr HT | Percentage change % | Percentage change % adjusted [a] |
|---|---|---|---|---|
| **Trans women** | | | | |
| Bone turnover markers | | | | |
| P1NP, µg/L (median, IQR) | 50 (42 – 65) | 48 (38 – 62) | -3 (-9 ; 3) | -8 (-17 ; 1) |
| 18-30 years | 61 (49 – 74) | 52 (47 – 75) | -2 (-10 ; 6) | n.a. |
| 30-50 years | 48 (38 – 52) | 46 (35 – 54) | +2 (-10 ; 14) | n.a. |
| ≥50 years | 40 (33 – 43) | 29 (22 – 39) | -15 (-29 ; -1) | n.a. |
| ALP, U/L (mean ± SD) | 70 ± 17 | 57 ± 17 | -19 (-21 ; -16) | -21 (-25 ; 18) |
| 18-30 years | 72 ± 19 | 60 ± 18 | -17 (-21 ; -13) | n.a. |
| 30-50 years | 69 ± 16 | 53 ± 13 | -23 (-27 ; -19) | n.a. |
| ≥50 years | 67 ± 13 | 58 ± 19 | -14 (-24 ; -4) | n.a. |
| CTx, ng/L (median, IQR) | 428 (306 – 538) | 329 (265 – 442) | -11 (-18 ; -4) | -11 (-23 ; 1) |
| 18-30 years | 507 (387 – 658) | 351 (309 – 476) | -17 (-26 ; -9) | n.a. |
| 30-50 years | 371 (275 – 500) | 313 (265 – 452) | -1 (-17 ; 14) | n.a. |
| ≥50 years | 287 (198 – 369) | 224 (165 – 279) | -12 (-32 ; 7) | n.a. |
| Sclerostin, pmol/L (median, IQR) | 10.4 (8.6 – 14.9) | 8.8 (7.3 – 13.5) | -8 (-13 ; -4) | -9 (-16 ; -2) |
| 18-30 years | 8.8 (7.7 – 11.0) | 7.7 (6.6 – 9.4) | -8 (-15 ; -0) | n.a. |
| 30-50 years | 11.4 (9.4 – 15.0) | 11.0 (8.1 – 13.4) | -9 (-15 ; -2) | n.a. |
| ≥50 years | 17.7 (16.0 – 21.9) | 17.9 (14.1 – 18.5) | -10 (-22 ; 2) | n.a. |
| DXA | | | | |
| BMD TH g/cm$^2$ (mean ± SD) | 0.938 ± 0.137 | 0.947 ± 0.137 | +1.0 (0.5 ; 1.5) | +0.8 (0.1 ; 1.6) |
| BMD FN g/cm$^2$ (mean ± SD) | 0.797 ± 0.127 | 0.812 ± 0.129 | +1.9 (1.3 ; 2.5) | +1.6 (0.7 ; 2.5) |
| BMD LS g/cm$^2$ (mean ± SD) | 0.968 ± 0.139 | 1.004 ± 0.138 | +3.8 (3.1 ; 4.6) | +3.2 (2.0 ; 4.4) |
| **Trans men** | | | | |
| Bone turnover markers | | | | |
| P1NP, µg/L (median, IQR) | 56 (43 – 71) | 71 (49 – 100) | +33 (24 ; 42) | +29 (11 ; 48) |
| 18-30 years | 60 (50 – 77) | 85 (67 – 111) | +42 (30 ; 54) | n.a. |
| 30-50 years | 40 (36 – 52) | 53 (37 – 60) | +21 (10 ; 33) | n.a. |
| ≥50 years | 46 (41 – 66) | 41 (29 – 55) | -19 (-35 ; -4) | n.a. |
| ALP, U/L (mean ± SD) | 67 ± 19 | 76 ± 22 | +16 (12 ; 20) | +15 (7 ; 23) |
| 18-30 years | 68 ± 20 | 80 ± 23 | +19 (14 ; 24) | n.a. |
| 30-50 years | 62 ± 18 | 68 ± 17 | +14 (5 ; 24) | n.a. |
| ≥50 years | 72 ± 21 | 65 ± 23 | -12 (-24 ; 1) | n.a. |
| CTx, ng/L (median, IQR) | 423 (323 – 533) | 432 (313 – 529) | +3 (-4 ; 10) | -5 (-19 ; 8) |
| 18-30 years | 448 (384 – 590) | 442 (364 – 586) | +3 (-4 ; 11) | n.a. |
| 30-50 years | 297 (222 – 386) | 313 (215 – 387) | +12 (-6 ; 30) | n.a. |
| ≥50 years | 427 (305 – 547) | 222 (193 – 381) | -32 (-50 ; -13) | n.a. |
| Sclerostin, pmol/L (median, IQR) | 8.7 (6.8 – 13.1) | 10.3 (7.9 – 13.2) | +15 (10 ; 20) | +10 (-0 ; 20) |
| 18-30 years | 7.6 (6.5 – 9.5) | 8.8 (7.4 – 11.3) | +20 (13 ; 26) | n.a. |
| 30-50 years | 13.9 (8.8 – 16.8) | 14.3 (10.8 – 18.1) | +10 (1 ; 19) | n.a. |
| ≥50 years | 15.9 (14.8 – 17.8) | 15.3 (13.2 – 16.3) | -10 (-19 ; -0) | n.a. |

**Table 2.** (continued)

| | Baseline | 1 yr HT | Percentage change % | Percentage change % adjusted [a] |
|---|---|---|---|---|
| DXA | | | | |
| BMD TH g/cm² (mean ± SD) | 0.948 ± 0.113 | 0.956 ± 0.114 | +0.9 (0.4 ; 1.4) | +0.0 (-0.9 ; 0.9) |
| BMD FN g/cm² (mean ± SD) | 0.833 ± 0.116 | 0.825 ± 0.116 | -0.9 (-1.6 ; -0.1) | -2.5 (-3.7 ; -1.2) |
| BMD LS g/cm² (mean ± SD) | 1.026 ± 0.125 | 1.036 ± 0.129 | +1.0 (0.4 ; 1.7) | +2.1 (0.9 ; 3.4) |

HT = gender-affirming hormonal treatment, IQR = interquartile range, ALP = alkaline phosphatase, n.a. = not applicable.

[a] adjusted for changes in BMI, alcohol and tobacco use, 25OHD, creatinine, AST, ALT, and -GT. Data only shown for the total adjusted group, as separate adjusted age groups resulted in too small groups for multivariable analyses.



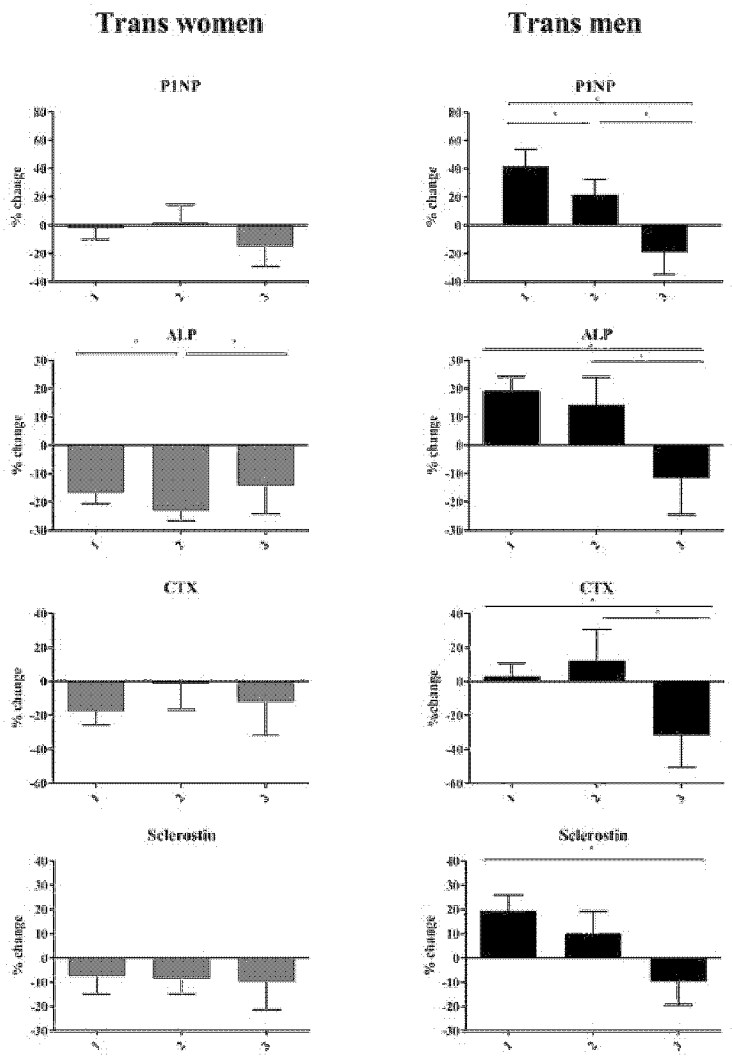



**Figure 2.** Percentage change in bone turnover markers in trans women and trans men after 1 year of HT, stratified for age groups. Group 1 = 18 to 30 years (trans women mean age 24 (2.9 SD), n = 61, trans men mean age 23 (3.0 SD), n = 91). Group 2 = 30 to 50 years (trans women mean age 39 (5.2 SD), n = 42, trans men mean age 39 (5.9 SD), n = 32). Group 3 = group ≥50 years (trans women mean age 56 (5.8 SD), n = 18, trans men mean age 54 (4.1 SD), n = 9). ALP = alkaline phosphatase. * p ≤ 0.05.

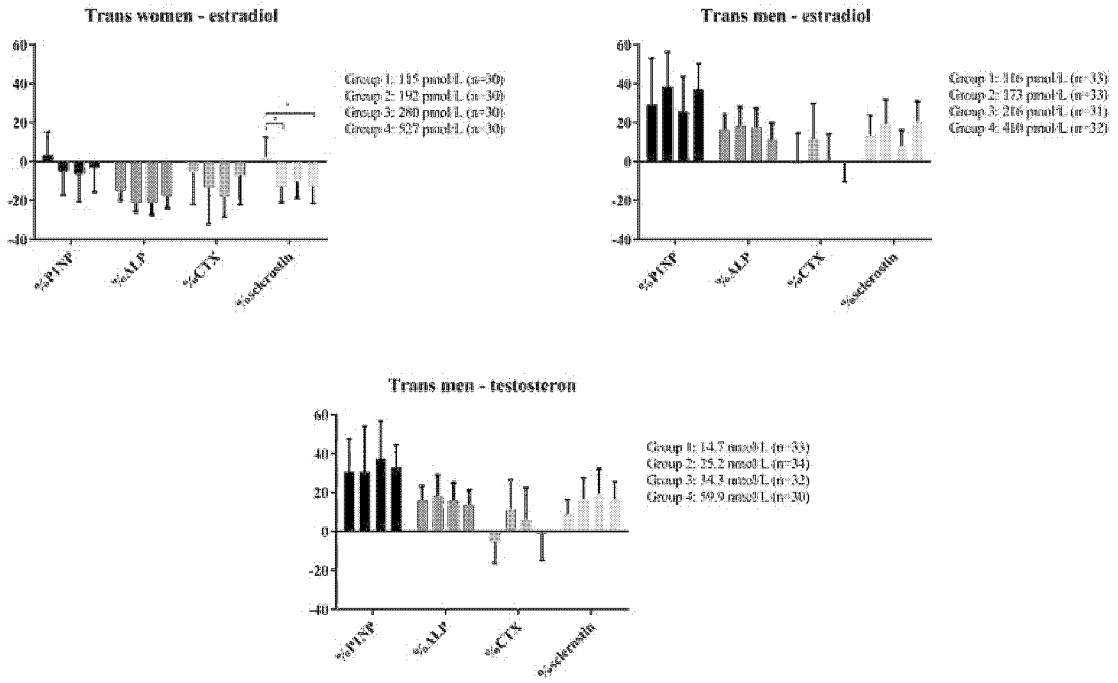



**Figure 3.** Percentage change in bone turnover markers by quartiles of average estradiol and testosterone concentrations measured at 3 and 12 months after baseline. Testosterone concentrations in trans women were <2 nmol/L, and therefore this group was not further divided into subgroups. ALP = alkaline phosphatase. * $p \leq 0.05$.

concentrations of testosterone and estradiol concentrations during HT did not result in different effects in the course of BTMs during 1 year of HT (Figure 3).

## Correlations between BTMs and BMD

Correlations between percentage change in BTM and percentage change in BMD for trans women and trans men are displayed in Table 3. The changes in BMD after 1 year of HT in this transgender population was described extensively in earlier research (5). In trans women, an increase in sclerostin was associated with a decrease in TH BMD. No correlations between change in BTMs and FN BMD were seen. Furthermore, P1NP, ALP, and CTx showed a modest negative correlation with LS BMD after 1 year of HT. In trans men, only P1NP showed a modest negative correlation with TH and FN BMD. Lastly, CTx showed a modest negative correlation with LS BMD in trans men (Table 3). Lastly, subgroup analyses were performed based on the baseline LS BMD data, which we divided into tertiles. This resulted in a mean (± SD) BMD of group 1 (0.817 ± 0.065), group 2 (0.972 ± 0.036), and group 3 (1.120 ± 0.082) in trans women. In trans men, this resulted in a mean BMD (± SD) of group 1 (0.893 ± 0.050), group 2 (1.017 ± 0.029), and group 3 (1.167 ± 0.075). Based on these tertiles, we evaluated changes of bone turnover markers per tertile. This resulted in a decrease of P1NP, CTx, ALP, and sclerostin in trans women, which was similar for all tertiles. In trans men, an increase of all markers except CTx was found, which was similar for the tertiles. In CTx, an increase was found in all but the highest BMD group (group 3).

**Table 3.** Correlation between percentage change in bone turnover markers and percentage change in BMD (mean and 95%CI), separately for trans women and trans men..

| Trans women | TH BMD % | FN BMD % | LS BMD % |
|---|---|---|---|
| P1NP % | -0.10 (-0.27 ; 0.09) | -0.15 (-0.32 ; 0.03) | **-0.28 (-0.44 ; -0.11)** |
| ALP % | -0.02 (-0.19 ; 0.16) | 0.00 (-0.18 ; 0.18) | **-0.22 (-0.38 ; -0.04)** |
| CTx % | -0.08 (-0.25 ; 0.10) | -0.17 (-0.34 ; 0.01) | **-0.27 (-0.43 ; -0.10)** |
| Sclerostin % | **-0.21 (-0.38 ; -0.03)** | -0.02 (-0.20 ; 0.17) | 0.03 (-0.15 ; 0.21) |
| **Trans men** | **TH BMD %** | **FN BMD %** | **LS BMD %** |
| P1NP % | **-0.21 (-0.37 ; -0.04)** | **-0.20 (-0.36 ; -0.02)** | -0.15 (-0.32 ; 0.02) |
| ALP % | -0.12 (-0.29 ; 0.06) | -0.05 (-0.23 ; 0.12) | -0.12 (-0.29 ; 0.05) |
| CTx % | -0.09 (-0.26 ; 0.09) | -0.11 (-0.28 ; 0.06) | **-0.21 (-0.38 ; -0.04)** |
| Sclerostin % | 0.08 (-0.10 ; 0.25) | -0.04 (-0.21 ; 0.14) | -0.08 (-0.25 ; 0.10) |

ALP = alkaline phosphatase

Bold text indicates p ≤ 0.05



# Discussion

This study evaluates changes in a variety of BTMs, sclerostin, and its correlation with changes in BMD in transgender people during the first year of HT. In trans women, a decrease in bone turnover was seen during the first year of HT, irrespective of age. In trans men, bone turnover increased in the younger groups, and decreased in the oldest trans men. No differences were seen between the different estrogen concentrations and percentage change in BTMs. Lastly, BTMs showed some modest negative correlations with predominantly changes in LS BMD of trans women.

## Effects on bone turnover after one year of HT

### Trans women

This is the first study to evaluate sclerostin concentrations in transgender people. It is known that sclerostin concentrations are higher in men than women and sclerostin increases gradually with age in both sexes [41]. We found that sclerostin decreased in trans women after 1 year of HT. Previous research suggested that estrogen results in a decrease in sclerostin, which is thought to result in an increase in BMD, as sclerostin is an important inhibitor of the anabolic Wnt/−catenin signaling pathway in osteoblasts [28,29]. An earlier study in premenopausal estrogen-sufficient women did not show changes in serum concentrations of sclerostin during their menstrual cycle and also did not show a relationship with estradiol concentrations [42]. Withdrawal of estrogens however, resulted in an increase in sclerostin in both post-menopausal women and elderly men, suggesting an inverse association between sclerostin and estrogen concentrations [43]. A longitudinal study in Japanese women also showed a decrease in estrogen and increase in sclerostin concentrations during menopause, which resulted in increased bone resorption [7]. The current study indeed showed a decrease in sclerostin in trans women after 1 year of HT. This finding provides additional evidence that estrogen treatment results in a decrease in sclerostin concentrations which has beneficial

effects on bone turnover. This finding aligns well with another study showing that treatment of postmenopausal women with the SERM raloxifene suppresses sclerostin [44].

The finding that CTx decreased during HT is also in line with the hypothesis that the increase in estrogen concentrations reduces osteoclast activity and thereby inhibits bone resorption. Although one study found no change in CTx due to HT in trans women [22], two other studies also found a decrease in CTx concentrations within 2 years of HT and lower CTx concentrations compared to control men after 8 years of HT [23,45]. Furthermore, ALP decreased during HT. A decrease in ALP was earlier found within the first year of HT [15] and during longer follow up [12]. Earlier studies also showed a decrease in P1NP within 2 years of HT [45], and lower P1NP concentrations after 8 years of HT compared with control men [23], while one study showed no changes in P1NP after 3 years of HT [22]. Lastly, the lowest estradiol quartile showed opposite or even no changes in BTMs compared to the other three estradiol quartiles in trans women, which implies that the estrogen concentrations in the lowest quartile might be too low to result in a decrease in bone turnover. Overall, the decrease in BTMs in trans women further support the bone preserving role of estrogens.



## Trans men

Sclerostin increased in the younger trans men after 1 year of HT. The effect of androgens on sclerostin concentrations are not fully elucidated yet. An earlier study found a possible direct androgen receptor-mediated effect on the production of sclerostin and negative correlation between sclerostin concentration and testosterone concentrations in birth-assigned men [46]. However, the current study did not show a decrease in sclerostin in trans men who had higher testosterone concentrations after 1 year of HT. On the other hand, another study showed that predominantly estrogen and not testosterone mediated the decrease of sclerostin [43]. However, as both testosterone and estradiol concentrations changed in trans men, we were not able to determine the isolated effect of testosterone. Also, this result can be explained by the use of different sclerostin assays in previous literature with sometimes high variability between various sclerostin assays [47].

With regard to bone formation, an increase in P1NP and ALP was seen after 1 year of HT. An earlier prospective study also showed an increase in P1NP, in respect of no changes in control women [24]. Furthermore, P1NP concentrations in trans men aged 37±8 years were approximately 25% higher compared to control women after 10 years of HT [24], which is comparable to the 21% change reported in our study in this age group. Regarding ALP, earlier studies showed an increase of approximately 13% in ALP within the first year of HT in trans men aged 16-40 years [15], which is also in line with the 13% change reported in our study. A previous study in postmenopausal women showed that ALP concentrations were higher compared to premenopausal women, and that ALP was negatively correlated with the estradiol concentration of the postmenopausal group [48]. The bone specific alkaline phosphatase fraction (BALP) instead of total ALP is a more sensitive parameter to evaluate bone formation, as increased serum ALP can also result from liver or gallbladder disease [38]. However, the trans men did not show signs of liver disease as all liver parameters besides ALP did not change during 1 year of HT, so it is not expected that this affected current results. As muscle

mass increased in trans men, which is resembled by an increase in creatinine after 1 year of HT, mechanical loading on bones increased, which possibly explains the increase in bone formation markers [31]. Concerning bone resorption, current study showed no increase in CTx in trans men after 1 year of HT. Earlier studies in trans men showed an increase in CTx after 1 year HT, compared to no changes in control women [20]. Also, trans men had higher CTx concentrations compared to control women after 10 years of HT [24]. CTx was measured in fasting state, just as in the studies mentioned before. As CTx is cleared by the kidney [38] higher concentrations of CTx can be found in case of impaired kidney function, yet our study population had no impaired kidney function. Alternatively, fasting state was based on self-report of the participants during follow-up. Therefore it is possible that some participants did not apply to the instructions to draw blood in fasting state. This might have masked the increase of CTx as CTx decreases due to food ingestion [38]. Lastly, when comparing age groups, the oldest trans men group showed a decrease in all BTMs and sclerostin in contrast to the younger trans men. The older group of trans men benefited most of HT as they were assumed to be estrogen deficient due their postmenopausal state at baseline (mean age 54 years, SD 4.1). In most studies in trans men, estradiol concentrations either remain stable or decrease slightly. However, two studies investigating the effect of testosterone in combination with an aromatase inhibitor found that estradiol concentrations remained stable in trans men using testosterone only, but decreased to great extent in trans men using both testosterone and aromatase inhibitor [49,50]. This indicates that the estradiol concentrations mainly result from aromatization of testosterone into estradiol. This is also supported by our finding that estradiol concentrations increased in trans men who were postmenopausal and therefore estrogen deficient before the start of HT. The increase in estrogen concentration after aromatization of testosterone resulted in decreased bone resorption, which further strengthens the beneficial role of estrogen on bone health.

### Associations between BTMs and BMD

Modest negative correlations were found between changes in BTMs and changes in BMD during 1 year of HT. This finding is in line with previous research in trans women, where no correlations between CTx and P1NP and volumetric BMD [vBMD] of the radius or tibia where found [22]. In trans men, only an inverse relationship with CTx and P1NP and vBMD at radius and tibia was found [24]. Changes in BTMs were predominantly correlated to the LS BMD of trans women. LS consists mainly of trabecular bone which is more metabolic active compared to the hip, that mainly consists of cortical bone [51,52]. This was not seen in the FN, although this region also contains significant trabecular bone albeit less compared to LS. This finding further emphasize the role of estradiol in maintaining adequate bone homeostasis, which is already studied well in men [53,54]. Also, earlier research about estrogen supplementation therapy in postmenopausal women showed an increase in BMD and a decrease in BMD after discontinuation of estrogen supplementation in a large female cohort [55]. Next to this, a murine model studying ovariectomized mice showed that estrogen therapy had more beneficial effects on bone architecture compared to mice treatment with testosterone alone [56]. Summarized, the increase in BMD after 1 year of HT in both trans women and trans men found in this study emphasizes the beneficial effect of estrogen on bone further.

### Strengths and limitations

Data for this study was collected during patient care following a standardized treatment protocol. As a result, this prospective study consisted of a large study population compared to other studies in transgender people, thereby ensuring a study population with a broad variation in age. Other strengths of this study were the use of the same BTM assays, all samples were thawed simultaneously, and all analyses were performed using one lot number. Moreover, this study is the first to evaluate the BTM sclerostin in transgender people. In addition, the same DXA scanner was used both at baseline and during follow-up.

This study also has some limitations. First, no control group was included and therefore changes in time as cause for changes in BTMs or BMD could not be evaluated. However, as the study population consisted of different age groups, almost all participants already had reached their peak bone mass and this would have resulted in decreasing BMD through time and increased bone turnover especially in postmenopausal women. From earlier literature it is known that bone turnover increases with age, after the initial high levels that are reached during puberty [57-59]. As part of standard patient care, participants were advised on healthy lifestyle and maintaining adequate calcium and 25OHD intake and physical activity. This resulted in changes in 25OHD concentrations during 1 year HT, but adjustments for these changes did not affect our results. No full data on earlier dietary calcium intake, weight-bearing exercise, steroid use, fractures, or family history were available. Furthermore, a three month measurement of bone turnover markers was not available. Lastly, due to the observational character of this study, this study was not designed to evaluate possible causal relationships. Nevertheless, this study contributes further to the current hypothesis that sclerostin indeed is a mediating factor in the anabolic effect of estradiol on bone turnover and BMD. Lastly, follow-up data regarding fractures were lacking.

To conclude, this study provides additional knowledge regarding the effect of HT on bone metabolism and BMD in transgender people and emphasizes the importance and beneficial effect of estrogen by decreasing bone turnover and increasing BMD. Summarized, this study shows that 1 year of HT does not result in deleterious effects on bone health in transgender people. Despite these results, effects after multiple years of HT, particularly for younger trans men, are of great interest to study in the future. Given the still increasing incidence and need for treatment of transgender people, additional studies should therefore be performed to evaluate the longer term relationships between change in bone turnover, BMD, and fracture risk during HT in transgender people.

## Disclosures

This work was supported by an unrestricted grant from Abbott diagnostics (Chicago, IL, United States of America) to authors Mariska Vlot (MV) and Annemieke Heijboer (AH). Sclerostin kits were provided by Diasorin, Saluggia, Italy. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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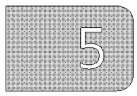
Authors' roles: Study design: MV, CW, GS, AH, MdH. Study conduct: MV, CW. Data collection: MV, CW. Data analysis: MV, CW. Data interpretation: MV, CW. Drafting manuscript: MV, CW. Revising manuscript content: MV, CW, GtS, RdJ, AH, MdH. Approving final version of manuscript: MdH takes responsibility for the integrity of the data analysis.



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# Part III

## EFFECTS OF INFLAMMATION AND AUTO-IMMUNE DISEASE ON BONE TURNOVER

# Chapter 6

# Effect of antiretroviral therapy on bone turnover and bone mineral density in men with primary HIV-1 infection

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# Abstract

## Introduction

Previous studies indicate that human immunodeficiency virus (HIV)-infection and combination antiretroviral therapy (cART) can affect bone turnover. Furthermore, HIV-infected patients have lower bone mineral density (BMD) compared to a healthy reference population.

## Objective

To evaluate the longitudinal effect of HIV-infection and cART on bone turnover markers (BTMs) and BMD in men with primary HIV-infection (PHI).

## Design, methods

Thirty-five PHI-men were divided into two groups, those that received cART for the first time (n=26) versus no-cART (n=9). Dual-energy X-ray absorptiometry (DXA) was performed on femoral neck (FN), total hip (TH) and lumbar spine (LS) and BTMs (P1NP, alkaline phosphatase, osteocalcin, ICTP and CTX) were measured at baseline and follow-up.

## Results

At baseline, the median CD4+ T-cell count was 455 cells/mm3 (IQR 320-620) and plasma viral load 5.4 log10 copies/mL (IQR 4.7-6.0) in the cART treated group, compared to 630 (IQR 590-910) and 4.8 (IQR 4.2-5.1) in the untreated group. The median follow-up time was 60.7 weeks (IQR 24.7-96.0). All BTMs, except ICTP, showed a significant increase during cART versus no changes of BTMs in the untreated group. FN and TH BMD showed a significant decrease in both groups. LS BMD did not change in both groups.

## Conclusion

Bone turnover increased in PHI-men treated with cART which was accompanied by a decrease in FN and TH BMD. No increase of bone turnover was seen in untreated PHI-men. Our study suggests that cART results in increased bone turnover and decreased BMD of the hip in PHI-men.

# Introduction

Studies have shown that HIV-infected patients often have a decreased bone mineral density (BMD) compared to NHANES reference groups, with an estimated prevalence of around 67% [1–4]. The accompanying higher risk of osteopenia, osteoporosis and fractures implies that monitoring their BMD is highly important [5–9]. Bone loss in HIV-infected patients seems to result from a combination of several contributing factors such as a lower vitamin D status, lower BMI and higher usage of alcohol and tobacco [7,10–12]. Also, combination antiretroviral therapy (cART) is associated with an even stronger decrease in BMD in HIV-infected patients [3,4,10,13]. A decline of 2-4 % of the total bone mass upon starting cART is known to occur within the first 2 years of cART [14,15]. Furthermore, a recent review showed that patients on cART were up to 2.5 times more prone to have T-scores <1 compared to untreated HIV-patients [11].



BMD measurements are assessed by a dual energy X-ray absorptiometry (DXA) scan. However, this method allows to estimate changes in bone mineral content which occur over years. In contrast, bone turnover markers (BTMs) reflect dynamic and short-term changes in bone remodeling. Therefore, the actual bone turnover is best reflected by measurements of BTMs as bone remodelling is a continuous and variable process. Bone resorption is displayed by resorption markers such as c-telopeptide crosslink of type 1 collagen (CTX) that is produced by osteoclasts and bone formation is reflected by formation markers such as procollagen type 1 N-terminal propeptide (P1NP) that is produced by osteoblasts. With regard to bone turnover, inflammatory cytokines and possibly viral HIV-proteins are thought to increase the activity of osteoclasts resulting in increased bone resorption [2,16,17]. Simultaneously, osteoblast activity is negatively influenced by HIV-proteins [18] and treatment with cART may result in increased bone turnover [4,9,14,19–21]. Altogether, these factors contribute to a lower BMD in HIV-infected persons.

A previous study in therapy-naïve primary HIV-infected (PHI) men, showed that bone turnover did not differ between those with a normal or a reduced BMD [10]. However, most other studies show higher BTMs in PHI-men [3,4,6,10,22–24]. These heterogeneous data might be caused by the choice of BTM, pre-analytical differences (for instance time of the blood withdrawal, fasting sample or not), the applicable reference interval of the BTM, or differences in patient characteristics (for instance males versus females). To date, the course and duration of the increase of BTMs during cART is not fully elucidated [20,25]. Therefore, the aim of this study is to assess the course of bone turnover and BMD in treated and untreated PHI-men.

# Methods

## Subjects and treatment protocol

Patients were selected from the PRIMO-SHM trial, a prospective multicenter cohort study in PHI-men from the Amsterdam Medical Center (AMC) in Amsterdam, the Netherlands, between February 2008 and October 2009, trial number ISRCTN59497461 [10,26]. The main inclusion criteria of this trial were age over 18 years and laboratory evidence of PHI, defined as having detectable plasma HIV-1 RNA with a negative or indeterminate Western blot, or in case of a positive Western blot, a documented negative HIV-RNA test within the previous 180 days [10,26]. The study was approved by the Ethical Committee of the AMC. All patients provided written informed consent.

For the current study, patients were selected if they had (i) measurement of the BTMs P1NP, alkaline phosphatase (ALP), osteocalcin (OC), and/or cross-linked carboxyterminal telopeptide of type I collagen (ICTP), CTX, at baseline and/or follow-up, and (ii) DXA-scan performed at baseline and/or follow-up. Patients were excluded in case they (A) suffered from medical conditions that possibly affected bone metabolism such as hypercalcaemia or recent corticosteroid therapy for at least three months, (B) reported earlier use of cART before entering the study or (C) if they needed to (re)start cART during the study because of decreasing CD4+ T-cell counts. Of the total of 43 patients, 35 eligible patients were included in this study.

The patients were divided into two groups, those who had initiated cART during PHI and those who remained untreated. Patients were treated with cART in case of confirmed CD4+ T-cell count < 350 cells/mm$^3$ or symptomatic HIV-disease. Twenty-six patients received early cART and nine patients remained untreated. Patients whom received cART were treated according to the PRIMO-SHM trial protocol [26]. Briefly, patients received a combination of emtricitabine (FTC), a nucleoside-analogue reverse transcriptase inhibitor (NRTI), tenofovirdisoproxil (TDF), a nucleotide-analogue transcriptase inhibitor, efavirenz (EFV) a non-nucleoside reverse-transcriptase inhibitor, and a combination of lopinavir and ritonavir, both protease inhibitors (PI). Lopinavir and ritonavir were discontinued when plasma viral load reached <50 $\log_{10}$ copies/mL. In case of drug resistance or side effects an adjusted cART combination was prescribed.

## Measurements

### *General*

Body weight and height were measured when performing the DXA-scan. Viral load and CD4+ T-cell count were measured at baseline. Fractures, calcium or vitamin D supplementation, smoking and alcohol use were also evaluated at baseline.

### *Bone turnover markers*

The formation markers P1NP, ALP and OC and resorption ICTP and CTX and were measured in serum after an overnight fast. P1NP and ICTP were measured using a radioimmunoassay (RIA) (both from Orion Diagnostica, Espoo, Finland) with an intra-assay coefficient of variation (CV) of 4-8% and 4-6%, respectively, and a lower limit of quantitation (LOQ) of 5 µgram/L and 1 µgram/L, respectively. CTX was measured using an electro-chemiluminescence immunoassay (ECLIA) (Roche diagnostics, Almere, the Netherlands) with a CV of <8% and LOQ of 10 ng/L. OC was measured using an immunometric assay (Biosource, Nivelles, Belgium) with an intra-assay CV of <5% and LOQ of 0.4 nmol/L. ALP was measured using a spectrophotometric assay (Roche Diagnostics, Almere, the Netherlands) with an interassay CV of 3% and LOQ of 5 IU/L.

### *Bone densitometry (DXA-scan)*

A DXA-scan (Hologic QDR 4500W, Hologic Inc, Bedford, MA, USA) was used to measure BMD of the lumbar spine (L1-L4) (LS), femoral neck (FN) and total hip (TH) of the non-dominant hip. Follow-up data on DXA-scans were available until December 2011. To evaluate osteopenia or osteoporosis status, T-scores and Z-scores were calculated based on the NHANES reference database. Based on

WHO criteria a T-score between minus 1 and 2.5 SD reflects osteopenia and minus 2.5 SD or less reflects osteoporosis. As this study comprehends generally younger patients who are supposed to have reached BMD values around the peak bone mass the Z-score is also displayed. The Z-score reflects the BMD SD of the patient compared to healthy age-matched controls with a Z-score of minus 2 SD or less reflecting osteoporosis.

### Statistics

Stata/SE 14.0 software (StataCorp, LP) was used for statistical analysis. Normality was tested by normality plots. Wilcoxon, Mann-Whitney U, Kruskall-Wallis or T-tests were used based on whether parametric or nonparametric tests were applicable. The median and interquartile range (IQR) were described, unless otherwise specified. Correlations were performed using a Spearman model. Furthermore, a regression model based on linear regression was used. Changes in absolute values between baseline and follow-up of BMD and BTM values were calculated as deltas (Δ) with corresponding 95% confidence intervals (CI) and *p*-values. A *p*-value of ≤0.05 was regarded as significant.



## Results

### Study population

The baseline characteristics of the 35 included patients are displayed in Table 1. The CD4+ T-cell count at baseline was lower in the treated versus the untreated group (95% CI 38 – 433, $p = 0.02$). To adjust for the difference in CD4+ levels between the two groups separate linear regression analyses were performed containing the outcome measures BMD, T- and Z-scores and all bone turnover markers. These analyses however did not affect the results (all *p*-values > 0.05, therefore data not shown). Only one patient in the treated group used vitamin D supplementation. All treated patients received a regimen containing TDF; 76.9% (n=20) received the triple-class drug regimen according to the study protocol. The total median follow-up time of our study was 60.7 weeks (IQR 24.7 – 96.0), with a longer follow-up time of the untreated group (median 96.6 weeks (IQR 37.7 – 97.4)) compared to the treated group. No hip or vertebral fractures were reported in both groups and no bisphosphonates were used. In the treated group an increase in median BMI was seen from 22.4 (IQR 21.3 – 25.6) to 23.4 (IQR 21.9 – 26.5) ($p = 0.02$), during follow-up. The untreated group showed a slight but not significant increase of BMI during follow-up.

### Bone turnover markers

All BTMs except ICTP increased during cART. P1NP had a mean increase of 22 µg/L (95% CI 9.9 – 33.3, $p = 0.0003$), ALP of 13.7 U/L (95% CI 5.3 – 22.1, $p = 0.0006$), OC of 6.9 nmol/L (95% CI 2.2 – 11.6, $p = 0.0022$), and lastly, CTX showed a mean increase of 225 ng/L (95% CI 113.6 – 337, $p = 0.0012$). Only the CTX concentration was significantly higher in the treated group compared to the untreated group at follow-up ($p = 0.02$). In the untreated group no changes in the BTMs were found. All changes between baseline and follow-up of BTMs are displayed in Fig 1 and 2.

**Table 1.** Baseline results of PHI-men.

| Total n = 35 | cART treated, n = 26 | Untreated, n = 9 | *p*-value |
|---|---|---|---|
| General | | | |
| Age (y) mean/SD | 39.0 (9.9) | 39.5 (9.1) | 0.97 |
| BMI | 22.4 (21.3 – 25.6) | 23.4 (21.2 – 24.0) | 0.94 |
| Ethnicity (%) Caucasian | 84.6 | 100 | 0.22 |
| MSM (%) | 88.5 | 100 | 0.29 |
| Current smoking (%) | 38.5 | 44.4 | 0.76 |
| Excessive alcohol use* (%)* defined as using ≥ 3 U/day | 11.5 | 0 | 0.24 |
| Vitamin D deficiency (≤50 nmol/L)(%) | 30.8 | 44.4 | 0.46 |
| Vitamin D supplementation use (%) | 3.9 | 0 | 0.56 |
| HIV parameters | | | |
| CD4+ T-cell count (cells/$mm^3$) | 455 (320 – 620) | 630 (590 – 910) | 0.02* |
| Plasma HIV-RNA ($log_{10}$ copies/mL) | 5.4 (4.7 – 6.0) | 4.8 (4.2 – 5.1) | 0.40 |
| Duration of cART therapy (weeks) | 60.3 (24.4 – 90.9) | N.A. | N.A. |
| Bone turnover markers | | | |
| P1NP (µg/L) | 37 (28 – 46) | 41 (38 – 53) | 0.27 |
| ALP (U/L) | 66 (60 – 72) | 71 (59 – 88) | 0.85 |
| OC (nmol/L) | 7.3 (4.3 – 10.3) | 8.8 (2.7 – 11.2) | 0.76 |
| ICTP (µg/L) | 3 (2.6 – 4.2) | 3 (2.7 – 3.1) | 0.52 |
| CTX (ng/L) | 304.5 (146 – 384) | 191 (124 – 358) | 0.57 |
| Bone mineral density | | | |
| BMD Femoral neck (g/$cm^2$) | 0.840 (0.771 – 0.941) | 0.806 (0.736 – 0.879) | 0.48 |
| BMD Total hip (g/$cm^2$) | 0.968 (0.886 – 1.068) | 1.00 (0.904 – 1.074) | 0.61 |
| BMD Lumbar spine (g/$cm^2$) | 0.962 (0.872 – 1.111) | 0.995 (0.931 – 1.023) | 0.73 |
| T–score Femoral neck | - 0.7 ( - 1.2 to 0.1) | - 1.0 ( - 1.5 to 0.4) | 0.48 |
| Z-score Femoral neck | - 0.3 ( - 0.9 to 0.3) | - 0.4 ( - 1.0 to 0.1) | 0.70 |
| T–score Total hip | - 0.4 ( - 1.0 to 0.2) | - 0.3 ( - 0.9 to 0.3) | 0.64 |
| Z-score Total hip | - 0.2 ( - 0.8 to 0.3) | - 0.1 ( - 0.5 to 0.5) | 0.49 |
| T–score Lumbar spine | - 1.2 ( - 2.0 to 0.2) | - 0.9 ( - 1.5 to - 0.6) | 0.81 |
| Z-score Lumbar spine | - 1.1 ( - 1.9 to 0.3) | - 0.8 ( - 1.4 to - 0.6) | 0.73 |
| Osteopenia (%)**in either hip or spine | 38.5 | 44.4 | 0.63 |
| Osteoporosis (%)**in either hip or spine | 15.4 | 11.1 | 0.76 |

Data are expressed as median/IQR, or percentages unless specified otherwise. Abbreviations: PHI = Primary HIV Infection, cART = combination anti-retroviral therapy, N.A. = not applicable, BMI = body mass index, MSM = men who have sex with men, BMD = bone mineral density, P1NP = procollagen type I propeptide, ALP = alkaline phosphatase, OC = osteocalcin, ICTP = cross-linked carboxyterminal telopeptide of type I collagen, CTX = C-terminal telopeptide of type 1 collagen,

* = $p \leq 0.05$

## BMD measurements

53.9 % of the patients in both treated and untreated group had either osteopenia or osteoporosis of the hip or spine at baseline. Osteopenia was more prevalent than osteoporosis in both groups. At baseline, no differences were observed between the different anatomical BMD sites in the treated verses untreated group. During treatment FN and TH BMD decreased with - 0.044 g/cm2 (95% CI - 0.067 to - 0.020, $p = 0.002$) and - 0.042 (95% CI - 0.067 to - 0.020, $p = 0.0003$). In the untreated group a decrease of FN of - 0.019 (95% CI - 0.038 to 0.000, $p = 0.05$) and decrease of TH of - 0.039

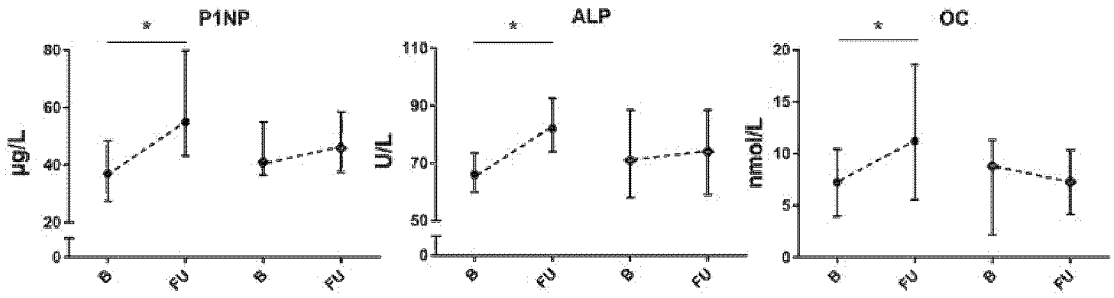


**Figure 1.** Bone formation markers in PHI-men, * = significant difference p ≤ 0.05 of follow-up versus baseline, ● = cART treated group, ♦ = untreated group, B = baseline, median and IQR, FU = follow-up, median and IQR.



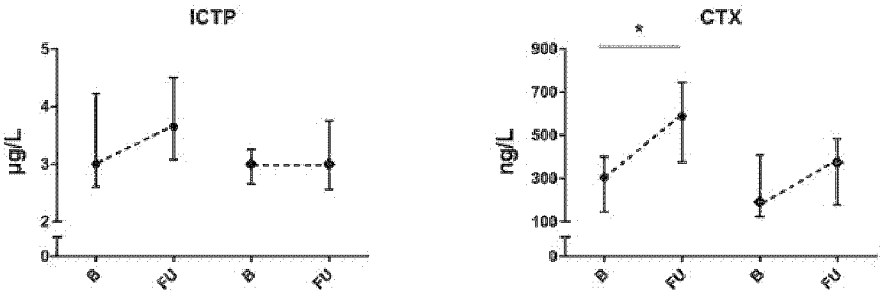


**Figure 2.** Bone resorption markers in PHI-men, * = significant difference p ≤ 0.05 of follow-up versus baseline, ● = cART treated group, ♦ = untreated group, B = baseline, median and IQR, FU = follow-up, median and IQR.

(95% CI - 0.063 to - 0.014, $p$ = 0.03) was seen. LS BMD did not change in both groups. In line with the BMD, also T- and Z-scores changed during follow-up. T-scores of FN and TH decreased with - 0.32 (95% CI - 0.50 to - 0.15, $p$ = 0.002) and - 0.28 (95% CI - 0.38 to - 0.18, $p$ = 0.0002) in the treated group. The Z-score of FN decreased with - 0.28 (95% CI - 0.45 to - 0.10, $p$ = 0.006) and the Z-score of TH decreased with - 0.25 (95% CI - 0.36 to - 0.14, $p$ = 0.0003) in the treated group. In the untreated group T-scores decreased as well in the FN with - 0.15 (95% CI - 0.31 to 0.01, $p$ = 0.05) and in the TH with - 0.23 (CI 95% - 0.42 to 0.05, $p$ = 0.04). Only the Z-score of TH decreased with - 0.25 (95% CI - 0.41 to - 0.09, $p$ = 0.03) Again, LS T- and Z-scores did not change in both groups. No significant differences between treated and untreated group were found at follow-up. All measurements at baseline and follow-up of BMD, T- and Z-scores are displayed in Table 2. Only TH Z-score and OC at baseline displayed a negative association (rho - 0.36, $p$ = 0.04). No other significant correlations were found between any of the BTMs and BMD measurements or T-scores in the treated and untreated PHI-men.

## Discussion

Our study explored the effect of HIV-infection and the use of cART on bone turnover and BMD in PHI-men. In men treated with cART, bone turnover increased accompanied by a decrease in BMD of the FN and TH. In the untreated group a slight decrease in FN and TH BMD was observed as well. Nevertheless, bone turnover did not change.

**Table 2.** Bone mineral density in PHI-men, median and IQR

| | cART treated group | | Untreated group | |
|---|---|---|---|---|
| | baseline | follow-up | baseline | follow-up |
| Femoral neck | | | | |
| BMD (g/cm$^2$) | 0.840 (0.771 - 0.941) | 0.781 (0.727 - 0.902)* | 0.806 (0.736 - 0.879) | 0.785 (0.688 - 0.892)* |
| Z-score | - 0.3 (- 0.9 - 0.3) | - 0.5 (- 1.0 - 0.3)* | - 0.4 (- 1.0 - 0.1) | - 0.4 (- 1.1 - 0.1) |
| T-score | - 0.7 (- 1.2 - 0.1) | - 1.1 (- 1.5 to - 0.2)* | - 1.0 (- 1.5 to - 0.4) | - 1.1 (- 1.8 to - 0.3)* |
| Total hip | | | | |
| BMD (g/cm$^2$) | 0.968 (0.886 - 1.068) | 0.936 (0.839 - 1.017)* | 1.0 (0.904 - 1.074) | 1.0 (0.884 - 1.042)* |
| Z-score | - 0.2 (- 0.8 - 0.3) | - 0.5 (- 1.2 - 0.1)* | - 0.1 (0.5 to - 0.5) | - 0.1 (- 0.7 - 0.2)* |
| T-score | - 0.4 (- 1.0 - 0.2) | - 0.6 (- 1.3 to - 0.1)* | - 0.3 (- 0.9 - 0.3) | - 0.3 (- 1.0 - 0.1)* |
| Lumbar spine | | | | |
| BMD (g/cm$^2$) | 0.962 (0.872 - 1.111) | 0.959 (0.876 - 1.012) | 0.995 (0.931 - 1.023) | 0.970 (0.915 - 1.031) |
| Z-score | - 1.1 (- 1.9 - 0.3) | - 1.2 (- 2.0 to - 0.2) | - 0.8 (- 1.4 to - 0.6) | - 1.1 (- 1.3 to - 0.5) |
| T-score | - 1.2 (- 2.0 - 0.2) | - 1.2 (- 2.0 to - 0.6) | - 0.9 (- 1.5 to - 0.6) | - 1.1 (- 1.6 to - 0.5) |

* = significant difference $p \leq 0.05$ of follow-up versus baseline.



## Bone turnover in PHI-men

P1NP, ALP, OC and CTX levels increased in the treatment group, only ICTP did not change. ICTP is also secreted by other tissues than bone which have masked the effect of cART. Literature reports that the same [7,14,19,21,22,27] and also other BTMs such as bone specific alkaline phosphatase (BALP), pyridoline and deoxypyridinoline crosslinks, may increase during cART [9,24,28]. A decrease of CTX was also described in one study [28], although CTX measurements were performed after several years of cART treatment in this study. Peak levels of BTMs are generally seen with 12-48 weeks after initiation of cART therapy, followed by a plateau phase, which persists for several years during ongoing cART therapy [4,19,20,24,25,27]. In our study, both bone resorption and formation markers increased within the median treatment period of 60 months of cART. The increased bone turnover in the treated group could result from TDF containing cART which is known to mediate mitochondrial dysfunction resulting in cytokine production that stimulate osteoclast maturation and therefore promotes bone resorption [16,18,23]. TDF containing cART is associated with higher levels of CTX, P1NP and OC and BALP compared to cART without TDF [18,20,]. Furthermore, it is known that the activity of osteoclasts can increase while osteoblastogenesis decreases due to PI containing cART, resulting in higher concentrations of pyridoline and deoxypyridinoline crosslinks, which resembles a state of increased bone turnover [28]. On the other hand, PHI itself is thought to limit the function of osteoblasts and stimulate the osteoclasts which may have influenced the bone turnover and BMD as well [17,26]. However, our study questions this direct effect of chronic inflammation due to HIV itself on bone turnover, since we found no changes in BTMs during follow-up in the untreated PHI group.

## Bone mineral density in PHI-men

At baseline, BMD and T-scores were lower in treated and untreated PHI-men compared to the reference group of NHANES, which is in line with a previous study [11]. The lower BMD can result from several risk factors that are known to attribute to lower BMD such as decreased physical activity, drug use, smoking and alcohol consumption, which were described more prevalent in men who have sex with men (MSM) than hetero-sexual men, and dispose high prevalence in our study population as well [11,18,26,29]. Interestingly, Grijsen et al. described a lower BMD in both HIV-positive, but also HIV-negative MSM, indicating that the lower BMD could be pre-existent in MSM and therefore be unrelated to PHI itself [10,30].

During follow-up BMD and T-scores of the hip decreased significantly in both groups. This raises concern as baseline BMD and T-scores were already low. The hip BMD decreased less in the untreated group compared to the treated group. Several underlying mechanisms could result in the further decrease of BMD such as persistent vitamin D deficiency, continued immune activation and cytokine release induced by the HIV-infection itself, and cART effects in the treated group [6,10,12,20,23,26, 28,31]. Currently, the European HIV guidelines indicate to screen only persons who are at risk for vitamin D deficiency and not all PHI-patients [32]. Nevertheless, almost 40% of our total study population had a vitamin D deficiency at baseline. With regard to the effect of HIV-infection itself on bone, high levels of viral load which are often seen in PHI, are known to decrease osteoblast activity and increase osteoclast function, both resulting in decrease of BMD [10,23,33,34]. This mechanism could explain the decreased BMD in both the treated and untreated group of PHI-men. In contrast, other studies argue that inflammation cannot be seen as a key mediator of decreasing BMD in a group of cART receiving HIV-men [7]. Interestingly, the decrease of BMD in the treated group was more pronounced in hip region compared to the LS, where only a slight but not significant decrease of BMD was found. Several other studies reported a similar pattern of decrease in BMD, even after several years of cART therapy [17,19,20,28]. Other studies, however, describe a decrease of both FN and LS BMD [4,8,16,22,30] or did not report a reduction in BMD after start of cART [23,28]. The timing of the DXA-scan might play a role in explaining these differences as a decrease of BMD is seen especially after 24 weeks and up to 96 weeks after start of cART [9,24,35]. The decrease of BMD is generally attenuated between week 48 and 96 [4,8,11,15]. This stabilization or even slight increase of BMD is thought to result mainly from a recovered balance in bone turnover during continuous long-term cART use [27]. Therefore, in our study the maximum effect of cART on the hormonal active trabecular bone of the LS might have not been detected yet as the follow-up DXA-scan was repeated relatively early after start of cART and furthermore the number of included patients might have been too small to be able to detect changes.

Finally, with regard to the impact of cART on BMD, it is known that TDF is associated with a up to 1-3% stronger decrease of BMD compared to other NRTI containing cART, both in already treated and untreated PHI-men [8,11,35,36]. In our study, all patients in the treated group received a TDF containing cART regime. TDF is thought to cause proximal tubulopathy in the kidneys which results in renal phosphate wasting, osteomalacia and is therefore considered as mediator of the detrimental effect on BMD in HIV [13,15,28,36]. This is reinforced by the fact that TDF is also thought to change vitamin D homeostasis by increasing vitamin D binding protein and decreasing 1.25diOHD levels

and osteoblast function [15,18,36], both resulting in increased bone turnover and lower BMD as well. Recently, tenofovir alafenamide (TAF) containing cART, which is the successor of TDF, was shown to display less bone toxicity and result in a smaller decrease of BMD in HIV-patients resulting in better long-term bone safety [37,38,39]. Based on these findings we suggest to be cautious starting TDF containing cART in HIV-patients particularly in those patients who already have osteopenia, osteoporosis or high fracture risk due to other causes.

Currently almost all PHI-patients start cART therapy directly in accordance with recent guidelines [32]. Therefore, this study offers an unique overview of the effect of HIV-infection and cART on bone turnover and BMD as our study shows data of both treated but also non-treated PHI-men. Other strengths of this study are the use of a wide panel of both bone resorption and formation markers, all measured in fasting samples with a standardized method, and a homogenous study population of patients who were all included from a highly specialized academic medical center. This study also has several limitations: data is lacking about the physical activity or possible sedentary lifestyle of our patients. In addition, follow-up data of vitamin D supplementation use or smoking was not available. We describe a relatively small group of almost all Caucasian men, which limits the extrapolation of findings to women or older MSM, in which osteopenia, osteoporosis and increased fracture risk might be even more prevalent. Lastly, the study design did not allow testing for possible causality of HIV itself with regard to bone turnover and BMD, as a control group of MSM with equal behavioral risk factors but who were HIV-negative was lacking.



In conclusion, bone turnover increases and FN and TH BMD decreases in PHI-men during treatment with TDF containing cART. These findings reinforce the matter to strongly consider alternatives for TDF containing cART in men with PHI who already have osteopenia or osteoporosis at time of diagnosis of HIV. In addition, this research stresses the need of evaluation of vitamin D levels and consideration of calcium and vitamin D supplementation to ensure the best possible bone health during TDF containing cART in PHI-men. Finally, as the incidence of HIV and need of cART are still increasing, this will result in more patients with osteopenia and osteoporosis and therefore studies regarding the effect of HIV and its treatment on bone turnover, BMD and fracture risk are warranted.

## Author contributions

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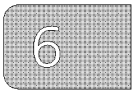

# Chapter 7

# Multiple sclerosis patients show lower bioavailable 25(OH)D and $1,25(OH)_2D$, but no difference in ratio of $25(OH)D/24,25(OH)_2D$ and FGF23 concentrations

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## Abstract

Vitamin D (VitD) insufficiency is common in multiple sclerosis (MS). VitD has possible anti-inflammatory effects on the immune system. The ratio between VitD metabolites in MS patients and severity of the disease are suggested to be related. However, the exact effect of the bone derived hormone fibroblast-growth-factor-23 (FGF23) and VitD binding protein (VDBP) on this ratio is not fully elucidated yet. Therefore, the aim is to study differences in total, free and bioavailable VD metabolites and FGF23 between MS patients and healthy controls (HC). FGF23, vitD (25(OH)D), active vitD (1,25$(OH)_2$D), inactive 24,25(OH)D and VDBP were measured in 91 MS patients and 92 HC. Bioavailable and free concentrations were calculated. No difference in FGF23 ($p$=0.65) and 25(OH)D/24.25$(OH)_2$D ratio ($p$=0.21) between MS patients and HC was observed. Bioavailable 25(OH)D and bioavailable 1.25$(OH)_2$D were lower ($p$<0.01), while VDBP concentrations were higher in MS patients ($p$=0.02) compared with HCs, specifically in male MS patients ($p$=0.01). In conclusion, FGF23 and 25(OH)D/24.25$(OH)_2$D did not differ between MS patients and HC, yet bioavailable VitD concentrations are of potential clinical relevance in MS patients. The possible immunomodulating role of VDBP and gender-related differences in the VD-FGF23 axis in MS need further study.

# Introduction

Multiple sclerosis (MS) is a chronic, progressive disease of the central nervous system characterized by an inflammatory, demyelinating and neurodegenerative process which can result in varying levels of disability. Consequently, MS has a negative effect on the daily life of patients, resulting from fatigue, muscle weakness and imbalance to immobility, which all negatively affect bone health [1-4]. Earlier studies showed that decreased bone mineral density (BMD) is prevalent already shortly after clinical onset even in physically active patients with MS [2, 4-6]. Lower BMD combined with impaired mobility can result in an increased fracture risk in MS patients and accompanying disability and economic burden [7]. The specific aetiology of MS is still unknown, but strong evidence exists regarding viral, genetic and immunological causes of the disease [8, 9].

Vitamin D is thought to play a role in the pathogenesis of MS, as it is known that the prevalence of MS increases with latitude, which in turn is associated with lower serum concentrations of vitamin D (25(OH)D) [10, 11]. Vitamin D possibly modulates T-lymphocyte subset differentiation and therefore a lower concentration is thought to lead to an increased risk of MS [12]. In line with this, associations between lower serum concentrations 25(OH)D and an increased risk of MS are shown in several studies [13-15]. As a result, vitamin D supplementation is often advised to MS patients, although contra-dictionary effects regarding the beneficial effects of increased 25(OH)D concentrations on for example, the recurrence rate of relapses, deterioration of number of brain laesions, and improvement of disability are described [16-20]. Because of the potential role of vitamin D in MS, other vitamin D metabolites have been studied in MS patients. 25(OH)D needs to be metabolized to the biologically active 1.25$(OH)_2$D, and both metabolites are predominantly bound to their carrier vitamin D binding protein (VDBP). Previous research showed a higher plasma VDBP concentration in MS patients compared with healthy controls [21]. VDBP is known to play an important role in the intracellular actin scavenging system by removing actin derived from damaged tissue and also promotes inflammation [22-26]. Polymorphisms of both VDBP and also of the vitamin D receptor (VDR) can result in a changed equilibrium between active and inactive vitamin D metabolites [24, 27, 28]. Interestingly, lower concentrations of the vitamin D metabolite 24.25$(OH)_2$D were associated with a higher grade of disability based on the Expanded Disability Status Scale (EDSS) score in MS patients [29]. Moreover, the ratio of 25(OH)D/24.25$(OH)_2$D was strongly inversely associated with brain parenchymal function [29].

A key player in vitamin D metabolism is the bone-derived hormone fibroblast growth factor 23 (FGF23) as it inhibits the enzyme 1-alpha hydroxylase and stimulates the enzyme 24-hydroxylase resulting in the conversion of 25(OH)D into 24.25$(OH)_2$D instead of into 1.25$(OH)_2$D. We thus hypothesize that plasma FGF23 concentrations differ between MS patients and healthy controls.

The primary aim of this study is to further elucidate the vitamin D-FGF23 axis by measuring multiple D metabolites and FGF23 using accurate state-of-the art analytical methods in a well-defined cohort of MS patients and healthy controls. The second aim of our study is to assess bone turnover markers (BTMs) in our cohort MS patients and healthy controls and to study the possible associations of BTMs with vitamin D metabolites as vitamin D deficiency is associated with increased bone turnover and lower bone mineral density [30-32]. Lastly, it is known that MS affects more

women than men and that women have higher VDBP concentrations compared with men [13, 21, 33-36]. Therefore, possible gender-related differences of vitamin D metabolites and BTMs between MS patients and healthy controls will be studied.

# Materials and Methods

## Subjects and study protocol

The subjects and study protocol were described earlier by Kragt et al. [13].Patients were eligible to participate in the study if they provided informed consent and were between 18 and 75 years of age. Three subtypes of MS were identified: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS). Patients with all subtypes of MS were eligible to participate. In addition patients with clinically isolated syndrome (CIS) were also included, which refers to a single episode of symptoms that are suggestive for MS. Patients were recruited between July and September 2003, to ensure that all blood samples were drawn during summer season. Patients were asked to bring a healthy control with them to the outpatient clinic if possible, preferably their partner in order to match the patients based on age and environmental factors. If a healthy control was lacking, hospital personnel volunteered to participate as controls. Patients were excluded if they were diagnosed with osteomalacia, hyperparathyroidism, hyperthyroidism or hypercortisolism, or if they had been receiving glucocorticoid treatment in the previous three weeks (daily oral treatment or intravenous methylprednisolone treatment), anti-epileptic drugs or vitamin D supplementation of more than 200 IU/day. The controls were excluded if they had MS or had a first-degree family member with MS. In addition, study participants were excluded in the case of suspicion of concomitant bone disease based on their laboratory results. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Medical Ethical Committee of the Amsterdam UMC, location VU University Medical Center (code 2003.029).

## Measurements

### General

The functional disability of MS patients was evaluated using the Expanded Disability Status Scale (EDSS) [37]. EDSS quantifies disability in MS patients, which ranges from 0 to 10 with higher scores representing higher levels of disability. Blood samples were collected before noon and after an overnight fast. Samples were collected in 2003 and stored (both as serum and EDTA plasma in several aliquots) in minus 80 degrees Celsius. Analyses were performed in 2011 (parathyroid hormone (PTH), alkaline phosphatase (ALP), albumin, calcium, phosphate, estimated glomerular filtration rate (eGFR)) and in 2017 (25(OH)D, 24.25$(OH)_2$D, 1.25$(OH)_2$D, VDBP, FGF23, c-terminal telopeptide (CTX), osteocalcin, procollagen type 1 N-terminal propeptide (P1NP) in the various aliquots.

### Vitamin D metabolites

In the current study, 25(OH)D and 24.25$(OH)_2$D were measured in serum using a dedicated isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) [38]. Total serum

concentrations of 25(OH)D were calculated by the sum of $25(OH)D_2$ and $25(OH)D_3$. For $25(OH)D_2$ the lower limit of quantitation (LLOQ) was 0.36 nmol/L and the inter-assay coefficient of variation (CV) was 6%. For $25(OH)D_3$, LLOQ was 1.19 nmol/L and the inter-assay CV was 6%. For $24.25(OH)_2D$, LLOQ was 0.12 nmol/L and the inter-assay CV was 5% [37, 39]. A two-dimensional isotope dilution ultra-pressure liquid chromatography tandem mass spectrometry (2D ID-UPLC-MS/MS) was used to measure serum $1.25(OH)_2D$ with an LLOQ of 3.4 pmol/L and inter-assay CV of 11% [40]. It is of note that, in the earlier study of Kragt et al., a radioimmunoassay was used for the measurement of 25(OH)D and $1.25(OH)_2D$ [13], but the concentrations shown in the current study are obtained using an LC-MS/MS method. VDBP was measured using a polyclonal ELISA (Immundiagnostik AG) with a LLOQ of 2.2 µg/L and an inter-assay CV of <13%.

### Free and bioavailable vitamin D metabolites

Vitamin D metabolites bound to protein are not biologically active, whereas the unbound hormones are. Free 25(OH)D was calculated using equations and affinity constants according to Malmstroem et al. [41] and free $1.25(OH)_2D$ was calculated using equations and affinity constants according to Bikle et al. [42]. Bioavailable 25(OH)D and $1.25(OH)_2D$ were calculated using equations adapted from Vermeulen et al. [43] and the supplement of Powe et al. [44].



### Bone turnover markers (BTMs)

FGF23 was measured in EDTA plasma using a c-terminal immunoassay (Immutopics) with a LLOQ of 20 RU/mL and inter-assay CV of <10% [45]. CTX and P1NP were measured in EDTA plasma using an immunoassay (Cobas, Roche Diagnostics, Almere, The Netherlands), with a LLOQ of 10 ng/L and inter-assay CV of <6.5% for CTX and a LLOQ of 5 µg/L and inter-assay CV of <8% for P1NP respectively. Osteocalcin was measured in EDTA plasma using an immunometric-assay (Biosource, Nivelles, Belgium) with an LLOQ of 0.4 nmol/L and inter-assay CV of 8%-15%.

### Other measurements

PTH was measured in EDTA plasma using an immunoassay (Architect, Abbott Diagnostics, Chicago, IL, USA) with an LLOQ 0.5 pmol/L and inter-assay CV of <9%. ALP, eGFR, calcium, phosphate and albumin were measured in heparin plasma using the chemistry module of the Cobas (Roche Diagnostics).

## Statistical analysis

Baseline characteristics between MS patients and healthy controls were compared using a Student's t-test or chi-square test. The Mann-Whitney U test was used in the case of non-parametric variables. To detect differences between patients and controls in biochemical indices of the vitamin D metabolites and BTMs, the Mann-Whitney U test was used as well. In addition, separate analyses for men and women were performed. Lastly, correlations between serum vitamin D metabolites, BTMs, and EDSS were calculated using Spearman correlations owing to non-parametric variables. All statistical analyses were carried out using SPSS software, version 23.0.

# Results

## General

A total of 213 participants were eligible to be included in this study. After applying the in- and exclusion criteria, a total of 30 participants were excluded: see Figure 1. The final study population that was included in the analyses consisted of 183 participants based on 91 MS patients and 92 healthy controls. Table 1 summarizes the baseline characteristics of all participants. In total, 61% of the MS patients were female. Relapsing remitting MS was the most predominant subtype of MS (57%).

## Vitamin D metabolites, FGF23, and bone turnover markers

Biochemical indices of vitamin D metabolism and bone turnover of the study population are displayed in Table 1. Again, it is of note that, in contrast to the earlier study of Kragt et al., current 25(OH)D and 1.25(OH)$_2$D measurements are reported based on the ID-LC-MS/MS measurements [13]. Overall, no significant difference between total serum concentrations of 25(OH)D ($p$ = 0.06) was found between MS patients and the healthy controls. MS patients had significant lower serum concentrations of free, albumin bound, and bioavailable 25(OH)D and 1.25(OH)$_2$D compared with healthy controls ($p$ < 0.01). In addition, MS patients had higher concentrations of phosphate and VDBP compared with controls, whereas no significant differences in BTMs were found. Plasma FGF23 concentrations did not differ between MS patient and the healthy controls ($p$ = 0.65) (Figure 2).

Table 2 shows the significant results of separate analyses for men and women. In contrast to male patients, female MS patients had lower serum concentrations of total 25(OH)D, 25(OH)D$_3$, 24.25(OH)$_2$D, free 25(OH)D, and free 1.25(OH)$_2$D compared with healthy female controls ($p$ = 0.04). Male MS patients had higher serum concentrations of VDBP compared with male controls ($p$ = 0.01). No other significant differences were found between male MS patients and healthy male

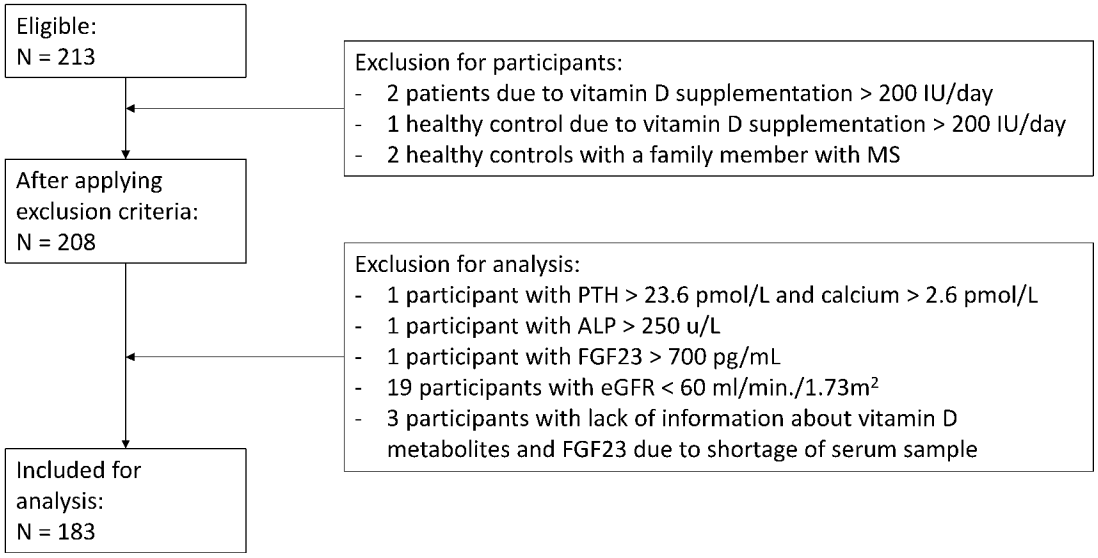



**Figure 1.** Flow chart of study population selection. PTH = parathyroid hormone, ALP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, FGF23 = fibroblast growth factor 23

**Table 1.** Baseline characteristics, vitamin D metabolites, FGF23 and bone turnover markers of multiple sclerosis (MS) patients and controls displayed as median with corresponding interquartile range (IQR), unless specified otherwise, of MS patients and controls.

| | Patients ($n$ = 91) | Controls ($n$ = 92) | $p$ value [a] | *Reference range* |
|---|---|---|---|---|
| Age, yr (mean ± SD) | 45 ± 11 | 42 ± 11 | 0.30 | |
| Female of total population (%) | 67 | 41 | **<0.01*** | |
| Postmenopausal of total population (%) | 34 | 16 | **0.04*** | |
| Caucasian (%) | 98 | 95 | 0.44 | |
| eGFR, mL/min/1.73m$^2$ (median, IQR) | 71 (66 – 81) | 71 (67 – 78) | 0.42 | >60 |
| MS subtype (%) | | n.a. | | |
| RRMS | 57 | | | |
| SPMS | 24 | | | |
| PPMS | 14 | | | |
| CIS | 1 | | | |
| Disease duration, yr (median, IQR) | 10 (5 – 16) | n.a. | | |
| EDSS (median, IQR) | 4 (3 – 6) | n.a. | | |
| Use of vitamin D supplements [b], (%) | 40 | 14 | **<0.01 *** | |
| Use of using disease modifying therapy, (%) | 32 | n.a. | | |
| Total 25(OH)D, nmol/L | 75 (59 – 93) | 77 (67 – 98) | 0.06 # | >50 |
| Total 1.25(OH)$_2$D, pmol/L | 105 (74 – 143) | 99 (79 – 133) | 0.57 | 59 – 159 |
| 25(OH)D$_2$, nmol/L | 1.1 (0.8 – 1.5) | 1.2 (0.9 – 1.5) | 0.66 | |
| 25(OH)D$_3$, nmol/L | 73 (58 – 92) | 76 (66 – 96) | 0.05 # | |
| 24.25(OH)D, nmol/L | 6.5 (4.4 – 9.0) | 7.1 (5.4 – 9.6) | 0.08 # | 0.4 – 8.9 |
| Free 25(OH)D (*10$^{-2}$), nmol/L | 1.2 (1.0 – 1.5) | 1.4 (1.2 – 1.7) | **<0.01 *** | |
| Albumin bound 25(OH)D, nmol/L | 4.5 (3.5 – 5.6) | 5.1 (4.1 – 6.1) | **<0.01 *** | |
| Bioavailable 25(OH)D, nmol/L | 4.5 (3.5 – 5.6) | 5.1 (4.1 – 6.1) | **<0.01 *** | |
| Free 1.25(OH)$_2$D (*10$^{-1}$), pmol/L | 2.0 (2.2 – 2.8) | 2.5 (0.2 – 3.1) | **<0.01 * #** | |
| Albumin bound 1.25(OH)$_2$D, pmol/L | 7.3 (5.7 – 9.1) | 8.4 (6.7 – 9.9) | **<0.01 *** | |
| Bioavailable 1.25(OH)$_2$D, pmol/L | 7.5 (5.9 – 9.4) | 8.6 (7.0 – 10.2) | **<0.01 *** | |
| Ratio total 25(OH)D/24.25(OH)$_2$D | 11.2 (9.9 – 13.6) | 11.4 (9.5 – 12.9) | 0.21 | 10 – 33 |
| VDBP, µg/L | 408 (374 – 445) | 388 (361 – 427) | **0.02 * #** | 200 – 550 |
| Albumin, g/L | 42 (39 – 44) | 42 (40 – 43) | 0.86 | 35 – 52 |
| Calcium, mmol/L | 2.4 (2.3 – 2.4) | 2.4 (2.3 – 2.4) | 0.73 | 2.2 – 2.6 |
| Corrected calcium, mmol/L | 2.3 (2.3 – 2.4) | 2.3 (2.3 – 2.4) | 0.81 | |
| FGF23, RU/mL | 88 (72 – 113) | 89 (69 – 106) | 0.65 | <125 |
| PTH, pmol/L | 5.2 (4.0 – 6.6) | 5.3 (3.8 – 6.7) | 0.94 | <10 |
| Phosphate, mmol/L | 1.0 (0.9 – 1.1) | 0.8 (0.8 – 0.9) | **<0.01 *** | 0.7 – 1.4 |
| CTX, ng/L | 256 (183 – 379) | 307 (212 – 418) | 0.10 | <580 |
| P1NP, µg/L | 37 (27 – 54) | 39 (29 – 56) | 0.37 | 22 – 87 |
| ALP, U/L | 74 (53 – 89) | 67 (56 – 79) | 0.14 | <115 |
| Osteocalcin, nmol/L | 1.5 (1.1 – 2.1) | 1.5 (1.1 – 2.0) | 0.99 | 0.4 – 4.0 |

eGFR = estimated glomerular filtration rate, RRMS = relapsing remitting MS, SPMS = secondary progressive MS, PPMS = primary progressive MS, CIS = clinically isolated syndrome, SP + R = secondary progressive + remitting, EDSS = Expanded Disability Status Scale. VDBP = vitamin D binding protein, PTH = parathyroid hormone, ALP = alkaline phosphatase, FGF23 = fibroblast growth factor 23, CTX = c-terminal telopeptide, P1NP = procollagen type 1 N-terminal propeptide. [a] $p$ values of Student's t-test, chi-square test or Mann-Whitney U test. [b] Maximum allowed dose of vitamin D supplements was 200 IU/day. * Bald, significance level $p \leq 0.05$. # Significant difference between male and female, details can be found in detail in Table 2.

**Table 2.** Vitamin D metabolites of MS patients and controls stratified for gender, only shown in the case of significant differences between male and females, displayed as median with corresponding interquartile range (IQR).

| | Men (n = 84) | | | Women (n = 99) | | |
|---|---|---|---|---|---|---|
| | Patients (n = 30) | Controls (n = 54) | p values[a] | Patients (n = 61) | Controls (n = 38) | p values [a] |
| Total 25(OH)D, nmol/L | 74 (56–97) | 75 (66–90) | 0.62 | 77 (60–90) | 88 (68–106) | 0.02 * |
| 25(OH)$D_3$, nmol/L | 73 (55–96) | 74 (64–89) | 0.64 | 75 (58–89) | 86 (67–104) | 0.01 * |
| 24.25$(OH)_2D$, nmol/L | 6.5 (4.5–8.8) | 6.9 (5.4–8.7) | 0.48 | 6.5 (4.4–9.1) | 7.1 (5.6–11.0) | 0.04 * |
| Free 25(OH)D ($*10^{-2}$), nmol/L | 0.013 (0.010–0.017) | 0.014 (0.012–0.017) | 0.17 | 0.012 (0.010–0.015) | 0.013 (0.011–0.017) | 0.03 * |
| Free 1.25$(OH)_2D$ ($*10^{-1}$), pmol/L | 0.23 (0.17–0.30) | 0.25 (0.22–0.31) | 0.18 | 0.21 (0.17–0.27) | 0.24 (0.21–0.31) | 0.03 * |
| Phosphate, mmol/L | 1.0 (0.9–1.0) | 0.8 (0.8–0.9) | <0.01 * | 1.0 (0.9–1.1) | 0.8 (0.8–0.9) | <0.01 * |

VDBP = vitamin D binding protein, PTH = parathyroid hormone. a p values of Mann-Whitney U test are shown as medians with interquartile ranges in parentheses. * significance level $p \leq 0.05$.

controls. Regarding BTMs, no significant differences were found between males and females (data not shown). Lastly, EDSS scores did not differ between male and female MS patients ($p$ = 0.77, data not shown).

## Associations

### Associations between vitamin D metabolites, FGF23, bone turnover markers and EDSS in MS patients

In MS patients, all vitamin D metabolites correlated strongly with each other (all $r$ > 0.77; $p$ < 0.01, data not shown). EDSS showed negative correlations with bioavailable 1.25(OH)$_2$D, bioavailable 25(OH)D and 24.25(OH)$_2$D ($r$ -0.30; $p$ < 0.01, $r$ -0.30; $p$ < 0.01 and $r$ -0.23; $p$ = 0.03 respectively). No correlations between EDSS and FGF23 or BTMs in MS patients were found (data not shown).

As shown in Table 3, FGF23 correlated positively with serum 25(OH)D and serum 24.25(OH)$_2$D ($r$ 0.22; $p$ = 0.04 and $r$ 0.22; $p$ = 0.04 respectively), and CTX correlated negatively with serum 24.25(OH)$_2$D and 25(OH)D ($r$ -0.31; $p$ < 0.01 and $r$ -0.23; $p$ = 0.03 respectively) in MS patients. Phosphate showed a positive correlation with osteocalcin ($r$ 0.22; $p$ 0.04). Negative correlations for osteocalcin with 1.25(OH)$_2$D, and P1NP with 24.25(OH)$_2$D were observed in MS patients only ($r$ -0.24; $p$ = 0.02 and $r$ -0.25; $p$ = 0.02 respectively), as were positive correlations of the ratio between total serum 25(OH)D and 24.25(OH)$_2$D with P1NP and CTX ($r$ 0.27; $p$ = 0.01 and $r$ 0.31; $p$ < 0.01 respectively). In addition, in MS patients ALP showed a positive correlation with osteocalcin, CTX and P1NP ($r$ 0.36; $p$ < 0.01; $r$ 0.44; $p$ < 0.01 and $r$ 0.43; $p$ < 0.01, respectively), P1NP showed a positive correlation with osteocalcin and CTX ($r$ 0.75; $p$ < 0.01 and $r$ 0.74 and $p$ < 0.01 respectively), and CTX correlated positively with osteocalcin ($r$ 0.67; $p$ < 0.01).

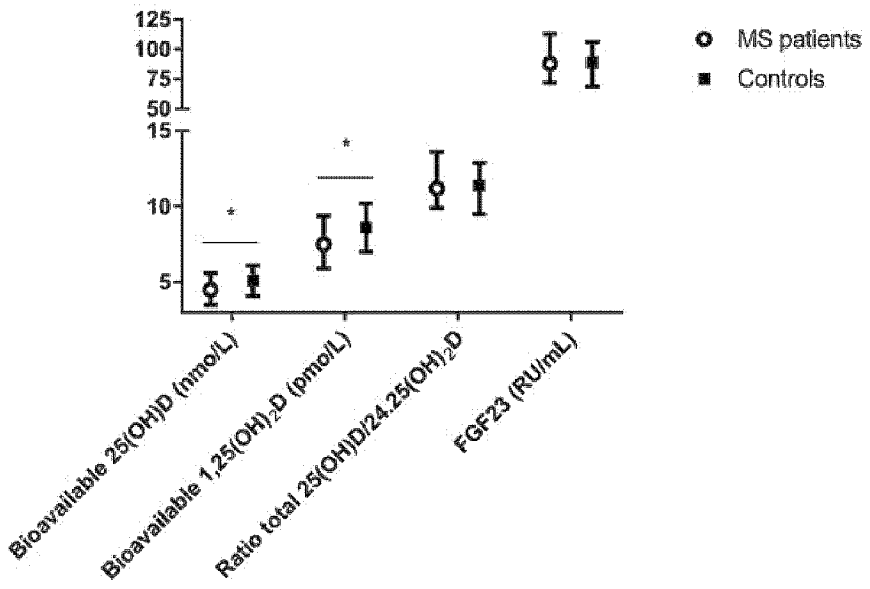



**Figure 2.** Vitamin D metabolites and FGF23 in MS patients versus healthy controls, * = p ≤ 0.05

## Associations based on gender of MS patients

Looking at gender differences, in female MS patients, the same correlations were found as described above, except for the correlations of FGF23 with serum 25(OH)D and 24.25(OH)$_2$D ($r$ 0.12; $p$ = 0.37 and $r$ 0.10; $p$ = 0.44 respectively). In male MS patients however, FGF23 correlated positively with total ($r$ 0.50; $p$ < 0.01), free ($r$ 0.43; $p$ = 0.02), bioavailable 25(OH)D ($r$ 0.43; $p$ = 0.02), free ($r$ 0.43; $p$ 0.02) and bioavailable 1.25(OH)$_2$D ($r$ 0.43; $p$ 0.02) and 24.25(OH)$_2$D ($r$ 0.46; $p$ = 0.01), respectively. Furthermore, EDSS showed negative correlations with CTX ($r$ -0.53; $p$ < 0.01), P1NP ($r$ -0.48; $p$ < 0.01 ) and osteocalcin ($r$ -0.40; $p$ 0.03) in male MS patients only.

## Associations in healthy controls

Lastly, in healthy controls, similar correlations of osteocalcin, CTX and P1NP were found (data not shown). FGF23 correlated significantly with serum 1.25(OH)$_2$D ($r$ -0.35; $p$ < 0.01, data not shown), and CTX correlated significantly with serum 25(OH)D ($r$ -0.24; $p$ = 0.05, data not shown). In addition, PTH showed a positive correlation with ALP (r 0.21; p 0.04, data not shown) and phosphate showed a negative correlation with P1NP (r -0.27; p 0.03, data not shown) in the healthy control group.



**Table 3.** Correlation between vitamin D metabolites, bone turnover markers (BTMs), and EDSS in MS patients.

| *N* = 90 | ALP | FGF23 | Osteocalcin | CTX | P1NP | EDSS |
|---|---|---|---|---|---|---|
| Free 1.25(OH)$_2$D | *r* −0.03 | *r* 0.10 | *r* 0.01 | *r* −0.10 | *r* −0.07 | ***r* −0.28** |
| | *p* 0.78 | *p* 0.33 | *p* 0.95 | *p* 0.33 | *p* 0.54 | ***p* 0.01** |
| Bioavailable 1.25(OH)$_2$D | *r* −0.04 | *r* 0.10 | *r* 0.04 | *r* −0.06 | *r* −0.03 | ***r* −0.30** |
| | *p* 0.71 | *p* 0.33 | *p* 0.72 | *p* 0.59 | *p* 0.77 | ***p* < 0.01** |
| Total 1.25(OH)$_2$D | *r* −0.12 | *r* −0.05 | ***r* −0.24** | *r* −0.09 | *r* −0.19 | *r* −0.08 |
| | *p* 0.25 | *p* 0.65 | ***p* 0.02** | *p* 0.41 | *p* 0.08 | *p* 0.47 |
| Free | *r* −0.02 | *r* 0.10 | *r* 0.01 | *r* −0.10 | *r* −0.06 | ***r* −0.28** |
| 25(OH)D | *p* 0.82 | *p* 0.34 | *p* 0.93 | *p* 0.36 | *p* 0.57 | ***p* 0.01** |
| Bioavailable 25(OH)D | *r* −0.03 | *r* 0.11 | *r* 0.05 | *r* −0.05 | *r* −0.02 | ***r* −0.30** |
| | *p* 0.77 | *p* 0.33 | *p* 0.66 | *p* 0.67 | *p* 0.84 | ***p* < 0.01** |
| Total | *r* −0.16 | ***r* 0.22** | *r* −0.12 | ***a* −0.23** | *r* −0.19 | ***r* −0.23** |
| 25(OH)D | *p* 0.14 | ***p* 0.04** | *p* 0.28 | ***p* 0.03** | *p* 0.08 | ***p* 0.03** |
| 24.25(OH)D | *r* −0.20 | ***r* 0.22** | *r* −0.16 | ***r* −0.31** | ***r* −0.25** | ***r* −0.22** |
| | *p* 0.06 | ***p* 0.04** | *p* 0.14 | ***p* < 0.01** | ***p* 0.02** | ***p* 0.04** |
| Ratio 25(OH)D/ 24.25(OH)$_2$D | *r* 0.18 | *r* −0.13 | *r* 0.16 | ***r* 0.31** | ***r* 0.27** | *r* 0.10 |
| | *p* 0.09 | *p* 0.23 | *p* 0.13 | ***p* <0.01** | ***p* 0.01** | *p* 0.34 |
| PTH | *r* 0.09 | *r* −0.04 | *r* −0.04 | *r* 0.04 | *r* −0.08 | *r* 0.16 |
| | *p* 0.40 | *p* 0.69 | *p* 0.97 | *p* 0.69 | *p* 0.46 | *p* 0.13 |
| Phosphate | *r* 0.10 | *r* 0.03 | ***r* 0.22** | *r* 0.13 | *r* 0.16 | *r* −0.03 |
| | *p* 0.35 | *p* 0.78 | ***p* 0.04** | *p* 0.24 | *p* 0.15 | *p* 0.76 |

ALP = alkaline phosphatase, FGF23 = fibroblast growth factor 23, CTX = c-terminal telopeptide, P1NP = procollagen type 1 N-terminal propeptide, EDSS = Expanded Disability Status Scale, PTH = parathyroid hormone. Bold text indicates a significance level of p ≤ 0.05. *r*: Spearman correlation coefficient.

## Discussion

This study examined differences in total, free and bioavailable vitamin D,FGF23 and bone turnover markers in patients with MS compared with healthy controls and possible gender differences. Although positive correlations between FGF23 and total 25(OH)D and $24.25(OH)_2D$ in MS patients were seen, no differences in plasma FGF23 concentrations between MS patients and healthy controls were observed. No differences between serum concentrations of BTMs in MS patients and healthy controls were found. Yet we did observe a negative correlation between CTX and total 25(OH)D and $24.25(OH)_2D$. EDSS showed negative correlations with bioavailable 1.25(OH)2D, bioavailable 25(OH)D, and 24.25(OH)2D. We found gender differences in vitamin D metabolism: serum concentrations of total 25(OH)D, $25(OH)D_3$, $24.25(OH)_2D$, free 25(OH)D and free $1.25(OH)_2D$ were lower in female MS patients compared with female healthy controls. Serum concentrations of VDBP were higher in male MS patients compared with healthy male controls.

The main aim of this study was to further elucidate the vitamin D-FGF23 axis by measuring multiple D metabolites and FGF23 using accurate state-of-the art analytical methods in a well-defined cohort of MS patients versus healthy controls. We confirmed the finding of an earlier study that the serum $24.25(OH)_2D$ concentration is negatively correlated with EDSS [29]. Although we found a significant positive correlation between FGF23 and 25(OH)D and $24.25(OH)_2D$, we did not observe a difference in FGF23 concentration between MS patients and healthy controls. A previous study described comparable plasma concentrations of FGF23 in MS patients compared with healthy controls as well [46], although two other studies found higher serum concentrations of FGF23 in MS patients compared with healthy controls [47, 48]. However, the latter study was performed in patients with RRMS only and these differences were found during autumn (September-November) and winter time, respectively. Therefore, seasonal effects or the use of different FGF23 assays measuring either intact or c-term FGF23 could have resulted in these different findings. We found a higher phosphate concentration in MS patients; nevertheless, no differences in PTH or eGFR compared with healthy controls were found. Lastly, no correlations between FGF23 and $1.25(OH)_2D$ or EDSS were found in MS patients, which is in line with two other recent studies [46, 48]. Summarized, these findings do not support our hypothesis that FGF23 differs between MS patients and healthy controls.

Regarding the various vitamin D metabolites, we found similar concentrations of 25(OH)D and $1.25(OH)_2D$ between MS patients and healthy controls, as some showed earlier [13, 49, 50], whereas others found lower serum concentrations of 25(OH)D or $1.25(OH)_2D$ in MS patients compared with HCs, respectively [15, 51-57]. These differences between studies might be caused by differences in sample size, analysis of both male and female participants in different seasons of the year, use of different assays to measure vitamin D and its metabolites and possible VDBP polymorphisms [24, 27, 28]. In addition, a number of studies did report a relationship between lower serum concentrations of $1,25(OH)_2D$ and an increased risk of MS [51, 52]. Furthermore, our study showed that MS patients were using supplementation of vitamin D more often compared with their healthy controls. However, the maximal supplementation dosage was <200 IU of vitamin D per day, of which no clinical relevant effect on the vitamin D metabolites is expected, as a higher supplementation dosage is normally advised [18, 19, 29]. Moreover, controls had similar baseline concentrations of 25(OH)D.

As suggested in recent studies, bioavailable 25(OH)D might be preferred above total 25(OH)D as marker for vitamin D status, and might thus be a better marker of mineral metabolism [58-62]. The sum of the albumin bound fraction of 25(OH)D plus the freely circulating 25(OH)D results in the bioavailable 25(OH)D, which can be calculated similarly for bio-available $1.25(OH)_2D$ respectively [58, 60]. In the circulation, VDBP and albumin bind over 99% of the 25(OH)D and $1.25(OH)_2D$ [60], whereas the binding affinity of vitamin D to albumin is much lower than the binding affinity to VDBP [63]. We found lower serum concentrations of both free and bioavailable 25(OH)D and $1.25(OH)_2D$, respectively, in MS patients compared with healthy controls, in contrast to comparable serum concentrations of total 25(OH)D and $1.25(OH)_2D$ between MS patients and healthy controls. In contrast to our findings, Behrens et al. found no difference in free and bioavailable 25(OH)D between MS patients and controls, yet $1.25(OH)_2D$ was not measured in this study [64]. However, their study included patients with CIS only (not yet diagnosed with MS), used non-parametrical statistical tests, and a de-seasonalized concentration of vitamin D was calculated afterwards. Our study measured summer concentrations of vitamin D only and included all subtypes of MS, which may explain the different findings.

The second aim of our study was to assess BTMs in MS patients versus healthy controls and to study the possible associations of BTMs with vitamin D metabolites as vitamin D deficiency is associated with increased bone turnover [32]. Our study did not show any differences between BTMs and FGF23 in MS patients and their healthy controls. This finding is in line with earlier studies, where similar serum concentrations of CTX, P1NP, or osteocalcin between MS patients and healthy controls were found, and similar concentrations of cross-linked N terminal telopeptide type 1 collagen (NTX) and bone ALP in newly diagnosed MS patients and healthy controls were found [18, 32, 65-67]. The current study shows that despite differences in vitamin D metabolites, bone turnover in MS patients seems to not be affected compared with healthy controls.

Lastly, possible gender-related differences of vitamin D metabolites and BTMs between MS patients and healthy controls were studied; as known, MS affects more women than men and, that in general, women have higher VDBP concentrations compared with men [13, 21, 33-36]. Indeed, our study showed more female MS patients of which the largest part was post-menopausal. Further analyses based on pre- or postmenopausal status was not possible because of small groups. Our study showed lower total and free 25(OH)D, free $1.25(OH)_2D$ and $24.25(OH)_2D$ only in female MS patients compared with healthy female controls, but no difference in this respect between male MS patients compared with healthy men. No differences between FGF23 in male and female MS patients were found. Interestingly, in our study VDBP was higher in MS patients compared with controls, but after further analysis this difference was found in male MS patients only. An earlier study found that serum VDBP concentrations were higher in MS patients compared with healthy controls as well, however, this study included both female and males [21]. In contrast, other studies showed no difference in VDBP concentrations between MS patients and controls [64, 68]. The differences between VDBP concentrations in the various studies could be the result of variation between numbers of male and female patients and controls included in these studies or by using either a polyclonal or monoclonal assay. It is known that VDBP can affect inflammatory processes [22, 23, 25, 26, 61, 69, 70]. As we found higher VDBP in male MS patients, in the presence of a similar

EDSS score as in female MS patients, this suggests a possible modulating effect of VDBP in male MS patients. It was shown before that higher concentrations of VDBP restrict the uptake of free vitamin D metabolites and reduce anti-inflammatory responses of immune cells [71-74]. Moreover, VDBP can act as a chemotactic cofactor which enhances chemotaxis of neutrophils and macrophages by complement factor C5a [23]. These macrophages are known to contribute to laesion formation and axonal damage [22, 26]. Previous experimental studies showed that T-lymphocytes, glia cells and neurons express 1-α-hydroxlyase and VDR, which enables them to convert 25(OH)D to 1.25(OH) [75-77]. Normally, in the central nervous system, focal inflammation is initiated by auto-reactive T-helper cells type 1 (Th 1) and T-helper cells type 17 (Th 17) [78]. The activated vitamin D is thought to induce anti-inflammatory effects in glia cells and neurons and affects vitamin D responsive genes in T-helper cells [76, 79-82], thereby inhibiting pro-inflammatory T-helper cell activity (Th1 and Th 17) and promoting anti-inflammatory T-helper cell activity (Th2 and regulatory T-cells) [51, 83]. However, whether vitamin D affects the T-cell response also depends on concentrations of VDBP [84, 85]. On the basis of these studies, the elevated concentrations of VDBP found in our study strengthen the pro-inflammatory role of VDBP in enhancing neuronal damage in MS.



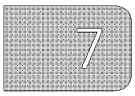
Lastly, EDSS scores were comparable between male and female MS patients, which is consistent with previous research [86, 87]. In female MS patients, however, EDSS showed negative correlations with free and bioavailable $1.25(OH)_2D$, free, bioavailable, and total 25(OH)D and $24.25(OH)_2D$, which is in line with earlier studies which reported a negative correlation between EDSS and $1.25(OH)_2D$ or 25(OH)D respectively [46, 88]. In contrast, in male MS patients, negative correlations between EDSS and CTX, P1NP, and osteocalcin were observed, which differs from previous studies, which found no correlations between EDSS and BTMs or only a positive correlation between EDSS and CTX [67, 89].

The strengths of this study are the relative large study population and the broad spectrum of vitamin D metabolites, FGF23 and BTMs that was measured. Accurate and well-standardized LC-MS/MS assays were used to measure vitamin D metabolites. In addition, to minimize seasonal changes known to affect vitamin D metabolism [48], only blood samples drawn during summertime were used. There are also some possible limitations of this study. First, owing to the cross-sectional study design, the described differences between MS patients and controls in serum concentrations of vitamin D metabolites are not necessarily causal. In addition, 97% of our study population is Caucasian, which reduces the generalizability of the results, as it is known that vitamin D metabolism differs between races [53]. Moreover, our study was not primarily designed to study gender difference, so the differences we found in this respect should be studied further in other cohorts. No data on fractures were available. Lastly, dual energy X-ray absorptiometry (DXA) scans were not available to compare vitamin D metabolites and bone turnover markers with BMD.

# Conclusion

In conclusion, this study provides additional knowledge of vitamin D-FGF 23 axis and bone turnover markers in MS patients compared with healthy controls as well as gender-related differences. Similar serum concentrations of total 25(OH)D and $1.25(OH)_2D$ in MS patients and healthy controls were found. Furthermore, no differences in plasma FGF23 concentrations and other bone turnover

markers between MS patients and healthy controls were observed. The ratio total 25(OH) D/ 24.25$(OH)_2$D did not differ between MS patients and healthy controls. However, this study suggested a relevant gender difference as serum concentrations of total 25(OH)D, 24.25$(OH)_2$D, free 25(OH)D and free 1.25$(OH)_2$D were lower in female MS patients compared with female healthy controls, while serum concentrations of VDBP were higher in male MS patients compared with male controls. This study thus shows that only a total 25(OH)D measurement probably does not reflect all changes in vitamin D metabolism in MS patients. The exact role of VDBP and its polymorphisms in MS needs further studies. Finally, given the high incidence of reduced bone mineral density and the still partially unknown mechanisms that affect bone turnover in MS patients [66], further studies should be performed to evaluate the relationship between change in the vitamin D-FGF23 axis, BMD, and fracture risk in both male and female MS patients.



## Author contributions

Authors' role: Study design: MV, LB, JKragt, MdH, and AH, Data collection: MV, LB, and JKragt, Data analysis: MV, and LB. Drafting manuscript: MV, and LB. Revising manuscript content: MV, LB, JKragt, JK, BvA, RdJ, MdH, and AH. Approving final version of manuscript: AH. AH takes responsibility for the integrity of the data analysis. Funding: This research received no external funding. Conflicts of Interest: The authors declare no conflict of interest.

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# Part IV

## POSSIBLE ROLE OF FGF23 AS TUMOR MARKER IN MALIGNANT DISEASE

# Chapter 8

# Plasma FGF23 is not elevated in prostate cancer

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# Letter to the editor

## Dear editor

Prostate cancer (PCa) is the most frequently diagnosed cancer in men in developed countries, with increasing incidence and mortality rate worldwide [1,2]. The diagnosis of PCa is based on measurement of serum prostate specific antigen (PSA) in blood, digital rectal examination (DRE) and prostate biopsies. Currently, no accurate non-invasive biomarker is available to differentiate between benign prostate conditions such as benign prostate hyperplasia and PCa. Recently, the protein fibroblast growth factor 23 (FGF23) was found to play a role in PCa development and possible progression [3–6]. FGF23 is secreted predominantly by osteocytes and displays hormonal activity in regulating phosphate and vitamin D metabolism [3–5]. FGF23 exerts its function via the FGF receptor and the membrane-bound protein klotho [4,5].

Earlier studies show that FGF23 might have autocrine, paracrine and endocrine effects in PCa [3–7] and that FGF23 increases growth factor activity in PCa [3,4,7]. Furthermore, it was postulated that FGF23 stimulates proliferation of PCa cells and can increase the formation of bone metastases [3,4]. Recently, it was suggested that high dietary intake of phosphorus possibly increases the risk of aggressive PCa due to increased FGF23 production, while no association was seen in low risk PCa [3]. These studies all point towards a role for FGF23 in PCa diagnosis and prognosis. However, little information is available on the actual FGF23 concentration in plasma of PCa patients up to now. Therefore, the aim of this case-control study was to compare the plasma FGF23 concentrations in men with biopsy proven PCa to men without prostate cancer (confirmed by negative biopsy (BN)) and to age-matched controls.

This study was performed with approval of the Local Medical Ethical Committee of the VU University Medical Center and samples were collected after signed informed consent. Three groups were included: (i) PCa patients (n=19), (ii) BN patients (n=7) and (iii) control men (n=17). The PCa group consisted of intermediate risk (Gleason-score 7, n=13) or high risk (Gleason-score 8-10, n=6) patients. The men in the intermediate risk group had a mean prostate volume of 42.8 cc (SD 15.4). Furthermore, within this group 7 patients had stage T2 and 6 patients stage T1 PCa. A mean prostate volume of 47.8 cc (SD 7.8) applied to the men in the high risk group which included 1 patient with stage T1, 1 patient with stage T2 and 4 patients with a T3 stage PCa, respectively. The BN group consisted of patients with normal or elevated PSA without the detection of cancerous tissue in the biopsies. It should be noted, that despite multiple biopsies, the presence of malignant cells in the prostate cannot be ruled out completely as biopsies only provide information of the specific biopsy locations in the prostate. The control group consisted of a random group of males ≥55 years, without self-reported history of prostate problems, malignant disease, use of bisphosphonates, or decreased kidney function and who needed a venipuncture for other reasons. PSA, FGF23, phosphate and creatinine concentrations were measured in serum or plasma and glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula in all subjects. Serum PSA was measured using the Architect 2000 SR immunoanalyser (Abbott Diagnostics, Hoofddorp, the Netherlands), plasma FGF23 using a c-terminal immunoassay (Immutopics) in one run with an intra-assay coefficient of variation of <5 % [8], and serum phosphate and creatinine were measured using a Cobas 8000 modular analyser series (Roche Diagnostics, Almere, the Netherlands).

Statistical analyses were performed with Mann-Whitney U tests, T-tests, Kruskal-Wallis tests and Spearman correlations and were displayed with corresponding *p*-values. The mean age of the total group (n= 43) was 67.1 (SD 6.6) years and the median GFR was 76 (IQR 68 - 90) ml/min/1.73m$^2$. Age ($p$=0.71) and GFR ($p$=0.75) did not differ between the groups. Serum PSA concentrations were higher in the PCa group (mean 25.6 ng/mL, SD 39.7, $p$=0.0001), compared to the BN group (mean 8.8 ng/mL, SD 6.1) and control group (mean 2.1 ng/mL, SD 1.5). The median phosphate concentration was lower in the BN group (median 0.6 mmol/L, IQR 0.6 – 0.8, $p$=0.0017) compared to the PCa group (median 0.9 mmol/L, IQR 0.8–1.0) and control group (median 1.1 mmol/L, IQR 1.0–1.2). With regard to phosphate, the BN group in our study had statistically lower phosphate levels, which we cannot explain. Since the differences between the phosphate levels were very small and the FGF23 concentration was not significantly higher in the BN group, this measurement should be repeated in another study as our study contained a low number of patient in each group. The median plasma FGF23 concentration was 72 RU/mL (IQR 64 – 90) in the PCa group, 72 RU/mL (IQR 69 – 88) in the BN group and 83 RU/mL (IQR 73 – 113) control group, respectively. Plasma FGF23 concentrations did not differ between the groups (Figure 1.). Also after merging the BN group and control group, the PCa negative and PCa group did not differ in plasma FGF23 concentrations. A negative correlation between plasma FGF23 and GFR was found (*rho* – 0.33, $p$=0.04, n=43), which was consistent throughout all three groups that were included in the study (Figure 2.).

In our study, plasma FGF23 concentrations were not increased in PCa patients, even in spite of inclusion of patients with high Gleason-scores. Previous research showed that PCa cells have higher FGF23 mRNA levels compared to normal prostate cells [4]. Furthermore, higher mRNA levels of FGF23 in tissues were associated with worse prognosis of PCa [6]. We observed similar plasma FGF23 concentrations in PCa patients compared to a recent study [9]. In that study, no control groups were included. Furthermore, plasma FGF23 concentrations of PCa patients were compared to patients with other types of cancer only and found no significant differences [9]. Therefore, despite indications of the relation between cellular and mRNA level that FGF23 to PCa, plasma FGF23 was not increased in patients with PCa, other types of cancer [9], negative prostate biopsies nor random aged matched males. This implies that plasma FGF23 is not a promising biomarker to detect PCa. The higher FGF23 levels in the control group were found in presence of normal GFR and PSA levels in this group. As the controls were assessed anonymously, no further clinical background information was available which limits further elaboration on the this finding. We also showed a significant association between the GFR and the plasma FGF23 concentration, even in a group with a GFR>60 ml/min. This is in agreement with previous study that demonstrated that the kidneys filter FGF23 [10]. New studies investigating plasma FGF23 should therefore take into account the kidney function, which is strengthened by the negative correlation between FGF23 and GFR in our study.

To conclude, our study does not show differences in plasma FGF23 concentrations between patients with prostate cancer, biopsy negative patients and age matched controls. Therefore, plasma FGF23 is not a promising biomarker for prostate cancer in vivo.

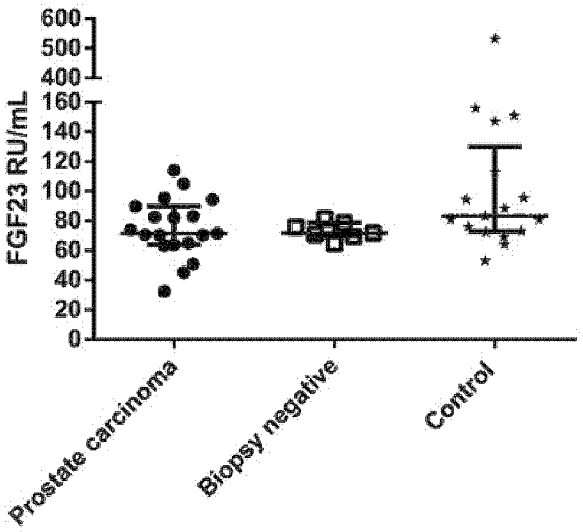


**Figure 1.** Plasma FGF23 concentrations in men with prostate cancer (PCa) (n = 19), men with a negative biopsy (BN) (n = 7) and control group (n = 17), no significant differences



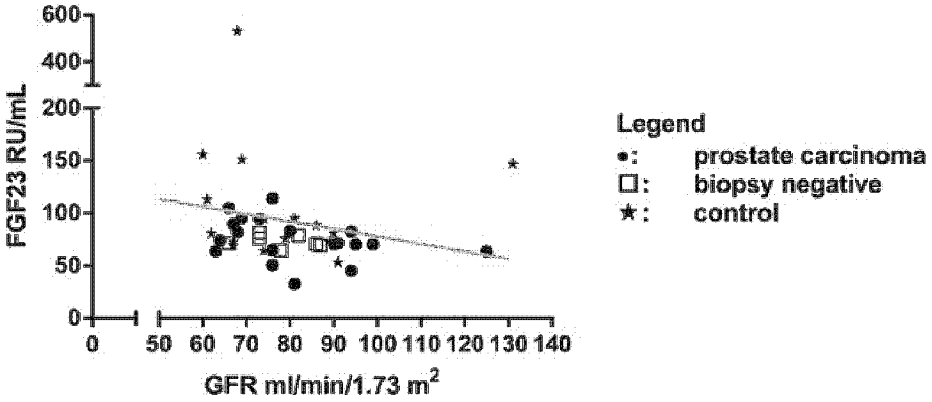


**Figure 2.** Correlation between plasma FGF23 concentrations and GFR (rho – 0.33, p = 0.04, n = 43).

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## Conflict of interest and author contributions

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# Part V

## SUMMARY AND DISCUSSION

# Chapter 9

# Summary and discussion (English and Dutch)

This part of the thesis summarizes the main findings of the studies of this thesis and discusses these results. In addition, strengths and limitations of our studies and future perspectives are provided.

# Part I: Introduction

The clinical utility of BTMs in various diseases is assessed in our review (**chapter 2**). Regarding the definition of clinical utility of BTMs, as described in the Introduction section of this thesis, our review shows that: several BTMs are of use in clinical practice in 1) diagnosing bone (related) disease in case of tumor induced osteomalacia (FGF23, ALP), van Buchem disease (sclerostin, ALP, P1NP, osteocalcin), Paget's disease ((B)ALP, and PINP), and hypophosphatemic rickets (ALP, FGF23). Next, we show that BTMs are of use in clinical practice in 2) assisting in making a prognosis of either improvement or deterioration of bone (related) disease in chronic kidney disease ((B)ALP, trimeric P1NP, TRAP5B, FGF23), Paget's disease ((B)ALP, P1NP) and that in osteoporotic patients the treatment effects can be monitored based on BTMs (CTX, P1NP), which is in line with current IOF and IFCC guidelines [1–4]. Last, we show that in case of 3) definition of high risk patients e.g. patients with low BMD or high fracture risk, no single BTM has proven to be suitable for prediction of fracture risk in individual patients up until now.

Our review further emphasizes that (pre)analytical variability, use of different assays with variable cut-off values and heterogeneous study results are still challenging the clinician in requesting and interpreting BTM measurements. We also discuss that, especially in bone resorption markers, except for TRAP5B, the circadian rhythm is important to keep in mind, with higher concentrations at nighttime and early morning. Furthermore, CTX needs to be measured in fasting state as concentrations decrease after food intake. In case of decreased kidney function, trimeric PINP, BALP and TRAP5B are BTMS can be reliably interpreted. BTMs can be elevated 6 months to 1 year after a fracture. Last, age and sex are important to keep in mind when interpreting BTMs as concentrations are higher in men and at younger age.



# Part II: Effects of sex steroids on bone health in transgender persons

In **chapter 3 to 5** of this thesis, changes of BTMs and bone mineral density (BMD) during gender affirming hormone treatment (HT) in transgender persons are evaluated. **Chapter 3** shows a decrease of P1NP and ICTP and volumetric bone mineral apparent density (BMAD) Z-score of the lumbar spine (LS) during gonadotrophin releasing hormone antagonist (GnRHa) treatment in adolescent transgender persons. 24 months after HT was started, P1NP and ICTP concentrations were even lower, whereas the BMAD and Z-scores returned to normal. In **chapter 4** it is shown that BMD increases in both trans women and trans men after 1 year of HT. In trans women, an increase of BMD in LS, total hip (TH) and femoral neck (FN) was found. In trans men a smaller increase of TH BMD was seen, whereas no changes were observed in FN BMD. In **chapter 5**, bone turnover decreased in trans women and increased in trans men after 1 year of HT. In addition, a striking age difference was seen looking into more detail into the trans men aged > 50 years, as they all showed a decrease of all BTMs, which was opposite to the trans men group aged <50 years which showed an increase of BTMs.

In adolescent transgender persons, a deleterious effect of GnRHa on bone health was seen in **chapter 3,** which was also shown in other patient groups receiving GnRHa such as patients with prostate cancer [5,6]. **Chapter 3 to 5** all show the beneficial effect of estradiol on bone as a decrease of BTMs and increase of BMD are described during HT treatment in transgender persons. Estradiol exerts its anabolic effect on bone either direct or indirect after the aromatization of testosterone into estradiol [7–9]. Estradiol reduces osteoclast activity, shown by a decrease in BTMs (**chapter 3 and 5**) and thereby results in less bone resorption and an increase of BMD **(chapter 4).** Testosterone itself does not decrease BTMs as shown in **chapter 5**. Testosterone concentrations are positively associated with muscle mass [10–12] leading to higher mechanical loading which is thought to result in higher BMD [13]. In contrast, a recent study showed that hand grip strength in adult trans men increased, but that this change was not associated with changes in BMD after 1 year of HT [14]. The studies in **chapter 3, 4 and 5** show more differences between bone health in trans women and trans men, with lower BMD before and during HT and vitamin D deficiency being more prevalent in trans women.

As the three chapters show only short term results on bone health, it is important to ensure that bone health of transgender persons is preserved on long term as well. A recent study from Wiepjes et al. [15] shows that mean estradiol concentrations are associated with changes of LS BMD in adult trans women, where the highest estradiol concentration tertile shows an increase, the middle tertile shows no change and lowest estradiol tertile shows a decrease of LS BMD respectively during 10 years of HT. This study also emphasizes the anabolic effect of estradiol on bone and concluded that there is no negative effect of long-term HT on BMD after 10 years of HT in transgender persons, when using adequate HT continuously [15]. Compared to our short-term results, it is reassuring that this large prospective study did not show deleterious effects on BMD in adult transgender persons.



Summarized, our three studies **(chapter 3, 4, and 5)** show that, at least on the short term, HT is not harmful for bone health. Furthermore, our studies show no additional value of BTM measurements in transgender persons in clinical practice, as on the short term BTMs decreased and BMD increased, so no clinical consequences can be based on these results. To monitor bone health, we therefore do not advice that BTMs should be embedded as a standard part of the adolescent or adult transgender persons patient care. In addition, it is important for the clinician to provide lifestyle advices regarding physical activity (to increase mechanical loading of bones), cessation of smoking and adequate calcium and vitamin D intake [16,17] in transgender persons to ensure adequate bone health.

## Part III: Effects of inflammation and auto-immune disease on bone turnover

Our study in **chapter 6** shows that combined antiretroviral therapy (cART) results in increased bone turnover and decreased BMD of the hip in adult men with primary human immuno-deficiency virus (HIV) infection. All BTMs, except ICTP, showed a significant increase versus no changes of BTMs in an untreated group of men with primary HIV infection. DXA revealed a significant decrease of FN and TH BMD in both groups, however LS BMD did not change in both groups. Previous studies also

showed that HIV-infected persons have a lower BMD compared to a healthy reference population [18,19]. Inflammatory factors such as interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) are known to negatively affect bone health [20] and this study emphasizes the deleterious effects of both HIV and cART on bone health. We feel that the outline of this study has become even more interesting as nowadays, patients infected with HIV start cART therapy directly after the diagnosis has been made and therefore studies in cART naïve primary HIV infected men are scarce.

Based on our study we suggest that BTMs should not be routinely incorporated in bone health assessments of male HIV patients. We suggest that DXA scans should be performed routinely given the high prevalence of a lower BMD in HIV-infected persons. In addition, patients require lifestyle advice on physical activity to improve mechanical loading on bones and to stop using cigarettes and alcohol and need to ensure adequate calcium and vitamin D intake [21]. Furthermore, especially tenofovir containing cART should be reconsidered in men with HIV who already have osteopenia or osteoporosis, due to the phosphaturic effects of this drug [21,22]. In this case, BTMs can be of additional value to evaluate if bone turnover decreases after the cART regimen is changed from a tenofovir containing cART regimen to another cART regimen.

Next to viral inflammation, also auto-immune diseases are known to affect bone metabolism. In case of the auto-immune disease multiple sclerosis (MS), both vitamin D insufficiency and lower BMD are common [23,24]. Vitamin D deficiency is associated with increased bone turnover and FGF23 is known to play a key role in vitamin D metabolism. However, **chapter 7** shows that no differences in plasma FGF23 concentrations, ratio of 25(OH)D/24.25$(OH)_2$D or BTMs between MS patients and healthy controls were observed. MS patients had lower bioavailable 25(OH)D and 1.25$(OH)_2$D concentrations compared to healthy controls. In addition, serum concentrations of total 25(OH)D, 25$(OH)D_3$, 24.25$(OH)_2$D, free 25(OH)D and free 1.25$(OH)_2$D were lower in female MS patients compared to female healthy controls, while serum concentrations of vitamin D binding protein (VDBP) were higher in male MS patients compared to male controls. Our study therefore shows that a single 25(OH)D measurement probably does not reflect all changes occurring in vitamin D metabolism in MS patients.



Based on our study, BTM measurements are not indicated in MS patients to evaluate bone health. MS patients should receive lifestyle advice on physical activity to increase mechanical loading of the bone, vitamin D supplementation to ensure adequate 25(OH)D concentrations and quit smoking, as the latter is associated with worse bone health especially in elderly people [16]. Last, the exact role of VDBP in MS and the clinical relevance of the gender related differences in vitamin D metabolites in MS patients need to be assessed further in future studies.

## Part IV: Possible role of FGF23 as tumor marker in malignant disease

In **chapter 8** plasma FGF23 concentrations in prostate cancer (Pca) were evaluated. In case of malignant disease, tumor derived factors such as tumor growth factor beta (TGF-β) can negatively affect bone metabolism [5,6]. These factors often result in an increase of bone resorption and lead to possible deleterious effects on BMD and bone strength. FGF23 is thought to have autocrine, paracrine and

endocrine effects in PCa and to increase growth factor activity in PCa [25–27]. However, in our study in PCa patients, FGF23 was not increased, even in spite of inclusion of patients with high Gleason-scores. We did show a significant association between the glomerular filtration rate (GFR) and the plasma FGF23 concentration, even in a group with a GFR > 60 mL/min, which was also seen in previous studies [28–30].

We showed that plasma FGF23 is not a promising biomarker in prostate cancer. We emphasize the importance of keeping the kidney function in mind when interpreting FGF23 concentrations as these increase when kidney function declines. Future studies are needed to evaluate whether BTMs are of additional clinical value to monitor malignant disease with risk of bone metastases, as discussed earlier in our review in **chapter 2.**

# Strengths and limitations

## Strengths

The studies in this thesis investigated various factors affecting bone health such as changes in sex steroids, presence of prostate cancer, infectious disease or auto-immune disease. Most of the studies contain large study groups, which allowed us to perform sub group analyses e.g. based on bone age or pre- and postmenopausal state. We were also able to include large cohorts of rare patient groups such as adolescent transgender persons and male primary HIV patients who were not treated with cART yet. The measurements of BTMs were all performed using state-of-the-art analytical methods using the same assays in the same laboratory throughout the different studies in this thesis. BTMs were measured in all studies in fasting state, except for the study in **chapter 3**. For this reason the marker CTX was not included in this study, as CTX concentrations are highly affected by food intake. Last, several studies of this thesis contained both BTM and DXA measurements, which allowed us to compare both short- and longer term effects of therapy or disease on bone health. DXA scans were included in the studies only if they were performed on the same DXA machine, to ensure that BMD data were comparable.



## Limitations

This thesis contains both longitudinal and cross-sectional studies. The cross-sectional aspect of some studies limits us to discuss causality of the associations we found. In addition, the natural course through time of changes in BTMs or BMD could not be evaluated in the cross-sectional studies. A possible bias due to confounding can be expected as all studies are observational studies. Not all studies contained a control group, especially in the studies in transgender persons as a control group was not ethical to include. In the transgender person studies it can be questioned which sex is the appropriate sex to us as reference sex when evaluating BTMs and BMD; we used the reference ranges of sex assigned at birth, but this needs further study. In the transgender studies, progestagens were also used by some trans women, which results in a decrease of testosterone itself [31]. This might have had a small effect on the changes in BMD and BTMs that we described as being a estradiol effect alone. Furthermore, the effects of lifestyle advice in the transgender studies during HT, but also in the HIV and MS studies were not possible to evaluate. In general, no data on

fractures, bone histomorphometry or bone geometry (such as Trabecular Bone Score (TBS)) were available. Last, most studies had a relatively short follow-up time.

## Summary and conclusion

We show that BTMs are useful in clinical practice to diagnose tumor induced osteomalacia, van Buchem disease, Paget's disease, or hypophosphatemic rickets. In addition, BTMs are useful to monitor disease activity in chronic kidney disease and Paget's disease which assists the clinician to assess the prognosis of his/her patient. Moreover, although measuring BTMs is not useful to predict the fracture risk of the individual patient, BTMs are useful to monitor treatment effects in patients with osteoporosis. Based upon the results of the studies of this thesis we can further conclude that there is no need to routinely measure BTMs in transgender persons, HIV patients and MS patients. FGF23 is not recommended to measure as biomarker in prostate cancer patients. However to assess bone health in these patient groups, DXA can be performed in case of low vitamin D status, suspicion of high fracture risk, or previous lower BMD.

The studies in this thesis attribute to the knowledge of BTMs from both clinical and biochemical perspective. As it remains a clinical challenge to choose the best BTMs to use and to interpret them with all possible (pre)analytical pitfalls, we underline the need for the clinician to inform oneself about these pitfalls one can come across when measuring BTMs. For clinical practice, it would be of great help if the clinicians and clinical chemists collaborate when requesting BTM measurement(s), in order to choose the right BTM and assay for the patient and to minimize the possibility of interpreting BTM concentrations falsely. Keeping in mind these challenges, we have shown that BTMs can contribute to a direct evaluation of bone health compared to longer term evaluation by DXA.



By all means, adequate standardized sample collection and analysis are the cornerstones of correct interpretation of BTMs. When measuring BTMs, the preferred timing is in the morning after an overnight fast, to limit effects of both circadian rhythm and food intake on bone turnover as much as possible. In addition, the age, sex, kidney function, liver function and previous fractures of the patient should all be taken into account when interpreting BTM concentrations.

For future research, it would be of great interest if changes in BTMs in large patient groups would be studied combined with additional information on bone health such as bone histomorphometry, fracture risk and rates and trabecular bone score as outcome measures. We also stress the urge to improve assay standardization, due to the high variety of different assays used, often with their own standardization. In addition, we emphasize the need for well described reference ranges for BTMs, preferably linked to sex and age of the patient.

To conclude, BTMs are useful to diagnose and to monitor primary bone disease. The additional value of BTM measurements in other diseases are limited, despite the effects these diseases may have on bone tissue. Further research is needed, with standardized assays, under standardized (pre-analytical) conditions, using also newer bone related proteins such as FGF23 and sclerostin, to show whether there are more possibilities to use BTMs in clinical care to improve the bone health of our patients.

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# Nederlandse samenvatting (Dutch summary)

## Botmarkers

Gedurende het gehele leven wordt oud en beschadigd bot afgebroken en nieuw bot gevormd. Dit continue gekoppelde proces wordt botremodellering genoemd. Osteoclasten, osteoblasten en osteocyten zijn de regulerende cellen van dit proces, waarbij de eerste stap botafbraak is, gevolgd door botaanmaak. De zogenaamde bot turnover markers (BTMs) zijn factoren die door de eerder genoemde cellen zelf geproduceerd worden of die vrijkomen bij bot aanmaak en bot afbraak. Deze BTMs kunnen vervolgens in bloed of urine gemeten worden. Aangezien BTMs een afspiegeling zijn van de botafbraak en botaanmaak in het lichaam geven zij het actuele bot metabolisme weer.

In de kliniek worden BTMs gemeten om botziekten en ziekten die effect hebben op bot vast te kunnen stellen, het beloop van een bot (gerelateerde) ziekte te evalueren of het effect van een behandeling te kunnen monitoren. Het is voor de clinicus echter vaak lastig om de meest geschikte BTM voor zijn/haar patiënt te kiezen. In de eerste plaats vanwege de grote verscheidenheid aan BTMs die gemeten kunnen worden. Ten tweede zijn bij het bepalen en interpreteren van een BTM vele factoren van belang om rekening mee te houden; onder andere geslacht, nierfunctie, leverfunctie, recente botbreuk en voedingsstatus van de patiënt, het dag-nacht ritme van sommige BTMs en de (pre) analytische variabiliteit van de gekozen assay waarmee de BTM gemeten wordt. Al deze punten dienen daarom te worden meegenomen bij de aanvraag en interpretatie van een BTM. Voor de clinicus is idealiter de beste BTM om te meten een marker die:

- alleen in botweefsel zelf geproduceerd wordt;
- via een gestandaardiseerde laboratorium methode tegen lage kosten, met lage variabiliteit en hoge nauwkeurigheid gemeten kan worden;
- vaststaande referentiewaardes heeft;
- die geassocieerd is met uitkomstmaten die van belang zijn voor de botgezondheid zoals de botsterkte, weergegeven als botmineraaldichtheid (BMD) of het risico op een botbreuk.

In dit proefschrift wordt het begrip "clinical utility" oftewel klinische toepasbaarheid van BTMs gebruikt. Hierbij wordt bedoeld dat een BTM geschikt is voor:

1. het stellen van een diagnose van een bot (gerelateerde) ziekte;
2. het maken van een prognose t.a.v. verbetering of verslechtering van een bot (gerelateerde) ziekte of
3. het classificeren van hoog risico patiënten ten aanzien van bijvoorbeeld hun risico op een botbreuk.

Alle genoemde punten kunnen de clinicus helpen de patiënt te adviseren en hierdoor een optimale botgezondheid te waarborgen. Dit resulteert in een algeheel betere gezondheid voor de patiënt en uiteindelijk ook in een reductie van gezondheidszorgkosten. Dit proefschrift zal daarom onder andere ingaan op de vraag wanneer en welke BTM toegevoegde waarde heeft in de dagelijkse kliniek.

## Bot en botcellen

De voornaamste functie van bot is om de interne organen te beschermen en om structuur aan het lichaam te geven. Verder bevat bot beenmerg, waaruit stamcellen worden gevormd, is bot een reservoir voor calcium en fosfaat, functioneert bot als een buffer voor zuur-base evenwichten en is het betrokken bij meerdere hormonale processen zoals de glucose huishouding in het lichaam.

Qua anatomie is bot in te delen in trabeculair en corticaal bot. Trabeculair bot bevindt zich voornamelijk in de wervelkolom en bevat vele botbalkjes waartussen beenmerg aanwezig is. Corticaal bot is compact bot, wat 80% van het totale botvolume inneemt en zich vooral in de heup bevindt. Botweefsel zelf bestaat uit een botmatrix, waarin zich de bot afbrekende en bot vormende cellen bevinden. Deze botmatrix zorgt voor de rigiditeit van bot en bestaat vooral uit anorganisch calcium hydroxyapatiet en type I collageen. Dit type collageen is het hoofdbestanddeel van osteoid, waarmee nieuw gevormd ongemineraliseerd bot wordt bedoeld. Type I collageen zit vooral in botweefsel, maar in mindere mate ook in de huid en pezen.

De volgende cellen zijn van belang bij het botmetabolisme:

1. Osteoclasten: bot afbrekende cellen die afkomstig zijn van de monocyt-cel reeks en daardoor qua functie lijken op macrofagen. Osteoclasten resorberen de botmatrix enerzijds door zure substanties uit te scheiden en anderzijds door gebruik te maken van bepaalde enzymen, waardoor collageen type I wordt geresorbeerd. Carboxy-terminaal telopeptide van type I collageen (CTX), amino-terminaal telopeptide van type I collageen (NTX), deoxypyridinoline (DPD), tartrate resistant alkaline phosphatase 5b (TRAP5b) en carboxy-terminaal telopeptide van type I collageen (ICTP) zijn voorbeelden van BTMs die de mate van botafbraak weergeven.
2. Osteoblasten: bot aanmakende cellen die afkomstig zijn van osteoprogenitor cellen van stamcellen. Osteoblasten vormen de botmatrix door type I collageen te maken en daarnaast mineraliseren zij het osteoid. Amino-terminaal propeptide van type I procollageen (PINP), osteocalcine (OC) en bot specifiek alkalische phosphatase (BALP) zijn hier voorbeelden van. Bij mineralisatie van het osteoid spelen vooral BALP en OC een belangrijke rol.
3. Osteocyten: oudere, geïnactiveerde osteoblasten, die liggen ingebed in de botmatrix. Deze cellen staan via kleine kanaaltjes met elkaar in verbinding, waardoor de osteocyten het botmetabolisme reguleren via onder andere stoffen als fibroblast growth factor-23 (FGF23) en sclerostine die zij produceren. FGF23 resulteert onder andere in fosfaat uitscheiding via de nier door het parathormoon (PTH) te verlagen en door het enzym 1-alpha-hydryxolase in de nier te remmen. Sclerostine remt de Wnt-signalling cascade in de cel, hetgeen resulteert in verminderde botaanmaak.

## Factoren van invloed op botmetabolisme en botmineraaldichtheid

Het botmetabolisme wordt onder andere beïnvloed door geslachtshormonen, insuline-like growth factor (IGF-1), PTH, cortisol, calcium, vitamine D en mechanische belasting van het skelet via spierkracht en door gewicht. De geslachtshormonen (met name oestrogeen) spelen een prominente rol in de botontwikkeling. Oestrogenen zorgen voor remming van de osteoclasten en dus remming

van de afbraak van bot. Bij mannen vindt omzetting van het testosteron naar oestrogeen plaats, door een enzymatisch proces dat aromatisatie genoemd wordt. In de puberteit groeien de botten in de lengte en dikte. Nadat aan het einde van de puberteit de lengte groei van bot stopt, gaat het proces van botmineralisatie door, waardoor de piek bot massa rond de leeftijd van 30 jaar bereikt wordt; deze is bij mannen hoger dan bij vrouwen. Op volwassen leeftijd neemt vervolgens de bot afbraak meer toe dan de bot aanmaak, waardoor er een afname van BMD plaatsvindt. Het verlies van botmassa vindt vooral plaats in het corticale bot en wordt bij vrouwen grotendeels veroorzaakt door afname van de eigen lichaamseigen oestrogeen productie als gevolg van de menopauze. Andere factoren die het botmetabolisme beïnvloeden en in dit proefschrift worden beschreven zijn bijvoorbeeld medicatie, zoals geslachtshormoontherapie of puberteitsremmers (gonadal trophin releasing hormone analoog (GnRHa)) in transgender personen. Verder zijn ontstekingsfactoren bij auto-immuun of infectieuze ziekten of van tumor afkomstige factoren van negatieve invloed op het botmetabolisme. Al deze stoffen resulteren vaak in toegenomen botafbraak, waardoor de BMD en dus botsterkte afnemen en de patiënt een groter risico op een botbreuk heeft.

## Evaluatie botdichtheid middels DXA



In de huidige klinische praktijk wordt bot gezondheid vooral geëvalueerd door het verrichten van een twee-dimensionele X-ray absorptiometrie (DXA) scan waarbij BMD wordt weergegeven in gram per vierkante centimeter ($g/cm^2$). DXA wordt vooral gebruikt om de botziekte osteoporose te diagnosticeren. Osteoporose wordt gekarakteriseerd door een achteruitgang van de botstructuur en botkwaliteit, hetgeen resulteert in verminderde botsterkte en hierdoor hoger risico op botbreuken. De DXA houdt echter geen rekening met vorm en grootte van het bot, waardoor de werkelijke BMD kan worden onder- of overschat, wat vooral in jonge, groeiende personen die hun piek bot massa nog niet bereikt hebben van belang is. Verder onderscheid DXA geen trabeculair bot van corticaal bot en wordt alleen het gemineraliseerde bot weergegeven, waarbij het van belang is dat nieuw gevormd bot 1-3 jaar nodig heeft om uiteindelijk volledig gemineraliseerd te worden.

Door genoemde punten is een DXA waardevol om lange termijn veranderingen van BMD weer te geven, echter geeft een DXA geen informatie over het actuele botmetabolisme. BTMs geven wel het actuele botmetabolisme weer en kunnen dus gebruikt worden om korte termijn veranderingen in bot weer te geven. In dit proefschrift worden daarom indien mogelijk veranderingen van zowel BTMs als DXA uitslagen aan elkaar gerelateerd om zowel korte- als lange termijn veranderingen van het botmetabolisme te onderzoeken in verschillende patiëntengroepen.

## Doel van het proefschrift

Het doel van dit proefschrift is om inzicht te verkrijgen in het gebruik van verschillende BTMs in de dagelijkse praktijk, om hun beperkingen in het gebruik te kwantificeren en om veranderingen van BTM concentraties door ziekte of medicatie gebruik in verschillende patiënt groepen te bestuderen. Dit proefschrift draagt hierdoor bij aan extra kennis over de:

- klinische toepasbaarheid en aandachtspunten bij de bepaling van verschillende BTMs;

- effecten van geslachtsaanpassende hormoontherapie op het botmetabolisme van adolescente en volwassen transgender personen;
- effecten van inflammatie met het humaan immuno deficiëntie virus (HIV-1) op het botmetabolisme in volwassen mannen;
- effecten van de auto-immuun aandoening multiple sclerose (MS) op het vitamine D – en botmetabolisme in volwassen patiënten versus hun gezonde controles;
- potentiële rol van FGF23 als tumor marker bij patiënten met prostaatkanker.

Om bovenstaande punten te onderzoeken hebben wij in dit proefschrift veelal grote studiegroepen kunnen includeren waardoor subgroep analyses mogelijk waren. Ook van zeldzamere patiëntengroepen (adolescente transgender personen en HIV patiënten die nog niet met medicatie behandeld zijn) hebben wij aanzienlijke groepen kunnen bestuderen. Verder hebben wij gebruik gemaakt van state-of-the-art laboratorium technieken voor de bepaling van BTMs in hetzelfde laboratorium.

## Hoofdbevindingen



**Hoofdstuk 2** bevat een review waarin een overzicht van recente literatuur wordt weergegeven met betrekking tot de klinische toepasbaarheid van BTMs in verschillende ziekten. Tevens worden hierin de potentiële valkuilen bij het aanvragen en interpreteren van BTMs beschreven. Met betrekking tot klinische toepasbaarheid zijn BTMs geschikt voor:

1. het stellen van de diagnose van een bot (gerelateerde) ziekte in geval van tumor geïnduceerde osteomalacie (FGF23, ALP), ziekte van Van Buchem (sclerostine, ALP, P1NP, osteocalcine), ziekte van Paget ((B)ALP en P1NP) en hypofosfatemische rachitis (ALP, FGF23).
2. het maken van een prognose t.a.v. verbetering of verslechtering van een bot (gerelateerde) ziekte in het geval van chronische nierziekten ((B)ALP, trimerisch P1NP, TRAP5B en FGF23), ziekte van Paget ((B)ALP, P1NP). In geval van osteoporose kunnen behandeleffecten worden geëvalueerd door het meten van CTX en P1NP.

Verder zijn BTMs niet geschikt voor

1. het classificeren van individuele patiënten met een hoog risico op een botbreuk.

Verder toont ons review dat met name bij bot afbraakmarkers (behalve TRAP5B) het dag-nacht ritme van belang is, waarbij hogere concentraties 's nachts en 's ochtends vroeg gemeten worden. De resorptie marker CTX dient in nuchtere staat te worden bepaald, aangezien concentraties dalen na voedselinname. In het geval van verminderde nierfunctie kunnen trimerisch P1NP, BALP en TRAP5B betrouwbaar geïnterpreteerd worden. Het is verder van belang dat BTMs 6 maanden tot een jaar na een botbreuk verhoogd kunnen blijven en tenslotte zijn leeftijd en geslacht van de patiënt relevant, aangezien BTMs bij mannen en op jongere leeftijd hogere concentraties bereiken.

**Hoofdstuk 3 tot en met 5** beschrijven studies naar het botmetabolisme en de BMD van transgender personen. Er is sprake van genderdysforie als er een verschil bestaat tussen het geboortegeslacht en het geslacht waarmee iemand zich identificeert, wat gepaard gaat met lijdensdruk. Een trans vrouw (man naar vrouw) identificeert zich als vrouw, maar krijgt bij de geboorte het mannelijk geslacht toegewezen. Hormoontherapie (HT) bestaat bij een trans vrouw uit anti

androgenen (onderdrukken de mannelijke testosteron productie) plus oestrogenen (vrouwelijk geslachtshormoon) om de gewenste lichaamsverandering te realiseren. Een trans man (vrouw naar man) identificeert zich als man, maar heeft bij de geboorte het vrouwelijk geslacht toegewezen gekregen. HT bij een trans man bestaat uit testosteron om het lichaam naar het gewenste geslacht aan te passen. Behalve HT kunnen er ook geslachtsaanpassende operaties worden uitgevoerd. Onderstaande studies werden uitgevoerd om het effect van veranderingen in geslachtshormonen en puberteitsremming op het botmetabolisme en BMD te kunnen evalueren.

In **hoofdstuk 3** hebben wij onderzocht wat het effect is van puberteitsremming en HT op het botmetabolisme van adolescente transgender personen. In deze studie werden adolescente transgender personen bestudeerd en hebben wij zowel afbraak als aanmaak BTMs (ICTP, P1NP en osteocalcine) gemeten en de volumetrische bot mineraal dichtheid (BMAD) berekend op drie verschillende momenten. De BTMs daalden tijdens puberteitsremming en de BMD van met name de wervelkolom nam ook af. Deze studie toont het negatieve effect van lage concentraties geslachtshormonen (met name oestrogenen), veroorzaakt door puberteitsremmers (GnRH-analogen), op het botmetabolisme. Twee jaar na start van de hormoontherapie zagen wij een verdere daling van de BTMs, behalve bij oudere trans mannen. Verder zagen wij in alle groepen een normalisatie van de BMD. Deze uitkomsten passen bij het positieve effect van geslachtshormonen (met name oestrogenen) op bot, waardoor de osteoclasten en dus de botafbraak wordt geremd. Deze remming gebeurt enerzijds direct, dan wel na de enzymatische omzetting van testosteron in oestrogenen.



**Hoofdstuk 4** beschrijft een studie naar de veranderingen in BMD bij volwassen transgender personen gedurende 1 jaar HT. In zowel trans vrouwen als trans mannen neemt de BMD van de wervelkolom toe na 1 jaar HT. In trans vrouwen werd verder een toename van de BMD in de totale heup regio en heup nek regio gevonden, waarbij in trans mannen een kleinere toename van de totale heup regio en geen verschil in de heup nek regio werd gezien. Trans vrouwen hadden vaker een lager vitamine D concentratie en indien zij vitamine D suppletie gebruikten tijdens HT werd bij hen een grote toename van de BMD gevonden in vergelijking met trans vrouwen die dit niet gebruikten.

In **hoofdstuk 5** hebben wij gekeken naar het verschil in botmetabolisme gedurende 1 jaar HT in volwassen transgender personen en associaties hiervan met veranderingen in BMD. In trans vrouwen namen de BTMs af. Trans vrouwen met hogere oestrogeen concentraties hadden een grotere daling van de BTMs en een grotere toename van BMD, hetgeen komt doordat oestrogenen de botafbraak remmen. In trans mannen stegen de BTMs, waarbij de BMD ook toenam. Dit komt waarschijnlijk doordat de bot aanmaak markers naar verhouding meer toe namen dan de bot afbraak markers. De groep trans mannen > 50 jaar had een grotere daling van BTMs en toename van BMD in vergelijking met jongere trans mannen. Dit verschil wordt waarschijnlijk veroorzaakt door het feit dat de oudere transmannen een sterkere stijging in de oestrogenen concentraties hadden door HT bij een lage uitgangs-oestrogenen concentratie doordat zij postmenopauzaal waren voor de start van HT. Het verschil in oestrogenen concentraties was namelijk een stuk kleiner in de jonge trans mannen groep. Overigens verschilden de veranderingen in testosteron concentraties tussen

beide groepen evenveel, waardoor dit ook past bij het feit dat van de geslachtshormonen vooral oestrogenen een positief effect hebben op bot.

**Hoofdstuk 6 en 7** beschrijven studies die effecten van inflammatie en auto-immuun ziekte op het botmetabolisme hebben onderzocht. Hiertoe zijn een mannelijke HIV populatie en MS populatie met hun controles bestudeerd.

In **hoofdstuk 6** hebben wij onderzocht hoe BTMs en BMD in volwassen mannen met een recent vastgestelde HIV-infectie worden beïnvloed. Hiertoe werden twee groepen bestudeerd waarin de ene groep wel behandeling met gecombineerde anti retrovirale therapie (cART) kreeg en de andere groep nog niet met cART behandeld werd. De groep mannen die wel werden behandeld met cART hadden toegenomen BTMs in vergelijking met de onbehandelde mannen. In beide groepen bleef de wervelkolom BMD stabiel en nam de BMD van de heup af. Dit verschil kan verklaard worden door actievere ziekte in de behandelde groep en anderzijds ook door het effect van cART zelf (met name het middel tenofovir) op bot. Dit medicijn zorgt dat er meer fosfaat wordt uitgescheiden via de urine, hetgeen een negatief effect heeft op de botkwaliteit. Tegenwoordig worden HIV patiënten direct na diagnose stelling met cART behandeld, dit maakt dat deze studie een schaarse patiënten groep beschrijft.



**Hoofdstuk 7** bevat een studie waarin wij hebben gekeken naar verschillen in vitamine D metabolieten, FGF23 en BTMs in patiënten met MS in vergelijking met hun gezonde controles. Aangezien vitamine D deels gekoppeld zit aan eiwitten hebben wij ook deze eiwitten gemeten (onder andere vitamin D binding protein (VDBP)) en vervolgens de vrije en biologisch beschikbare fractie van de verschillende vitamine D metabolieten berekend. Wij vonden geen verschil tussen FGF23 concentraties, de ratio 25(OH)D/24.25$(OH)_2$D of BTMs in MS patiënten en hun controles. MS patiënten hadden echter wel lagere biologisch beschikbare 25(OH)D en 1.25$(OH)_2$D concentraties vergeleken met de controles. Er was verder een verschil tussen mannelijke en vrouwelijke patiënten, waarbij VDBP hoger was in mannelijke MS patiënten. Deze studie toont aldus een verschil in vitamine D metabolisme tussen MS en gezonde controles.

Ten slotte bevat **hoofdstuk 8** een pilot studie waarin wij hebben uitgezocht of FGF23 een potentiele biomarker kan zijn in patiënten met prostaatkanker. Eerdere literatuur suggereerde dat op cel niveau de groeifactor activiteit in prostaatkanker cellen toenam onder invloed van FGF23. Echter in onze klinische studie zagen wij dat niet terug, aangezien FGF23 niet verhoogd was in de prostaatkanker groep in vergelijking met gezonde mannen en mannen met een vergrote prostaat zonder dat hierin kanker aanwezig was. Wij toonden in deze studie aan dat de nierfunctie van belang is bij de interpretatie van FGF23 concentraties, aangezien deze concentratie toeneemt bij afnemende nierfunctie. Concluderend vinden wij FGF23 geen geschikte biomarker in prostaatkanker.

## Klinische implicaties van dit proefschrift

Ten eerste hebben wij middels **hoofdstuk 2** uit dit proefschrift kennis bij elkaar gebracht over het nut en de beperkingen van BTM bepalingen in de kliniek. Clinici hebben nu meer handvatten welke BTM wanneer kan worden aangevraagd en hebben wij inzicht verschaft over de potentiele valkuilen per bepaling en bij de interpretatie van de uitslagen.

Ten tweede, op basis van hoofdstuk **3 tot en met 5** zien wij geen meerwaarde van het bepalen van BTMs als onderdeel van standaard transgender zorg, noch in adolescenten noch in volwassenen. Het is voor met name trans vrouwen van belang een eventueel vitamine D tekort na te gaan en indien nodig suppletie te starten. Verder dienen leefstijladviezen zoals voldoende lichaamsbeweging, zonlichtexpositie, calcium inname en het staken van roken en liefst ook alcohol besproken te worden. Aangezien vooral trans vrouwen lagere BMD hebben, dient in deze groep laagdrempelig een DXA verricht te worden, bijvoorbeeld in geval van botbreuken op jonge leeftijd. In de adolescenten studie **(hoofdstuk 3)** illustreren wij de nadelige effecten van puberteitsremmers op de botkwaliteit. Concluderend zien wij in zowel adolescenten als volwassenen transgender personen geen nadelige effecten van HT op de botgezondheid in deze korte-termijn studies. Tenslotte is het van groot belang dat de therapietrouw ten aanzien van de hormoontherapie wordt nagegaan, gezien de positieve rol van oestrogenen op de botgezondheid, die uit al onze studies naar voren komt.

Als derde punt noemen wij dat op grond van **hoofdstuk 6** er geen noodzaak is tot routinematig bepalen van BTMs tijdens behandeling van mannelijke HIV patiënten. BTMs kunnen wel gemeten worden na wisselen van cART tenofovir voor een andere vorm van cART, om zodoende het effect op de botgezondheid te evalueren, aangezien dit via een DXA scan pas op veel later moment betrouwbaar kan worden bekeken. Aangezien er in deze patiëntgroep vaker een lage BMD wordt gevonden met een hoger risico op botbreuken, raden wij ook bij deze patiëntgroep leefstijladviezen ten aanzien van lichaamsbeweging, zonlicht, calcium, vitamine D, staken van roken en alcohol aan. Een DXA scan dient laagdrempelig verricht te worden in deze groep, bijvoorbeeld in geval van meerdere botbreuken.



Als vierde punt zien wij in MS patiënten geen redenen om BTMs te meten als onderdeel van hun periodieke controles. Op basis van **hoofdstuk 7** zien wij wel meerwaarde van het bestuderen van andere vitamine D metabolieten in patiënten met MS in toekomstige studies. Ook in deze patiëntgroep raden wij de eerder genoemde leefstijladviezen aan, met specifieke aandacht voor vitamine D suppletie indien nodig. Ook dient bij deze patiënten laagdrempelig een DXA scan verricht te worden om de botkwaliteit te evalueren.

Tenslotte zien wij op grond van **hoofdstuk 8** geen rol voor FGF23 als biomarker weggelegd in prostaatkanker patiënten. In deze studie komt verdere noodzaak van de interpretatie van FGF23 in het licht van de nierfunctie van de patiënt duidelijk naar voren.

## Toekomstperspectieven

In de studies beschreven in dit proefschrift hebben wij de BTMs zoveel mogelijk gerelateerd aan uitslagen van DXA. Er zijn echter ook grote studies nodig die naast vergelijking met DXA ook specifiek naar het risico op botbreuken in relatie tot de BTMs en veranderingen hiervan kijken, aangezien botbreuken een klinische uiting (kunnen) zijn van verminderde botkwaliteit. Verder dienen studies te worden verricht waarin bothistomorphometrie en geometrische verschillen in bot bijvoorbeeld door middel van het berekenen van trabecular bone score (TBS) via DXA worden meegenomen als uitkomstmaten om zodoende een beter beeld van de totale botgezondheid te verkrijgen.

Ten aanzien van de studies in de transgender personen, zijn er grote studies nodig om na te gaan of het bij geboorte toegewezen geslacht als referentie geslacht moet worden blijven gebruikt. In MS patiënten lijkt er een immuun systeem modulerende rol van vitamine D en VDBP te bestaan, welke nog verder bestudeerd dienen te worden. Tevens dienen de gevonden geslachtsverschillen ten aanzien van het vitamine D-FGF23 metabolisme in deze patiëntgroep verder uitgezocht te worden. Ten aanzien van prostaatkanker, maar ook bij andere vormen van kanker is het wenselijk dat er studies gedaan worden om na te gaan of andere BTMs kunnen worden gebruikt ter monitoring van kanker met risico op botuitzaaiingen.

Tenslotte is er op klinisch chemisch gebied noodzaak om de standaardisatie van de BTM te verbeteren vanwege de grote variatie tussen verschillende assays die per BTM worden gebruikt in de verscheidene laboratoria wereldwijd. Hierdoor blijft het vergelijken van studie uitkomsten tot op heden nog moeizaam. Verder dienen er adequate referentiewaardes per leeftijd en geslacht per BTM worden vastgesteld. Naar verwachting zullen alle bovenstaande punten verder bijdragen aan de verbetering van de bot gezondheid van onze patiënten.

## Conclusie



Studies in dit proefschrift dragen bij aan kennis over BTMs vanuit zowel klinisch als klinisch chemisch perspectief. Wij hebben in dit proefschrift onderzocht wanneer BTMs van klinisch toegevoegde waarde zijn. BTMs zijn te gebruiken als diagnosticum van tumor geïnduceerde osteomalacie, ziekte van Van Buchem, ziekte van Paget en hypofosfatemische rachitis. Verder zijn zij te gebruiken ter monitoring van de ziekte activiteit in chronische nierziekte en ziekte van Paget. Tenslotte zijn BTMs geschikt ter evaluatie van het behandeleffect in patiënten met osteoporose. Momenteel zijn de BTMs (nog niet) geschikt als indicator van het risico op botbreuken in de individuele patiënt.

Op grond van de resultaten uit de studies in dit proefschrift achten wij het niet nodig om BTMs te meten als onderdeel van standaard patiëntenzorg in transgender personen (adolescenten en volwassenen), HIV, MS of prostaatkanker patiënten. Het verdient aanbeveling in deze groepen een DXA te verrichten in geval van botbreuken en/of hoog risico op een botbreuk, lage vitamine D concentraties en ter evaluatie van eerder bekende lage BMD. Al met al hebben wij wel aangetoond dat BTMs geschikt zijn voor een directe evaluatie van het botmetabolisme ten opzichte van de langere termijn DXA meting.

Gezien de verscheidene kanttekeningen bij de bepaling en interpretatie van een BTM blijft het een uitdaging voor de clinicus welke BTM de beste keuze is. Wij benadrukken op grond van onze studies de noodzaak voor de clinicus zich te informeren over de potentiele valkuilen per BTM. Wij adviseren daarom om laagdrempelig met een klinisch chemicus te overleggen om zodoende de meest geschikte BTM aan te kunnen vragen en onjuiste interpretatie te voorkomen. Ten aanzien van de bepaling van een BTM zijn een gestandaardiseerde sample afname, verzameling en analyse de hoeksteen van iedere BTM meting. Verder dient een BTM meting liefst in de ochtend na een nacht vasten te worden afgenomen, om zo de invloed van het dag-nacht ritme en voedselinname op de BTM concentratie te reduceren. Bij de interpretatie dient ten slotte altijd rekening gehouden te worden met het geslacht, de leeftijd van patiënt, recente botbreuken en de lever- en nierfunctie.

Concluderend draagt het meten van BTMs bij in de diagnostiek en monitoring van primaire botziekten. Er is weinig toegevoegde waarde voor het meten van BTMs bij andere ziekten, ook al hebben die effect op het bot. Nieuw onderzoek naar BTMs met gestandaardiseerde assays onder gestandaardiseerde afname condities, met speciale aandacht voor nieuwe bot gerelateerde markers zoals FGF23 en sclerostine, is nodig. Hierdoor kan worden onderzocht of deze markers toegevoegde waarde hebben in andere klinische situaties.

# Part VI

## APPENDICES

# List of publications

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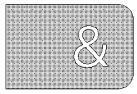

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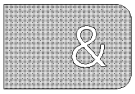

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# Portfolio

## Presentations at (inter)national conferences and symposia

### 2020

*Multiple sclerosis patients show lower bioavailable 25(OH)D and 1,25(OH)$_2$D, but no difference in ratio of 25(OH)D/24,25(OH)$_2$D and FGF23 concentrations,* Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands, poster pitch

### 2019

*Gender-affirming hormonal treatment decreases bone turnover in transwomen and older transmen*, Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands, oral presentation

### 2018

*Bone turnover markers and bone mineral density in transgender people treated with gender-affirming hormones: a prospective study,* World Professional Association for Transgender Health (WPATH), Buenos Aires, Argentina, oral presentation

*Estrogen Decreases Bone Turnover and Increases Bone Mineral Density in Transwomen: a Prospective Study,* American Society for Bone and Mineral Research (ASBMR), Quebec, Canada, poster presentation

### 2017

*Effect of HIV status and antiretroviral therapy on bone turnover and bone mineral density in HIV positive men*, Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands, poster pitch

*Effect of HIV status and antiretroviral therapy on bone turnover and bone mineral density in HIV positive men,* yearly conference of the Netherlands Society for Clinical Chemistry and Laboratory Medicine (NVKC), Scheveningen, the Netherlands, poster pitch

### 2016

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands, oral presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, yearly conference of the NVKC, Papendal, the Netherlands, oral poster presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, World Professional Association for Transgender Health (WPATH), Amsterdam, the Netherlands, oral presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, Science exchange day, Amsterdam University Medical Center, location VUmc, Amsterdam, the Netherlands, poster presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, European Young Endocrine Scientist meeting (EYES), Moscow, Russia, oral presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers and BMAD in transgender adolescents*, European Calcified Tissue Society (ECTS), Rome, Italy, poster pitch

2015

*Effect of one year cross-sex hormonal treatment on bone mineral density of lumbar spine and hip in transgender patients*, European Calcified Tissue Society (ECTS), Rotterdam, the Netherlands, poster presentation

*Effect of one year cross-sex hormonal treatment on bone mineral density of the lumbar spine in transgender patients,* European Society for Endocrinology (ESE) Summerschool, Bregenz, Austria, poster presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents,* European Society for Endocrinology (ESE) Summerschool, Bregenz, Austria, poster presentation

*Effect of one year cross-sex hormonal treatment on bone mineral density of lumbar spine and hip in transgender patients*, European Congress of Endocrinology (ECE), Dublin, Ireland, guided poster presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, young Dutch Endocrine Society (JNVE), Leiden, the Netherlands, oral presentation

*Effect of postponing puberty and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents*, Dutch Society for Calcium and Bone Metabolism (NVCB), Zeist, the Netherlands, oral presentation

*Effect of one year cross-sex hormonal treatment on bone mineral density of lumbar spine and hip in transgender patients*, European Network for the Investigation of Gender Incongruence (ENIGI) meeting, Amsterdam, the Netherlands, poster pitch

*Effect of one year cross-sex hormonal treatment on bone mineral density of the lumbar spine in transgender patients*, Science exchange day, Amsterdam University Medical Center, location VUmc, Amsterdam, the Netherlands, poster presentation

## Invited presentations

### 2020

*Hypogonadism in adult men,* presentation of Dutch guideline, on behalf of the Gonadal Endocrinology network group of the Dutch Endocrinology Society (NVE), Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands

### 2019

*Bone in balance: the clinical utility of bone turnover markers,* Dutch Endocrine Meeting, Noordwijkerhout, the Netherlands

*Additional options of using DXA-scans,* Dutch course on bone health, Amsterdam University Medical Center, location VUmc, Amsterdam, the Netherlands

### 2018

*Bone health in transgender persons,* European Society for Endocrinology (ESE), Summerschool, Berlin, Germany

### 2017

*Secondary osteoporosis*, European Society for Endocrinology (ESE) post-graduate course, Lviv, Ukrain

*Effect of pubertal suppression and cross-sex hormone therapy on bone turnover markers (and BMAD) in transgender adolescents,* European Society for Endocrinology (ESE) post-graduate course, Moscow, Russia

*Auto-immune related infertility?* European Society for Sexual Medicine (ESSM), Nice, France

## Awards and grants

### 2016

RYES award (Russian Young Endocrinologist Society) at European Young Endocrine Scientist meeting (EYES), Moscow, Russia, best oral presentation

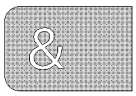
Travel grant European Society of Endocrinology (ESE)

### 2015

Travel grant European Society of Endocrinology (ESE)

## Extracurricular activities

| | |
|---|---|
| 2018 – current | Member of the Gonadal Endocrinology network group, part of Dutch Endocrine Society (NVE), one of main authors of Dutch guideline "Hypogonadism in adult men". |
| 2014 - 2019 | Board member of the Young Dutch Endocrine Society (JNVE), treasurer and conference organization |
| 2014 – 2019 | Supervisor of several students e.g. research minor students and honour programme students |
| 2018 | Co-organizer of the track Endocrinology within the minor of the study of Medicine, Amsterdam University Medical Center, location VUmc, the Netherlands |
| 2017 | Mellanby Center training course: Clinical use of bone turnover markers, Salzburg, Austria |
| 2014 – 2016 | Chair and organizer of the monthly interdisciplinary bone research meeting, Amsterdam University Medical Center, location VUmc, the Netherlands |
| 2015 | Course Clinical Epidemiology, EpidM, Rolduc, the Netherlands "Epidemiological research, design and interpretation", EpidM Vrije Universiteit, Kerkrade, the Netherlands |
| 2015 | Course Scientific Writing, Vrije Universiteit, Amsterdam, the Netherlands |
| 2014 | Course Biostatistics, Amsterdam University Medical Center, location VUmc, the Netherlands |
| 2013 – 2014 | SPSS and SPSS advanced course, Linnaeus Institute, Spaarne Gasthuis, location Haarlem, the Netherlands |



# About the author

Mariska Caroline Vlot was born in Leiden, the Netherlands, on the 12th of December 1984. She completed her pre-university education (gymnasium) at the Herbert Vissers College in Nieuw-Vennep, the Netherlands in 2003. In 2003, she started studying medicine at the Vrije Universiteit (VU) in Amsterdam, the Netherlands, after successfully completing a selection procedure for medical students. During her studies she finished a scientific internship at the andrology department of the Concord Repatriation General Hospital of Sydney, Australia. Her interest in endocrinology was raised further by completing several endocrine based teaching classes and by a voluntary internship at the endocrinology department of the Leiden University Medical Center (LUMC), the Netherlands.

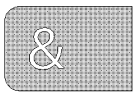
In 2010, Mariska graduated Cum Laude after which she directly proceeded with her further career as resident Internal Medicine being supervised by prof. dr. R.T. ten Kate and dr. W.de Ronde in the Kennemer Gasthuis hospital (currently named Spaarne Gasthuis), Haarlem, the Netherlands, from 2010 to 2014. She travelled to Bonaire, the Netherlands Antilles, in the last year of her residency to complete a facultative junior-internist supervision internship. She finished the first four years of her residency Internal Medicine in 2014. Mariska then temporarily discontinued her residency to perform PhD research.

From 2014 to 2017, Mariska worked at the Endocrine laboratory and Endocrinology department of the Amsterdam University Medical Center (Amsterdam UMC), location VUmc, the Netherlands, where she was supervised by prof. dr. M. den Heijer, prof. dr. M.A. Blankenstein (until he became professor emeritus), prof. dr. R. de Jonge and dr. A.C. Heijboer. During her PhD research, she was board member of the young Dutch Endocrine Society (JNVE) as treasurer and conference organizer from 2015 until 2019.

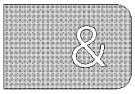
In 2017 Mariska resumed her residency Internal Medicine and started her specialty in Endocrinology at the Amsterdam University Medical Center, location VUmc, the Netherlands. She continued her PhD research in the same period. She became a member of the Gonadal Endocrinology network group of the Dutch Endocrine Society (NVE) in 2018. She completed her specialization in Endocrinology at the end of 2019. Mariska is currently working as internist-endocrinologist at the St Jansdal hospital, Harderwijk and Lelystad, the Netherlands.

# List of abbreviations

| | |
|---|---|
| ALP | alkaline phosphatase |
| ALT | alanine transaminase |
| AST | aspartate transaminase |
| BALP | (bone specific) alkaline phosphatase |
| BMAD | bone mineral apparent density |
| BMD | bone mineral density |
| BMI | body mass index |
| BTM | bone turnover marker |
| cART | combination antiretroviral therapy |
| CI | confidence interval |
| CIS | clinically isolated syndrome |
| CHT | cross-sex hormonal treatment = gender-affirming hormonal treatment |
| CSHT | cross-sex hormone therapy = gender-affirming hormonal treatment |
| CTX | carboxy-terminal telopeptide of type I collagen |
| DPD | deoxypyridinoline |
| DXA | dual-energy X-ray absorptiometry |
| EDSS | expanded disability status scale |
| eGFR | estimated glomerular filtration rate |
| ENIGI | European network for investigation of gender incongruence |
| FGF23 | fibroblast growth factor-23 |
| FN | femoral neck |
| FtM | female to male = trans men |
| yGT | gamma-glutamyltransferase |
| GnRHa | gonadotropin-releasing hormone analogue |
| HIV | human immunodeficiency virus |
| HT | hormone therapy = gender-affirming hormonal treatment |
| ICTP | cross-linked carboxy-terminal telopeptide of type I collagen |
| IQR | interquartile range |
| LH | luteinizing hormone |
| LS | lumbar spine |
| MS | multiple sclerosis |
| MSM | men who have sex with men |
| MtF | male to female = trans women |
| NTX | amino-terminal telopeptide of type I collagen |
| OC | osteocalcin |
| PCa | prostate cancer |
| PHI | primary HIV-infection |
| PICP | carboxy-terminal propeptide of type I procollagen |
| PINP | amino-terminal propeptide of type I procollagen |



| | |
|---|---|
| PPMS | primary progressive MS |
| PSA | prostate specific antigen |
| PTH | parathyroid hormone |
| PYD | pyridinoline |
| RANKL | receptor activator of nuclear factor kappa-B ligand |
| RRMS | relapsing remitting MS |
| SPMS | secondary progressive MS |
| SP + R | secondary progressive + remitting |
| T | testosterone |
| Trans men | assigned with female gender at birth, but identifying himself as male |
| Trans women | assigned with male gender at birth, but identifying herself as female |
| TRAP5b | tartrate resistant alkaline phosphatase 5b |
| VDBP | vitamin D binding protein |



Amsterdam Movement Sciences

Amsterdam Movement Sciences conducts scientific research to optimize physical performance in health and disease based on a fundamental understanding of human movement in order to contribute to the fulfillment of a meaningful life.

COSMETIC
Outcomes Article

# Patient Satisfaction with Breasts and Psychosocial, Sexual, and Physical Well-Being after Breast Augmentation in Male-to-Female Transsexuals

Romain Weigert, M.D.
Eric Frison, M.D.
Quentin Sessiecq, M.D.
Khalid Al Mutairi, M.D.
Vincent Casoli, M.D., Ph.D.

*Bordeaux, France*

**Background:** Satisfaction with breasts, sexual well-being, psychosocial well-being, and physical well-being are essential outcome factors following breast augmentation surgery in male-to-female transsexual patients. The aim of this study was to measure change in patient satisfaction with breasts and sexual, physical, and psychosocial well-being after breast augmentation in male-to-female transsexual patients.

**Methods:** All consecutive male-to-female transsexual patients who underwent breast augmentation between 2008 and 2012 were asked to complete the BREAST-Q Augmentation module questionnaire before surgery, at 4 months, and later after surgery. A prospective cohort study was designed and postoperative scores were compared with baseline scores. Satisfaction with breasts and sexual, physical, and psychosocial outcomes assessment was based on the BREAST-Q.

**Results:** Thirty-five male-to-female transsexual patients completed the questionnaires. BREAST-Q subscale median scores (satisfaction with breasts, +59 points; sexual well-being, +34 points; and psychosocial well-being, +48 points) improved significantly ($p < 0.05$) at 4 months postoperatively and later. No significant change was observed in physical well-being.

**Conclusions:** In this prospective, noncomparative, cohort study, the current results suggest that the gains in breast satisfaction, psychosocial well-being, and sexual well-being after male-to-female transsexual patients undergo breast augmentation are statistically significant and clinically meaningful to the patient at 4 months after surgery and in the long term. (*Plast. Reconstr. Surg.* 132: 1421, 2013.)

**CLINICAL QUESTION/LEVEL OF EVIDENCE:** Therapeutic, IV.


Breast augmentation in male-to-female transsexuals is part of gender reassignment surgery. Hormonal feminization might not be sufficient to induce mammogenesis,[1] so many patients seek surgery for their chests to resemble the female gender. Literature describes techniques and results.[2–6] The World Professional Association for Transgender Health, in the seventh version of the Standards of Care for the Health of Transsexual, Transgender, and Gender Nonconforming People emphasizes that, although breast augmentation can be labeled as an aesthetic procedure, this operation can be medically necessary, depending on the unique clinical situation of a given patient's condition and life situation.[7] No studies report sexual, psychosocial, and health-related quality-of-life changes after breast augmentation in male-to-female transsexuals. The purpose of this study was to evaluate the impact of breast augmentation on patient-reported satisfaction with breasts and sexual, physical, and psychosocial well-being.

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*Presented at the French Society of Plastic, Reconstructive and Aesthetic Surgery Annual Meeting, in Paris, France, November 19 through 21, 2012.*

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**Disclosure:** *The authors have no financial interest to declare in relation to the content of this article.*

## PATIENTS AND METHODS

### Study Sample

Research ethics board approval was granted for this study from the University of Bordeaux Segalen, Bordeaux, France. Patients were recruited from the Hospital Transgender Health Network (Bordeaux University Hospital, Bordeaux, France) from July of 2008 through July of 2012. Breast augmentation procedures were all paid for by health insurance after a medical counselor granted approval. Inclusion criteria were age 18 years or older, capacity to make a fully informed decision and to consent for treatment, sex reassignment surgery already performed, at least 12 months of feminizing hormone therapy, no previous breast surgery, and primary augmentation mammaplasty with the same surgeon.

### Data Collection

In accordance with the European Directive 95/46/EC of the European Parliament and of the European Council of October 24, 1995, on the protection of individuals with regard to the processing of personal data and on the free movement of such data, the database has been declared at the Commission Nationale de l'Informatique et des Libertés, which is the independent French administrative authority protecting privacy and personal data. After being informed on the study, verbal consent was obtained from the patients. They were asked to complete the BREAST-Q Augmentation module at three time points: (1) at 3 weeks preoperatively and (2) at 4 months and (3) at least 12 months after completion of breast augmentation. Patient and treatment data were collected at baseline and after the procedure. Patient information included age, height, weight, body mass index, employment, tobacco status, date of beginning of hormone therapy, date of sex reassignment surgery, sternal notch–to-nipple distance, and breast width. Treatment information included date of surgery, position of incision, pocket plane, and size and shape of the implants. After surgery, surgical information was obtained from the electronic patient record on operative procedure, significant postoperative complications (e.g., hematoma, infection, and capsular contracture), and hospitalization stay.

### BREAST-Q

The BREAST-Q Augmentation module is a patient-reported outcome measure that was specifically designed to assess the health-related quality of life and patient satisfaction after breast augmentation.[8] This instrument was developed and validated with strict adherence to recommended international guidelines[9–11] to remedy the lack of instruments for breast surgery patients.[12] In the original development study, all scales were found to fulfill criteria for good measurement.[8,13] The BREAST-Q was further validated to be appropriately used in clinical research and practice.[14] Four subscales measure well-being and satisfaction before and after augmentation:

1. The Augmentation module's 17-item subscale, satisfaction with breasts, addresses issues such as satisfaction with breast volume and shape; feel to touch; and one's appearance clothed, unclothed, and in a bra.
2. The Augmentation module's nine-item subscale, psychosocial well-being, addresses issues such as feelings of beauty, self-confidence, and self-worth.
3. The Augmentation module's five-item subscale, sexual well-being, addresses issues such as feelings of sexual attractiveness and sexual self-confidence.
4. The Augmentation module's seven-item subscale, physical well-being, addresses issues such as chest pain, sleeping discomfort, and physical activity discomfort.

Five additional subscales measure postaugmentation outcomes related to the satisfaction with outcome, information, medical staff, and office staff. However, because we were assessing the changes in health-related quality of life and patient satisfaction after breast augmentation, only the four subscales that included preaugmentation scores and postaugmentation scores were analyzed. Good psychometric properties have been reported for the BREAST-Q subscales used in the study (Cronbach α, 0.83 to 0.96). Good test-retest reliability has been reported (intraclass correlation coefficient, 0.90 to 0.96).[14] All raw questionnaire data were transformed into BREAST-Q scores using the Q-Score program.[15] Then, scores were computed in summary scores for each BREAST-Q subscale that range from 0 to 100, with higher values representing a more favorable outcome.

### Surgery

#### Preoperative Evaluation

Current cross-sex hormone substitution was not standardized, even if almost all were treated by the same endocrinologist, and consisted of estrogens (100 μg of ethinyl estradiol per day orally).

The selection of implant volume was based both on the patient's chest anatomy and on preference. Evaluation included the expectations of the patient and other features such as the patient's height, weight, chest morphology, existing breast appearance, asymmetries, and thickness of the subcutaneous tissue in the upper and lower poles of the future breast. Information to patient emphasizes the difficulty to attenuate the wide intermammary cleft usually present in the masculine thorax. In our practice, we always choose anatomical implants to fulfill the lack of an axillary process.

All breast augmentations were performed with Perthèse Esthea anatomical breast implants (Perouse Plastie, a Mentor Company, Bornel, France) filled with a cohesive silicone gel with a microtextured surface. The Perthèse Esthea mammary anatomical implant line differentiates itself from other devices on the market through its original microtextured surface—the envelope consists of high-mechanical-resistance medical grade silicone elastomer vulcanized during the manufacturing process—and through specific different base shapes. Photographs were taken at the initial consultation and at the follow-up evaluation.

### Operative Technique

All operations were performed with the patient under general anesthesia. Intercostal nerve block with 15 ml of 7.5 mg/ml ropivacaine was performed for blocking both sides. Preoperative antibiotics were given (cefazolin, 2 g intravenously). All patients had a 45- to 50-mm-long inframammary incision (Fig. 1). Dissection was performed using the electrocautery knife under direct vision. During the procedure, the centerline of the anatomical implant was positioned parallel to the inferior margin of the pectoralis major muscle so that it filled superolateral region toward the axilla. Wound closure was completed in layers using running 3-0 Monocryl (Ethicon GmbH, Norderstedt, Germany) for the fascia and subcutaneous fat because of its softness. Skin closure was performed with 3-0 monofilament resorbable Monocryl suture, placed intracutaneously at the mid-dermis level.

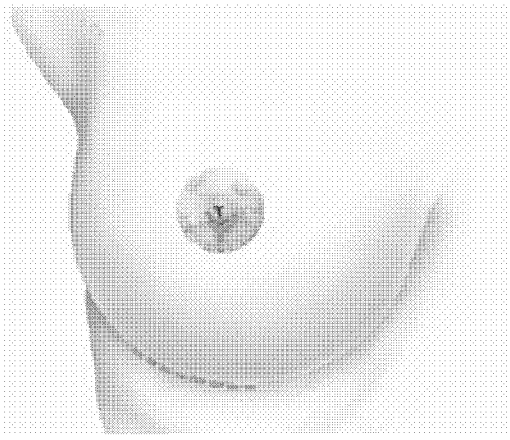

**Fig. 1.** Schematic diagram of the inframammary incision.

### Postoperative Care

Breasts were immobilized for 2 days in a semicompressive dressing. Patients were given a specific supportive, properly sized bra with a front clasp. Drains were removed at the time of discharge from the hospital.

### Postoperative Controls

Postoperative control assessments were performed at 2 days, 21 days, 4 months, 12 months, and later. Control assessments were performed for all patients by the operating surgeon (R.W.). At 4 months and after 12 months, the patients had to complete the postoperative BREAST-Q Augmentation module, which was followed by a physical examination and photographs.

### Sample Size Calculation

According to previous research, we defined a clinically relevant change in the health-related quality of life as a difference that exceeds half a standard deviation of the baseline value.[16,17] Because greater standard deviation of all scores at baseline is approximately 25 for the sexual well-being BREAST-Q subscale in our study population, the minimum significant difference for each subscale is estimated as 13. When the power is set at 80 percent with a standard $\alpha$ of 0.05 and a minimum difference of 13, a minimum sample size of 32 patients was calculated for this study, using the equation for a one-sample paired *t* test.

### Statistical Analysis

Descriptive data were calculated for continuous variables (i.e., mean, standard deviation, median, interquartile range, minimum, and maximum) and categorical variables (i.e., number and frequency). The responsiveness of the BREAST-Q scales was examined at the group level by testing the difference between scores at baseline and after breast augmentation. Because of nonnormal distribution of BREAST-Q scores, we described them with median and interquartile range, and we used the nonparametric Wilcoxon signed rank

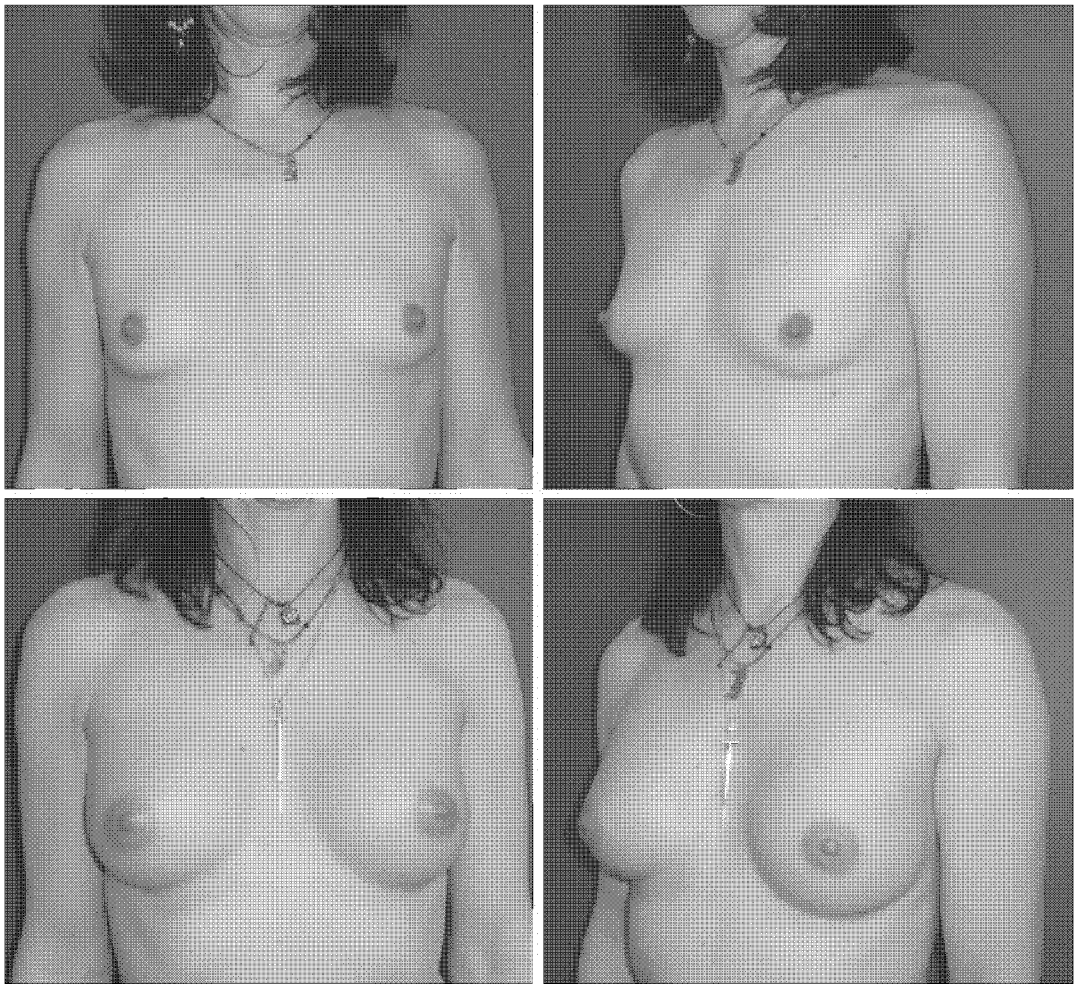

**Fig. 2.** (*Above*) Preoperative views. (*Below*) Four months after implantation with Esthea moderate-profile 325-cc implants.

test for repeated-measures analysis. We then calculated the standard indicator Kazis effect size calculation, defined as the difference between means of presurgery and postsurgery scores divided by the standard deviation for the data.[18] Larger effect sizes indicate greater responsiveness, and it is standard practice to interpret the magnitude using Cohen's arbitrary criteria, where 0.2 to 0.5 indicates a small effect size, 0.5 to 0.8 indicates a medium effect size, and greater than 0.8 indicates a large effect size.[19]

Outcomes assessed by the BREAST-Q were also described at the individual patient level. This was achieved by classifying each patient according to score change between visits. We used our study definition of "minimum significant difference" of 13 (see earlier under Sample Size Calculation) to define five categories: significant improvement (change $\geq +13$ points), nonsignificant improvement ($0 < \text{change} < +13$), no change (change $= 0$), nonsignificant worsening ($-13 < \text{change} < 0$), and significant worsening (change $\leq -13$). We then counted the number and frequency of people achieving each level of change.

Finally, we compared BREAST-Q scores and changes according to implant breast volume using the nonparametric Kruskal-Wallis test. All analyses were performed with a significance level of 0.05, using the SAS Statistical Package (version 9.2; SAS Institute, Inc., Cary, N.C.).

## RESULTS

Figures 2 and 3 show postoperative results over time.

### Patient Characteristics

A total of 35 patients were recruited for participation. Table 1 lists characteristics of the study sample. In the study, male-to-female transsexual

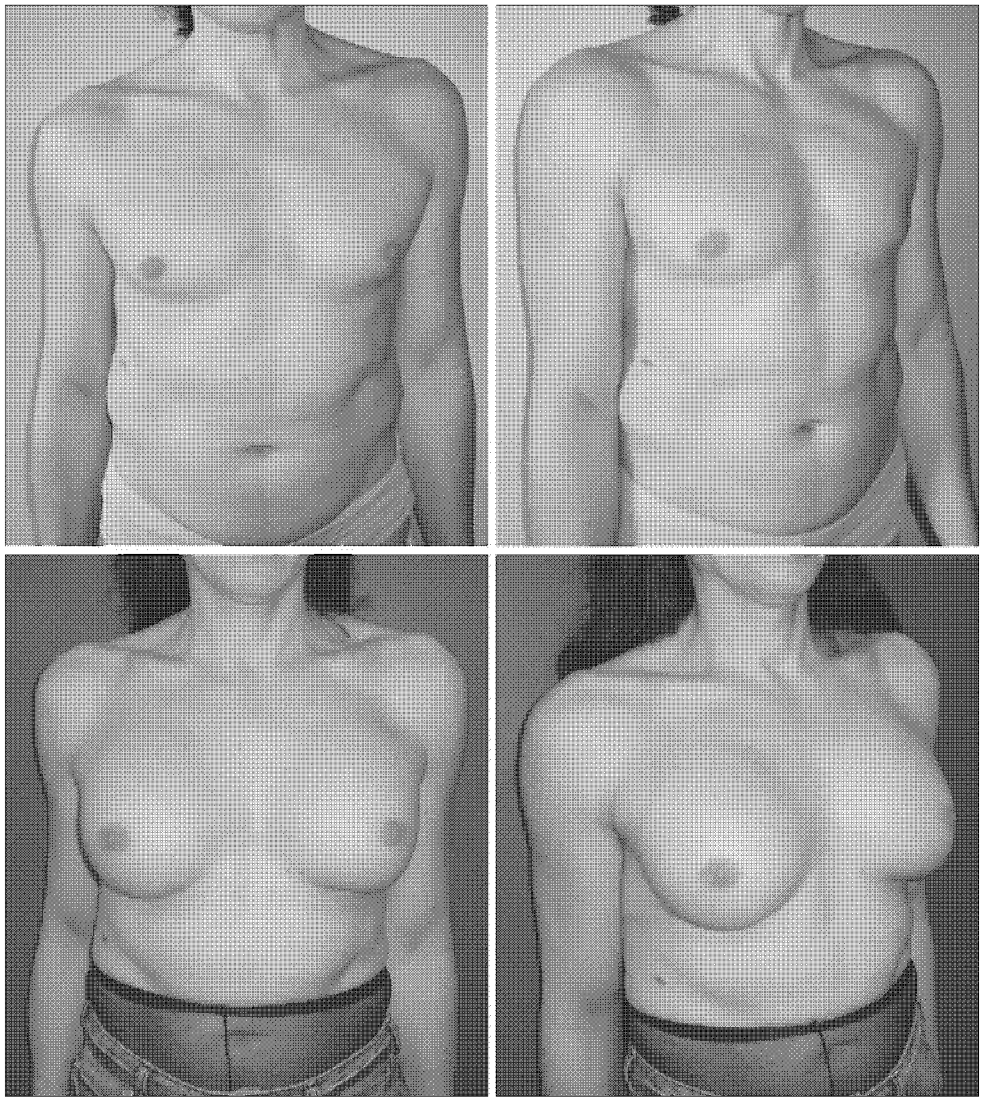

**Fig. 3.** (*Above*) Preoperative views. (*Below*) Eighteen months after implantation with Esthea high-profile 375-cc implants.

patients were most often patients who had begun late transition and therefore had a high average age at surgery (42.2 years). The high average size (173.9 cm) regarding masculinity of the patients is counterbalanced by a normal body mass index. Similarly, breast width (13.1 cm) was large regarding masculine chest anatomy. Hormone therapy was started long before the procedure; the World Professional Association for Transgender Health recommends waiting at least 12 months before breast augmentation. Breast surgery was performed on average 16 months after sex reassignment surgery, in relation to vaginoplasty recovery time. All patients completed the BREAST-Q both 3 weeks preoperatively and at a median of 4.0 months (interquartile range, 4.0 to 4.0 months; range, 3.8 to 4.0 months) following augmentation. Twenty-one patients completed the BREAST-Q again at a median of 20.7 months (interquartile range, 8.1 to 28.3 months; range, 12.0 to 39.6 months).

Table 2 lists treatment characteristics. Patient morphology induced high-volume implants (327 ml). In addition, pocket implant was most often located in the retropectoral position (77 percent). Hospitalization stay was long because patients were kept until drains were removed (5.5 days). None of the patients had significant postoperative complications after breast augmentation (Table 2).

### BREAST-Q Scores

Evolution of BREAST-Q subscales scores is illustrated in Figure 4. BREAST-Q subscale scores for

**Table 1. Characteristics of Male-to-Female Transsexual Patients Undergoing Breast Augmentation Surgery***

| | Mean ± SD | Range | No. (%) |
|---|---|---|---|
| Age at time of BA, yr | 42.2 ± 12.6 | 18.9–62.6 | |
| Length, cm | 173.9 ± 6.6 | 159.0–184.0 | |
| Weight, kg | 68.6 ± 11.9 | 49.0–89.0 | |
| BMI, kg/m$^2$ | 22.7 ± 3.5 | 17.0–29.4 | |
| Age of hormonal therapy, yr | 4.9 ± 4.2 | 1.3–16.7 | |
| Age of SRS, mo | 15.9 ± 17.1 | 4.5–81.0 | |
| Sternal notch–to-nipple distance, cm | 21.6 ± 3.6 | 17.0–34.0 | |
| Breast width, cm | 13.1 ± 2.7 | 9.0–20.0 | |
| Socioprofessional group | | | |
| Artisan, storekeeper | | | 12 (34) |
| Employed full time or part time | | | 10 (29) |
| Retired | | | 4 (11) |
| Unemployed/ students/others | | | 9 (26) |
| Active smoking | | | 12 (34) |

BA, breast augmentation; BMI, body mass index; SRS, sex reassignment surgery.
*July 30, 2012, end of follow-up, Bordeaux University Hospital, Bordeaux, France ($n = 35$).

**Table 2. Surgical Characteristics of Breast Augmentation Surgery Performed on Male-to-Female Transsexual Patients***

| | Mean ± SD | Range | No. (%) |
|---|---|---|---|
| Breast implant volume, ml | 327 ± 61 | 190.0–425.0 | |
| Procedure length, min | 86 ± 20 | 60.0–120.0 | |
| Hospitalization stay, days | 5.5 ± 1.5 | 4.0–10.0 | |
| Pocket used | | | |
| Subglandular | | | 8 (23) |
| Subpectoral | | | 27 (77) |
| Type of implant | | | |
| ELP | | | 11 (31) |
| EHP | | | 18 (51) |
| ESHP | | | 6 (17) |
| Complications | | | |
| Hematoma | | | 0 (0) |
| Infection | | | 0 (0) |
| Capsular contracture | | | 0 (0) |

ELP, Esthea Low profile; EHP, Esthea High profile; ESHP, Esthea Super–high profile.
*July 30, 2012, end of follow-up, Bordeaux University Hospital, Bordeaux, France ($n = 35$).

satisfaction with breasts, psychosocial well-being, and sexual well-being were significantly higher at both postoperative times than the baseline values (Tables 3 and 4). Satisfaction with breast increased by 59 points ($p < 0.0001$) at 4 months and 47 points ($p < 0.0001$) later. A significant improvement in psychosocial well-being was assessed at 4 months (48 points, $p < 0.0001$) and later (37 points, $p < 0.0001$). Sexual well-being increased by 34 points ($p < 0.0001$) at 4 months and 33 points ($p = 0.0003$) later. BREAST-Q subscale physical well-being had a nonsignificant change at both postoperative times: −10 points ($p = 0.1131$) at 4 months and +6 points ($p = 0.3265$) later.

Satisfaction with breast Kazis effect size was very large at 4 months and later, matching with a significant improvement in 97 percent of patients at 4 months and 95 percent later. Kazis effect sizes were large for psychosocial well-being and sexual well-being matching, respectively, with a significant improvement in 85 percent and 86 percent at 4 months and 76 percent and 70 percent later (Tables 3 and 4).

When comparing BREAST-Q subscale between subjects with breast implant volume lower or higher than the mean, only sexual well-being at 4 months after surgery is different. Subjects with breast volume implant below average ($n = 17$) were more sexually satisfied than subjects with volume implant above average ($n = 14$) (median, 100 versus 65; mean, 86.7 versus 61.4; $p = 0.001$).

## DISCUSSION

Although breast augmentation in male-to-female transsexuals was studied extensively in the late 1990s, the impact on well-being has never been measured before. Acquiring a female phenotype through hormonal and surgical treatments is essential for male-to-female transsexuals to undo the incongruity between their mind and their body. Transsexual patients perceive the breasts as a strong image of the feminine gender and seek feminization through breast surgery. The study's goal was to measure changes in patient satisfaction level with breasts and sexual, psychosocial, and physical well-being after breast augmentation in male-to-female transsexuals using a valid, reliable, and responsive patient-reported outcome measure (i.e., BREAST-Q). The current results indicate that gains are statistically significant and clinically meaningful as early as 4 months after surgery and later. In this study, procedure length was longer than in native women because we mostly had to choose a retropectoral pocket and the pectoralis muscle was strong.

The high rate of satisfaction with breasts might be explained by the large average volume of implanted prostheses in this study (327 ml) that is, to us, adequate with anatomical characteristics of male-to-female transsexual patients' chests. In 1999, Kanhai et al. had noticed that the average volume had doubled between 1979 and 1996, going from 165 ml to 287 ml, without mentioning a correlation between volume and patients' physical characteristics.[4] In their study, they observed a satisfaction rate of 75 percent at 4.8 years, consistent with the

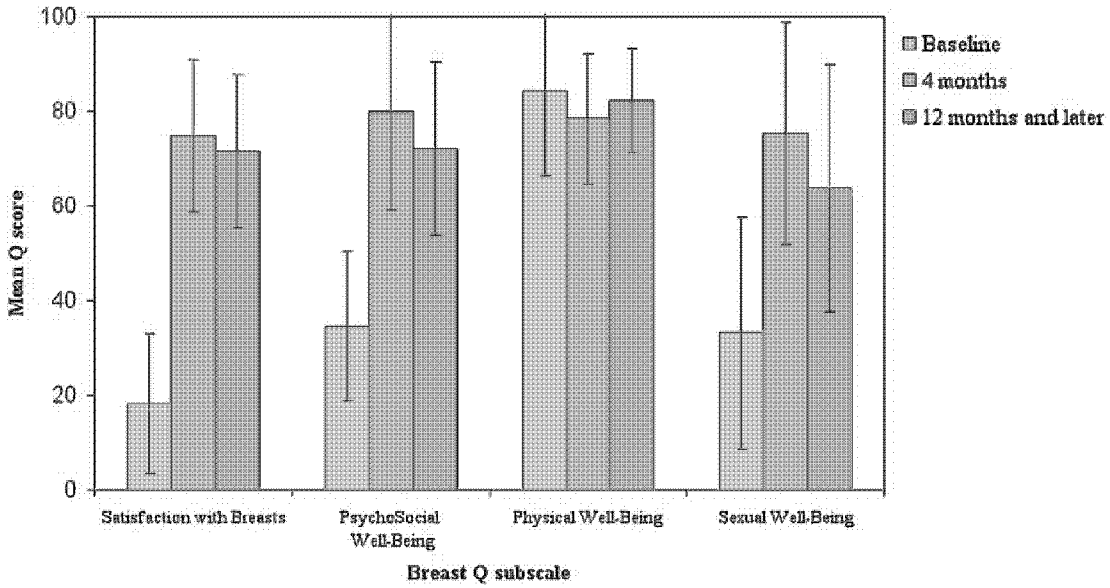


**Fig. 4.** BREAST-Q subscale scores at baseline and at 4 months and 12 months after surgery ($n = 35$, $n = 35$, and $n = 21$, respectively; except for sexual well-being, where $n = 29$, $n = 31$, and $n = 21$, respectively).

**Table 3. Distribution of BREAST-Q Augmentation Module Subscales and Change from before Surgery to 4 Months after Surgery among Male-to-Female Transsexual Patients Undergoing Breast Augmentation Surgery***

| | Satisfaction with Breasts | Psychosocial Well-Being | Sexual Well-Being | Physical Well-Being |
|---|---|---|---|---|
| No. | 35 | 35 | 27 | 35 |
| Before surgery | | | | |
| Mean ± SD | 18 ± 15 | 35 ± 16 | 33 ± 25 | 84 ± 18 |
| Median (IQR) | 19 (0–26) | 36 (23–48) | 29 (20–42) | 100 (70–100) |
| After surgery | | | | |
| Mean ± SD | 75 ± 16 | 80 ± 21 | 75 ± 24 | 79 ± 14 |
| Median (IQR) | 77 (61–85) | 85 (58–100) | 72 (63–100) | 79 (76–84) |
| Change | | | | |
| Mean ± SD | 57 ± 25 | 46 ± 29 | 40 ± 26 | −6 ± 19 |
| Median (IQR) | 59 (42–74) | 48 (16–64) | 34 (20–59) | −10 (−24–6) |
| *p*† | <0.0001 | <0.0001 | <0.0001 | 0.1131 |
| Kazis effect size‡ | 3.8 | 2.9 | 1.6 | −0.3 |
| Change, no. (%)§ | | | | |
| Significant improvement | 34 (97) | 30 (85) | 23 (86) | 6 (17) |
| Nonsignificant improvement | 1 (3) | 2 (6) | 2 (7) | 6 (17) |
| No change | 0 (0) | 2 (6) | 0 (0) | 4 (11) |
| Nonsignificant worsening | 0 (0) | 0 (0) | 2 (7) | 2 (6) |
| Significant worsening | 0 (0) | 1 (3) | 0 (0) | 17 (49) |

IQR, interquartile range.
*July 30, 2012, end of follow-up, Bordeaux University Hospital, Bordeaux, France ($n = 35$).
†*p* value for Wilcoxon signed rank test.
‡Mean change/SD before surgery (0.2–0.5 = small; 0.5–0.8 = medium; >0.8 = large).
§Significant improvement (change ≥ +13 points), nonsignificant improvement (0 < change < +13), no change (change = 0), nonsignificant worsening −13 < change < 0), and significant worsening (change ≤ −13).

satisfaction rate of our study of 67 percent at 21 months. So far, interpretation of sexual satisfaction with breast implantation is biased by outcomes of sexual reassignment[20] and brings into light complex considerations, making interpretation difficult. However, sexual well-being was more improved in patients with implanted volumes under the mean. Although biases could exist (e.g., selection of patients, confusion with other variables), this consideration allows surgeons to propose smaller implants and then improve satisfaction.

Whereas changes in physical well-being are nonsignificant in this study, it would be interesting to measure the impact of breast augmentation in male-to-female transsexual patients at work and during sport practice. No such study was considered in native women.

**Table 4. Distribution of BREAST-Q Augmentation Module Subscales and Change between before Surgery and 12 Months after Surgery and Later among Male-to-Female Transsexual Patients Undergoing Breast Augmentation Surgery***

| | Satisfaction with Breasts | Psychosocial Well-Being | Sexual Well-Being | Physical Well-Being |
|---|---|---|---|---|
| No. | 21 | 21 | 17 | 21 |
| Before surgery | | | | |
| Mean ± SD | 23 ± 14 | 40 ± 10 | 39 ± 28 | 77 ± 18 |
| Median (IQR) | 26 (19–31) | 40 (34–48) | 29 (20–49) | 72 (59–100) |
| After surgery | | | | |
| Mean ± SD | 72 ± 16 | 72 ± 18 | 64 ± 26 | 82 ± 11 |
| Median (IQR) | 68 (65–77) | 76 (62–85) | 72 (45–85) | 79 (76–90) |
| Change | | | | |
| Mean ± SD | 49 ± 21 | 32 ± 22 | 29 ± 23 | 6 ± 23 |
| Median (IQR) | 47 (39–69) | 37 (28–49) | 33 (12–39) | 6 (0–25) |
| *p*† | <0.0001 | <0.0001 | 0.0003 | 0.3265 |
| Kazis effect size‡ | 3.3 | 2.0 | 1.2 | 0.3 |
| Change, no. (%)§ | | | | |
| Significant improvement | 20 (95) | 16 (76) | 12 (70) | 8 (38) |
| Nonsignificant improvement | 1 (5) | 2 (10) | 3 (18) | 6 (29) |
| No change | 0 (0) | 2 (10) | 0 (0) | 2 (9) |
| Nonsignificant worsening | 0 (0) | 0 (0) | 2 (12) | 0 (0) |
| Significant worsening | 0 (0) | 1 (4) | 0 (0) | 5 (24) |

IQR, interquartile range.
*July 30, 2012, end of follow-up, Bordeaux University Hospital, Bordeaux, France ($n = 35$).
†*p* value for Wilcoxon signed rank test.
‡Mean change/SD before surgery (0.2–0.5 = small; 0.5–0.8 = medium; >0.8 = large).
§Significant improvement (change ≥ +13 points), nonsignificant improvement (0 < change < +13), no change (change = 0), nonsignificant worsening (−13 < change < 0), and significant worsening (change ≤ −13).

McCarthy et al. have already confirmed the positive psychological effects of breast augmentation in native women, with similar effect sizes.[21] Whereas native women seek satisfaction with their breasts through breast augmentation, male-to-female transsexual patients seem to look for better social integration. Evaluation of this parameter appears to be of utmost importance for proposing this procedure to male-to-female transsexual patients. This study detects positive psychosocial changes associated with surgery. Murphy et al., in 2009, in a psychosocial quality of life after breast augmentation study, referred to the fact that the Short Form-36 Health Survey currently used to determine the impact of an intervention on the quality of life was especially weighted with questions regarding physical health problems,[22] whereas the BREAST-Q enables change in psychosocial well-being to be measured.

Despite meaningful results on quality of life, this study has some significant limitations. A significant number of questionnaires were missing in the long term (14 of 35 patients). Eight patients were interviewed too early, as less than 6 months had elapsed since the intervention, and six patients were lost to follow-up. Quantitative data (i.e., age, size, weight, body mass index, sternal notch–to-nipple distance, and breast width) at inclusion of loss to follow-up were on average higher, resulting in a selection of larger prostheses (350 ml versus 325 ml) and implant insertion in the prepectoral pocket in more than 60 percent of cases (unlike 75 percent of cases for patients reinterviewed later). Patients lost to follow-up had better improvement in satisfaction with breasts at 4 months (+91 versus +46). It can be assumed that this improvement of satisfaction is extended in time, which supports the significant findings of this study in the long term. Besides, it has been suggested that satisfaction associated with breast augmentation may be compromised by postoperative complications,[23] and none of the patients had complications during the follow-up period. Furthermore, only anatomical implants were used in this study. A single-blind prospective study comparing anatomical versus round implants should evaluate the impact of implant selection on outcomes.

It is necessary to analyze these findings not only exclusively based on their statistical significance but also in view of their clinical significance. Effect sizes, such as Kazis effect size, can measure the strength of change and then can help interpret the data. Nevertheless, Kazis effect size calculation is based on the hypothesis of normal distribution of variables that is not satisfied in our study. However, the magnitude of the results is strong enough to support this. Effect sizes were large for the three scale mean change scores, and the vast majority of individual patients underwent highly significant improvement. This finding strongly supports the hypothesis that breast augmentation in male-to-female transsexuals can have a significant and wide positive impact on a

patient's satisfaction with breasts, psychosocial well-being, and sexual well-being.

According to this study, breast augmentation in male-to-female transsexual patients significantly improves satisfaction with breasts and global psychosocial well-being. However, improvement of sexual well-being is to be balanced with outcomes of sexual reassignment, marital status, and probably other complex personal situations. Finally, physical abilities are not altered significantly, which could have worried some patients who were in stereotypical male trades.

On the basis of our findings, demand exists at all ages, in all occupations, and with all physical aspects regarding height, weight, and body mass index. In France, once approval is granted by a medical insurance counselor, all of the procedure is paid for by the national health insurance; the results of our study support this policy.

In addition, these results could be affected by the onset of capsular contractures in following years and suggests an extended follow-up study. With this aim in mind, we continue the inclusion of patients to gain perspective and increase the power of the study.

Through breast augmentation, the male-to-female transsexual patient improves identification with the female gender and therefore is socially integrated. Regarding results, this study supports breast augmentation in this population. It would be interesting to measure how the surgery affects the patient's work and artistic production.

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