Full Length Article

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

Mariska C. Vlot\textsuperscript{a,b}, Daniel T. Klink\textsuperscript{c,d}, Martin den Heijer\textsuperscript{b,c}, Marinus A. Blankenstein\textsuperscript{a}, Joost Rotteveel\textsuperscript{c,d}, Annemieke C. Heijboer\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a} Department of Clinical Chemistry, Endocrine Laboratory, VU University Medical Center, de Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands
\textsuperscript{b} Department of Internal Medicine, section Endocrinology, VU University Medical Center, de Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands
\textsuperscript{c} Center of Expertise on Gender Dysphoria, VU University Medical Center, de Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands
\textsuperscript{d} Department of Pediatric Endocrinology, VU University Medical Center, de Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands

\textsuperscript{*} Corresponding author.

E-mail addresses: d.klink@vumc.nl (D.T. Klink), m.denheijer@vumc.nl (M. den Heijer), ma.blankenstein@vumc.nl (M.A. Blankenstein), j.roteveel@vumc.nl (J. Rotteveel), a.heijboer@vumc.nl (A.C. Heijboer).

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A B S T R A C T

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 \( \leq 15 \) years in transwomen or \( \leq 14 \) in transmen) or old group (bone age of \( \geq 15 \) years in transwomen or \( \geq 14 \) 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-\( \beta \)-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 ICTP 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.

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1. 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.

2. Methods

2.1. 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 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/m² body surface area IM every two weeks and doses are increased every 6 months until an adult maintenance dosage of 250 mg every 4 weeks is reached. The transwomen are treated with 17β-

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].

2.2. Measurements

2.2.1. 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.

2.2.2. 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 an RIA (Orion Diagnostica, Espoo, Finland) with an intra-assay CV of 4–6%, inter-assay CV of 7% and LOQ of 1 μg/L.

2.2.3. 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/cm² 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/cm³ was 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 de-sired 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 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].

2.3. 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.

3. Results

3.1. 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.

3.2. 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.

3.2.1. 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. Young transmen showed 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 and old transwomen groups. P1NP concentrations decreased further in all but the young transmen group 24 months after CSHT (Fig. 1).

3.2.2. 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 (Fig. 2).

3.2.3. 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 transmen and transwomen. ICTP concentrations decreased especially in the young transmen group 24 months after CSHT (Fig. 3).

3.3. BMAD and Z-scores

3.3.1. 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 (Fig. 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).

3.3.2. 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 (Fig. 5).

Regarding the LS BMAD Z-scores, young transmen showed a lower Z-score compared to old transmen (p = 0.002) 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 transmen groups increased after 24 months of CSHT (young transmen 95% CI = 0.099 to 0.642, p = 0.01 and old transmen 95% CI = 0.316 to 0.753, p = 0.04), but the BMAD Z-score did not reach the zero level. In general, after 24 months of CSHT the median LS

| Table 1 | Baseline characteristics of transmen and transwomen adolescents receiving GnRHa and CSHT. |
|-----------------|-----------------|-----------------|-----------------|
|                | 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. | N.A. | N.A. | N.A. |
| Tanner stage pubic hair, median (range) | 5 (3–5) | 5 (3–5) | 6 (4–5) | 3 (2–5) | 4 (2–5) | 4 (3–5) |
BMAD Z-score of the transwomen were still lower than the median BMAD Z-score of the transmen.

4. 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.

4.1. 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 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

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<td>Overview of BTMs and BMAD and Z-scores of transmen and transwomen adolescents receiving GnRHa and CSHT, including legend.</td>
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** NS.
†/‖ trend (p ≤ 0.1).
††/‖‖ p ≤ 0.05.
†††/‖‖‖ p ≤ 0.01.
Fig. 1. PINP measurements per patient, the red star represents the median, D0 = 100%, with mean Δ, 95% CI and corresponding p-value.

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

4.2. 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 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 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].

4.3. 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
Fig. 4. BMAD of the hip (FN) measurements per patient, the red star represents the median, D0 = 100%, with mean $\Delta$, 95% CI and corresponding $p$-value.

Fig. 5. BMAD of the lumbar spine measurements per patient, the red star represents the median, D0 = 100%, with mean $\Delta$, 95% CI and corresponding $p$-value.
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 250HD 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) BMAD 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 standardized 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 naive 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 calcitriol) 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 ICTP 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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