

BONE HEALTH IN TRANSGENDER PEOPLE

Short-term and long-term effects of hormonal treatment on bone mineral density, metabolism, geometry, and fractures

CHANTAL WIEPJES

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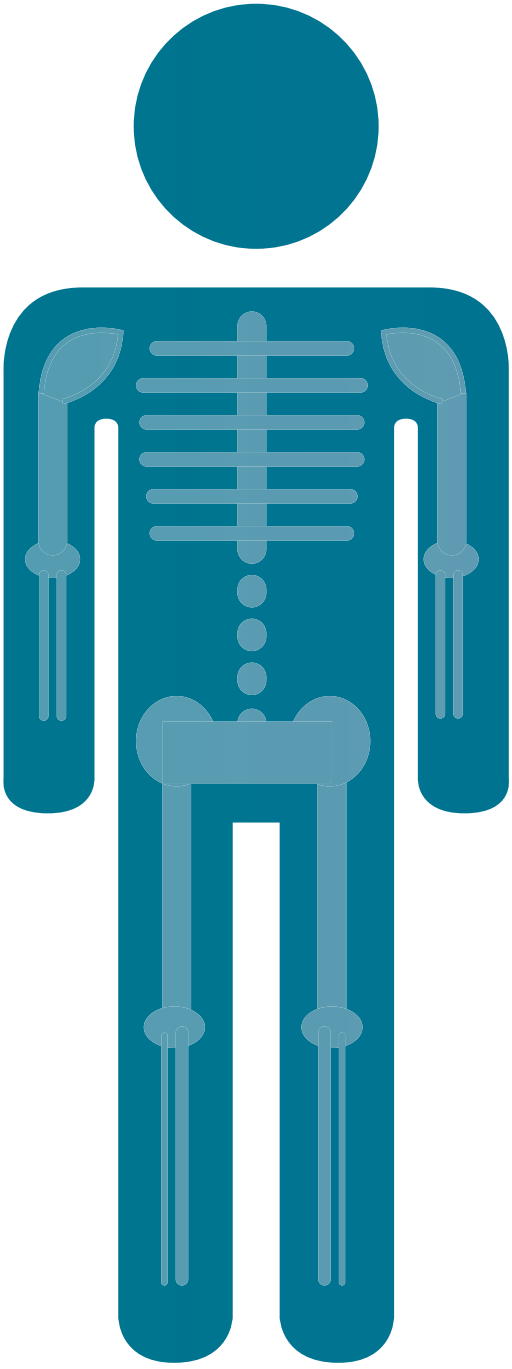
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Chapter 1

General introduction

In this chapter, a general introduction about the topic 'Bone health in transgender people' will be given. First, some information about the diagnosis and treatment of transgender people will be given. Then, bone physiology will be explained, also describing the differences between cis (non-transgender) men and women. Thereafter, different methods for bone measurements are described. Earlier literature about bone health in transgender people will be described, with the remaining gaps in these studies. This chapter ends with describing the aims, study populations, and outline of this thesis.

Transgender people

Transgender people are people who are diagnosed with gender dysphoria. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, the diagnosis gender dysphoria can be made if there is an incongruence between one's sex assigned at birth and one's identified gender.[1] This incongruence has to be present for at least six months and leads to significant distress. People diagnosed with gender dysphoria may wish to receive treatment with gender-affirming hormonal treatment (HT) with or without gender-affirming surgery, in order to adapt their physical characteristics to their identified gender.

Trans women are people who have a male sex assigned at birth, but have a female gender identity. Trans women can receive treatment with anti-androgens, usually until orchiectomy, and estrogens.[2] The most commonly prescribed anti-androgens are cyproterone acetate, spironolactone, or gonadotropin-releasing hormone analogues. Estrogen treatment changed over the years. In the past, the most commonly prescribed estrogens were ethinyl estradiol, conjugated estrogens, or injected estrogens. In the more recent years, mostly oral estradiol valerate, estradiol patch, or estradiol gel was prescribed. This hormonal treatment will result in physical changes, for example change in body composition, breast growth, softness of the skin, and decrease in body hair growth.[2-4] After at least one year of HT and at the age of 18 years or older, surgery can be performed. The most performed surgery is vaginoplasty with orchiectomy, but they may also choose to undergo breast augmentation or facial feminization surgery.

Trans men have a female sex assigned at birth, but have a male gender identity. They can receive treatment with testosterone, either as testosterone gel, testosterone esters intramuscularly, or testosterone undecanoate orally or intramuscularly.[2] Physical changes that occur because of testosterone treatment include lowering of the voice, increased muscle mass, increased body hair growth, and cessation of the menses.[2,5,6] After at least one year of HT and at the age of 18 years or older, surgery can be performed. Mastectomy is the most commonly performed surgery, but also hysterectomy with or without oophorectomy, and colpectomy, phalloplasty, or metadoioplasty can be performed.

Besides the effects of hormonal treatment on the described physical changes, it can also influence bone health. However, before that topic will be described, first some information about bone physiology and bone measurements will be given.

Bone physiology

Bone consists of trabecular and cortical bone. Cortical bone forms 80% of the total bones of the body, is mainly located in the long bones [7], and consists of three parts. The periosteal surface is the outer surface of the bone, the endosteal surface is the inner surface of the cortex facing the medulla, and the intracortical envelope, which is the cortical thickness. Although long bones also contain trabecular bone, it is predominantly present in the vertebrae.[7]

Bone is constantly modeled to stimulate bone growth, and remodeled to maintain bone strength by damage repair. The cells primarily responsible for this modeling and remodeling are osteoblasts, osteoclasts, and osteocytes.[8] Osteoblasts are differentiated from mesenchymal stem cells and synthesize bone. In the bone formation process, they produce collagen and proteins to form the organic matrix of bone. In addition, they produce hydroxyapatite, which is deposited into the organic bone matrix to form a strong and dense mineralized tissue. Osteoclasts develop from macrophages and break down bone tissue, by secreting acids and collagenase. Osteocytes are derived from osteoblasts and are found in mature bone tissue. Although they do not have synthetic activity, they are involved in bone turnover through mechanosensory mechanisms.

The remodeling of bone is also called bone turnover. The balance between bone formation by osteoblasts and bone resorption by osteoclasts determines whether bone mass increases or decreases. In young childhood, during puberty, and late adolescence, the bone formation exceeds bone resorption.[9] The net balance is therefore positive, leading to an increase in bone mass. After the peak bone mass, which is around the age of 25-30 years, bone formation and bone resorption occur in roughly the same amount, with only slightly more bone resorption than formation.[9] The net balance is negative, leading to a small decrease in bone mass over time. In postmenopausal women, the bone resorption exceeds bone formation, leading to a larger net negative balance and therefore a larger decrease in bone mass.[9] These changes in bone mass are shown in Figure 1.

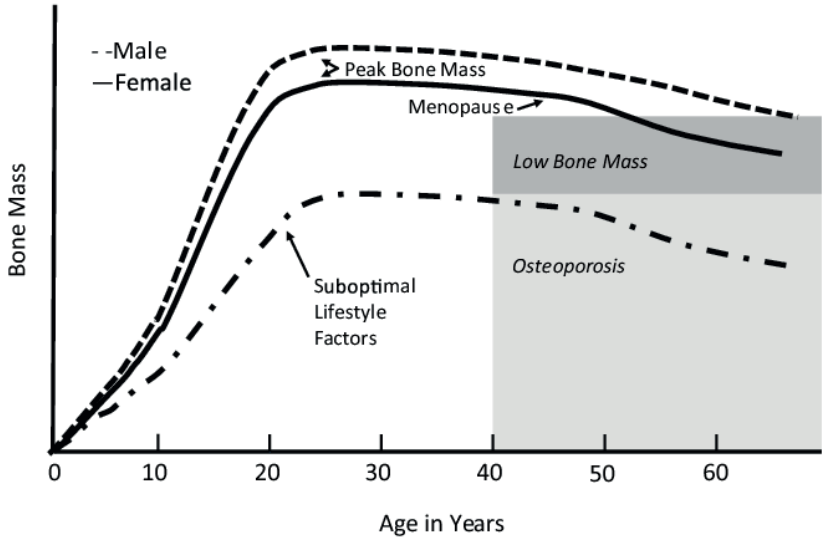


Figure 1.
Changes in bone mass during life in cis males and females. Re-used from Weaver C.M. et al.[10] This article is available under the Creative Commons CC-BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/) and permits non-commercial use, distribution and reproduction. No changes in this figure were made.

Bone measurement

Dual-energy X-ray absorptiometry

Different techniques are used in clinical practice that can contribute to estimate the bone strength. The most commonly used technique is the dual-energy X-ray absorptiometry (DXA) scan. This is a 2-dimensional scan using two X-ray beams with different energy levels, as shown in Figure 2. Bone mineral content (in grams) is measured by determining the bone absorption of both beams. This bone mineral content is thereafter divided by the bone area (in cm^2) that is scanned. In that way, the bone mineral density (BMD, in g/cm^2) is measured.[11]

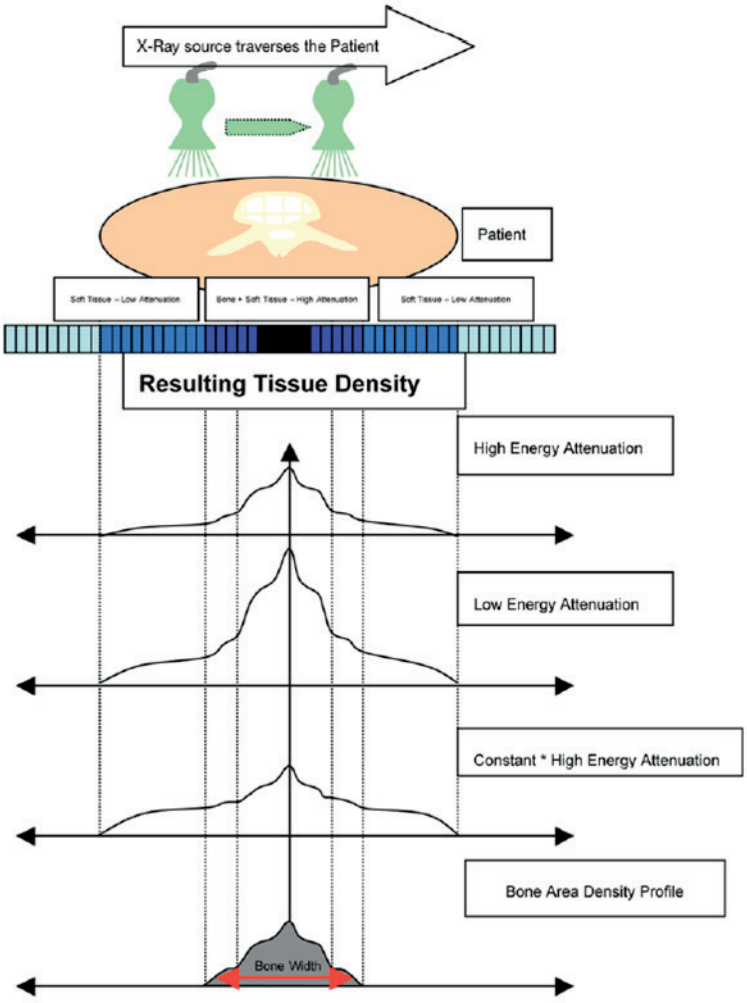


Figure 2.
A schematic explanation of the high-energy and low-energy level beams of the DXA. The bone profile, observed as the x-ray moves linearly across the patient, and the corresponding tissue density profiles. Re-used from Crabtree et al.[12], with permission from Springer Nature.

BMD is primarily used for diagnosis and treatment of osteoporosis. Osteoporosis is characterized by low BMD and deterioration of bone tissue.[13] This leads to bone fragility and therefore an increased fracture risk of particularly the spine, hip, and forearm. To diagnose osteoporosis, the person's BMD is compared with the BMD of a healthy young adult reference population. This difference is expressed as a T-score, which is calculated as the standard deviation (SD) difference between the person's BMD and the BMD of the young-adult reference population. A T-score of -2.5 or lower is classified as osteoporosis when using the female reference population, while a T-score of -2.8 or lower should be used to diagnose osteoporosis when using the male reference population.[14] Besides the T-scores, also the Z-scores can be calculated. This is calculated as the SD difference between the person's BMD and an age-matched BMD of the same sex and ethnicity. This Z-score is primarily used in children and adolescents who have not reached the peak bone mass yet.

A limitation of DXA is that BMD is measured in a 2-dimensional way. To accurately measure BMD, mass should be divided by volume. However, width of the bones is not measured by DXA. Therefore, BMD measured by DXA is also referred to as areal BMD (aBMD), while BMD measured by techniques with 3-dimensional view, such as quantitative computed tomography (qCT), is expressed as volumetric BMD (vBMD). People with wider bones will have a higher aBMD than people with smaller bones, even if vBMD is similar.[11] This difference in aBMD, vBMD, and bone size is shown in Figure 3.

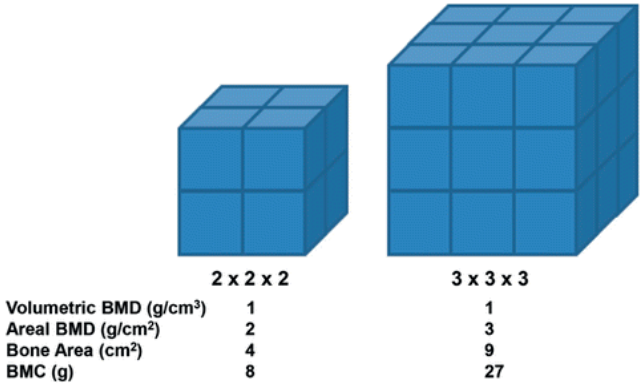


Figure 3.
The volumetric BMD, areal BMD, bone area, and bone mineral content (BMC) are shown for two different cubes, as an example of differences in bone size. It is shown that areal BMD is higher with wider bones, even if volumetric BMD is similar. Re-used from Logan et al.[15] with permission from Springer Nature.

Hip structural analysis and trabecular bone score

Another limitation of the general BMD images is that no specific information can be extracted about the cortical and trabecular bone. However, new techniques are available that can overcome this limitation. These techniques include hip structural analysis (HSA) and trabecular bone score (TBS). HSA uses the standard hip DXA images to obtain structural geometrical data.[11,16] It analyzes the distribution of the pixel mass across the regions in the hip to estimate different parameters, for example periosteal width, endocortical diameter, and cortical thickness, as shown in Figure 4. These measurements mainly include cortical bone, whereas TBS measures trabecular bone. TBS is measured from the standard lumbar spine DXA images [17], as shown in Figure 5. It analyzes the variations in grey-level of the pixels, to provide an index of the trabecular microarchitecture. TBS is associated with the number and connectivity of trabeculae. People with similar BMD can have different TBS values. A low TBS indicates poor microarchitecture and wide spaces between trabeculae.

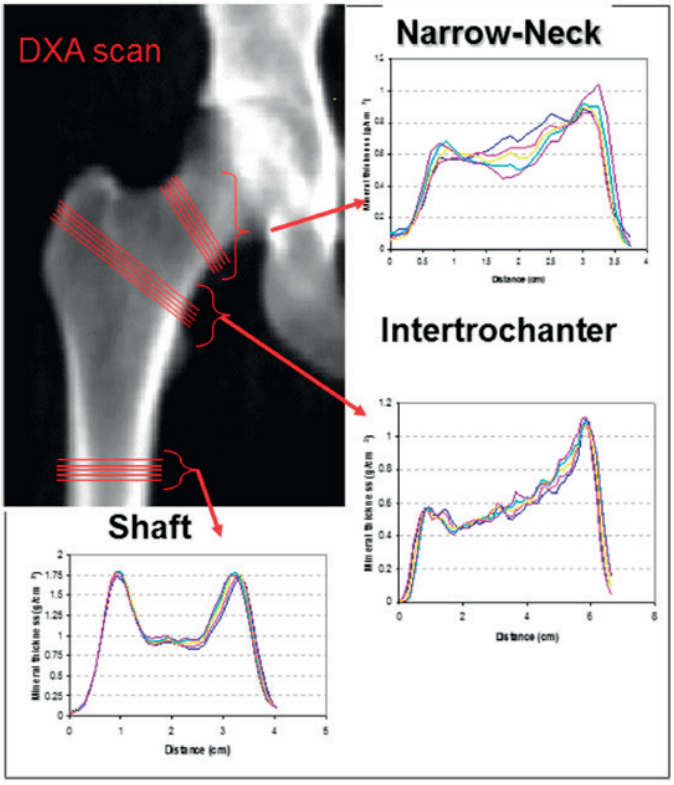


Figure 4.
Method of performing the hip structure analysis by using the DXA hip images, including locations of the cross-sections and the corresponding plots of mass profiles. Re-used from Beck et al.[18] with permission from Elsevier.

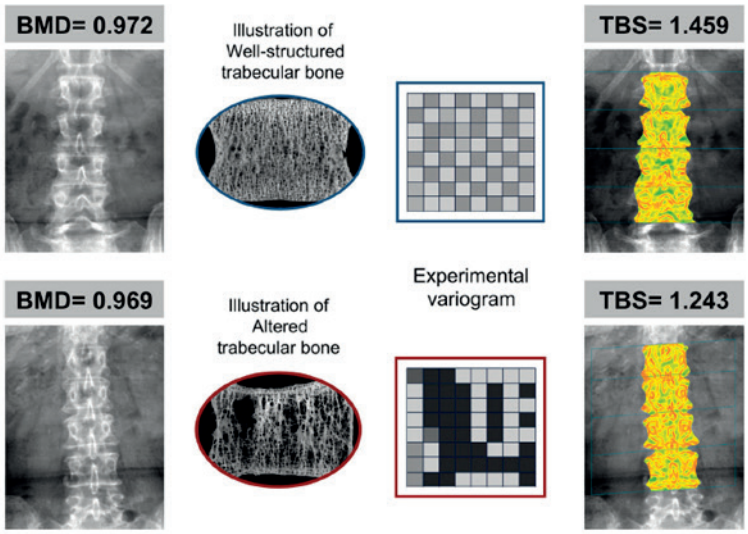


Figure 5.
Method of calculating trabecular bone score based on the DXA lumbar spine images. Representation of the TBS principles and an example where the TBS appears to be independent from BMD. Re-used from Silva et al.[17] with permission from John Wiley and Sons.

Bone turnover markers

Another technique to measure bone quality is measurement of bone turnover markers (BTMs). BTMs are collagen breakdown products and other molecules released by osteoblasts and osteoclasts, during the process of bone formation and bone resorption, respectively.[19] These BTMs reflect the changes in bone metabolism. Examples of BTMs are carboxy-terminal collagen crosslinks (CTX), procollagen type 1 N-propeptide (P1NP), alkaline phosphatase (ALP), and sclerostin. CTX is released by osteoclasts during bone resorption [19], whereas P1NP and ALP are bone formation markers produced by osteoblasts.[19] Sclerostin is primarily produced by osteocytes, but because it among others promotes apoptosis of osteoblasts, it can be interpreted as a bone resorption marker.[19,20]

Sex differences in bone health

Before puberty sets in, bone mass is similar in boys and girls. However, men achieve a higher peak bone mass than women.[21] This can be explained because men develop wider bones than women. In men, periosteal growth is stimulated, while it is inhibited in women, leading to larger bones in men than in women.[22,23] In women, endosteal growth is stimulated, leading to narrowing of the medullary cavity [24], while this does not occur in men. These differences are shown in Figure 6. Although men have wider bones

than women, the cortical thickness, expressed as the distance between the periosteum and endosteum, is similar in men and women.[25] However, as the bones are wider in men, the total bone mass is higher in men than in women.[21]

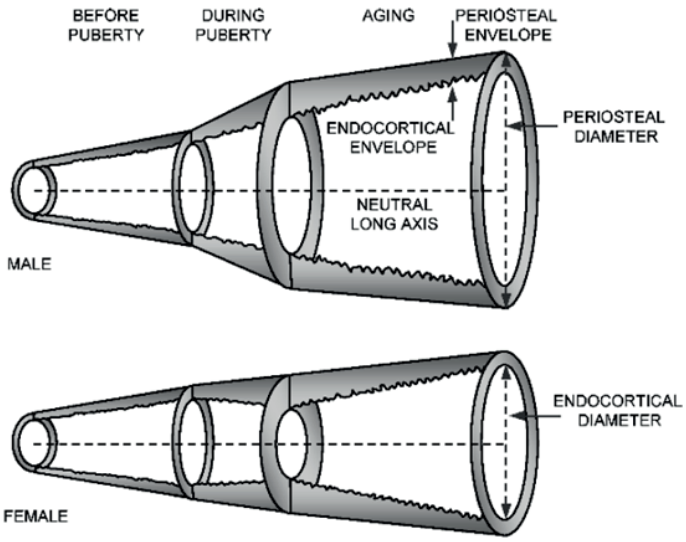


Figure 6.
Changes in periosteal surface (outer surface of cortex) and endocortical (endosteal) surface (inner surface of cortex) during life, different for cis males and cis females. Re-used from Duan et al.[25] with permission from John Wiley and Sons.

The increase in periosteal growth in men, but not in women, is thought to result from the higher testosterone concentrations in men than in women. This testosterone might act directly on the bone via androgen receptor (AR) activation.[26] The effect can also be indirectly, because of the increase in muscle mass caused by testosterone.[27-29] This increases mechanical loading on the bone, which can also increase the periosteal growth.[30]

The accelerated decrease in bone mass in women during menopause is the result of the decrease in estrogen concentrations. Estrogen is known to have positive effects on bone health, as shown in Figure 7. It stimulates the apoptosis of osteoclasts, leading to a decrease in bone resorption.[31] When estrogen deficiency occurs, as during menopause, an increase in osteoclastic bone resorption is found [32], leading to a decrease in bone mass.[33]

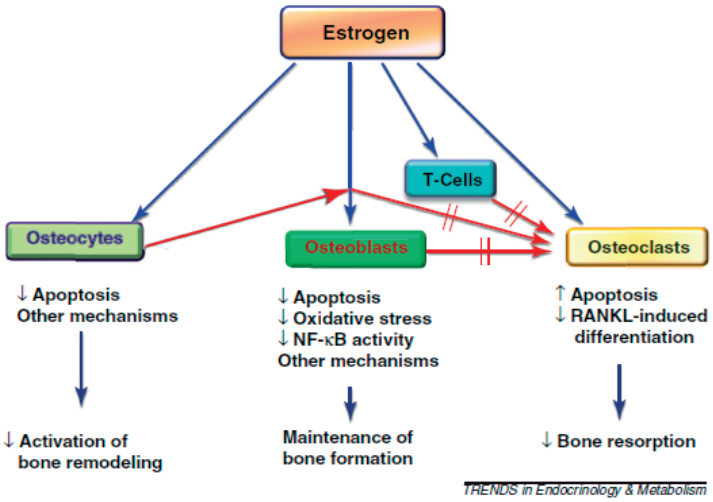


Figure 7.
Working model for estrogen regulation of bone turnover via effects on osteocytes, osteoblasts, and osteoclasts. Re-used from Khosla et al.[34] with permission from Elsevier.

Although estrogen is the main important factor of bone health in women, it is not clear yet whether this is also the case in men. After orchiectomy in men, or after chemical castration during prostate cancer treatment, testosterone concentrations decrease. Also, bone mass decreased in these people [35], indicating that decrease in testosterone concentrations influences bone mass in men. However, testosterone is aromatized into estradiol. Therefore, by decreasing the testosterone concentrations, also the estradiol concentrations decreased. One study by Finkelstein et al. [36] found that bone mass did not change in men with normal estradiol and normal testosterone levels, but decreased in men with normal testosterone but low estradiol levels. This indicates that maintenance of bone health, also in men, is mainly regulated by estrogens.

In addition to the direct effects of testosterone and estrogen on bone, also indirect effects can occur. Testosterone can increase muscle mass, which increases mechanical loading and can therefore influence bone remodeling and geometry, as described before. Besides the indirect effects on muscle mass, testosterone and estrogen can also influence the metabolism of vitamin D (25-hydroxy-vitamin D, 25[OH]D). Low vitamin D status is associated with high bone turnover and low bone mineral density.[37,38] Vitamin D deficiency can lead to osteoporosis, fractures, and mineralization defects.[39,40] In the circulation, more than 99% of the total 25(OH)D is bound to serum proteins, particularly to albumin and vitamin D-binding protein (DBP).[41] Contrary to DBP-bound 25(OH)D, albumin-bound 25(OH)D is available for metabolic processes. Albumin-bound and free circulating 25(OH)D together form the bioavailable 25(OH)D. Sex steroids can influence vitamin D metabolism, in particular the DBP

concentrations. DBP concentrations are higher in women than in men.[42] Also, the use of oral contraceptives [43] or pregnancy [44,45] leads to higher DBP concentrations, whereas postmenopausal women have lower DBP concentrations than premenopausal women.[46]

Fracture risk is also different in men and women. At younger ages (<50 years), men have a higher fracture risk than women.[47,48] This is mainly the result of accidents, particularly because of sporting and traffic accidents.[47,48] At older ages (≥50 years), women have a higher fracture risk than men, which is mainly the result of the decreased bone mineral density after menopause.[47]

Hormonal treatment effect on bones in transgender people

As explained above, there are differences in bone health between cis men and women, mainly caused by differences in testosterone and estrogens. Hormonal treatment in transgender people might therefore affect bone health.

A few studies investigated the BMD in trans women and trans men compared with cis men and women, respectively, before the start of HT. Trans women were found to have lower BMD than cis men.[49,50] This difference might be explained because trans women also had lower vitamin D concentrations and lower muscle mass than cis men, which can be due to a different life style.[49] Trans men had higher or similar BMD than cis women [6,50], with no differences in vitamin D concentrations and muscle mass.

After one year of HT, BMD increased in trans women [50-56]. In trans men, also an increase or a maintenance in BMD is described.[6,50,55,57] The long-term effects of approximately 10 years of HT on BMD have only been investigated in cross-sectional studies, with contradictory results. In trans women compared with cis men, either lower [58], similar [59], and higher BMD [60] was observed. Also, in trans men compared with cis women contradictory results are obtained: one study found no difference in BMD [61], while another study found higher whole body Z-scores.[59]

A few studies found no increased fracture risk before the start of HT in both trans women [49] and trans men.[6] In short-term follow-up studies, trans women, trans men, and their controls did not experience any fractures.[6,54] In studies after long-term HT, no increased fracture risk was found in trans women and trans men.[58,62]

However, all these studies about bone health in transgender people are limited by small sample sizes. Most studies included less than 50 people per group. Therefore, it was not possible to study possible mechanisms or predictors, for example concentrations of testosterone or estradiol, or age. In addition, the long-term effects of HT on bone health are only investigated in cross-sectional studies, taking baseline data not into account. Also, fracture risk was only determined in small populations and used questionnaires to identify fractures.

Aims, study populations, and outline of this thesis

Aims

This thesis has multiple aims.

1. To investigate the short-term effects of HT on bone health in trans women and trans men, focusing on short-term changes in bone mineral density, bone turnover markers, vitamin D metabolism, and grip strength and muscle mass. The influence of concentrations of sex steroids and age on these effects are investigated.

2. To investigate the long-term effects of HT on bone health in trans women and trans men, focusing on the long-term change in bone mineral density, change in bone geometry, and fracture risk.

Study populations

To investigate these aims, we used two different study populations. The first study population, to study the aims of (1), was part of the European Network for Investigation of Gender Incongruence (ENIGI). This is a large prospective observational multicenter study, performed in Amsterdam (the Netherlands), Ghent (Belgium), Oslo (Norway), and Florence (Italy). Data were collected before the start of HT and thereafter every three months during the first year of HT. DXA was performed at baseline and after one year of HT. Blood samples were collected at baseline, after three months of HT, and after 12 months of HT. Physical examinations, including body height, body weight, and grip strength, was performed every three months.

The second study population, to study the aims of (2), was collected in the Amsterdam Cohort of Gender dysphoria (ACOG) study. This study is a retrospective chart study, including data of all 6,793 people who once visited the gender identity clinic of the Amsterdam UMC between February 1972 and December 2015. During clinical patient care, blood examination and DXA scans were regularly performed as they were part of the treatment protocol. These data were analyzed to study the long-term effects of HT on bone mineral density and bone geometry. To study the fracture

risk, this cohort was linked to Statistics Netherlands (Central Bureau of Statistics, the Netherlands).

Outline

In **Chapter 2**, the ACOG study population will be introduced, with the total number of people seen at our clinic and the treatment trajectories they had undergone, including hormones and surgery, and regret. In **Chapter 3**, the first-year changes in BMD, measured by DXA, in trans women and trans men will be described. In **Chapter 4**, the first-year changes in bone turnover markers, including P1NP, CTX, ALP, and sclerostin, in trans women and trans men are described. In **Chapter 5**, the changes in vitamin D metabolism, including vitamin D binding protein, free and bioavailable 25(OH) D, and total 25(OH)D, during the first three months of hormonal treatment in trans women and trans men are evaluated. The changes in grip strength and muscle mass and its association with change in BMD during the first year of hormonal treatment are described in **Chapter 6**. In **Chapter 7**, the first ten-year effects of hormonal treatment on BMD, measured by DXA, are analyzed. In **Chapter 8**, the differences in bone geometry, measured by hip structure analysis and trabecular bone score, in trans women and trans men at baseline and after different durations of hormonal treatment, in different age groups, are analyzed. In **Chapter 9**, the fracture risk after long-term use of hormonal treatment in trans women and trans men, compared with age-matched control men and women, are described. In **Chapter 10**, the findings of this thesis will be discussed.

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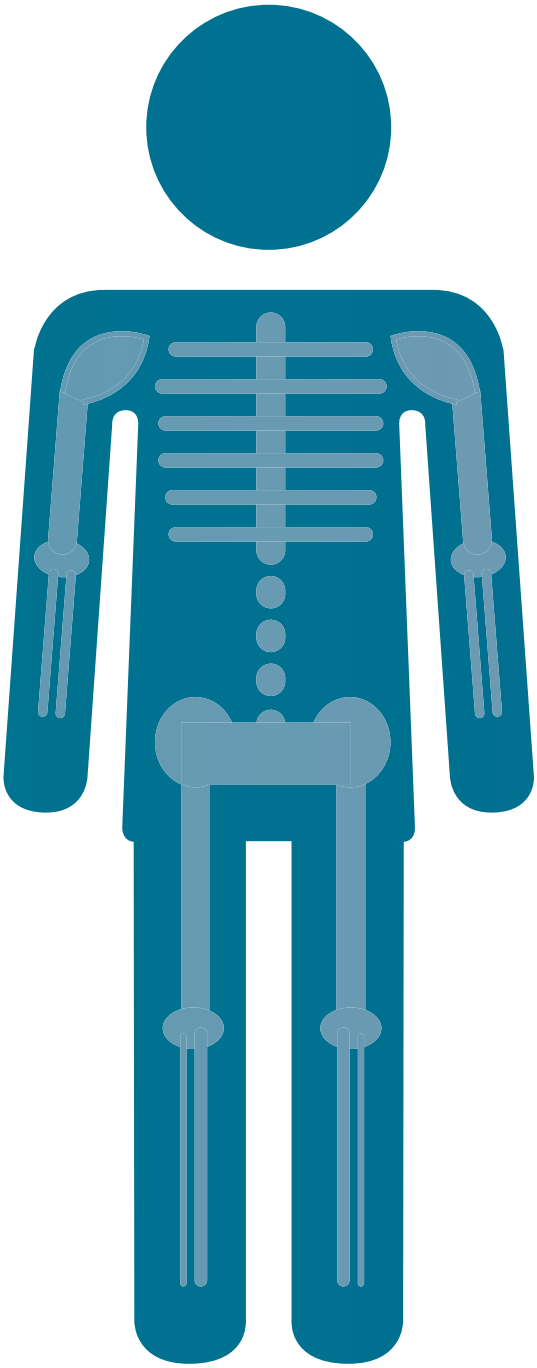
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Chapter 2

The Amsterdam Cohort of Gender Dysphoria study (1972-2015): trends in prevalence, treatment, and regrets

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Abstract

Background: Over the past decade, the number of people referred to gender identity clinics have increased rapidly. This raises several questions, especially concerning the frequency of performing gender affirming treatments with irreversible effects, and regret of such interventions.

Aim: To study the current prevalence of gender dysphoria, how frequently gender affirming treatments are performed, and the number of people experiencing regret of this treatment.

Methods: The medical files of all people who attended our gender identity clinic from 1972 to 2015 were reviewed retrospectively.

Outcomes: The (change in) number of people that applied for transgender healthcare, percentage of people starting with gender affirming hormonal treatment (HT), the estimated prevalence of trans people receiving gender affirming treatment, the percentage of people who underwent gonadectomy, and the percentage of people who regretted gonadectomy, specified for each year separately.

Results: A total of 6,793 people (4,432 birth-assigned males, 2,361 birth-assigned females) visited our gender identity clinic between 1972 and 2015. The number of people assessed per year increased 20-fold from 34 people in 1980 to 686 people in 2015. The estimated prevalence in the Netherlands in 2015 is 1:2,800 for men (trans women) and 1:5,200 for women (trans men). The percentage of people who started with HT within 5 years after the first visit decreased over time, with almost 90% in 1980 to 65% in 2010. The percentage of people who underwent gonadectomy within 5 years after starting HT remained stable over time (74.7% of the trans women and 83.8% of the trans men). Only 0.6% of the trans women and 0.3% of the trans men who underwent gonadectomy were identified as experiencing regret.

Clinical Implications: Because the transgender population is growing, a larger availability of transgender healthcare is needed. Other healthcare providers should familiarize themselves with transgender healthcare, as hormonal treatment may influence diseases and interact with medication. As not all people apply for the classical treatment approach, special attention should be given to those who choose less common forms of treatment.

Strengths & Limitations: This study was performed in the largest Dutch gender identity clinic, treating over 95% of the transgender population in the Netherlands. Due to the retrospective design, some data may be missing.

Conclusion: The number of people with gender identity issues seeking professional help increased dramatically in the last decade(s). The percentage of people who regretted gonadectomy remained low and did not show a tendency to increase.

Introduction

Gender Dysphoria (GD) refers to the distress related to a marked incongruence between one's assigned sex at birth and the experienced gender later in life.(1) In this study, we define trans women as birth-assigned males, and trans men as birth-assigned females, who may receive medical treatment to adapt their physical characteristics to their experienced gender. This treatment may include puberty suppression (PS), gender affirming hormonal treatment (HT), and gender affirming surgery (GAS).

It is widely observed that the transgender population is growing and broadening. [2,3] This increase in the transgender population raises several questions, especially concerning the frequency of performing gender affirming treatments with irreversible effects, and regret of such interventions.

There are no reliable estimations of the current prevalence of trans people who actually have received gender affirming treatment (including HT), as most recent studies are based on questionnaires [4,5] or data about GAS only.[6,7] In most countries transgender care is performed by multiple healthcare providers (e.g. university clinics or general practitioners), which makes it difficult to provide these numbers. In contrast, in the Netherlands, over 95% of the transgender population have received treatment in only one center – the gender identity clinic at the VU University medical center (VUmc) in Amsterdam, the Netherlands, currently known as the Center of Expertise on Gender Dysphoria.[8-10] This center started treating adults in 1972. Since 1987, children and adolescents were seen by a mental health specialist in the Utrecht University Medical Center, the Netherlands. After considered eligible, they could receive medical treatment in the VUmc, which consisted of PS (usually by gonadotropin-releasing hormone [GnRH] analogues), later followed by HT (see Kreukels & Cohen-Kettenis for the treatment protocol for adolescents diagnosed with GD [11]). After 2002, the Utrecht clinic stopped seeing adolescents and the diagnostics were performed in the VUmc. Adult people are referred to a psychologist or psychiatrist for the diagnostic phase after an initial screening. The people diagnosed with GD can start with HT if they are considered eligible. HT consists of testosterone in trans men, and oestrogens, often combined with anti-androgens, in trans women. In the first year of HT, check-ups are performed every three months. After a minimum of 12 months of HT, GAS can be performed, including mastectomy and hysterectomy with oophorectomy in trans men, and breast augmentation and vaginoplasty (including orchiectomy) in trans women. After gonadectomy (oophorectomy or orchiectomy), people are usually seen every one to two years for a clinical follow-up.

In the current study we have included the complete population seen at the gender identity clinic of the VUmc between 1972 and December 2015 to assess the current prevalence of trans people who received medical treatment, the frequency of specific medical treatments performed, and the numbers of people who received

hormonal treatment in line with their sex assigned at birth because they regretted gonadectomy.

Material and methods

Study design and patient selection

After approval of the local ethics committee, a retrospective medical record review was performed to identify all people seen in our gender identity clinic from 1972 until December 2015. Data were collected from the Hospital Registries of the VUmc. The total study population was defined by selecting people who had been diagnosed with one of the following International Classification of Diseases (ICD) diagnoses: 302.5 (transsexualism), 302.6 (gender identity disorder not otherwise specified), or 302.85 (gender identity disorder in adolescent or adult) for the 9th edition, or F64 (gender identity disorders) for the 10th edition.[12] In addition, the administrative employees of our gender identity clinic registered everyone who was referred to our gender identity clinic since the early seventies. People reported on this list were included in the study population as well. Some people of this study population have been described in earlier studies.[9,13-18] People were excluded from the study if they had been registered at our gender identity clinic but had actually never visited the clinic, or if they had presented themselves with other complaints than gender identity issues. Due to the retrospective design and the large study population, necessity for informed consent was waived by our local ethics committee.

Hospital Registries

The Hospital Registries stores clinical data obtained during regular patient care performed in our center, including medical diagnoses (since 1985), medication prescriptions (since 2000), surgical interventions (since 2006), laboratory test results (since 2004), radiology results (since 1993), and visit dates (since 2007). The first visit was defined as the first appointment with the psychologist, psychiatrist, pediatrician, endocrinologist, or gynecologist for gender identity related healthcare.

Clinical data collection

Not all data were available from the Hospital Registries, particularly older data or surgeries performed in other centers were missing. In order to generate the most reliable results, the medical records of all people who were reported as study population were checked. All people were classified as trans women or trans men (based on the sex assigned at birth), and date of birth and death were noted. The following categories were included: the individual was in diagnostic stage, the individual did not start with HT, or the individual was on HT. A start with HT was defined as the first date gender

affirming hormones were prescribed by a physician in our gender identity clinic after a confirmed GD diagnosis, irrespective of previous gender affirming hormone use. Of the people who started HT, baseline and follow-up data, including first visit, medical history, medication use, prior gender affirming hormone use, start date and type of PS and HT, and date of gonadectomy were collected. Some people regretted the interventions they had undergone. Trans women who started testosterone treatment after vaginoplasty or trans men who started estrogen treatment after oophorectomy and expressed regret were categorized as those who experienced regret. Reasons for regret as reported in their medical records were noted. Dates were set to the first of the month and personal identification data were removed from the research database.

Statistical analysis

The total number of people visiting the clinic each year and their median age were reported separately for trans women and trans men, and were stratified for age at the first visit: children <12 years, adolescents between 12 and 18 years, and adults ≥18 years. The percentage of people who started with HT within five years after the first visit was reported for each year. The prevalence was calculated for people aged ≥12 years, ≥16 years, 12 to 18 years, 18 to 30 years, 30 to 50 years, and ≥50 years, by using the total number of people in these age groups who received medical treatment in our center until 2015, excluding deceased people. The total population of these age groups in the Netherlands in 2015 were provided by the Central Bureau of Statistics (CBS) of the Netherlands. The percentage of people who underwent gonadectomy within five years after start with HT was reported. For calculation of the total percentage of the study population who had undergone gonadectomy, only people of 18 years and older who used HT for at least 1.5 years were included, as these were requirements for surgery. People who regretted their medical transition are reported as a percentage of the total population of trans women and trans men that underwent gonadectomy. In adults, time between first visit and start with HT or gonadectomy, if applicable, are expressed as median days with inter quartile range (IQR). Total follow-up time was calculated for every individual who started with HT and was expressed as years between the first visit and last visit. Prevalence with 95% confidence interval (CI) were calculated using OpenEpi.[19] All other analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 13.1.

Results

First visit

In total, 6,793 people had presented themselves for gender affirming treatment, with more trans women (65.2%) than trans men (34.8%) (Table 1). The number of people attending the gender identity clinic increased over time, while the median age of adults at the time of their first visit decreased, as shown in Figure 1. The median age at first visit is lower for adult trans men (25 years, IQR 21 – 35 years) than adult trans women (33 years, IQR 25 – 42 years). While historically more trans women than trans men presented themselves for treatment, in 2015 more trans men than trans women applied for treatment. This change in sex ratio is mainly due to the increase in adolescent trans boys, as the ratio of trans women:trans men in adults remained stable over time.

Table 1. Treatment patterns of the total study population, stratified for age groups, and for trans women and trans men.

	Trans women	Trans men	Total	Ratio trans women: trans men
Total number people (% of total)	4,432 (65.2%)	2,361 (34.8%)	6,793 (100%)	1.9:1
- Adults ≥18 years	3,809	1,624	5,433	2.3:1
• Age, yr (median, IQR; max)	33 (25 – 42; 81)	25 (21 – 35; 73)	31 (23 – 41; 81)	
• % start HT	68.9	72.9	69.9	
• % gonadectomy	75.3	83.8	77.7	
- Adolescents 12-18 years	330	482	812	0.7:1
• Age, yr (median, IQR)	16 (15 – 17)	16 (15 – 17)	16 (15 – 17)	
• % start PS	28.7	50.8	41.0	
◊ % who stopped PS	4.1	0.7	1.9	
• % start HT without PS *	33.9	30.8	32.2	
• % gonadectomy	79.5	77.2	78.2	
- Children <12 years	293	255	548	1.1:1
• Age, yr (median, IQR)	8 (7 – 10)	9 (8 – 11)	9 (7 – 10)	
• % start PS	33.6	49.1	40.3	
Regret	0.6% (n=11)	0.3% (n=3)	0.5% (n=14)	2.0:1

Percentage start with puberty suppression (PS): only in people after reaching the age of elig`ty (usually 12 years or older). Percentage start gender affirming hormonal treatment (HT): only in people aged 16 years or older. Percentage gonadectomy (orchiectomy in trans women and oophorectomy in trans men): only in people treated with cross-sex hormones for at least 1.5 years and 18 years or older. People with regret: only in people after gonadectomy. Age is defined as age at first visit in the VU University medical center (VUmc), Amsterdam. Between 1987 and 2002, children and adolescents were first seen in Utrecht University Medical Center and were only seen in the VUmc if they could start with medical treatment. * After diagnostic phase too old (≥18 years) to start with PS, could start directly with HT. Abbreviations: yr = years, IQR = inter quartile range, max = maximum.

Table 2. Description of the adult study population for every 5-years cohort

	Number first visit	% start HT	Age start HT	% Use HT past	% gonadectomy
Trans women ≥18 years					
1972-1979	119	89.9	33 (26 – 40)	16.8	79.4
1980-1984	189	88.4	33 (25 – 40)	12.6	71.9
1985-1989	319	75.9	31 (25 – 39)	15.3	76.5
1990-1994	392	65.8	30 (25 – 41)	20.5	76.7
1995-1999	522	65.5	34 (27 – 41)	26.6	78.7
2000-2004	605	56.0	38 (30 – 45)	29.2	67.3
2005-2009	476	61.6	39 (29 – 47)	22.9	68.6
2010-2014	926 (138*)	60.9*	32 (23 – 42)*	29.8*	na
Trans men ≥18 years					
1972-1979	30	96.7	24 (21 – 30)	10.3	72.4
1980-1984	69	84.1	24 (21 – 32)	3.5	82.8
1985-1989	105	84.8	24 (21 – 30)	1.1	79.8
1990-1994	142	69.0	27 (21 – 33)	7.1	88.8
1995-1999	177	65.0	29 (24 – 37)	7.0	88.7
2000-2004	207	63.3	32 (26 – 39)	5.3	87.0
2005-2009	185	63.8	29 (23 – 37)	3.4	81.4
2010-2014	518 (70*)	71.4*	24 (21 – 37)*	0*	na

Age is reported as median with interquartile range (IQR). Percentage of people starting with gender affirming hormonal treatment (HT) are people who started HT within 5 years after the first visit. Percentage of people who underwent gonadectomy are people who had this procedure within 5 years after start of HT. *Only in people who had their first visit 5 years before 31 December 2015 (n=138 for trans women, n=70 for trans men).

Prevalence and treatment

At the end of 2015, a total of 3,838 trans people aged 16 years or older had received medical treatment and were not deceased. As the total population of people aged 16 years or older in the Netherlands in 2015 was 13,870,426 people, the prevalence is 27.7 per 100,000 people (95%CI 26.8 – 28.6), or 1:3,600 people. Stratification for trans women and trans men leads to a prevalence of 36.4 (95%CI 35.0 – 37.8) per 100,000 people (or 1:2,800 people) for men (trans women), and 19.3 (95%CI 18.3 – 20.3) per 100,000 people (or 1:5,200 people) for women (trans men). Calculation of prevalence numbers of people aged 12 years or older and specific age groups are shown in Table 3.

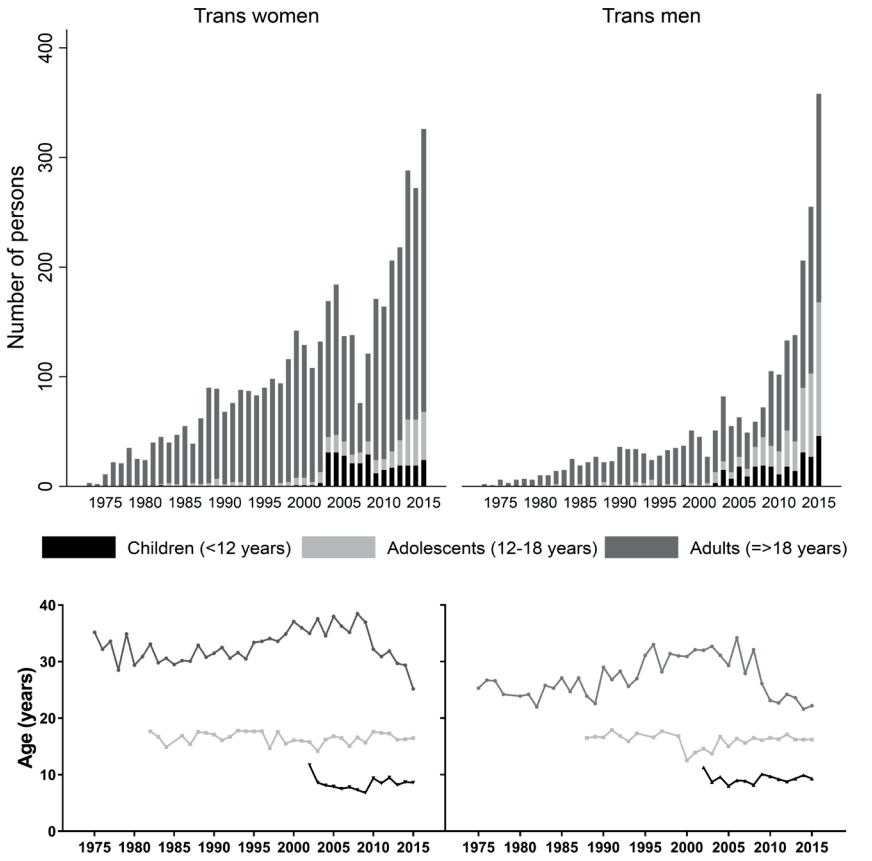


Figure 1.
Number of people with median age for each year, stratified for trans women and trans men, and stratified for children (<12 years), adolescents (12-18 years), and adults (≥18 years). Age is defined as age at first visit in the VU University medical center (VUmc), Amsterdam. Between 1987 and 2002, children and adolescents were first seen in Utrecht University Medical Center and were only seen in the VUmc if they could start with medical treatment.

The percentage of adult people who started with HT within 5 years after the first visit decreased over time, while the percentage of people who underwent gonadectomy within 5 years after start HT remained stable (Figure 2). Of the total study population treated with HT for at least 1.5 years and aged 18 years or older, 75.6% of the trans women (n=1,742) and 82.4% of the trans men (n=885) underwent gonadectomy. The median time between first visit and start of HT for adults was 327 days (IQR 36 to 570 days), and between first visit and gonadectomy 1029 days (IQR 679 to 1465 days). The median follow-up time for people treated with HT was 6.4 years (range 0.4 to 41.6 years).

Table 3. Prevalence numbers, specified for different age groups

	Total population		Male sex assigned at birth (trans women)		Female sex assigned at birth (trans men)	
	per 100,000	1 per	per 100,000	1 per	per 100,000	1 per
≥12 years	26.9 (26.1-27.8)	3,700	34.8 (33.5-36.2)	2,900	19.3 (18.3-20.3)	5,200
≥16 years	27.7 (26.8–28.6)	3,600	36.4 (35.0-37.8)	2,800	19.3 (18.3-20.3)	5,200
12-18 years	16.0 (13.9-18.4)	6,300	11.1 (8.8-14.1)	9,000	21.0 (17.7-25.1)	4,800
18-30 years	35.7 (33.5-38.2)	2,800	30.3 (27.4-33.4)	3,300	41.4 (37.9-45.1)	2,400
30-50 years	30.5 (29.0-32.2)	3,300	40.1 (37.6-42.8)	2,500	21.0 (19.2-23.0)	4,800
≥50 years	23.0 (21.9-24.2)	4,300	37.6 (35.5-39.8)	2,700	9.7 (8.7-10.8)	10,300

Data are shown as numbers with 95% confidence intervals.

Of the adolescents, 41.0% started with PS, while only 1.9% of these adolescents stopped with PS and did not start with HT (Table 1). 32.2% of the adolescents started directly with HT, as they were too old (≥18 years) to first start with PS after the diagnostic phase.

Regret

Regret was identified in 0.6% of the trans women and 0.3% of the trans men who underwent gonadectomy. The characteristics of these people are summarized in Table 3. Their ages at start of HT ranges from 25 to 54 years of age, and they expressed their regrets between 46 and 271 months after initiation of HT. Reasons for regret were divided into social regret, true regret, or feeling non-binary. Trans women who were classified as having social regret still identified themselves as women, but reported reasons as 'ignored by surroundings' or 'the loss of relatives is a large sacrifice' to life in the male role again. People who were classified as having true regret reported that they had thought gender affirming treatment was a 'solution' for, for example, homosexuality or personal acceptance, but, in retrospect, regretted the diagnosis and treatment.

Table 4. Characteristics of people with regret

Case	Type	Year start HT	Age start HT	Year of gonadectomy	Time after HT	Time after gonadectomy	Reversal surgery	Reason regret
1	M-F-M	1978	31 yr	1979	± 153 mo	± 130 mo	None	Social acceptance
2	M-F-M	1982	25 yr	1984	± 54 mo	± 27 mo	Mastectomy	Social acceptance
3	M-F-M	1986	47 yr	1988	± 216 mo	± 197 mo	Mastectomy	Social acceptance
4	M-F-M	1988	33 yr	1990	± 186 mo	± 167 mo	None	True regret
5	M-F-M	1988	38 yr	1990	± 70 mo	± 44 mo	Mastectomy	Social acceptance
6	M-F-M	1991	41 yr	1993	± 67 mo	± 49 mo	Mastectomy, vaginectomy, phalloplasty	Social acceptance
7	M-F-M	1991	38 yr	1995	± 271 mo	± 225 mo	Mastectomy	True regret
8	M-F-M	1993	30 yr	1994	± 79 mo	± 61 mo	None	Feels non-binary
9	M-F-M	1996	33 yr	1997	± 90 mo	± 73 mo	Mastectomy, phalloplasty	True regret
10	M-F-M	1997	43 yr	1999	± 46 mo	± 27 mo	Mastectomy	True regret
11	M-F-M	2004	54 yr	2007	± 130 mo	± 92 mo	Mastectomy, vaginectomy	True regret
12	F-M-F	1987	25 yr	1990	± 91 mo	± 50 mo	Breast augmentation, remove testicular implants	True regret
13	F-M-F	1990	34 yr	1993	± 102 mo	± 74 mo	Remove testicular implants	Feels non-binary
14	F-M-F	1993	31 yr	1997	± 258 mo	± 212 mo	None	True regret

Abbreviations: HT = gender affirming hormonal treatment; M-F-M = male-to-female-to-male; F-M-F = female-to-male-to-female; yr = years, mo = months

Discussion

The aim of this study was to generate a data set of all the individuals whom have presented to our clinic for gender affirming care from 1972 to 2015. We found that the number of people with gender identity issues who seek professional help increased dramatically in the last decade(s), and that the median age of adults at presentation decreased. The ratio of trans women:trans men remained stable over the years for adults, while in adolescents the population of trans boys increased relatively more than the population of trans girls. Currently, more trans boys than trans girls are seen. This phenomenon has also been described by Aitken et al.[17] The age at first visit was higher for adult trans women than trans men. The percentage of adult people starting with HT within five years after the first visit decreased over time, while the percentage of people who underwent gonadectomy within five years after starting HT remained stable. Of the total population treated with HT, 77.8% underwent a gonadectomy. Only a very small percentage of people who underwent gonadectomy regretted their decision, expressed as start of hormonal treatment in line with their sex assigned at birth.

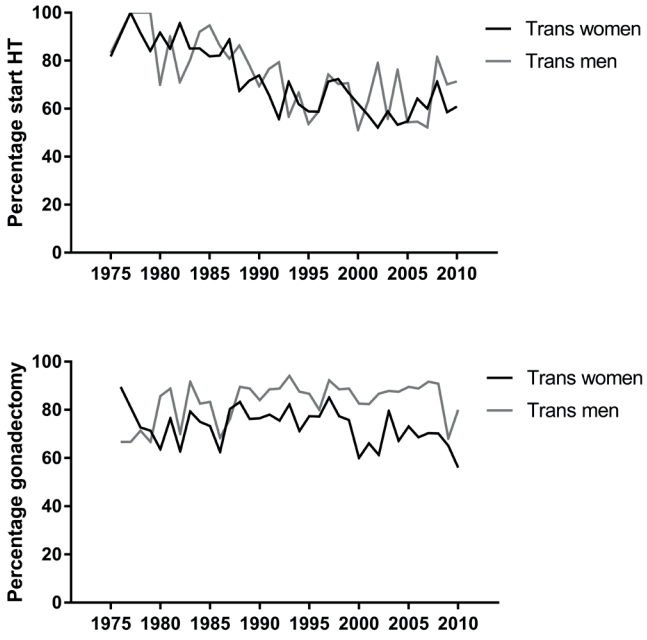


Figure 2.
Percentage of adult trans people starting with gender affirming hormonal treatment (HT, upper) within 5 years after first visit, or occurrence of gonadectomy (lower) within 5 years after start HT for each year, stratified for trans women and trans men. Year is defined as year of first visit.

An explanation for the increase in referrals may be the increased attention in society and media, which contributes not only to awareness of existence of GD and possibilities for medical treatment, but also to a higher social acceptance. In addition, information about trans identities are much more accessible via the internet within the last decade, which may lead to an earlier recognition of gender identity issues. Also, trans and gender non-binary individuals may be more willing to access care and more access to care made be available.

The increase in the prevalence of people with gender dysphoria who search for medical treatment in the Netherlands (1:11,900 trans women and 1:30,400 trans men in 1990 [8] versus 1:2,800 trans women and 1:5,200 trans men currently) suggests that the transgender population is dramatically increasing. The highest prevalence for trans women is found in the age group 30 to 50 years (1:2,500), while in trans men it is found in the age group 18 to 30 years (1:2,400). Trans people in the Netherlands seem to experience a reasonable degree of acceptance due to a tolerant social climate in contrast to many other countries.[20] For example, the medical costs are reimbursed by the medical insurance companies, and it is possible to change the legal sex status (even without gonadectomy). These points may lead to a lower threshold to seek help, making this study population useful for an adequate estimation of the current prevalence of people with gender dysphoria who wish medical treatment. Not all, but over 95% of the treated trans people are treated in our gender identity clinic. In addition, not all trans people seek medical help. Some use self-medication or go abroad for treatment. Therefore, these numbers may still be an underestimation of the real prevalence. Our data represent a population that actively had sought help in a medical setting. In 2012, a Dutch study of non-clinical people reported that 0.6% (1:167) of male sex assigned at birth and 0.2% (1:500) of female sex assigned at birth reported an incongruent gender identity with a wish for hormones or surgery.[21] However, that study was a population-based study with a response rate of 20.9% which could lead to nonresponse bias. In addition, the existence of incongruent gender identities was based on self-report and no detailed assessment of GD was performed, which could both have led to higher prevalence rates.

Interestingly is the percentage of children who were referred in childhood (before the age of 12) and who started puberty suppression when the GD persisted and eligibility criteria were fulfilled. This 40% of children who start with PS is almost identical to the 39% of persistence of childhood gender dysphoria reported in an earlier Dutch study (using a smaller cohort of children).[22] In addition, the finding that the persistence is higher in natal girls (49.1%) compared to the natal boys (33.6%) is also in line with the observations in previous follow-up studies on the persistence of gender dysphoria in children (see for an overview Ristori & Steensma [23]).

Remarkably, we found a decrease over time in percentage of referred adult people who did actually start with HT. This finding might be explained by the fact that

in the past, it was harder to find information about GD and its treatment, and only people suffering from extreme types of GD managed to visit our gender identity clinic for treatment. Currently, due to media attention and the internet, it is easier to access information about our gender identity clinic, making the threshold lower to search for help. This may have led to referrals of people with milder forms of GD and people who are not sure of their feelings and just want to explore these with a psychologist. Such people may eventually not pursue HT. Another explanation might be that not all trans people wish HT, for example trans men or people with a non-binary identity who only have a wish for a mastectomy.[24]

By contrast, we noticed that the percentage of people who underwent gonadectomy within five years after start of HT remained stable over time. At the start of the clinic in 1972, the knowledge about transgender care was limited and only people who wished a classical treatment, consisting of a diagnostic phase, hormonal treatment plus social transitioning, and surgery (in this order) were treated. There was no room for partial treatments. Since the publication of the Standards of Care version 6 in 2001, other types of treatment are also offered.[25] In addition, in 2014, a change in the Dutch law was made, making it possible for trans people without a wish for gonadectomy to alter the sex on their birth certificate with a statement of an expert who declared that the individual is diagnosed with GD (Dutch civil law, article 1:28). While these changes in clinical guidelines and the law might have led to a decrease in the number of trans people choosing for gonadectomy, the current results do not show this. However, the follow-up time of this study may be too short to notice such changes.

In the HT group, 22% of the people who were eligible for surgery had not undergone gonadectomy. These numbers are comparable with a study from Sweden [26], but higher than a study from Belgium [27], in which approximately 15% of both trans women and trans men did not undergo gonadectomy. A possible wish to carry a child may change these numbers in the future, as fertility is a more important issue currently.

Despite the large increase in treated trans people, the percentage of people who underwent gonadectomy but regretted their decision is still very low (0.5%). In a review of Pfäfflin in 1992, a regret percentage of <1% for trans men and 1-1.5% for trans women after gonadectomy was found.[28] More recent studies found regret percentages ranging from 0% [29,30] to 2% [7] and 6% [31] after gonadectomy. Thirteen of the 14 people who regretted gonadectomy had started HT between 1978 and 1997 and one in 2004. At best, this indicates that diagnostic and eligibility criteria for treatment have improved over the last decade. Another explanation might be the altered treatment protocol, which also allowed people to receive HT without gonadectomy. Possibly, our findings are an underestimation of people with regret after gonadectomy, as some may choose to go elsewhere for reversal therapy or may experience regret without pursuing reversal surgery or hormone treatment. Regret may not always result in a desire for reversal therapy, as it may be hidden for others. In addition, in our population the average time

to regret was 130 months, so it might be too early to examine regret rates in people who started with HT in the last 10 years.

The Center of Expertise on Gender Dysphoria of the VUmc Amsterdam is the largest gender identity clinic in the Netherlands, where people of all ages, including children and adolescents, are treated. Life-time follow-up is recommended, making it a useful study population for collection of epidemiologic data and future long-term effect studies of treatment effects. However, there are some limitations as well. Because this study is a retrospective chart review study, some data may be lacking. First, it is possible that not all people who once visited our clinic are reported in our database. However, we used several search strategies to identify the total study population, thereby reducing the possibility of missing people. Second, a high number of trans people who had initially received treatment in our center were lost to follow-up. Even though care for trans people is lifelong, a large group (36%) did not return to our clinic after several years of treatment. Therefore we may have missed some information on, for example, gonadectomies performed at other centers or people with regret.

Conclusions

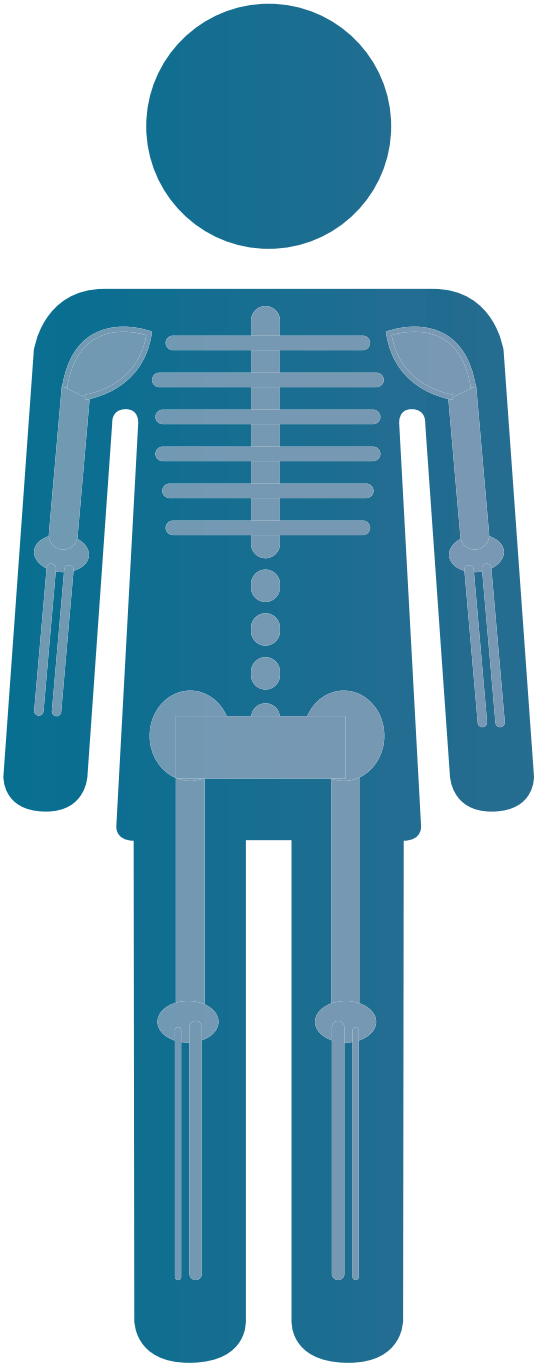
In conclusion, we found that the prevalence of treated trans people increased exponentially. Because of this growing population, it is necessary that healthcare providers outside university clinics also have knowledge about GD and its treatment, as HT can influence the course of several diseases [32,33] and interact with several types of medication.[34] We also found that of all trans people treated with HT, approximately 22% kept their gonads in situ. These people require special attention, as the long term effects of HT on testes, ovaries, and uterus are not established yet. These topics, as well as other possible complications such as cancer risks, are subjects for further research.

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Chapter 3

Bone mineral density increases in trans persons after one year 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.

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.

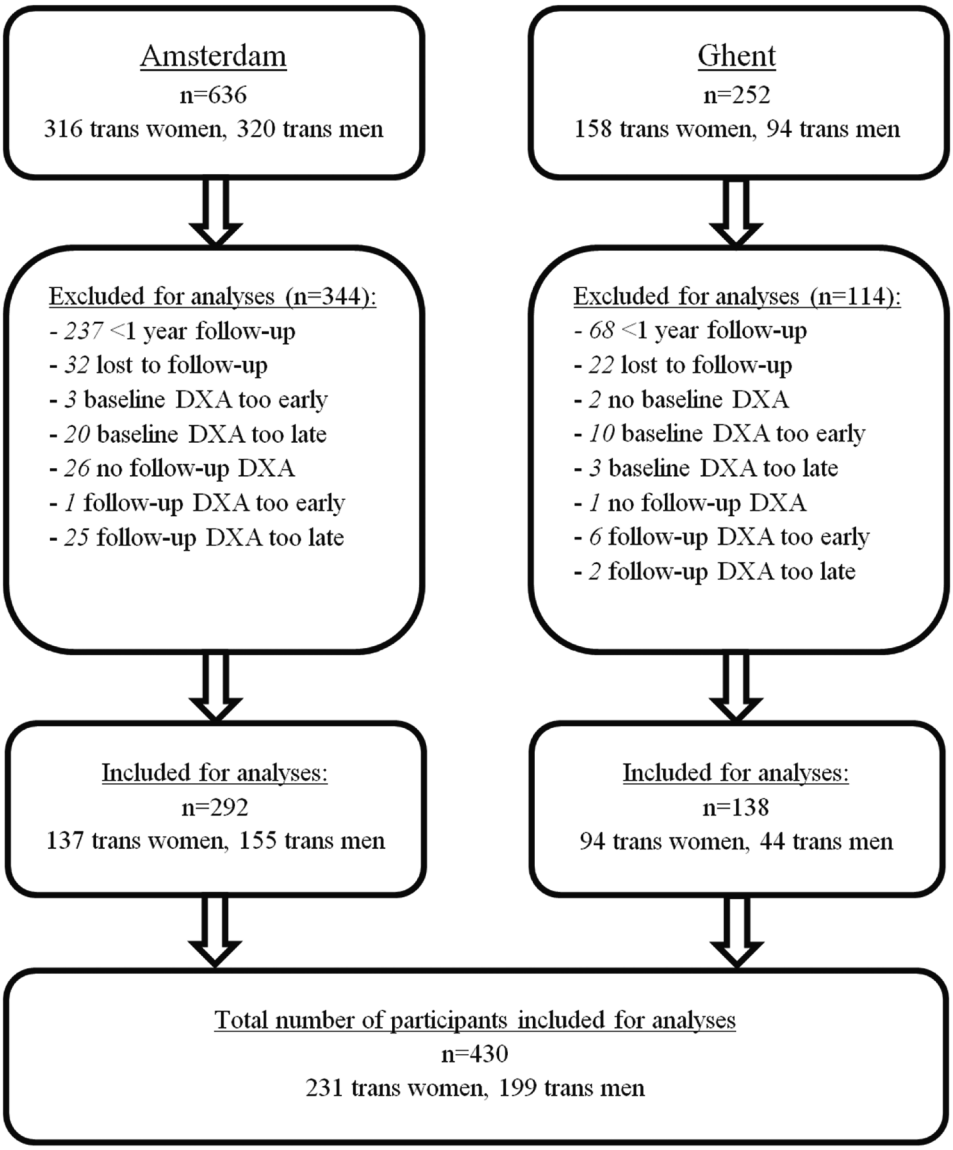


Figure 1.
Flow chart of the inclusion of participants in the present study. Abbreviations: DXA=dual-energy X-ray absorptiometry.

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) *		
- 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) #		
- 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. * Transwomen n=135, transmen n=137; # 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).

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

Numbers represent absolute bone mineral density in g/cm^2 (standard deviation). Independent t-tests were performed between the Amsterdam and Ghent data. Abbreviations: BMD=bone mineral density.

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.

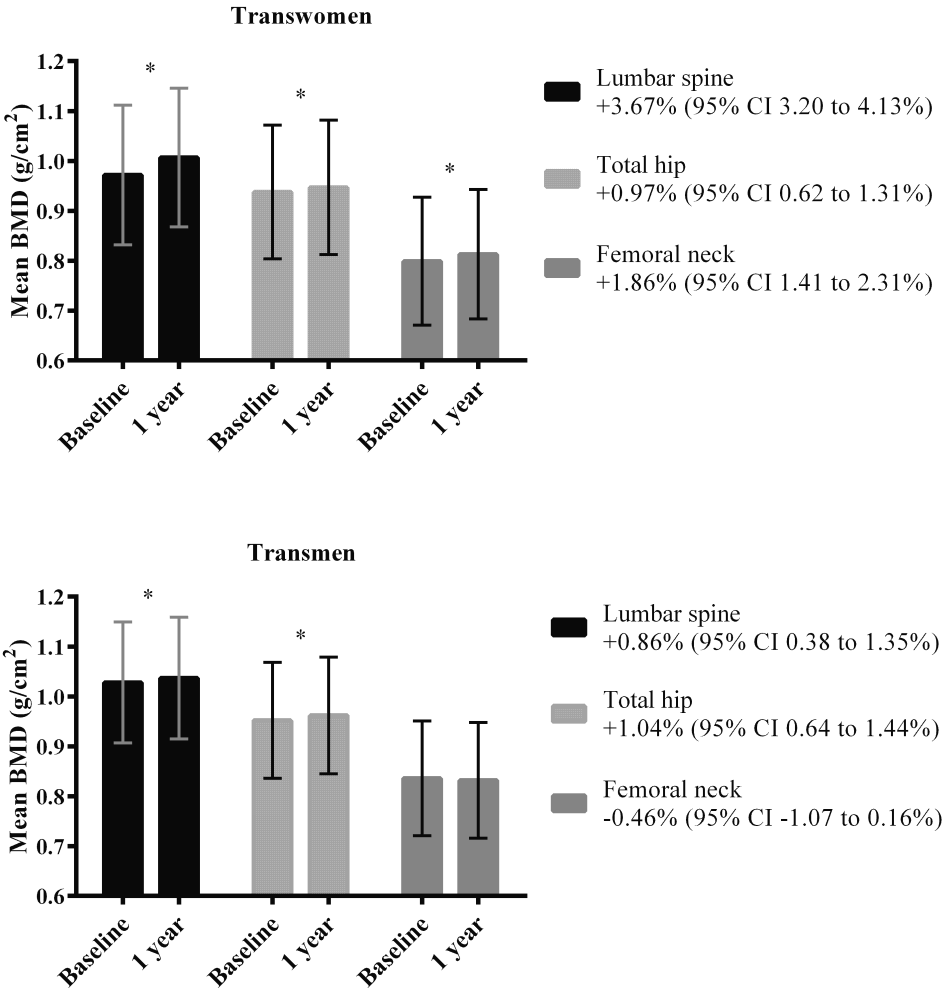


Figure 2.
Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen after one year cross-sex hormonal treatment. Data represent mean baseline and mean one-year BMD with standard deviation. Percentage changes in BMD were calculated for every person 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. Abbreviations: BMD=bone mineral density.

Effects of change in cigarette or alcohol use on BMD change

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).

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 three 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 three 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 three to 12 months were not correlated with BMD change. As testosterone levels after three to 12 months were suppressed in transwomen, no correlation analyses could be performed. In transmen, testosterone levels after three to 12 months were not correlated with LS, TH, and FN BMD change.

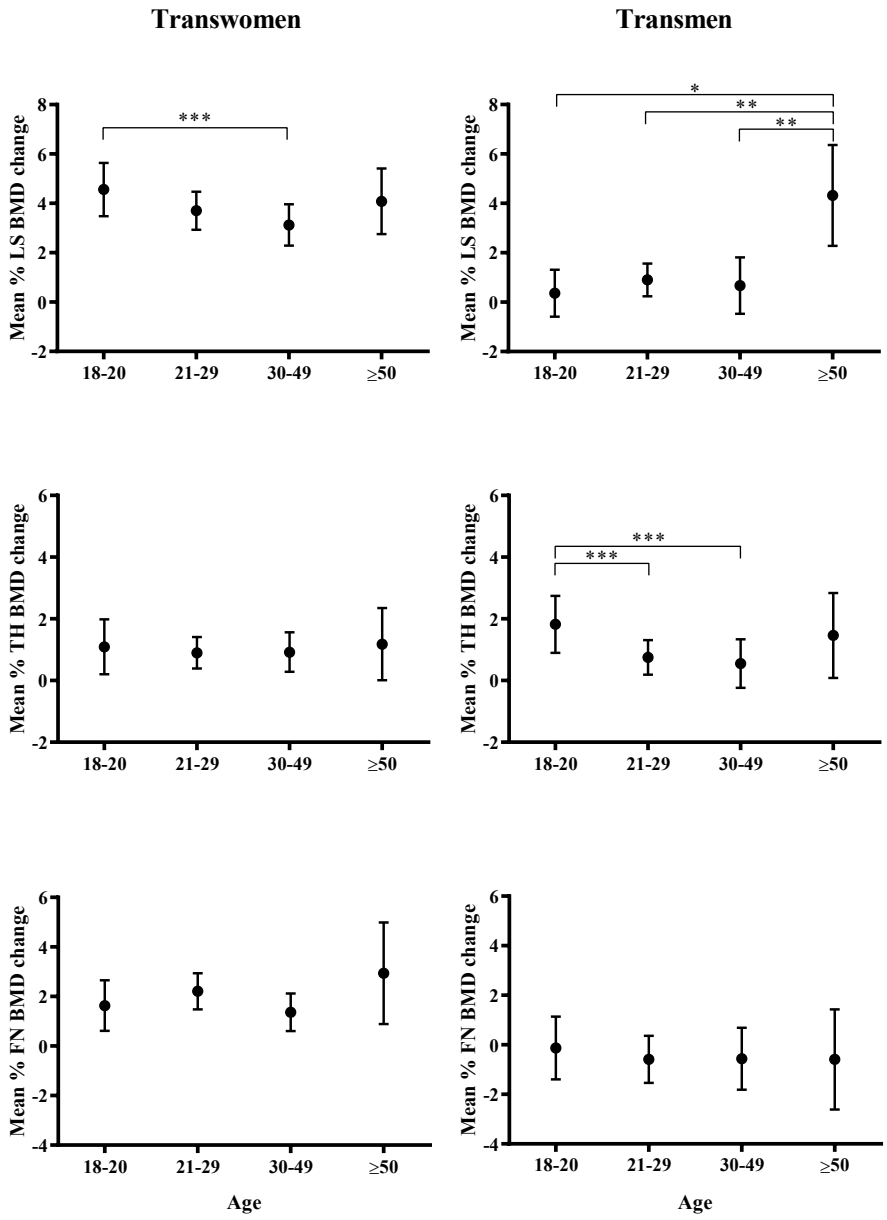


Figure 3.

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. * $p \leq 0.001$; ** $0.001 < p \leq 0.01$; *** $0.01 < p \leq 0.05$. Abbreviations: LS=lumbar spine; TH=total hip; FN=femoral neck; BMD=bone mineral density.

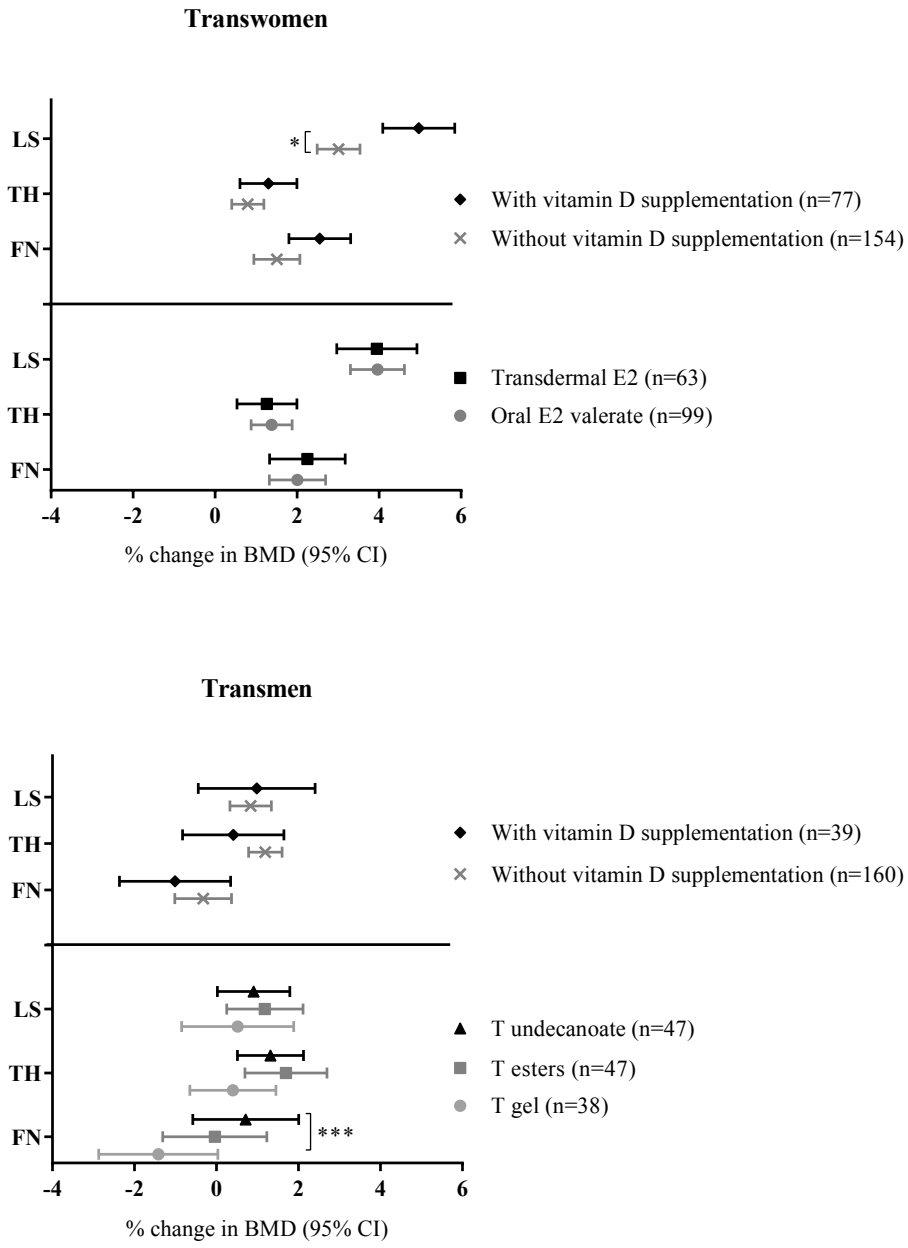


Figure 4.
Change in bone mineral density (BMD) of the lumbar spine, total hip, and femoral neck in transwomen and transmen, stratified for vitamin D supplementation or administration routes, respectively. Linear regression analyses with change in BMD as outcome variable and vitamin D supplementation 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.

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 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, as 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.

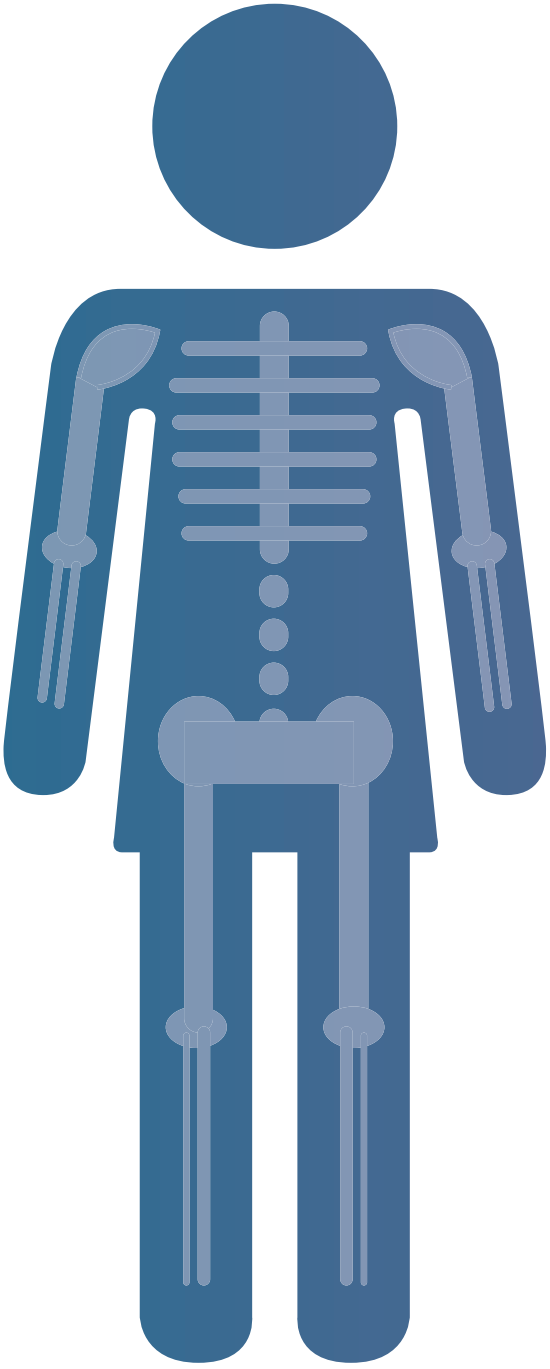
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Chapter 4

Gender-affirming hormonal 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. Especially 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 – 100 mg daily, oral) accompanied by estrogen treatment consisting of either estradiol valerate (2 – 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 after an overnight fast.

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.

Other

Last, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and gamma glutamyltransferase (γGT) concentrations were all measured using an immunoassay (Cobas, Roche Diagnostics, Mannheim, Germany).

DXA

DXA (Hologic Discovery A, Hologic Inc., Bedford, MA, USA) was used to measure BMD in g/cm^2 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 γGT 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 meandifferences 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/cm^2 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.

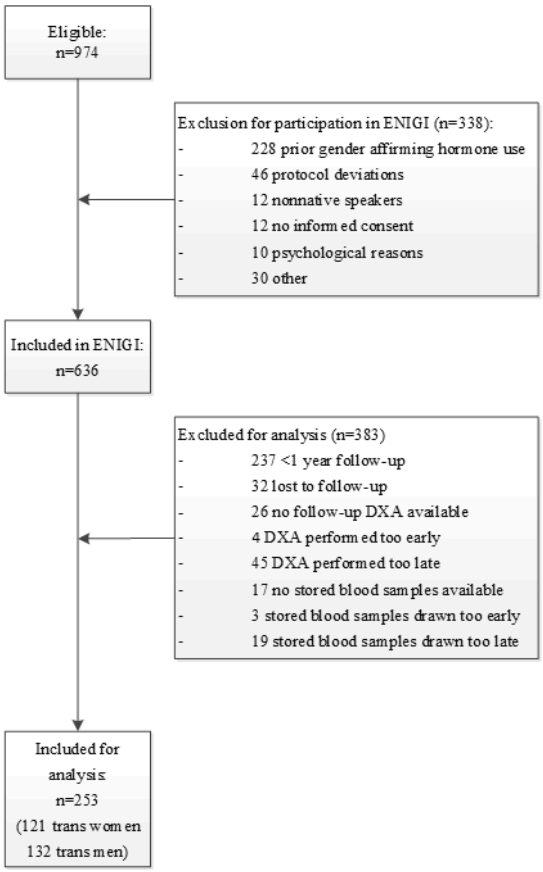


Figure 1.
Flowchart of in- and exclusion of participants. Abbreviation: ENIGI = European Network for Investigation of Gender Incongruence

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 – 3338) was seen during the first year of HT. In both groups BMI increased and tobacco use decreased during 1 year of HT (Table 1).

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)

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

Trans women

ALP, CTx, and sclerostin decreased with 19% (95% -21 to -16), 11% (95% -18 to -4), and 8% (95% -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).

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 *
Trans women				
Bone turnover markers				
P1NP, µg/L (median, IQR)	50 (42 – 65)	48 (38 – 62)	-3 (-9 ; 3)	-8 (-17 ; 1)
ALP, U/L (mean ± SD)	70 ± 17	57 ± 17	-19 (-21 ; -16)	-21 (-25 ; 18)
CTx, ng/L (median, IQR)	428 (306 – 538)	329 (265 – 442)	-11 (-18 ; -4)	-11 (-23 ; 1)
Sclerostin, pmol/L (median, IQR)	10.4 (8.6 – 14.9)	8.8 (7.3 – 13.5)	-8 (-13 ; -4)	-9 (-16 ; -2)
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)
ALP, U/L (mean ± SD)	67 ± 19	76 ± 22	+16 (12 ; 20)	+15 (7 ; 23)
CTx, ng/L (median, IQR)	423 (323 – 533)	432 (313 – 529)	+3 (-4 ; 10)	-5 (-19 ; 8)
Sclerostin, pmol/L (median, IQR)	8.7 (6.8 – 13.1)	10.3 (7.9 – 13.2)	+15 (10 ; 20)	+10 (-0 ; 20)
DXA				
BMD TH g/cm^2 (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/cm^2 (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/cm^2 (mean ± SD)	1.026 ± 0.125	1.036 ± 0.129	+1.0 (0.4 ; 1.7)	+2.1 (0.9 ; 3.4)

* Adjusted for changes in BMI, alcohol and tobacco use, 25OHD, creatinine, AST, ALT, and γGT. Abbreviations: HT = gender-affirming hormonal treatment, IQR = interquartile range, ALP = alkaline phosphatase

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 γGT 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 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).

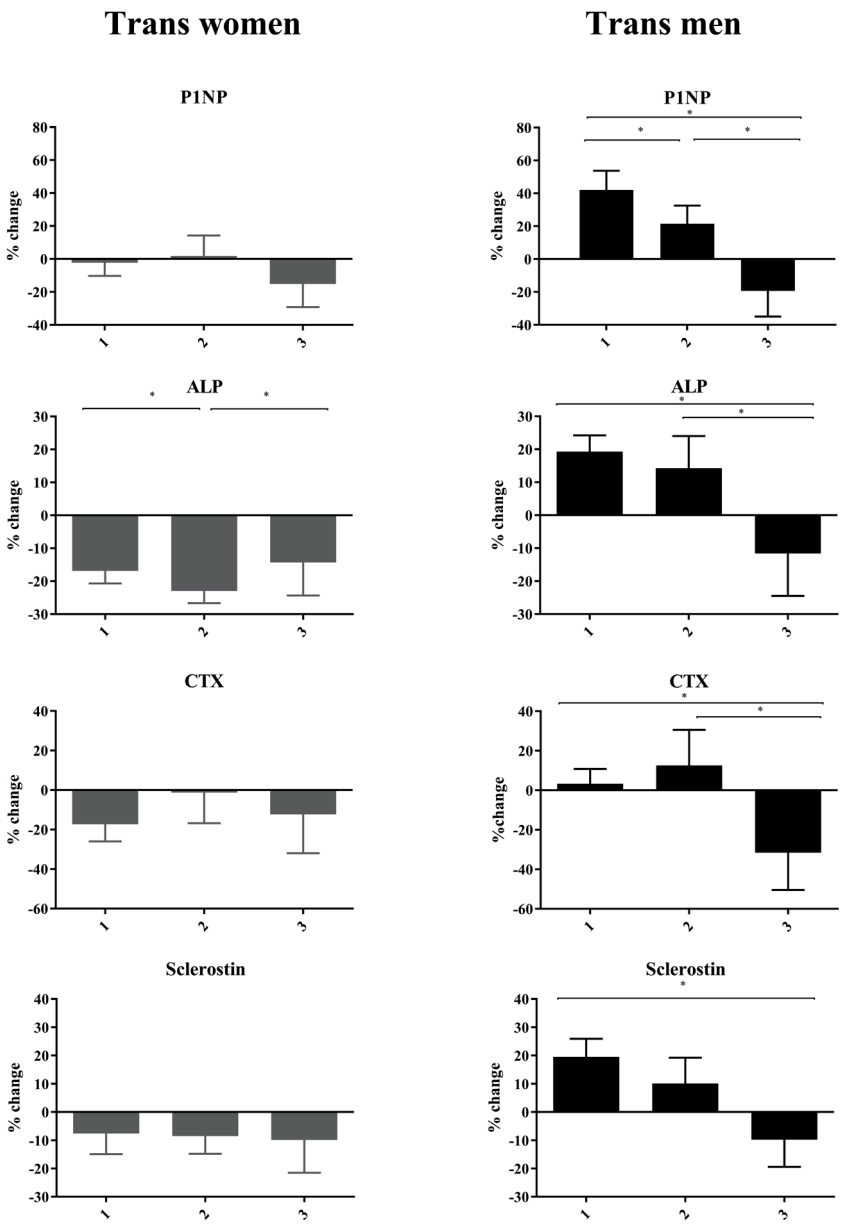


Figure 2.
Percentage change in bone turnover markers in trans women and trans men after 1 year of HT, stratified for age groups. * p = ≤ 0.05. Groups:
Group 1 = 18 to 30 years (trans women mean age 24 (2.9 SD), n = 61, trans men mean age 23 (3.0 SD), n = 91).
Group 2 = 30 to 50 years (trans women mean 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)
Abbreviations: ALP = alkaline phosphatase

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.

	TH BMD %	FN BMD %	LS BMD %
Trans women			
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			
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)

Abbreviation: ALP = alkaline phosphatase

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/β-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 confirm the bone preserving role of estrogens.

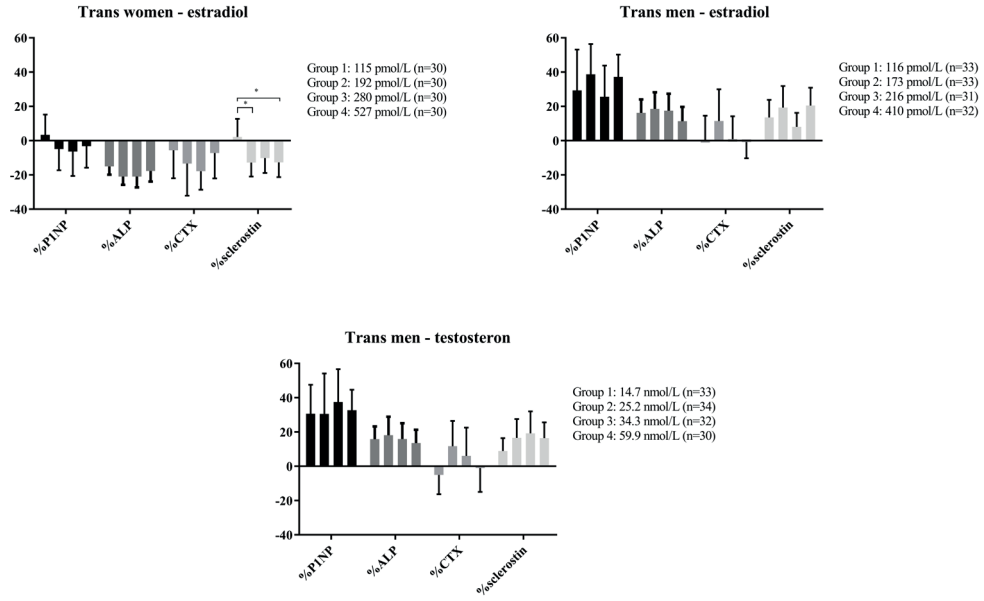


Figure 3.
Percentage change in bone turnover markers for mean different estradiol and testosterone concentrations during 1 year of HT. Testosterone concentrations in trans women were <2 nmol/L, and therefore this group was not further divided into subgroups, * $p = \leq 0.05$.
Abbreviation: ALP = alkaline phosphatase

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] Lastly, 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 were higher compared to control women after 10 years of HT.[24] Regarding ALP, earlier studies showed an increase in ALP within the first year of HT in trans men [15] and also during long-term follow-up of trans men.[12] 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). The

increase in estrogen concentration after aromatization of testosterone therefore 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.[49,50] 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.[51,52] 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.[53] 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.[54] 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.[55–57] 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.

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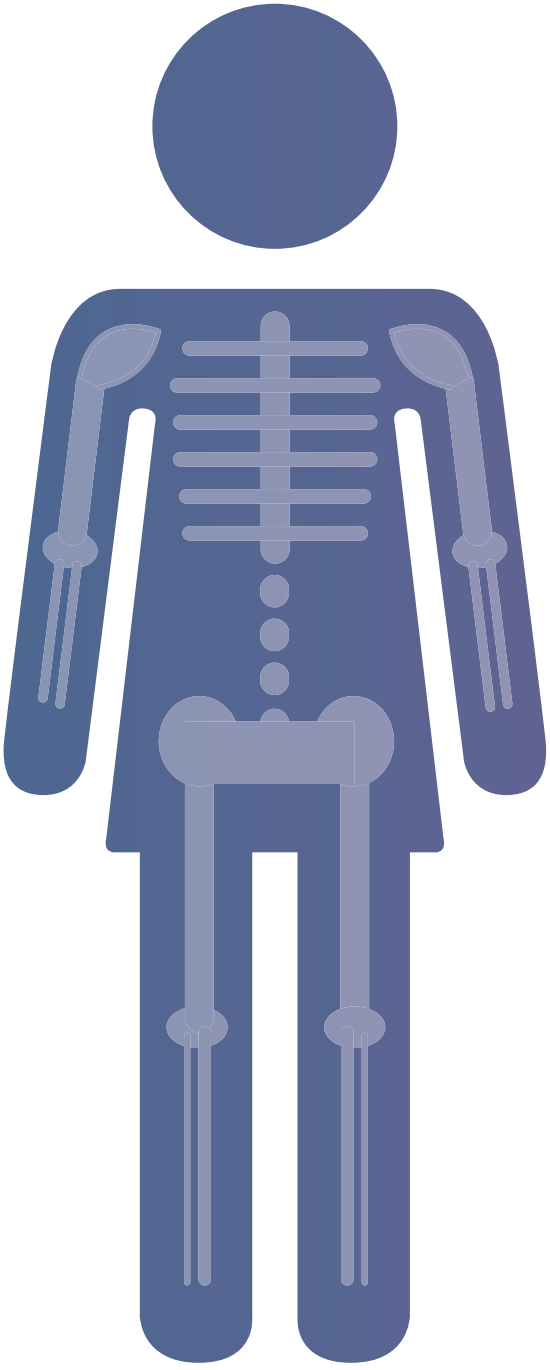
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Chapter 5

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

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Abstract

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

Introduction

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

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

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

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

Materials and Methods

Design and population

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

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

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

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

Biochemical assessment

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

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

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

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

Statistical analysis

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

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

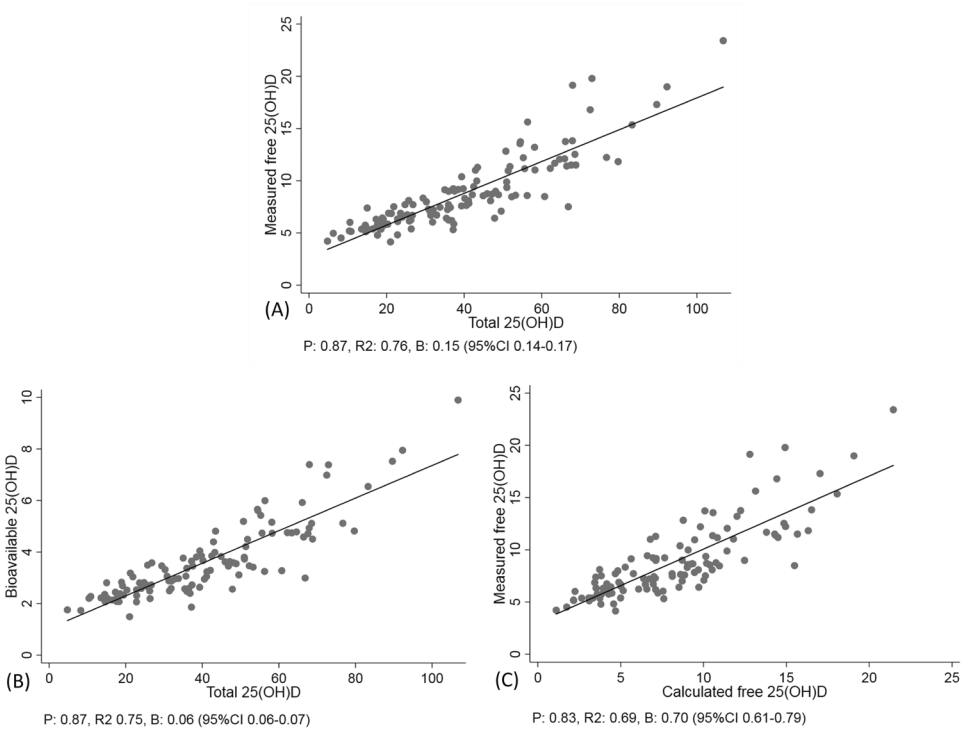


Figure 1.
Correlations between concentrations of (A) total 25(OH)D and measured free 25(OH)D, (B) total 25(OH)D and bioavailable 25(OH)D, and (C) measured and calculated free 25(OH)D. Abbreviations: P = Pearson correlation coefficient, R2 = R squared, B = beta

Discussion

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

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

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

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

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

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

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

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

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

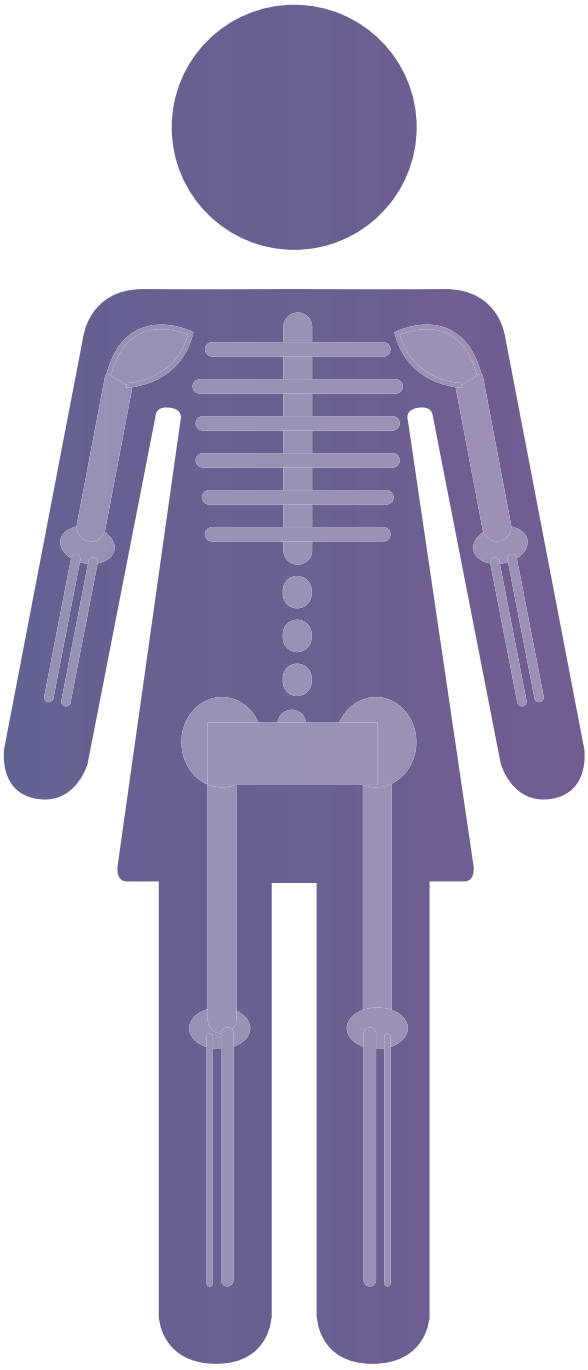
In conclusion, HT did not substantially influence DBP, free 25(OH)D, total 25(OH)D, and bioavailable 25(OH)D concentrations in transwomen and transmen, as the observed changes were small and not statistically significant. In addition, total 25(OH)D concentrations seem to reflect free and bioavailable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

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Chapter 6

Change in grip strength in trans people and its association with lean body mass and bone density

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Abstract

Objective: Gender-affirming hormonal treatment (HT) in trans people changes physical appearance. Muscle mass and strength are important aspects of physical appearance, but few data exist on the effect of HT on grip strength and muscle mass. This study aimed to investigate the change in grip strength in trans people during the first year of HT, to study possible determinants of this change, and to study the associations between changes in grip strength, lean body mass, and bone mineral density (BMD).
Design and methods: A multicenter, prospective study was performed, including 249 transwomen and 278 transmen. Grip strength, lean body mass, and BMD were measured at baseline and after one year.
Results: After one year of HT, grip strength decreased with -1.8kg (95%CI -2.6; -1.0) in transwomen and increased with +6.1kg (95%CI +5.5; +6.7) in transmen. No differences in grip strength change was found between age groups, BMI groups, hormonal administration routes, or hormone concentrations. In transmen, increase in grip strength was associated with increase in lean body mass (per kg increase in grip strength: +0.010 kg, 95%CI +0.003; +0.017), while this was not found in transwomen (per kg increase in grip strength: +0.004 kg, 95%CI -0.000; +0.009). Change in grip strength was not associated with change in BMD in transwomen and transmen.
Conclusions: After one year of HT, grip strength decreased in transwomen, and increased in transmen. In transmen only, change in grip strength was associated with change in lean body mass.

Introduction

According to the DSM-criteria, gender dysphoria (GD) is defined as the incongruence between a person's assigned sex at birth and the experienced gender.[1] GD often has a strong impact on the person's psychological wellbeing. Therefore, most trans people prefer treatment to change physical appearance, for example gender-affirming hormonal treatment (HT) with or without gender-affirming surgery.

Grip strength and muscle mass are good indications of the masculinity of the body. A decrease in grip strength and muscle mass could lead to a more feminine body for transwomen (male-to-female trans people), while an increase in grip strength and muscle mass indicates a more masculine body for transmen (female-to-male trans people). Besides the importance of physical appearance, a change in grip strength and muscle mass also might be important in the prevention of sarcopenia (the age-related loss of muscle mass) [2], and dynapenia (the age-related loss of muscle strength).[3]

Earlier studies on effects of HT in trans people focused on bone mineral density (BMD) and body composition. These studies found an increase in lean body mass in transmen and a decrease in lean body mass in transwomen.[4, 5] BMD increased in both transwomen and transmen.[6] A study in cis gender people found grip strength to be a predictor of bone mass.[7] Possibly, a change in BMD in trans people is associated with a change in grip strength. Some studies described the change in grip strength [8, 9], however, sample sizes of these studies were small and possible influences on these changes, for example age, body mass index (BMI), administration routes of HT, and sex hormone concentrations during HT, have not been studied.

The aim of this study is to investigate the time course of change in grip strength in trans people in the first year of HT, to study possible determinants of this change, and to study the reciprocal associations between changes in grip strength, lean body mass (as an approximation of muscle mass), and BMD.

Material and methods

Study design and study population

This study is part of the European Network for the Investigation of Gender Incongruence (ENIGI) study, a multicenter prospective cohort study, including treatment centers in Amsterdam, Ghent, Oslo, and Florence using the same treatment protocol. The study design is published previously [10,11] and the study is registered at https://clinicaltrials.gov/ct2/show/NCT01072825. In short, people were included from 2010 until April 2016. The included people were 18 years and older and gave informed consent. People could participate in this study when they started with HT, if they did not use gender-affirming hormones before the start of HT, and if they spoke the native language. During the first

year of treatment, the people were seen every 3 months. For the current study, only data from Amsterdam, Ghent, and Oslo were analyzed, as grip strength was not assessed in Florence. People were included if their grip strength was measured at baseline and after 12 months. For the analyses on lean body mass and BMD, people were included if a dual-energy X-ray absorptiometry (DXA) was performed at baseline (range 4 months before to 4 months after baseline) and after 12 months (range 10 to 14 months) of HT. People from Oslo were excluded from the analyses on lean body mass and BMD, because a different type of DXA scanner was used (Oslo: Lunar (GE Lunar, Madison, WI, USA); Amsterdam and Ghent: Hologic Discovery A (Hologic Inc, Bedford, MA, USA)). In total, 1,017 participants were included in the overall study. After exclusions due to unknown grip strength values at baseline or 12 months (n=60), a follow-up of less than one year (n=363), or lost to follow-up (n=57), a total of 249 transwomen and 278 transmen were included in our analyses (Figure 1).

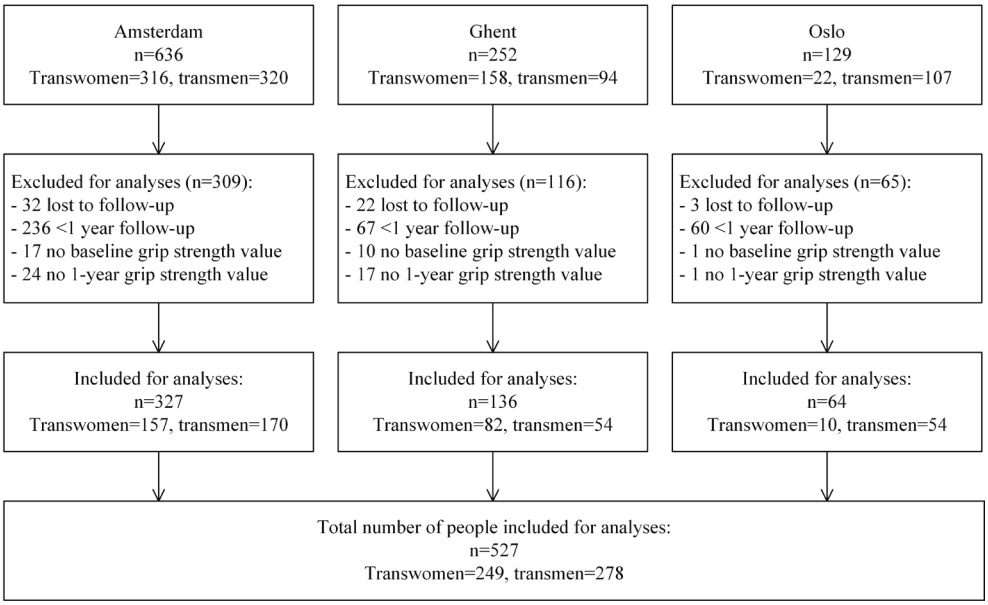


Figure 1.
Inclusion flowchart

Transwomen were treated with the anti-androgen cyproterone acetate (CPA) 50 mg daily, in combination with two to four mg oral estradiol valerate a day, or 100 mcg/24h estradiol patch twice a week. People older than 40 years were advised to be treated with transdermal estrogens, because of thrombosis risk.[12] Transmen were treated with testosterone. They could choose between testosterone gel (50 mg daily), testosterone esters (250 mg intramuscular every two to three weeks), or testosterone

undecanoate (1000 mg intramuscular every 12 weeks).

The Medical Ethics Review Committee of Ghent approved the study protocol. Local Ethical Review Committees approved participations in the other centers.

Clinical data collection

Grip strength was measured in kilograms (kg), using an adjustable hand-held standard grip device, a Jamar Dynamometer.[10,13] The grip strength of the dominant hand was measured twice and the highest value was noted. These measurements were performed at baseline and after three months, six months, nine months, and twelve months of HT. However, from Oslo only data at baseline and after 12 months were available.

Body weight and height were measured at every visit. People were measured without shoes and in light indoor clothes. BMI was calculated by weight divided by the square of body height.

DXA

A whole-body DXA was performed to measure lean body mass of the arms and the legs. Outcomes of body composition were determined using manufacturer-supplied algorithms. The specific arm and leg regions were defined with software from Hologic.

Absolute BMD values were obtained for lumbar spine (L1–L4, LS), total hip (TH), and femoral neck (FN). The same type of DXA device was used in Amsterdam and Ghent (Hologic Discovery A, Hologic Inc., MA, USA) and software version 13.5.3 was used.

Laboratory measurements

Venous blood samples were obtained in the morning at baseline, after three months of HT, and after 12 months of HT. As the results were also used during clinical care, assays with higher quality were implemented when they were available. In order to compare the new and old values likewise, conversion formulas were generated by the endocrine laboratory.

In Oslo, testosterone and estradiol were determined using a competitive immunoassay (ECLIA, Roche Diagnostic, Indianapolis, USA) with a lower limit of quantitation (LOQ) of 0.1 nmol/L (2.6 ng/dL) and 18.4 pmol/L (5.0 pg/mL), and a coefficient of variation (CV) of 5 and 7%, respectively. In Ghent, an E170 Modular (E2 Gen II, Roche Diagnostics, Germany) was used for testosterone (LOQ 0.4 nmol/L [11.5 ng/dL], CV 2.6%) and estradiol (LOQ 92 pmol/L [25.1 pg/mL], CV 3.2%). For estradiol, it was updated in March 2015 to an E170 Modular (E2 Gen III, Roche Diagnostics, Germany) with a conversion formula of Gen III=6.687940 + 0.834495*Gen II. In Amsterdam, estradiol was measured using a competitive immunoassay (Delfia, ParkinElmer, Wallac Oy, Turku, Finland) with a LOQ of 20 pmol/L (5.4 pg/mL) and a CV of <13% until July 2014. Thereafter, an LC-MS/MS (VUmc, Amsterdam, the

Netherlands; LOQ 20 pmol/L [5.4 pg/mL], CV <7%) was used, with a conversion formula of LC-MS/MS=1.60*Delfia-29. Testosterone was measured using a radioimmunoassay (RIA, Coat-A-Count, Siemens, Los Angeles, CA, USA; LOQ 1 nmol/L [28.8 ng/dL], CV <10%) until January 2013. After that, it was measured using a competitive immunoassay (Gen III, Architect, Abbott, Abbott Park, IL, USA) with a LOQ of 0.1 nmol/L (2.9 ng/dL) and a CV <10%. Two conversion formulas were generated: <8 nmol/L: Architect=1.1*RIA+0.2; >8 nmol/L: Architect=1.34*RIA-1.65.

Statistical analyses

Results are presented as mean with standard deviation (SD) in case of normal distribution, or median with interquartile range (IQR) for non-normally distributed data, and percentages. In case of non-normally distributed data, a log transformation was performed before further analyses.

To examine the course of the change in grip strength during the first year, linear mixed model analyses were performed. The influence of age, differences in BMI, different administration routes of HT, and serum hormone concentrations, were analyzed. Age was divided into groups (<25 years, 25-40 years, ≥40 years), to stratify for accrual of grip strength, peak grip strength, and age-related decrease of grip strength. BMI was defined as underweight (<18.5 kg/m^2), normal weight (18.5-25 kg/m^2), and overweight (≥25 kg/m^2). For analyses between differences in the administration route of HT, people who used the same administration route for at least nine months were included. Mean concentrations of estradiol and testosterone during HT were calculated by averaging the results of the measurements after three and after 12 months of HT. As different assays were used in the centers to determine estradiol and testosterone and no conversion formulas between the centers were available, it was not possible to analyze the estradiol and testosterone concentrations as absolute values. Therefore, center specific tertiles were created for estradiol and testosterone and were thereafter analyzed together. The center specific mean estradiol concentrations in transwomen were: first tertile 122 pmol/L (33 pg/mL, Amsterdam), 159 pmol/L (43 pg/mL, Ghent), and 167 pmol/L (46 pg/mL, Oslo); second tertile 233 pmol/L (63 pg/mL, Amsterdam), 248 pmol/L (68 pg/mL, Ghent), and 317 pmol/L (83 pg/mL, Oslo); and third tertile 405 pmol/L (110 pg/mL, Amsterdam), 572 pmol/L (156 pg/mL, Ghent), and 398 pmol/L (108 pg/mL, Oslo). The center specific mean testosterone concentrations for transmen were: first tertile 16 nmol/L (461 ng/dL, Amsterdam), 10 nmol/L (288 ng/dL, Ghent), and 15 nmol/L (432 ng/dL, Oslo); second tertile 28 nmol/L (806 ng/dL, Amsterdam), 16 nmol/L (461 ng/dL, Ghent), and 21 nmol/L (605 ng/dL, Oslo); and third tertile 54 nmol/L (1555 ng/dL, Amsterdam), 24 nmol/L (691 ng/dL, Ghent), and 33 nmol/L (950 ng/dL, Oslo).

The average of the right and left arm was calculated for the analyses on the lean body mass of the arms, and the average of the right and left leg was calculated to analyze the lean body mass of the legs. To evaluate the change of grip strength, lean body

mass, BMD, and serum creatinine after one year, the absolute and percentage difference between the baseline values and the values after 12 months were calculated. As these were normal distributed variables, linear regression analyses were performed to calculate the mean and 95% confidence interval (CI).

To study the association between changes in grip strength and changes in lean body mass, BMD, or creatinine concentrations, linear regression analyses were performed.

All analyses were performed separately for transwomen and transmen. Data was analyzed using STATA Statistical Software (Statacorp, College Station, Texas, USA, version 15.1). P-values <0.050 were considered statistically significant.

Results

Characteristics

The characteristics are shown in Table 1. No differences were found between included and excluded people in baseline grip strength, age, BMI, estradiol concentrations, testosterone concentrations, or smoking habits (Supplementary Table 1).

For the analyses on lean body mass, 171 transwomen and 154 transmen were included. For these analyses, excluded transwomen were younger (median age 26 years, IQR 21 to 34) than included transwomen (median age 31 years, IQR 23 to 42). Excluded transmen were younger (median age 22 years, IQR 19 to 25) and had a lower BMI (mean 24.3 kg/m^2, SD 5.2) than included transmen (median age 28 years, IQR 20 to 33, and mean BMI 26.0 kg/m^2, SD 5.5). No differences were found between included and excluded people in baseline grip strength, estradiol concentrations, testosterone concentrations, or smoking habits.

For the analyses on LS BMD, 207 transwomen and 229 transmen were included. For TH and FN, 206 transwomen and 216 transmen were included. No differences were found between included and excluded transwomen. Excluded transmen were younger (median age 21 years, IQR 19 to 25) than included transmen (median age 24 years, IQR 21 to 31).

Table 1. Characteristics of the study population.

	Transwomen (n=249)		Transmen (n=278)	
	Baseline	During HT	Baseline	During HT
Age, years	28 (23 – 40)		23 (20 – 30)	
BMI, kg/m^2	23.8 (4.5)	24.5 (4.4)	25.5 (5.6)	25.9 (4.8)
Smoking (% yes)	24.7	14.7	29.9	19.9
Alcohol (% yes)	44.8	44.6	55.3	55.8
Grip strength (kg)	41.8 (8.9)	40.0 (8.9)	33.1 (6.5)	39.2 (6.8)
Creatinine (μmol/L)	78.5 (10.8)	73.1 (10.6)	66.0 (9.0)	77.4 (10.6)
Hormone administration route*				
- Estradiol oral	-	134	-	-
- Estradiol transdermal	-	86	-	-
- Testosterone gel	-	-	-	52
- Testosterone undecanoate i.m.	-	-	-	130
- Testosterone esters i.m.	-	-	-	81
Estradiol concentrations, pmol/L				
- Amsterdam	105 (83 – 131)	225 (141 – 329)	142 (59 – 371)	180 (138 – 242)
- Ghent	109 (88 – 130)	246 (181 – 342)	166 (116 – 431)	134 (113 – 169)
- Oslo	120 (100 – 140)	257 (195 – 325)	280 (180 – 480)	168 (140 – 210)
Testosterone concentrations, nmol/L				
- Amsterdam	18.5 (14.0 – 23.0)	0.8 (0.6 – 0.9)	1.3 (1.0 – 1.7)	27.8 (19.5 – 39.0)
- Ghent	17.9 (13.5 – 21.5)	0.7 (0.5 – 1.1)	1.0 (0.7 – 1.3)	16.3 (11.2 – 21.1)
- Oslo	18.1 (12.2 – 20.4)	0.4 (0.4 – 0.6)	1.0 (0.8 – 1.3)	21.4 (16.7 – 28.5)

Data are presented as median with inter quartile range, mean with standard deviation, percentages, or absolute numbers. * Only in people who used the same hormone administration route for >75% of the follow-up time. Abbreviations: n=number of people, BMI=body mass index, kg/m^2=kilogram per square meter, i.m.=intramuscular.

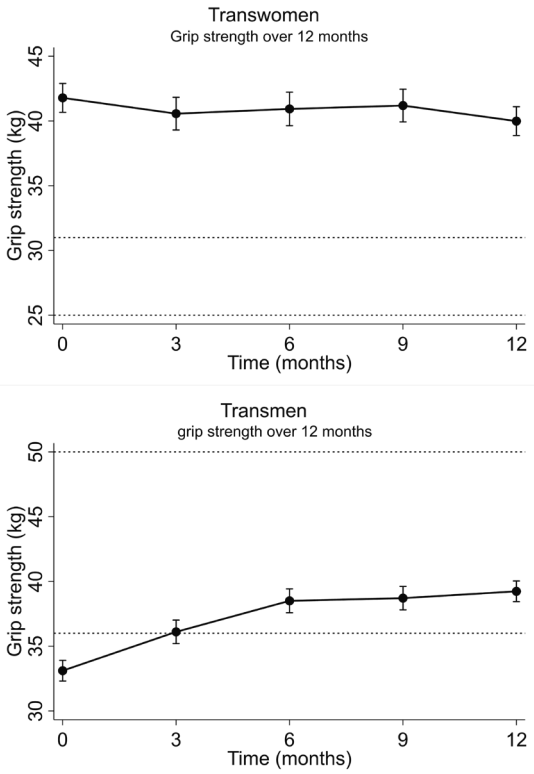


Figure 2.
Change in grip strength during the first 12 months of gender-affirming hormonal treatment in transwomen and transmen. Data are presented as means with 95% confidence interval. The 25th and 75th percentiles of the reference populations are shown with dashed lines. For transwomen the 25th percentile is 25 kg and the 75th percentile is 31 kg. For transmen the 25th percentile is 36 kg and the 75th percentile is 50 kg.[18] Abbreviations: kg= kilograms.

Grip strength

In transwomen, grip strength decreased with -1.8 kg (95% CI -2.6 ; -1.0), while in transmen, grip strength increased with +6.1 kg (95% CI +5.5 ; +6.7). The course of the grip strength change over time is shown in Figure 2. For transwomen, 66% of the decrease in grip strength (-1.2 kg) occurred in the last three months, while in transmen 49% of the increase (+3.0 kg) occurred in the first three months. Change in grip strength did not vary between different age groups (Figure 3a), different BMI groups (Figure 3b), and different administration routes of HT (Figure 3c), for both transwomen and transmen. Change in grip strength did not vary between different hormone concentrations, either for transwomen of transmen (Figure 3d). No analyses on testosterone concentrations in transwomen could be performed, as testosterone was suppressed (<2 nmol/L, <58 ng/dL) in the majority of the transwomen (94%).

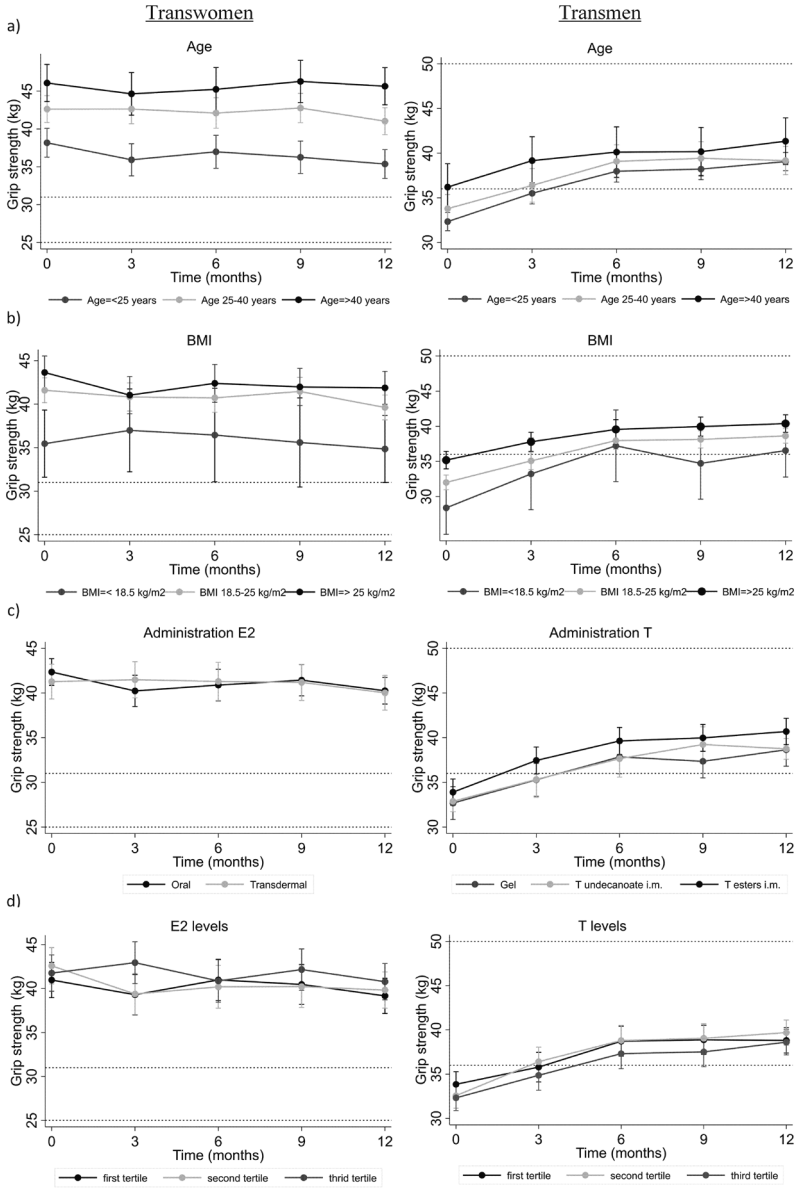


Figure 3.

Differences in change in grip strength for age groups, BMI groups, routes of hormonal administration, estradiol concentrations, and testosterone concentrations. Data are presented as means with 95% confidence intervals. Reference values (25th and 75th percentile) are based on the mean age of this population. For transwomen the 25th percentile is 25 kg and the 75th percentile is 31 kg. For transmen the 25th percentile is 36 kg and the 75th percentile is 50 kg.[18] a) Grip strength change over 12 months between age, adjusted for administration route. b) Grip strength change over 12 months between BMI groups. c) Grip strength change over 12 months between different administration routes, adjusted for age. d) Grip strength change over 12 months between different hormone concentrations. Abbreviations: BMI=body mass index, T=testosterone, E2=estradiol, kg/m^2= kilogram per square meter, i.m.=intramuscular.

Grip strength in relation to lean body mass

As reported previously in this study population [4], a decrease in lean body mass was observed in transwomen and an increase in lean body mass was observed in transmen during the first year of HT.

Associations of change in grip strength with change in lean body mass are presented in Table 2. In transwomen, no associations between change in grip strength and change in arm or leg lean body mass was seen. In transmen, increase in grip strength was associated with an increase in arm lean body mass (per kg increase in grip strength: +0.010 kg, 95% CI +0.003 ; +0.017), but not with change in leg lean body mass.

Table 2. Associations between change in grip strength (per kg increase) and lean body mass, bone mineral density, and creatinine, separately for transwomen and transmen

	Transwomen		Transmen	
	Mean change (95%CI)	p-value	Mean change (95%CI)	p-value
Lean body mass				
- Arm	+0.004 kg (-0.000; +0.009)	0.079	+0.010 kg (+0.003; +0.017)	0.003
- Leg	+0.009 kg (-0.003; +0.021)	0.161	+0.014 kg (-0.002; +0.030)	0.078
Bone mineral density				
- Lumbar spine	+0.02 % (-0.05; +0.10)	0.556	+0.01 % (-0.08; +0.09)	0.900
- Total hip	+0.05 % (-0.01; +0.10)	0.112	-0.02 % (-0.09; +0.05)	0.630
- Femoral neck	+0.05 % (-0.03; +0.12)	0.201	+0.04 % (-0.06; +0.15)	0.401
Creatinine	-0.1 µmol/L (-0.2; +0.1) *	0.535	+0.2 µmol/L (+0.0; +0.4)	0.035

* data shown is per kg decrease in grip strength

Grip strength in relation to bone mineral density

As reported previously in this study population [6], increases in LS BMD, TH BMD, and FN BMD were observed in transwomen, and increases in LS BMD and TH BMD, but not FN BMD, were observed in transmen during the first year of HT.

The associations of change in grip strength with change in bone mineral density are shown in Table 2. Change in grip strength was not associated with change in bone mineral density in both transwomen and transmen.

Grip strength in relation to creatinine

Serum creatinine concentrations decreased with -5.0 µmol/L (95%CI -6.2 ; -3.8) in transwomen and increased with +11.1 µmol/L (95%CI +10.1 ; +12.2) in transmen. The associations of change in grip strength with change in creatinine are described in Table 2. In transmen, the increase in creatinine was associated with an increase in grip strength (per kg increase in grip strength: +0.2 µmol/L, 95%CI +0.0 ; +0.4). In transwomen, a decrease in creatinine tended to be associated with a decrease in grip strength (per kg decrease in grip strength: -0.1 µmol/L, 95%CI -0.2 ; +0.1).

Discussion

In transwomen, a decrease in grip strength after one year of HT was found, while in transmen, an increase in grip strength after one year of HT was found. These changes were also found in the serum creatinine concentrations. Grip strength change did not vary between age groups, BMI groups, administration routes, and different hormone concentrations, for neither transwomen and transmen. Change in grip strength was associated with change in lean body mass in transmen but not in transwomen. Change in grip strength was not associated with a change in BMD.

In our study, transwomen decreased in grip strength, possibly due to the lack of testosterone. This is in agreement with a study in young men using gonadotropin-releasing hormone agonists, which found that muscle mass decreased by approximately one kilogram after 10 weeks.[14] The finding of an increase in grip strength in transmen is consistent with results from studies about testosterone replacement in hypogonadal men.[15] Testosterone has an effect on myoblast proliferation and myoblast differentiation, and testosterone increases the number of satellite cells, which promotes protein synthesis of muscle mass[16]. Thus, testosterone plays an important role in muscle mass and muscle strength. Accordingly, testosterone is not only important for muscle in men, but also for muscle in women. This is among others important for the prevention of dynapenia in older people.

The largest decrease in grip strength for transwomen took place in the last three months of HT, while the largest change for transmen took place in the first three months. The change in transmen is in line with a randomized controlled trial, that shows an increase in strength in the first 6 months, after the use of testosterone in healthy men ≥60 years old.[17] Interestingly, the time difference of the slow loss of grip strength in transwomen and the fast increase in transmen is opposite of what is observed for strength with training and detraining. It might possibly take longer for muscles to decrease in proteins due to lack of testosterone, than it is to increase due to administration of testosterone. However, to fully understand the difference, more research is necessary. After 12 months, the median grip strength of transwomen still falls into the 95th percentile for age-matched females. The median grip strength of transmen after 12 months falls into the 25th percentile of age-matched males.[18] Thus, transwomen are still stronger than average females and transmen are still weaker than average males. However, as this study is a follow-up of 12 months, transwomen and transmen might attain a grip strength value closer to the reference values of, respectively, females and males after a longer duration of HT.

No difference was found for grip strength change between age groups, BMI groups, and administration routes. A larger decrease in transwomen and a smaller increase in transmen was expected at age ≥40 years, because of the age related decrease of grip strength in combination with the lack of testosterone. However, our study

population of ≥40 years was not very old (mean age 49 years for transwomen and 47 years for transmen). Furthermore, our study suggests that change in grip strength was not related to mean serum hormone concentrations during the first year of HT. This finding is line with a study on endocrine determinants in sarcopenia in 518 men aged 40-79 years, that found no association between total and bioavailable testosterone and the annual change in grip strength in men, after a follow-up of 4.3 years.[19] The finding that concentrations of testosterone and estradiol were not associated with the change in grip strength can be used to inform trans people that a higher dosage does not lead to a larger increase or decrease in grip strength. Also, because no relationship was found between change in grip strength and administration routes, trans people can make a more well informed decision when choosing between administration routes.

In transwomen, no association was found between change in lean body mass and change in grip strength, while it was only weakly associated in transmen. This is in contrast to a large cross-sectional and longitudinal perspective study of 847 participants aged 20-100 years, which found a strong correlation between grip strength and lean body mass.[20] However, this correlation was age dependent, and it was the strongest at 60 years old. People younger than 60 years appeared to be stronger than predicted with muscle mass and people older than 60 years appeared to be weaker. They also showed a stronger correlation between age and grip strength compared to age and muscle mass. Our population has a mean age of 25 years, this is significantly younger than 60 years, thus the correlation might be lower. Furthermore, change in grip strength was not associated to change in BMD. Therefore, change in BMD cannot be predicted by change in grip strength.

This is a large, multicenter, prospective study. Standardized measurements were used to measure grip strength, and there is a widespread age range. To our knowledge, this is the first study that describes the change in grip strength over the course of a year, and that examines possible influences on this change. However, our study also had some limitations. First, data about physical activity was only available in smaller subgroup of the study population and was therefore not analyzed. Therefore we could miss data that can partially explain the change in grip strength. However, in previous studies no change in physical activity was found in transwomen [8] and transmen [9] during the first year of HT. Second, possibly a true impact of testosterone concentrations on grip strength cannot be detected, since circulating testosterone concentrations were evaluated, instead of available testosterone in muscle cells.[21] In addition, the laboratory measurements were performed differently at the study centers and changed in two sites during follow-up. Although conversion formulas were generated and tertiles were used instead of absolute values, it might be that the variability in hormone concentrations affected the results. Last, it is questionable whether a decrease in grip strength in transwomen of almost 2 kg is clinically relevant. Nonetheless, transwomen possibly feel more feminine due to the decrease in muscle mass.[22,23] One study reported on the desired effects of HT

in transwomen in Indonesia, including reduced muscle mass.[23] A study in associations among masculinity, strength, and attractiveness shows a positive correlation between masculinity and grip strength, and a negative correlation between masculinity and attractiveness in young women.[24] This might indicate that a decrease in grip strength in women could influence the feeling of attractiveness. However, this may be dissimilar in transwomen. A questionnaire for transwomen and transmen would be of interest to investigate a possible satisfaction with the change in grip strength.

In conclusion, grip strength and lean body mass decreases in transwomen and increases in transmen, after 12 months of HT. This is interesting for the prevention of sarcopenia and dynapenia, since testosterone has a positive effect on muscle mass and grip strength. In addition, knowing the effects of HT on grip strength, its association with hormone concentrations and hormonal administration routes, and its relation with change in muscle mass and BMD, can help care providers and trans people with their expectations of HT. For further research, it would be interesting to evaluate the subjective satisfaction related to changes in grip strength, and to evaluate the change of grip strength after a longer follow-up to see if the grip strength will reach the grip strength reference values for women or men, for transwomen and transmen, respectively.

Supplementary Table 1. Differences in characteristics between included and excluded transwomen and transmen

	Transwomen			Transmen		
	Included (n=249)	Excluded (n=247)	p	Included (n=278)	Excluded (n=243)	p
Age, yr	28.5 (23.0 – 40.2)	28.0 (22.4 – 44.0)	0.977	23.0 (20.3 – 30.2)	22.2 (20.1 – 26.6)	0.084
BMI, kg/m^2	23.8 (4.4)	23.5 (4.4)	0.478	25.3 (5.5)	25.3 (5.8)	0.945
Smoking, %yes	24.7	27.4	0.513	29.9	35.0	0.274
Alcohol, %yes	44.8	50.0	0.246	55.3	55.1	0.967
Baseline grip strength, kg	41.8 (8.9)	43.2 (9.1) (n=218)	0.084	33.1 (6.5)	34.0 (6.6) (n=215)	0.144
Estradiol, pmol/L						
- Amsterdam	105 (83 – 131)	96 (73 – 155)	0.907	142 (59 – 371)	397 (206 – 697)	0.053
- Ghent	109 (88 – 130)	93 (88 – 116)	0.468	166 (116 – 431)	128 (92 – 437)	0.721
- Oslo	120 (100 – 140)	100 (90 – 130)	0.307	280 (180 – 480)	275 (150 – 510)	0.810
Testosterone, nmol/L						
- Amsterdam	18.5 (15.0 – 23.0)	17.5 (15.0 – 22.5)	0.928	1.3 (1.0 – 1.7)	1.3 (0.5 – 1.7)	0.876
- Ghent	17.9 (13.5 – 21.5)	19.3 (13.2 – 23.6)	0.822	1.0 (0.7 – 1.3)	0.9 (0.7 – 1.3)	0.892
- Oslo	18.1 (12.2 – 20.4)	15.4 (11.6 – 20.1)	0.535	1.0 (0.8 – 1.3)	1.3 (0.8 – 1.6)	0.107
Creatinine (µmol/L)	78.5 (10.8)	77.0 (10.8)	0.266	66.0 (9.0)	65.0 (10.0)	0.363

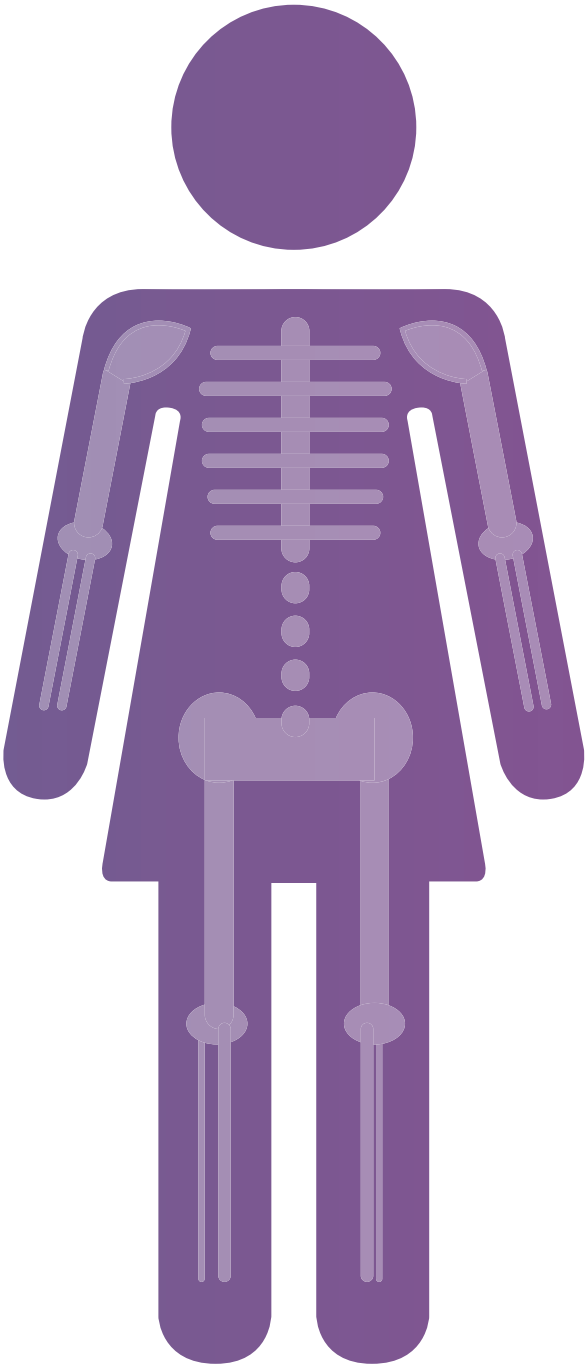
Data are presented as median with inter quartile range, mean with standard deviation, or percentages.

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Chapter 7

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

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Abstract

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

Introduction

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

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

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

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

Materials and Methods

Design and population

This study is part of the retrospective Amsterdam Cohort of Gender Dysphoria (ACOG) study, including all 6,793 people who once presented themselves at the gender identity clinic of the VU University medical center (VUmc), Amsterdam, the Netherlands, between 1972 and 2016. Study design is described previously.[27] Briefly, data was collected by reviewing medical records. Age, medical history, medication use, type and dose of HT, prior HT use, gender-affirming surgery, smoking habits (in cigarettes per day), body weight (in kilogram), body height (in centimeter), laboratory test results, and dual-energy X-ray absorptiometry (DXA) results were retrieved. HT consisted of oral or transdermal estrogens and, usually until gonadectomy, anti-androgens in transwomen. Transmen were treated with oral, transdermal, or intramuscular testosterone. After at least one to 1.5 years of HT, surgery could be performed. For the current study, only people of 18 years and older with a baseline DXA scan (range one year before to four months after start with HT) who started with HT after 1998 were included, as BMD measurements were available since then. People who had undergone gonadectomy (orchiectomy in transwomen or oophorectomy in transmen) or used gender-affirming hormones prior to HT were excluded. We calculated that with 711 included transwomen and 543 included transmen (Figure 1), a mean difference in lumbar spine BMD of 0.021 g/cm^2 and 0.022 g/cm^2 could be detected, respectively, with a power of 80% and an alpha of 0.05. Our local ethics committee reviewed the study and determined that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study, and necessity for informed consent was waived due to the retrospective design.

Bone mineral density

BMD measurements were performed during regular patient care before the start of HT and were usually repeated every two to five years during HT. BMD of the lumbar spine (LS), total hip (TH), and femoral neck (FN) were measured using a DXA Hologic Delphi, which was updated in July 2004 and replaced by a Hologic Discovery A in February 2011. Phantom calibration allowed for comparison of BMD values with a difference of <1.0% between the densitometers. Coefficients of variation were <1.0% for both densitometers and least significant change was 0.022 g/cm^2. Absolute BMD values (g/cm^2) were obtained and were converted into T-scores (standard deviation (SD) difference between the person's BMD and the BMD of a young-adult reference population of the same birth-sex and ethnicity) and Z-scores (SD difference between the person's BMD and an age-matched BMD of the same birth-sex and ethnicity), based on the National Health and Nutrition Examination Survey (NHANES). In addition, T-scores and Z-scores on baseline were also calculated using the identified sex as reference population. Osteoporosis was defined as a T-score below -2.5, and a Z-score below -2.0 was classified as low bone density for age.[28]

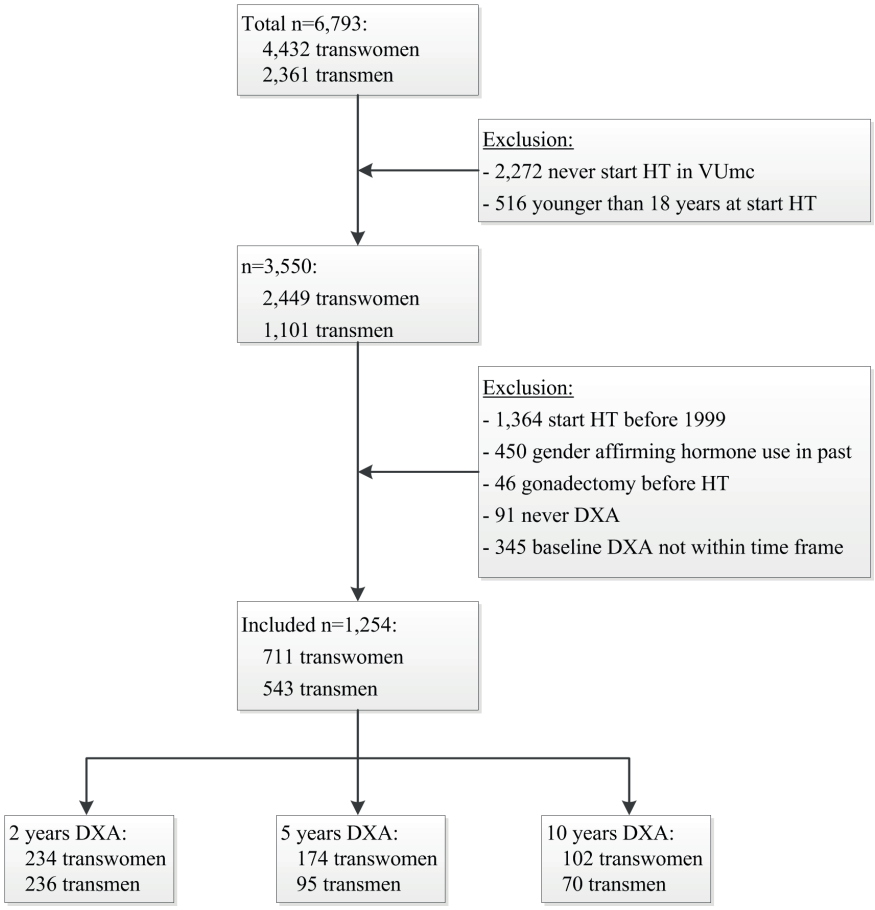


Figure 1.
Flowchart of inclusion of study population. DXA not within time frame: more than one year before start of gender-affirming hormonal treatment or more than 4 months after start of HT. The reported percentages are the percentages of transwomen or transmen with a DXA scan at that time point compared with the total included transwomen or transmen, respectively. Abbreviations: n=number, HT=gender-affirming hormonal treatment, VUmc=VU University medical center, DXA=dual-energy X-ray absorptiometry

Biochemical assessments

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

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

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

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

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

Statistical analyses

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

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

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

Results

Study participants

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

Lumbar spine

Transwomen on estradiol based treatment

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

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

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

Table 1. Baseline characteristics of the study population

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

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

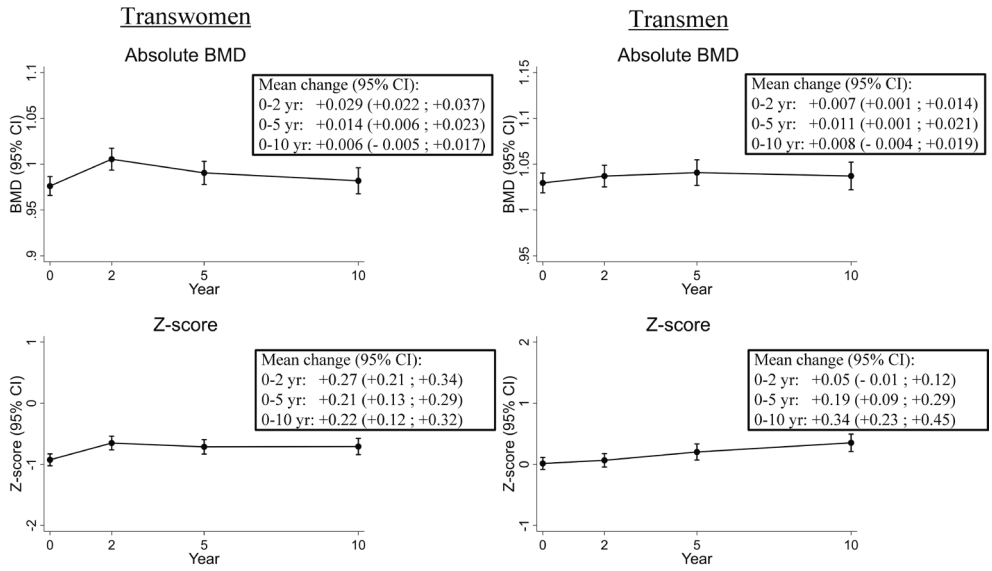


Figure 2.
Change in absolute BMD and Z-score in transwomen and transmen during the first ten years of gender-affirming hormonal treatment. Abbreviations: BMD = bone mineral density, 95% CI = 95% confidence interval, yr = year

Transmen on testosterone based treatment

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

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

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

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

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

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

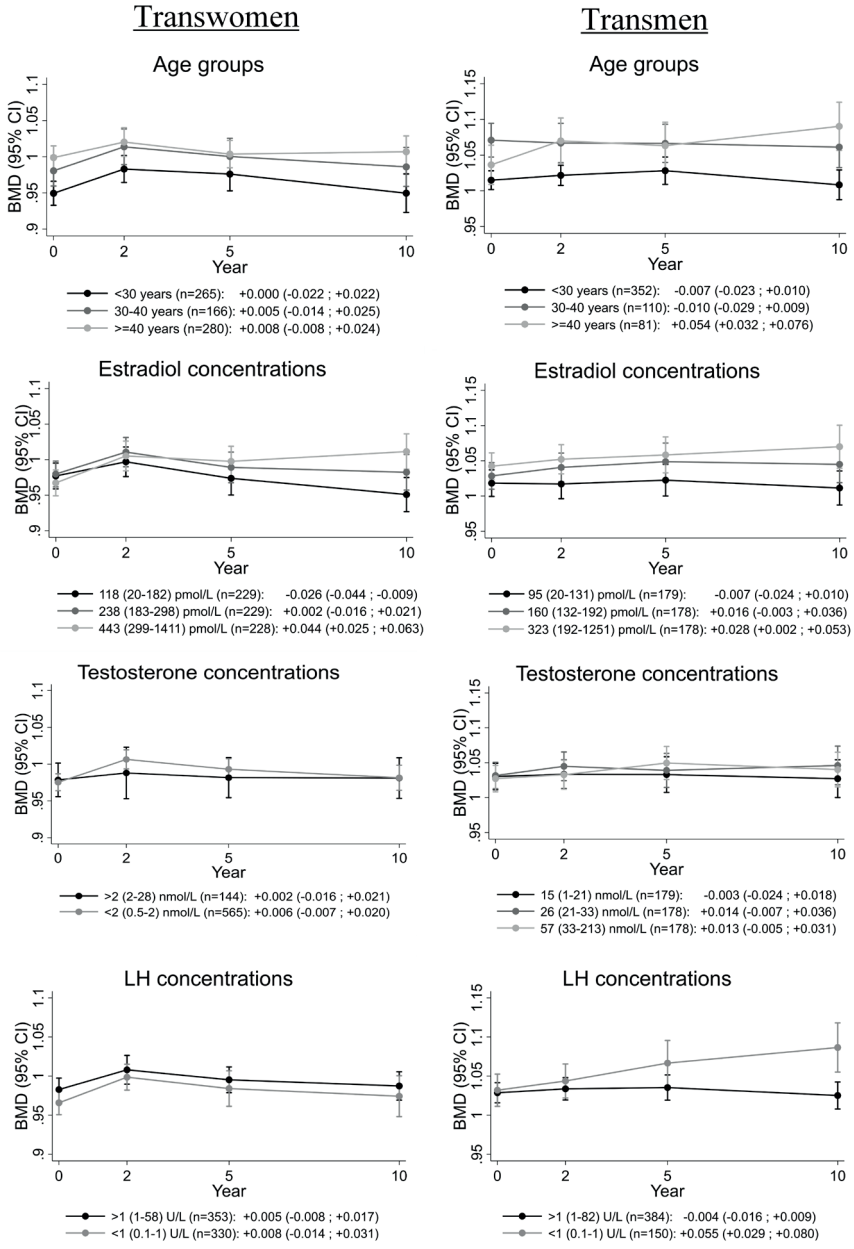


Figure 3.

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

Total hip and femoral neck

Only a small number of BMD measurements of TH and FN were performed after five years (n=24) and ten years (n=4) of HT. In the overall analyses, no changes in TH BMD (-0.034 g/cm^2, 95%CI -0.070 ; +0.002 g/cm^2) and FN BMD (+0.057 g/cm^2, 95%CI -0.036 ; +0.047 g/cm^2) were found in transwomen. Also in transmen, no changes in TH BMD (-0.024 g/cm^2, 95%CI -0.075 ; +0.026 g/cm^2) and FN BMD (-0.033 g/cm^2, 95%CI -0.092 ; +0.026 g/cm^2) were found. No subgroup analyses could be performed due to the limited number of people in each of the subgroups.

Discussion

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

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

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

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

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

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

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

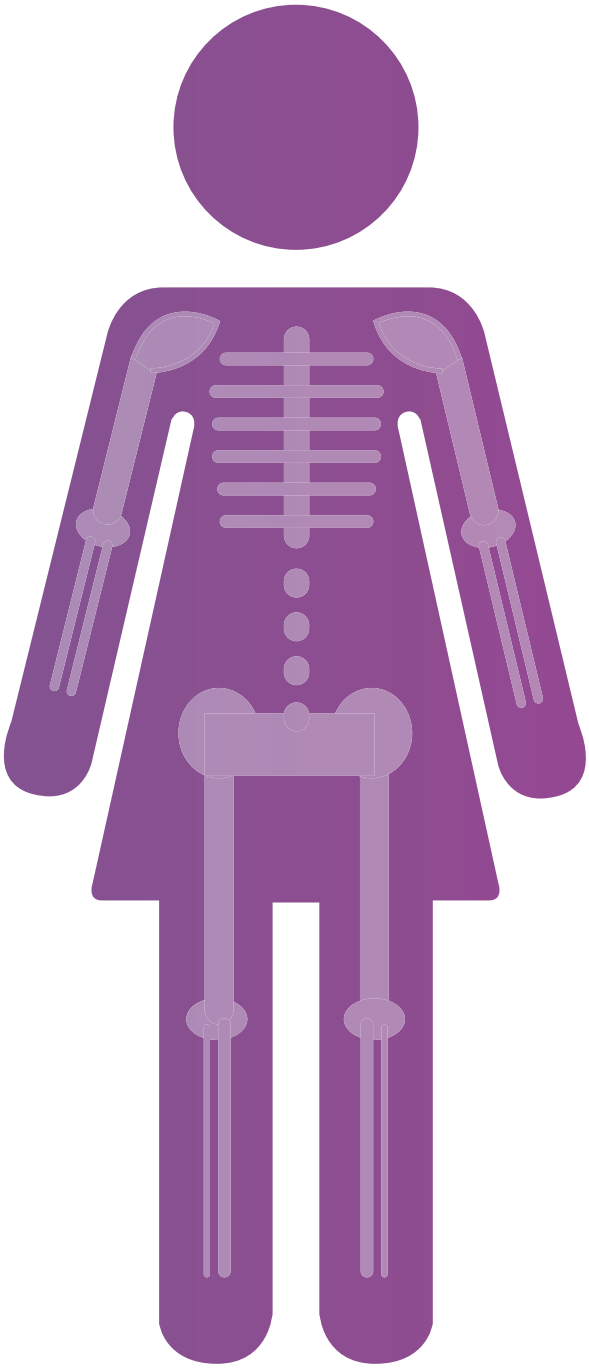
In clinical practice, concerns about the safety of HT on bone health are present, probably because earlier studies found low BMD after long-term HT in transwomen compared with control men, and because of fear that HT decreases estradiol concentrations in transmen, which could have negative effects on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

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Chapter 8

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

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Abstract

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

Introduction

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

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

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

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

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

Methods

Study design and population

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

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

Bone mineral density

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

Hip structure analysis

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

Trabecular bone score

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

Statistical analyses

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

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

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

Results

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

Table 1. Characteristics of trans women

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

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

Table 2. Characteristics of trans men

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

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

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

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

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

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

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

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

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

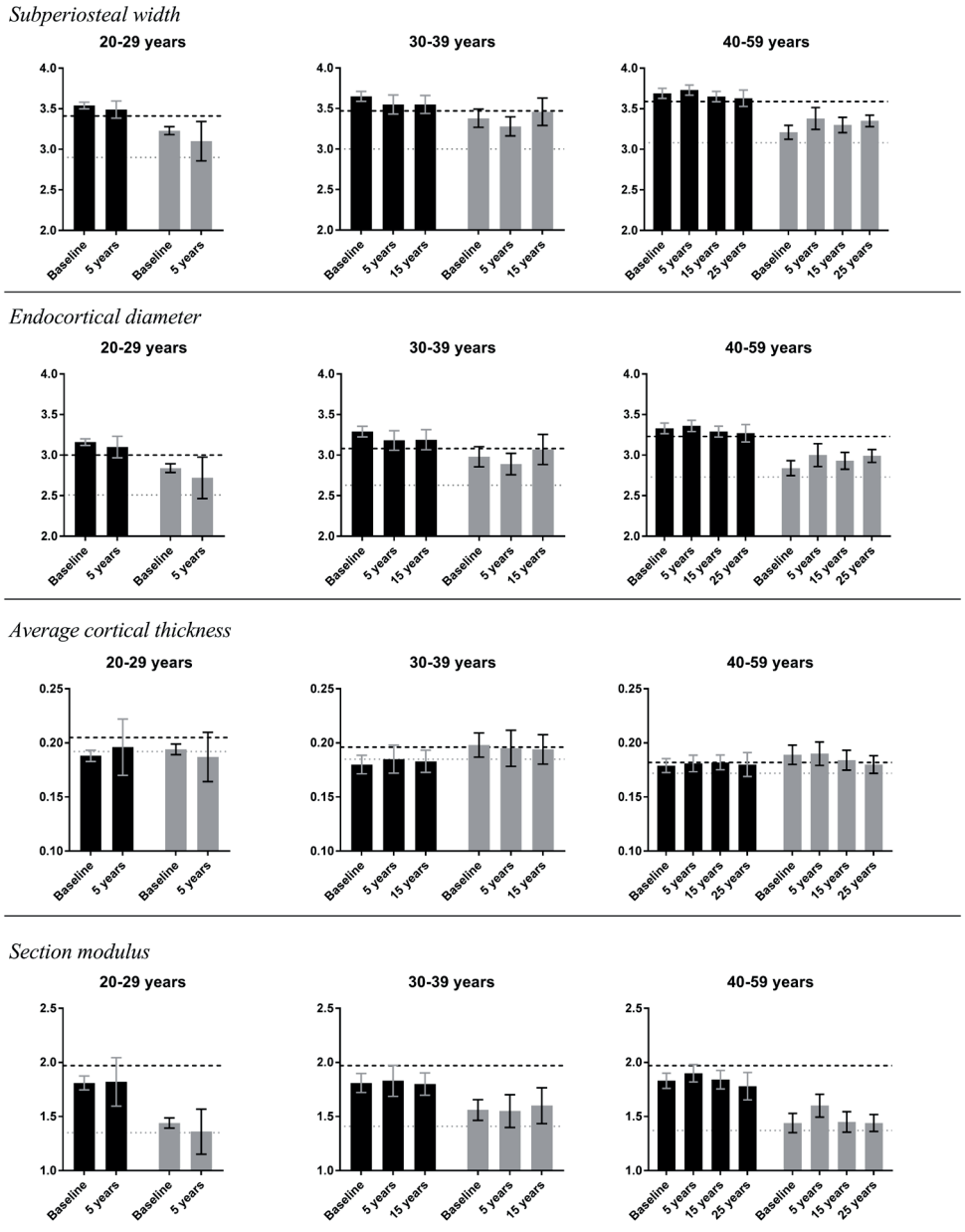


Figure 1.
Hip structure analyses of the narrow neck. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the y-axis the periosteal width, endocortical diameter, average cortical thickness, and section modulus in centimeters is shown for the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

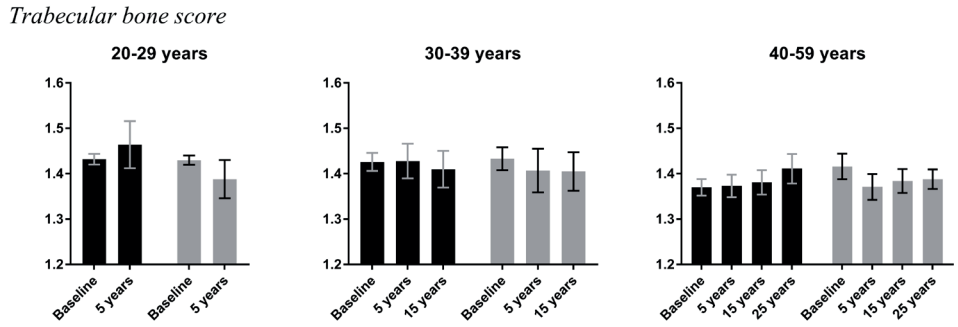


Figure 2.
Trabecular bone score. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the *y*-axis the trabecular bone score is shown. On the x-axis, the different durations of HT are shown.

Discussion

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

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

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

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

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

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

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

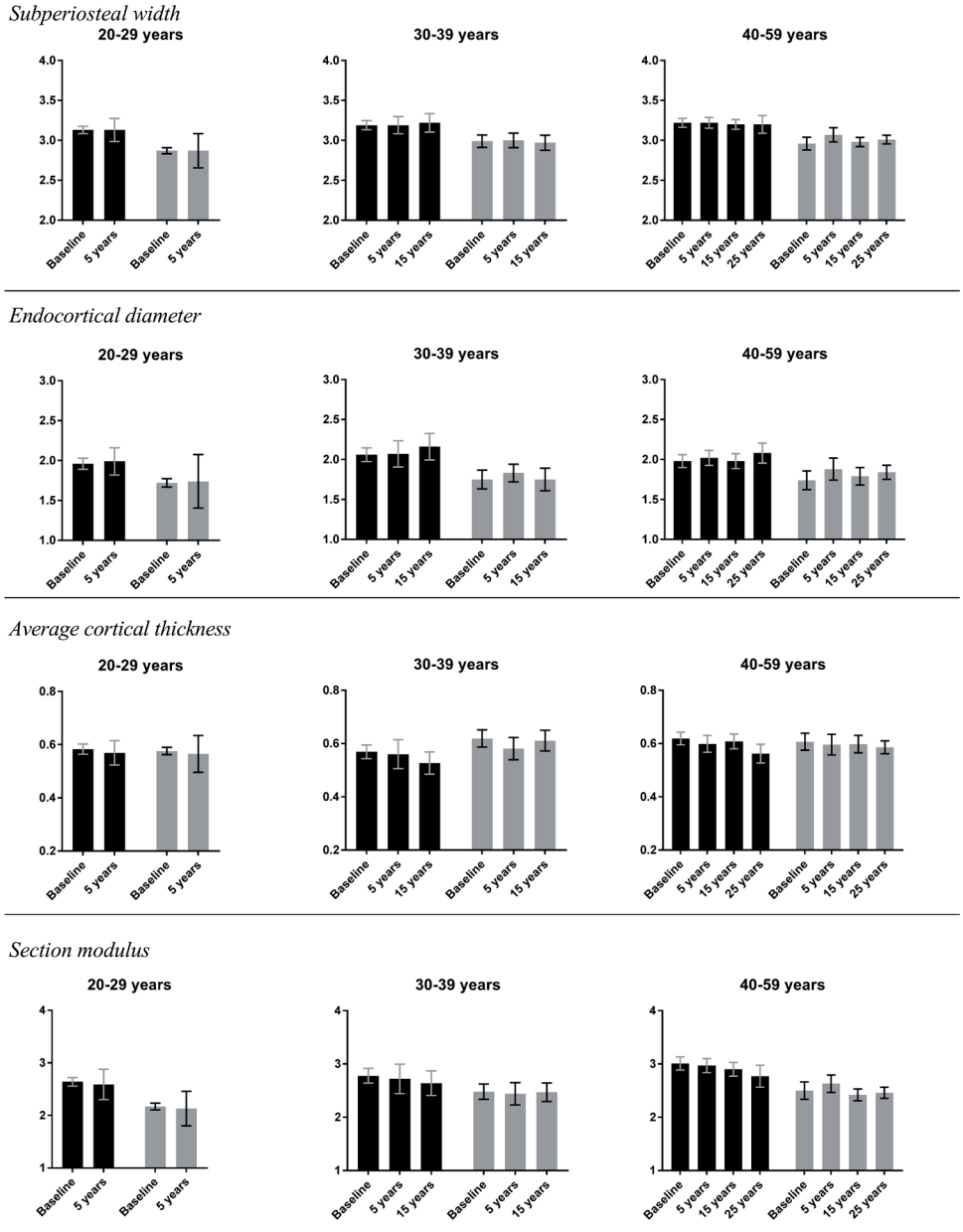
In clinical practice, DXA is the most commonly used technique to evaluate bone health. However, DXA is a 2-dimensional scan using two X-ray beams with different energy levels, to measure BMD (in g/cm^2) calculated as bone mineral content (in grams) divided by the bone area (in cm^2). In order to measure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

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

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

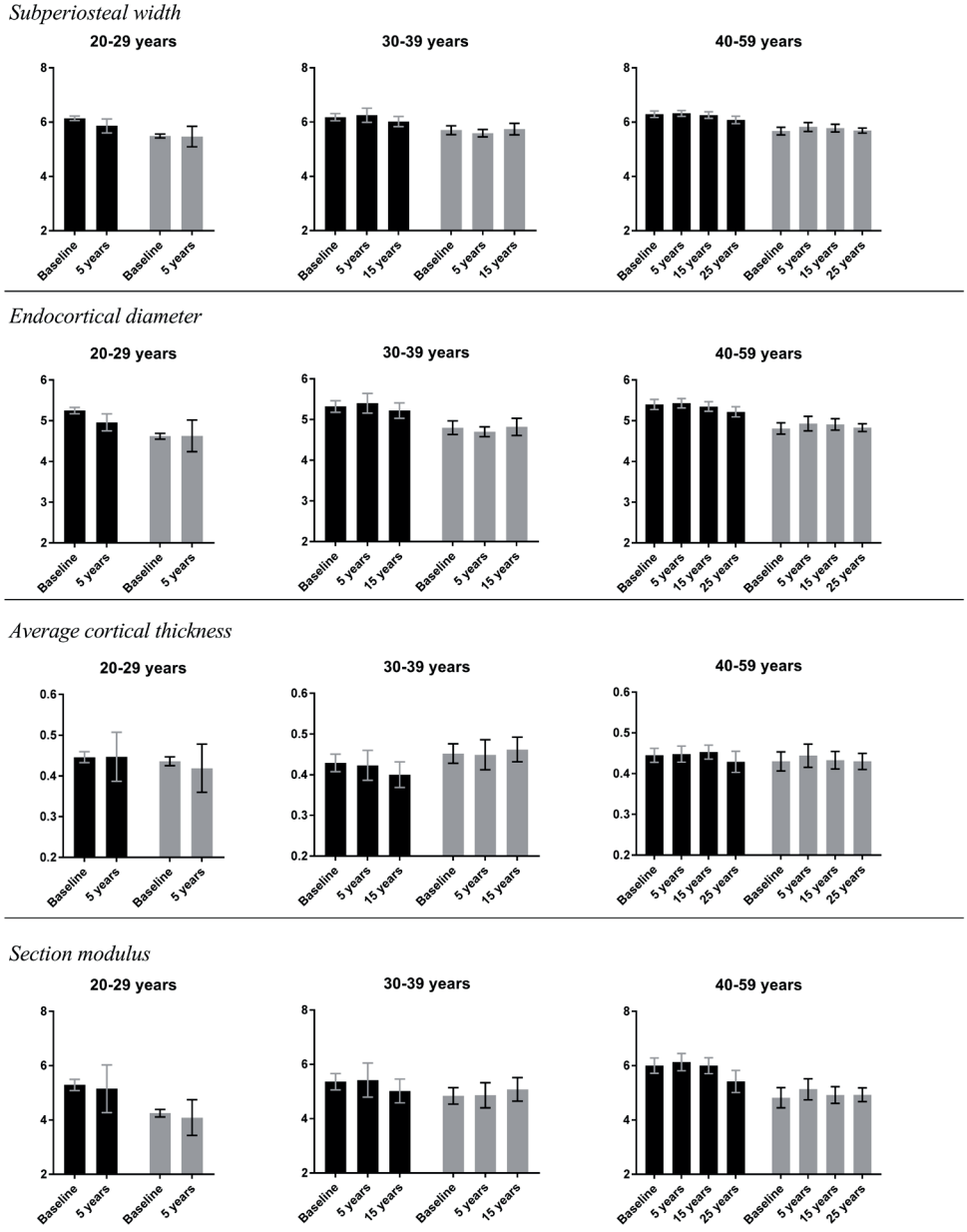
Conclusion

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



Supplementary Figure 1.

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



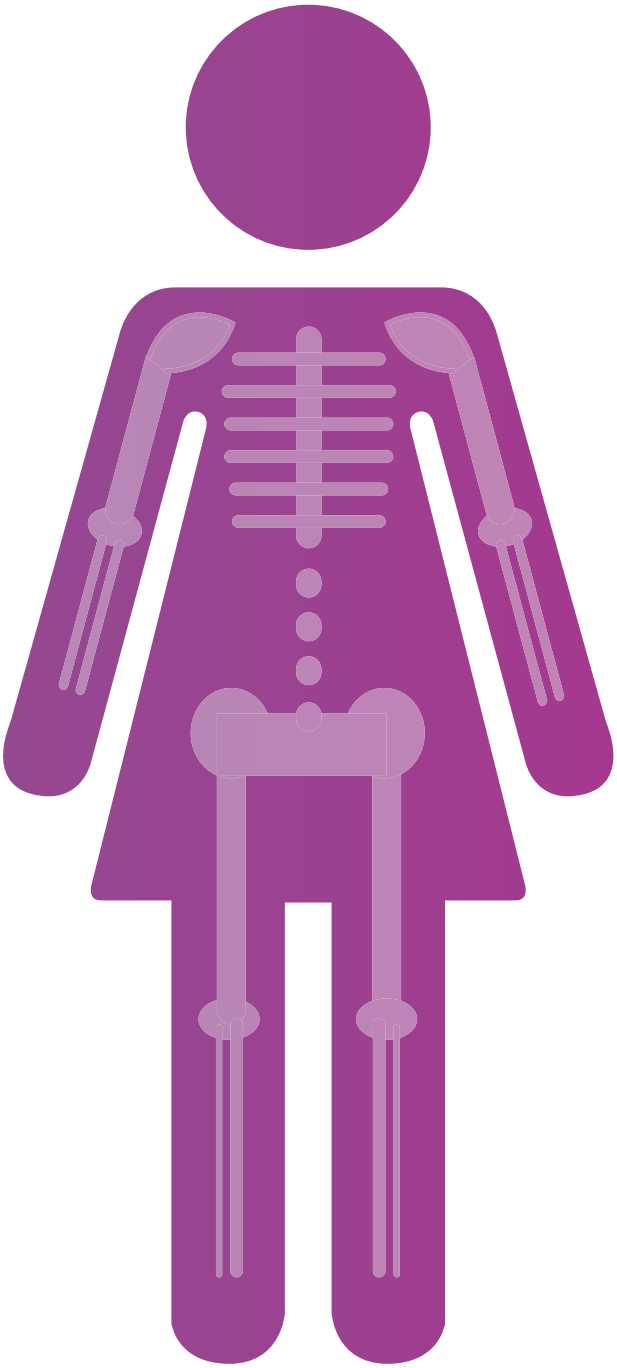
Supplementary Figure 2.
Bone geometry parameters of the intertrochanteric region. The black bars represent the mean values in trans women, the grey bars represent the mean values in trans men. On the *y*-axis the periosteal width, endocortical diameter, average cortical thickness, and section modulus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

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Chapter 9

Fracture risk in trans women and trans men using long-term gender-affirming hormonal treatment: a nationwide cohort study

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J Bone Miner Res. 2020;**35**(1):64-70

Abstract

Concerns about bone health in transgender people using gender-affirming hormonal treatment (HT) exist, but the fracture risk is not known. In this nationwide cohort study, we aimed to compare the fracture incidence in transgender people using long-term HT with a control population. All adult transgender people who started HT before 2016 at our gender identity clinic were included and were linked to a random population-based sample of 5 age-matched control men and 5 age-matched control women per person. Fracture incidence was determined using diagnoses from visits to hospital emergency rooms nationwide between 2013-2015. A total of 1,089 trans women <50 years (mean 38±9) and 934 trans women ≥50 years (mean 60±8) using HT for median 8 (IQR 3-16) and 19 (IQR 11-29) years, respectively, were included. 2.4% of the trans women <50 years had a fracture, while 3.0% of the control men (OR 0.78, 95%CI 0.51; 1.19) and 1.6% of the control women (OR 1.49, 95%CI 0.96; 2.32) experienced a fracture. In trans women ≥50 years, 4.4% experienced a fracture, compared with 2.4% of the control men (OR 1.90, 95%CI 1.32; 2.74) and 4.2% of the control women (OR 1.05, 95%CI 0.75; 1.49). A total of 1,036 trans men (40±14 years) using HT for median 9 (IQR 2-22) years were included. Fractures occurred in 1.7% of the trans men, 3.0% of the control men (OR 0.57, 95%CI 0.35; 0.94), and 2.2% of the control women (OR 0.79, 95%CI 0.48; 1.30). In conclusion, fracture risk was higher in older trans women compared with control men. In young trans women, fracture risk tended to be increased compared with control women. Fracture risk was not increased in trans men.

Introduction

Transgender people can receive gender-affirming hormonal treatment (HT) to change the physical characteristics belonging to their experienced gender. Trans women (birth-assigned males, female identity) receive estrogens to induce feminization, resulting for example into breast growth [1] and changes in body composition.[2] Trans men (birth-assigned females, male identity) are treated with testosterone, which among others stimulates lowering of the voice [3] and growth of body hair.[4] Besides changes in physical characteristics, HT also influences bone mineral density (BMD).

Earlier studies found either a maintenance [5-9] or increase [5-7,10-15] in BMD in both adult trans women and trans men after short-term HT. Long-term effects of HT have been investigated in a few small-sample cross-sectional studies in which trans women were compared with control men, and trans men with control women. Contradictory results were obtained from these studies: higher [16,17], similar [17,18], and lower [19] BMD than controls were found. One follow-up study found no change in BMD in trans women and trans men during the first ten years of HT.[20] However, before the start of HT, trans women were found to have relatively low BMD, possibly due to co-existing vitamin D deficiency and a different life style, leading to decreased muscle mass and therefore decreased mechanical loading on bone.[21]

In clinical practice there are concerns about bone health in transgender people, particularly regarding the low initial BMD in trans women and the lack of fracture data. A few studies described no increased fracture risk before the start of HT in trans women [13,21] and trans men.[9] In short-term follow-up studies, no fractures were observed in trans women [13], trans men [9], or their controls. In studies after long-term HT, no increased fracture risk was found in both trans women [19] and trans men.[22] However, all these studies had a small sample size (n<50) or were using questionnaires to define fractures.

Therefore, the aim of this study is to investigate the fracture incidence in a large cohort of adult trans women and trans men after long-term HT, based on diagnoses from visits to the hospital emergency rooms nationwide, and to compare this incidence with an age-matched male and female control population. In addition, we aimed to study whether the types of fractures differed between the transgender population and their control groups, and whether BMD or other characteristics were different in the transgender population with fractures compared to those without fractures.

Materials and Methods

Study design and population

This study is part of the Amsterdam Cohort of Gender Dysphoria study [23], including all 6,793 people who once visited the gender identity clinic of the Amsterdam University Medical Center, the Netherlands, between 1972 and 2016. Study design and population have been described previously.[23] In short, the medical files of these people were reviewed and clinical data were retrieved. For the current study, only people who started with HT and were not deceased at time of data collection were included. The cohort was linked to a random control sample of 5 age-matched males and 5 age-matched females per transgender person, provided by the Statistics Netherlands (Central Bureau of Statistics) based on the National Civil Record Registry. The study was approved by the Medical Ethics Committee of the Amsterdam University Medical Centers, location VUmc, and necessity for informed consent was waived due to the retrospective design and the absence of interventions.

Treatment

After the diagnostic process, people could start with HT when the diagnosis gender dysphoria was confirmed.[24] In trans women, HT consists of anti-androgens, which were usually continued until orchiectomy, in combination with estrogens. In trans men, HT consisted of testosterone only. After at least one year of HT and after the age of 18 years, surgery could be performed, including vaginoplasty with orchiectomy in trans women and hysterectomy with oophorectomy in trans men.

Fracture data

The total population of transgender people and their 10 age-matched controls were linked to a database from Statistics Netherlands, which stores all diagnoses made by medical doctors from visits to the hospital emergency rooms nationwide based on Diagnosis-Treatment-Combination trajectories (DTCs) for specialized medical care. These DTCs are used to calculate the insurance claim, which is sent to the individual's medical insurance company. Health insurance is obligatory for every inhabitant of the Netherlands. The diagnoses were available for the years 2013, 2014, and 2015.

Dual-energy X-ray absorptiometry (DXA)

BMD was regularly measured during patient care using a DXA Hologic Delphi. This densitometer was updated in July 2004 and replaced in February 2011 by a Hologic Discovery A (Hologic Inc., Bedford, MA, USA). For both machines, the coefficient of variation (CV) was <1%. Phantom calibration allowed for comparison of the absolute BMD values. Absolute BMD values (g/cm^2) of the lumbar spine (LS) were obtained and T-scores were calculated, based on the birth-assigned sex reference values of the

National Health and Nutrition Examination Survey (NHANES). If more than one BMD measurement was available, the most recent measurement was used for analysis. BMD measurements were available for 77% of the trans women and 84% of the trans men.

Laboratory measurements

Blood samples were frequently obtained during patient care. When improved quality assays were available, these were implemented and conversion formulas for comparison of the concentrations were generated. Until January 2010, estradiol was measured using a radioimmunoassay (Diasorin, Saluggia, Italy) with an inter-assay CV of 10% and a lower limit of quantitation (LOQ) of 18 pmol/L. Between January 2010 and July 2014, a competitive immunoassay (Delfia, Wallac, Turku, Finland) was used (inter-assay CV 10%, LOQ 20 pmol/L). For conversion, the formula Delfia=1.267*Diasorin–28.87 was used. Since July 2014, LC-MS/MS (VUmc, Amsterdam, the Netherlands) was used (inter-assay CV 7%, LOQ 20 pmol/L) and the formula LC-MS/MS=1.60*Delfia–29 was used for conversion. Testosterone was measured using a radioimmunoassay (RIA; Coat-A-Count, Siemens, USA) until January 2013 (inter-assay CV 7-20%, LOQ 1 nmol/L), hereafter a competitive immunoassay (Architect, Abbott, USA) was used (inter-assay CV 6-10%, LOQ 0.1 nmol/L). Two formulas were used for conversion: Architect=1.1*RIA+0.2 for testosterone concentrations <8 nmol/L; Architect=1.34*RIA–1.65 for testosterone concentrations >8 nmol/L. Luteinizing hormone (LH) was measured using an immunometric assay (Delfia, Wallac, Turku, Finland) until June 2011 (inter-assay CV <7%, LOQ 0.5 U/L). After June 2011, an immunometric assay (Architect, Abbott, USA) was used (inter-assay CV <6%, LOQ 2 U/L), using the formula Architect=0.91*Delfia–0.01 for conversion. Mean estradiol, testosterone, and LH concentrations per person were calculated by averaging the results from the measurements performed during HT. Laboratory measurements were available for 66% of the trans women and 72% of the trans men.

Statistical analysis

Characteristics of the transgender population are presented as mean with standard deviation (SD), median with inter quartile range (IQR), or percentages. First, the fracture incidence was calculated in trans women, trans men, and their age-matched control men and women. Thereafter, logistic regression analyses were performed to calculate odds ratios (OR) with 95% confidence intervals (CI), as an approximation of the relative risk for fractures. As age affects the risk of fractures, the trans women group was divided into two groups (<50 years and ≥50 years), and the analyses were repeated for both age groups separately. Fractures were divided into two groups: hip, spine, forearm, and humerus fractures (which are all an approximation of osteoporotic fractures), and other fractures [25]. Chi-square tests were performed to investigate whether the type of fractures differed

between the groups. To investigate whether age in 2015, age at start of HT, body mass index (BMI), smoking habits, T-score of the LS, estradiol concentrations, testosterone concentrations, and LH concentrations were associated with fracture risk, multivariable logistic regression analyses were performed in the transgender population. To protect the anonymity of the population, data are only shown if more than 10 individuals were present in each group. Subgroup analyses were therefore not performed if less than 20 individuals were present in one group. Analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 14.2.

Results

Study population

Of the 6,793 people who are included in the total cohort, 2,726 people were excluded for this study because they did not start with any treatment (yet), 442 started with treatment during adolescence, 15 received alternating testosterone and estradiol treatment, 319 could not be linked to the Statistics Netherlands database (e.g. because they were not registered in the Netherlands), and 232 people were deceased. This led to a total study population of 3,059 people, consisting of 2,023 trans women and 1,036 trans men. The characteristics are shown in Table 1.

Table 1. Characteristics of the study population.

	Trans women <50 years	Trans women ≥50 years	Trans men
Number of people	1,089	934	1,036
Age in 2015, yr	38 (9)	60 (8)	40 (14)
Age at start HT, yr	26 (22 – 33)	40 (31 – 48)	25 (21 – 33)
Duration HT, yr	8 (3 – 16)	19 (11 – 29)	9 (2 – 22)
BMI, kg/m^2 (n=2,756)	23.9 (4.2)	25.7 (4.6)	25.8 (4.9)
Smoking, % yes (n=2,614)	44.7	49.0	47.8
Gonadectomy, % yes	57.8	80.9	69.8
Laboratory *			
Estradiol, pmol/L	211 (132 – 308)	241 (138 – 391)	147 (102 – 205)
Testosterone, nmol/L	1.2 (0.7 – 1.4)	1.3 (1.0 – 1.3)	25.0 (17.1 – 36.5)
LH, IU/L	2.2 (0.2 – 9.7)	3.2 (0.3 – 8.4)	3.6 (0.9 – 11.5)

Characteristics are shown as mean with standard deviation, median with inter quartile range, or percentage. Associations are shown as odds ratios (OR) with 95% confidence intervals (CI). # Laboratory measurements were available of 66% of the trans women and 72% of the trans men. Abbreviations: yr=years, HT = hormonal treatment, BMI = body mass index, LH = luteinizing hormone

Trans women

Fractures occurred in 3.3% of the trans women (n=67) in the 3-year time period, while 2.7% of the control men (n=275) and 2.8% of the control women (n=283) had a fracture. The overall fracture incidence was not increased in trans women compared with control men (OR 1.23, 95%CI 0.93; 1.61) nor with control women (OR 1.19, 95%CI 0.91; 1.56). 41.8% of all fractures in trans women was a hip, spine, forearm, or humerus fracture, compared with 26.6% in control men (p=0.014) and 36.0% in control women (p=0.381).

After age stratification, trans women ≥50 years (n=934) had an increased fracture risk (4.4%, n=41) compared with control men ≥50 years (2.4%, n=110, OR 1.90, 95%CI 1.32; 2.74), but a similar fracture risk compared with control women ≥50 years (4.2%, n=195, OR 1.05, 95%CI 0.75; 1.49) (Figure 1). Trans women <50 years (n=1,089) did not have an increased fracture risk (2.4%, n=26) compared with control men <50 years (3.0%, n=165, OR 0.78, 95%CI 0.51; 1.19), but tended to have a higher fracture risk compared with control women <50 years (1.6%, n=88, OR 1.49, 95%CI 0.96; 2.32) (Figure 1).

In Table 2, the differences in characteristics between trans women with and without fractures are shown. In the multivariable analysis, age in 2015 (per year: OR 1.05, 95%CI 1.02; 1.08) and T-score of the lumbar spine (per 1.0 point: OR 0.75, 95%CI 0.59; 0.96) were associated with fracture risk. Smoking tended to be associated with a higher fracture risk (yes vs no: OR 1.68, 95%CI 0.93; 3.04), while mean BMI (per point: OR 1.04, 95%CI 0.97; 1.11) and age at start of HT (per year: OR 0.98, 95%CI 0.95; 1.01) were not associated with an increased fracture risk. Laboratory measurements were not included in this multivariable analysis, due to the smaller number of individuals with laboratory measurements available. However, in univariable analyses, no associations were found between estradiol (per 10 pmol/L: OR 0.99, 95%CI 0.97; 1.02), testosterone (per 1 nmol/L: OR 1.03, 95%CI 0.96; 1.10), and LH (per 1 IU/L: OR 1.00, 95%CI 0.97; 1.04) concentrations and fracture risk.

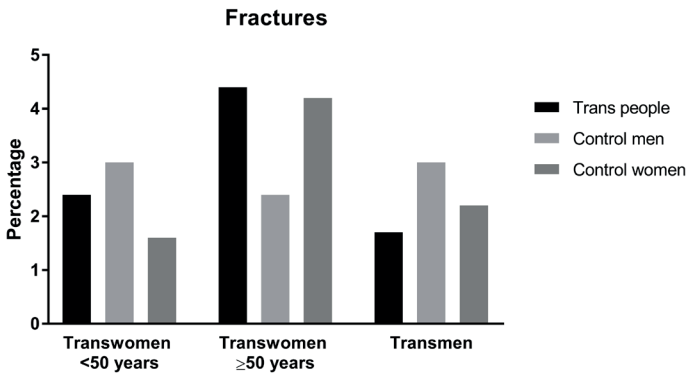


Figure 1.
Fracture incidence during the years 2013, 2014, and 2015 in the transgender population and control groups. Data is shown as percentages separately for trans women <50 years, trans women ≥50 years, trans men, and their age-matched control men and women. Due to the low number of fractures in trans men, no stratification for age could be performed.

Trans men

Eighteen trans men experienced a fracture (1.7%) in the 3-year time period, while 3.0% of the control men (n=155) and 2.2% of the control women (n=114) had a fracture (Figure 1). The fracture risk was similar to control women (OR 0.79, 95%CI 0.48; 1.30), but lower compared with control men (OR 0.57, 95%CI 0.35; 0.94). Table 2 displays the differences in characteristics between trans men with and without fractures. In the multivariable analyses, no associations were found between fracture risk and age in 2015 (per year: OR 1.02, 95%CI 0.97; 1.07), age at start of HT (per year: OR 1.00, 95%CI 0.93; 1.07), mean BMI (per point: 0.92, 95%CI 0.80; 1.06), smoking (yes vs no: OR 1.44, 95%CI 0.48; 4.30), and T-score of the LS (per 1.0 point: 0.99, 95%CI 0.62; 1.59). Due to the smaller number of individuals with laboratory measurements, these were not included in the multivariable analyses. However, in the univariable analyses it was found that estradiol concentrations were associated with fracture risk (per 10 pmol/L: OR 0.89, 95%CI 0.80; 0.99), but testosterone (per 1 nmol/L: 0.98, 95%CI 0.93; 1.02) and LH (per 1 IU/L: OR 1.03, 95%CI 0.98; 1.08) concentrations were not.

Due to the low number of fractures in trans men, no stratification for age or type of fracture could be performed.

Table 2. Differences in characteristics in the transgender population with and without fractures.

	Trans women		Trans men	
	No fracture	**Fracture**	**No fracture**	**Fracture**
Number of people	*1,956*	*67*	*1,018*	*18*
Age in 2015, yr	48 (14)	55 (13)	40 (14)	45 (14)
Age at start HT, yr	31 (24 – 41)	33 (26 – 45)	25 (21 – 33)	25 (21 – 34)
BMI, kg/m^2 (n=1,785)	24.6 (4.4)	25.8 (5.1)	25.8 (4.9)	24.4 (3.5)
Smoking, % (n=1,714)	46	63	48	56
T-score lumbar spine	-1.02 (1.28)	-1.34 (1.40)		
Laboratory #				
Estradiol, pmol/L	220 (135 – 337)	172 (116 – 299)	148 (103 – 206)	84 (65 – 133)
Testosterone, nmol/L	1.3 (0.8 – 1.3)	1.3 (0.9 – 2.2)	25 (17 – 37)	22 (17 – 29)
LH, IU/L	2.5 (0.3 – 9.3)	2.8 (0.7 – 9.6)	3.6 (0.9 – 11.4)	10.1 (2.2 – 15.2)

Characteristics are shown as mean with standard deviation, median with inter quartile range, or percentage.
* Laboratory measurements were available of 66% of the trans women and 72% of the trans men.
Abbreviations: HT = hormonal treatment, BMI = body mass index, LH = luteinizing hormone

Discussion

In this study, we found that fracture risk was higher in older trans women using long-term HT compared with control men but similar to control women. In young trans women, fracture risk tended to be increased compared with control women but not compared with control men. In addition, the type of fractures differed in trans women compared with control men, with relatively more hip, spine, forearm, and humerus fractures. Fracture risk was not increased in trans men using long-term HT. In trans women, older age and lower T-score of the LS were associated with an increased fracture risk, while this was not found in trans men.

In trans women <50 years, fracture risk tended to be increased compared with control women but not compared with control men. Earlier studies found that trans women, also at younger ages, had a high prevalence of osteoporosis or low BMD even before hormonal treatment.[5,21] Although earlier short-term and long-term studies did not show a detrimental effect of HT on BMD [7,20], higher fracture risk in young trans women compared with control women may be explained by lower initial BMD even before start of HT. In the general population, men have a higher fracture incidence than women at younger ages and these fractures usually occur as a result of an accident.[26,27] It can be speculated that, although BMD in trans women is lower than in control men, fracture risk is not increased because trans women often tend to have a less active life style than control men and are therefore less likely to suffer an accident which leads to a fracture.

In trans women ≥50 years, fracture risk was increased compared with control men but similar to control women. At older ages, control women have a higher fracture risk than control men, mainly due to a decreased BMD in control women because of the loss of estrogens after menopause.[26] The similar risk in trans women compared with control women at older ages can be thought to be the result of decreased estrogen concentrations, possibly because of decreasing or discontinuation of estradiol supplementation in older trans women. However, this is not part of our clinical protocol and we did not find a difference in mean estradiol concentrations in trans women <50 years and ≥50 years. Control women usually have normal BMD before menopause, but it decreases during menopause because the loss of estrogen. Trans women, however, have low BMD but, in our center, do not stop or lower estrogen therapy at the age of 50 years and therefore do not experience a decrease in BMD because of the loss of estrogen. This might explain why the fracture risk becomes similar in trans women compared with control women after the age of 50 years, but higher than control men.

It was also found that the type of fractures differed in trans women compared with control men, with relatively more hip, spine, forearm, and humerus fractures, while it was similar to control women. This might be explained by the fact that hip, spine, forearm, and humerus fractures usually occur because of low BMD, while other fractures mostly occur because of accidents. As trans women have lower BMD than control men, the risk of getting a hip, spine, forearm, or humerus fractures is higher, but because of a less active life style, the risk for other fractures is lower.

Trans men had a similar fracture risk compared with control women, but a lower risk than control men. Earlier studies found no increased fracture risk [4,9,22], a normal BMD at baseline [9], and no detrimental effects of HT on BMD.[9,14,20] Therefore, it was not expected that fracture risk would be increased in trans men. The finding that trans men had a lower fracture risk than control men but similar to control women might be explained that trans men are more careful or participate less in (sporting) activities than the control men, leading to less fractures.

This study is the first study investigating the fracture risk in a large population of adult trans women and trans men using long-term HT. This population was linked to a random sample of 10 age-matched control men and women per person, which makes the control population more accurate. The occurrence of fractures was retrieved from the database from Statistics Netherlands, which stores all diagnoses of the visits to the emergency rooms nationwide. As in the Netherlands, everybody with a (suspicion of a) fracture visits the emergency room, this is a reliable source for fracture data. However, there are also some limitations. Firstly, the time frame for fracture occurrence was only three years. Although it is long enough for a point prevalence of fractures, most ideally transgender people would be followed from the moment they start with HT. Secondly, we did not have any clinical data of the control population. Therefore, no other factors with effects on bone health and fractures, for example smoking habits and physical

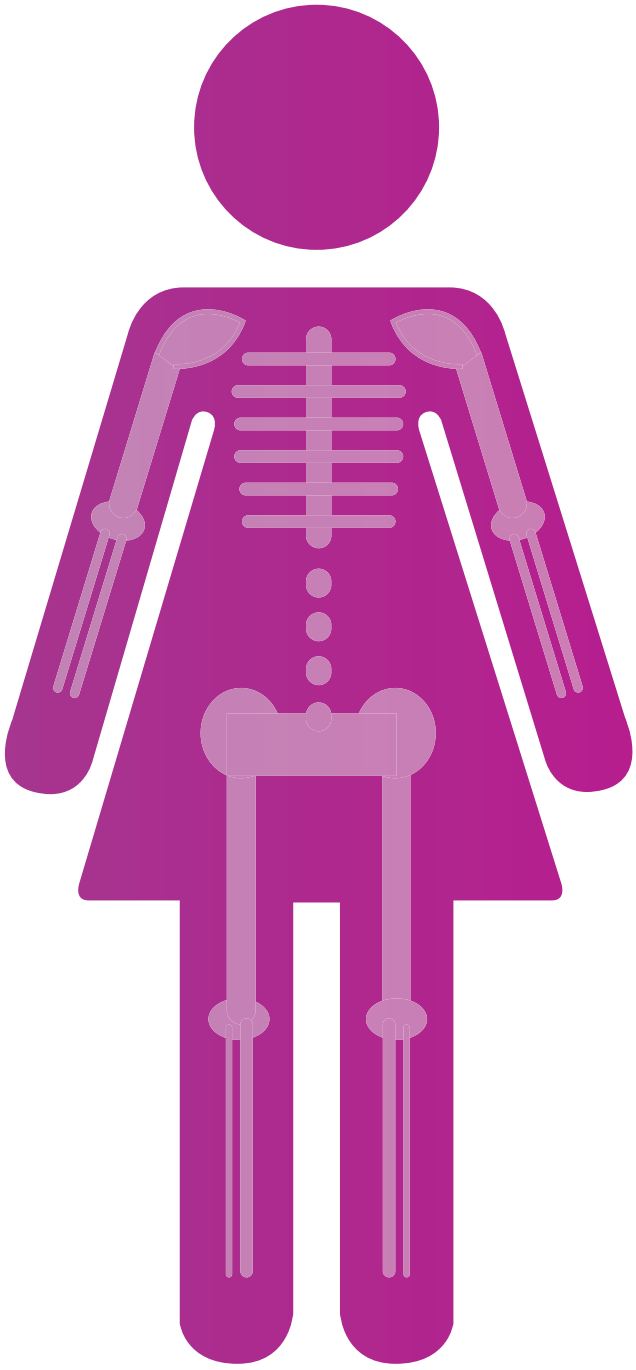
activity, could be analyzed in the control groups. However, in studies from our neighbor country Belgium, no differences (except smoking in trans men) were found in weight, smoking, and physical activity between the transgender population and control groups.[9,21] Thirdly, this study used data that were collected during clinical care. Therefore, not all data were available from the entire population, such as BMD and laboratory measurements. However, as they were known of the majority of the included population, these data were still analyzed, but should be interpreted cautiously. Fourthly, as this study was performed in collaboration with Statistics Netherlands, it was only allowed to show data if more than 10 individuals per group were present to protect the anonymity of the data. Therefore, only the overall fracture risk could be shown in trans men. Lastly, although the transgender population used HT for a long term, the population was still young of age. As most fractures occur at higher age, only the fracture risk at younger ages was assessed in this study. The effects of HT on fracture risk at old age therefore remains a topic for further research.

In conclusion, fracture risk was higher in older trans women using long-term HT compared with control men but similar to control women. In young trans women, fracture risk tended to be increased compared with control women but not compared with control men. Fracture risk was not increased in trans men using long-term HT. However, as type of fractures differed in trans women and the transgender population was still young, bone health remains an important health topic, particularly in trans women, and longer follow-up studies are needed.

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Chapter 10

General discussion

In this chapter, the main findings of the studies described in this thesis will be summarized (also described in Table 1 and Table 2) and will be discussed into the context of other studies, separately for trans women and trans men. A distinction will be made between short-term effects and long-term effects. Thereafter, the strengths and limitations of this thesis will be discussed and the implications for clinical practice will be given.

Population

The number of people with gender identity issues who seek professional help increased enormously in the last decades, as described in Chapter 2.[1-3] Possible explanations for this increase can be sought in the increased media and society attention. This can lead to more awareness of the existence of gender dysphoria and the possibility for medical treatment, and to higher social acceptance. In addition, the change in treatment protocols, including the option for a 'partial' treatment such as hormones without surgery, or mastectomy without hormones [4], may lead to an increase in referrals. However, these possible explanations are only hypotheses, as we did not study the reasons for the increase in referrals.

While the ratio trans women : trans men remained stable in adults, in adolescents the population of trans boys increased relatively more than the population of trans girls, leading to that currently more trans boys than trans girls are seen. This finding is in line with another study reporting this phenomenon [5], but a clear explanation is not elucidated yet.

Only a very small percentage of people who underwent gonadectomy regretted their decision, expressed as start of hormonal treatment in line with their sex assigned at birth. The numbers reported in our study (0.5%) are in line with other studies investigating the regret rates, which are <1% in trans men and 1-1.5% in trans women in an older study [6], and 0% [7,8], 2% [9], and 6% [10] in more recent studies.

Bone health
Trans women

Before start of HT

In trans women, we found that the mean Z-score before the start of HT was lower than expected **(Chapter 7)**. Also, 20% had a Z-score below -2.0, which indicates a low BMD for age. We also observed that the mean vitamin D concentrations in trans women at baseline were low, which might be one of the explanations for this low BMD. This observation was earlier described by Haraldsen et al. in 2007.[11] They found that at baseline trans women had a lower lumbar spine BMD than cis men. They also found that trans women had a lower lean body mass and lower total body fat than cis men. Van

Caenegem et al. [12] also described this finding in 2013. They found that trans women had lower areal BMD at the total hip, femoral neck, and lumbar spine, a thinner radial cortex and lower cortical area. In addition, they had lower muscle mass and strength, and lower vitamin D concentrations than cis men, which might be contributing factors to the lower BMD. Possible explanations for these baseline differences could be found in lifestyle factors. It can be thought that trans women, before any medical intervention, don't go out much and therefore have low vitamin D concentrations and low muscle mass (and therefore lower mechanical loading), which negatively influences the achievement of bone mass.

Short-term effects of HT

During the first year of HT, we observed that BMD increased **(Chapter 3)** and the BTMs decreased **(Chapter 4)** in trans women. The largest increase in BMD was observed in the lumbar spine, which mainly consists of trabecular bone.[13] Trabecular bone is thought to be more metabolic active compared to cortical bone, which is particularly present in the hip.[13] We also observed that the changes in BTMs were mainly correlated to the change in LS BMD. This indicates that an increase in estradiol concentration inhibits osteoclast activity [14], shown by a decrease in bone resorption, which leads to an increase in BMD. The latter is supported by the relationship found between the change in estradiol concentration during HT and both BMD and BTMs. Mean estradiol concentrations during HT were positively associated with change in BMD. In addition, we found that the estradiol concentrations in the lowest quartile did not show a decrease, or showed even an increase in BTMs, indicating that these estradiol concentrations might be too low to result in a decrease in bone turnover.

Vitamin D deficiency (<50 nmol/L) is very common in trans women. In our study described in **Chapter 5**, we found that almost 70% of the trans women were vitamin D deficient at baseline. Trans women with vitamin D deficiency received treatment with vitamin D supplements, which also has positive effects on bone health.[15] We observed that the trans women who received both HT and vitamin D supplementation had a larger increase in BMD than trans women who only received HT (**Chapter 3**). However, we still observed an increase in BMD in trans women without vitamin D supplementation, indicating that the increase in BMD is not solely the result of the vitamin D supplementation. In addition, estrogens can influence vitamin D metabolism. Earlier studies found that cis women using oral anticonceptives [16] or during pregnancy [17,18] had a higher concentration of vitamin D binding protein (DBP). If more total vitamin D is bound to DBP, less free or bioavailable vitamin D is available for metabolic processes. This might indicate that hormonal treatment in trans women can influence vitamin D metabolism and might hamper diagnostics of vitamin D deficiency, with possible negative effects on bone health. In our study (**Chapter**

5), we found that DBP only increased mildly in trans women during HT, and free and bioavailable vitamin D decreased slightly. Total vitamin D concentrations did not change and were well correlated with free and bioavailable vitamin D concentrations. This indicates that hormonal treatment in trans women does not negatively influence vitamin D metabolism and diagnostics of vitamin D deficiency.

Besides direct effects of estrogens on bone, also indirect effects might occur, for example effects on muscle mass. During hormonal treatment, the testosterone concentrations decrease. Testosterone concentrations are positively associated with muscle mass [19-21], and higher muscle mass and therefore higher mechanical loading, is associated with a higher BMD.[22] We observed in **Chapter 6** that in trans women, grip strength and muscle muss decreased during HT. However, BMD increased in these individuals. These findings indicates that, even though muscle mass decreases in trans women, one year of HT does not have deleterious effects on bone health.

Long-term effects of HT

As described in **Chapter 7**, we studied the change in BMD during the first ten years of HT. We found that BMD initially increased during the first two years of HT and decreased thereafter, to the same BMD after ten years of HT than at baseline. However, as the majority of the included trans women were after the age of peak bone mass, the natural course of BMD is to decrease over time. As we did not follow a cis control group for ten years, we also analyzed the change in Z-score during the first ten years of HT. This Z-score is the BMD of the study population compared with the BMD of someone of the same ethnicity, age, and birth-sex. We observed that Z-score increased initially and stabilized thereafter. This indicates that the decrease in BMD is due to the aging of the population and not due to HT. In line with the short-term study as described in **Chapter 3**, we found that change in BMD was associated with the mean estradiol concentrations during HT. Trans women who were in the highest estradiol tertile showed an increase in BMD. Trans women who were in the middle estradiol tertile showed no change in BMD, while trans women who were in the lowest estradiol tertile showed a decrease in BMD. This shows that estradiol concentrations are associated with change in BMD and that adequate hormone substitution and therapy compliance should be stimulated.

Differences in bone geometry exist between cis men and cis women. Cis men have a larger bone size than cis women, with a larger periosteal circumference.[23] In cis women, the endosteal circumference is smaller than in cis men [24], indicating that the bone of cis women growths towards the inside of the bone. Cortical thickness is however similar between cis men and cis women.[25] In the study described in **Chapter 8**, we studied whether bone geometry changed during HT. We found that subperiosteal width, endocortical diameter, cortical thickness, and section modulus were not different between hormone-naïve trans women at baseline, and trans women using HT for 5, 15,

or 25 years of HT. As these measurements of the hip are mainly of cortical origin, we also studied whether trabecular bone changes by investigating the change in trabecular bone score (TBS). We found that trans women who were using HT for either 5, 15, or 25 years, had a higher (or tended to be higher) TBS than trans women at baseline. This indicates that treatment with anti-androgens and estrogens does not influence cortical bone size, as measured by hip structure analysis, but it affects trabecular bone, as shown by the higher TBS values. As low TBS is associated with an increased fracture risk [26], the higher TBS in trans women using HT indicates that HT does not have detrimental effects on bone health, but may even be beneficial.

In the general population, cis men have a higher fracture risk than cis women at younger ages.[27,28] This is because cis men are probably more involved in accidents, for example sporting accidents, leading to an increased fracture risk.[27,28] At older ages, cis women have a higher fracture risk than cis men. This is the result of the lower BMD in cis women because of menopause [27], whereas this is not the case in cis men. In the last study described in **Chapter 9**, we investigated the fracture risk in trans women using HT. Trans women using long-term HT were divided into two groups: <50 years and ≥50 years. They were compared both with cis men and cis women. In trans women <50 years, we found that fracture risk was in between the risk of cis men and cis women: it was lower than the fracture risk of cis men, but tended to be higher than the fracture risk of cis women. The lower fracture risk of trans women than cis men can be thought to be the result of a less active life style than cis men, with also less sporting activities, leading to a decreased fracture risk. In trans women ≥50 years, we found that fracture risk was increased compared with cis men, but similar to cis women. Also the type of fractures differed: trans women experienced more often a hip, spine, forearm, or humerus fracture (as an approximation of osteoporotic fractures) than cis men, but similar to cis women. This indicates that fracture risk in trans women at older ages resembles the risk of cis women, while at younger ages the risk is in between the risk of cis men and cis women.

Table 1. Summarizing table of the main outcomes of the described studies in this thesis in trans women

	Number of trans women	Measurement	Time points	Outcome
Before start of hormonal treatment				
- Bone mineral density	711	DXA	0 year	↓
Short-term effects				
- Bone mineral density	231	DXA	0 and 1 year	↑
- Bone turnover markers	121	Blood samples	0 and 1 year	↓
- Vitamin D concentrations	29	Blood samples	0 and 3 months	=
- Grip strength/lean body mass	249	Dynamometer/DXA	0, 3, 6, 9, 12 months	↓
Long-term effects				
- Bone mineral density	711	DXA	0, 2, 5, 10 years	↑ / =
- Bone geometry				
• Trabecular bone score	535	DXA	0, 5, 15, 25 years	↑
• Hip structure analysis	535	DXA	0, 5, 15, 25 years	=
- Fractures				
• <50 years	1,089	National registry	8 years	↑ vs cis women ↓ vs cis men
• ≥50 years	934	National registry	19 years	= vs cis women ↑ vs cis men

Trans men

Before start of HT

Before the start of hormonal treatment, we found that trans men had a normal lumbar spine BMD, expressed as a Z-score of zero (**Chapter 7).** This is in agreement with earlier baseline studies in trans men. Haraldsen et al. [11] described in 2007 that trans men had a similar lumbar spine BMD than cis women, but a higher femoral neck BMD. Van Caenegem et al. [29,30] reported in 2012 and in 2015 that trans men at baseline had similar bone and body composition than cis women. This indicates that prior to HT, no decreased bone health is present in trans men.

Short-term effects of HT

During the first year of HT (**Chapter 3**), we observed small increases in BMD of the lumbar spine and total hip, and no change in femoral neck. However, the BTMs increased as well (**Chapter 4**). The changes in BMD and BTMs were not associated with the mean testosterone concentrations during HT. Interestingly, we found an age difference in change in BMD and BTMs. In people over 50 years, who were thought to be postmenopausal and therefore estrogen deficient at baseline, BMD increased

more compared to the younger trans men. BTMs decreased in this group, while it increased in the younger trans men. Because testosterone is aromatized into estradiol, supplementation with testosterone will not only increase the testosterone concentrations in this older group but also the estradiol concentrations. As the younger trans men had higher estradiol concentrations at baseline than older trans men, only an increase in testosterone concentrations was observed and no change in estradiol concentrations was seen. This indicates that, in trans men, estrogen is mainly responsible for the increase in BMD and the decrease in BTMs, because in trans men with no change in estrogen concentrations but only in testosterone concentrations, only a small increase in BMD was found, and BTMs increased. These findings are in line with the study performed by Finkelstein et al. [31] who included a large population of cis men and treated them with gonadotropin-releasing hormone agonists. Half of the group was treated with testosterone only, in different dosages, while the other half of the group also received an aromatase inhibitor to inhibit the aromatization of testosterone into estradiol. Therefore, the first group had normal testosterone and normal estradiol concentrations, while the other group had normal testosterone but low estradiol concentrations. They observed that BMD of the lumbar spine decreased in the group using an aromatase inhibitor, with low estradiol concentrations, and remained stable in the group without an aromatase inhibitor and normal estradiol concentrations. This also shows that the effect of testosterone on BMD maintenance mainly occurs through the indirect effects of aromatization of testosterone into estradiol, instead of direct effects of testosterone.

Also in trans men, vitamin D deficiency occurs frequently. In our study described in **Chapter 5**, we found a prevalence of almost 70% at baseline. Testosterone might influence vitamin D metabolism, as a study in hypogonadal cis men treated with testosterone demonstrated that DBP concentrations decreased.[32] However, we observed that DBP only tended to decrease minimally, and also free and bioavailable 25(OH)D concentrations tended to increase minimally. Total 25(OH)D concentrations did not change because of HT and were well correlated with free 25(OH)D concentrations. This indicates that hormonal treatment in trans men does not have negative effects on vitamin D metabolism.

Testosterone also influences muscle mass, as demonstrated in studies in hypogonadal cis men with testosterone replacement therapy.[19] Testosterone affects myoblast proliferation and myoblast differentiation, and testosterone increases the number of satellite cells, which promotes protein synthesis of muscle mass.[21] We observed that grip strength increased during the first year of HT in trans men and that this increase in grip strength was associated with an increase in lean body mass (as an approximation of muscle mass). Change in grip strength was not associated with change in BMD, while change in muscle mass was mildly associated with change in femoral neck BMD. This might indicate that the preserving effects of testosterone on bone health in trans men is, besides the estrogen-mediated effects after aromatization, also

partially mediated by an increase in muscle mass and therefore an increase in mechanical loading. The latter was earlier described by van Caenegem et al. [30], who also found a correlation between BMD and muscle mass in trans men.

Long-term effects of HT

The first ten-year effects of HT on BMD in trans men are described in **Chapter 7**. We found that BMD remained stable during the first ten years of HT. However, because of aging we would expect BMD to decrease. Therefore Z-score was also analyzed, which revealed a continued increase in Z-score during the first ten years of HT, indicating no negative effects of testosterone treatment on BMD. As observed in the short-term study, also in this study the oldest age group experienced a larger increase in BMD than the younger age groups, which is thought to be a result of the baseline postmenopausal status as explained above. Testosterone and estradiol concentrations were not associated with change in BMD, but LH concentrations showed a relationship with BMD. Trans men with suppressed LH concentrations (<2 U/L) had an increase in BMD during the first ten years of HT, while in trans men with not suppressed or elevated LH concentrations (>2 U/L) no change in BMD was found. This indicates that adequate hormone substitution and therapy compliance should be stimulated for bone health.

The differences in bone geometry in trans men with and without testosterone treatment were studied in **Chapter 8**. We found that overall, no differences in subperiosteal width, endocortical diameter, cortical thickness, and section modulus were found between hormone-naïve trans men at baseline and trans men who were using HT for 5, 15, or 25 years, except again for the postmenopausal baseline group. Changes in trabecular bone score were found in all age groups. Trans men at baseline had a higher TBS than trans men using testosterone. Estrogens are known to positively influence trabecular bone.[33,34] Decreasing concentrations of estrogen can therefore negatively influence trabecular bone. Although we found that TBS was lower in trans men using testosterone than trans men at baseline, we did not find a linear relationship with duration of HT. This indicates that trabecular bone score decreases because of HT, but does not continue to decrease.

As last step, we investigated the fracture risk in trans men using long-term HT (**Chapter 9**). Overall, we found that trans men had a similar fracture risk than cis women and a lower fracture risk than cis men. This might be explained by that trans men are more careful or participate less in (sporting) activities than cis men, leading to less fractures. As the total number of fractures in trans men was already low, we could not stratify the analysis for age groups or type of fracture.

To conclude, in the past, it was thought that testosterone treatment in trans men would be detrimental for bone health because of the decreasing estradiol concentrations. Loss of estrogen at menopause leads to an increased osteoclastic activity and therefore accelerated bone loss.[14] However, we found in trans men that, because of the aromatization of testosterone into estradiol, no large changes in estradiol concentrations were observed. Also, no changes or even increases in BMD were observed. Even larger increases in BMD and decreases in BTMs were found in trans men with larger changes in estradiol concentrations. This indicates that HT does not have deleterious effects on bone health in trans men, which might to be caused by the adequate estradiol concentrations after the aromatization of testosterone into estradiol.

Table 2. Summarizing table of the main outcomes of the described studies in this thesis in trans men

	Number of trans men	Measurement	Time points	Outcome
Before start of hormonal treatment				
- Bone mineral density	543	DXA	0 year	=
Short-term effects				
- Bone mineral density	199	DXA	0 and 1 year	↑ / = <50 years ↑↑ ≥50 years
- Bone turnover markers	132	Blood samples	0 and 1 year	↑ <50 years ↓ ≥50 years
- Vitamin D concentrations	30	Blood samples	0 and 3 months	=
- Grip strength/lean body mass	278	Dynamometer/DXA	0, 3, 6, 9, 12 months	↑
Long-term effects				
- Bone mineral density	543	DXA	0, 2, 5, 10 years	↑ / =
- Bone geometry				
• Trabecular bone score	473	DXA	0, 5, 15, 25 years	↓
• Hip structure analysis	473	DXA	0, 5, 15, 25 years	=
- Fractures	1,036	National registry	9 years	= vs cis women ↓ vs cis men

Strengths, limitations, and recommendations for further research

Strengths

In this thesis, we analyzed the short-term effects of HT on BMD, BTMs, vitamin D metabolism, and grip strength, and the long-term effects of HT on BMD, bone geometry, and fracture risk. These studies had several strengths. In the majority of our studies, we included a large population, considering the rarity of the diagnosis. This allowed us not only to answer our main research questions, but also to perform subgroup analyses, such as analyzing the effects of different hormone concentrations. We also included people with a wide age range, therefore we were able to stratify the analyses for different age groups. Some studies were part of a multicenter prospective study, in which measurements were performed before and during HT. All trans people were treated according to a defined treatment protocol, and standardized measurements were used for physical examination, laboratory measurements, and DXA. Also in the long-term studies, the same DXA scanner was used for all analysis and most measurements were performed before and during HT. Fracture data was obtained using a nationwide registry.

Limitations

However, there are also some limitations. First, we did not include control groups in the analyses, except for fractures. Trans people may change their life style during HT, such as becoming more physical active, developing healthier eating habits, and stop smoking cigarettes. It can therefore not be proven that the change in, for example BMD and BTMs, are solely the result of HT. However, it is ethically not possible to perform a randomized controlled trial where some trans people would be withhold to use HT to investigate whether the change in BMD and BTMs are solely the effect of HT. A possible, but not optimal, solution would be to include a cis control population during the same follow-up period.

Second, only the effect of the first ten years of HT on BMD are investigated. Although it is not likely that the effects of HT on BMD for longer terms, for example 30 or 40 years, would be different than the first ten years, it is interesting to investigate this, particularly in regard to therapy compliance. One may assume that therapy compliance becomes lower after longer use of HT.

Third, bone health was only assessed using DXA. Although in regular patient care BMD is also assessed using DXA, this does not give insight into whether changes occur in BMD, bone geometry, or other characteristics such as cortical porosity. We tried to overcome this limitation by using additional software such as HSA and TBS. However, the DXA was not developed to analyze these changes. Another possibility could be to use peripheral qCT. Some short-term studies already studied this issue, but no long-term longitudinal changes have been studied yet.

Fourth, we only investigated fracture risk after long-term HT. Most ideally, fracture risk would be studied before the start of HT, in order to investigate what the baseline fracture risk is in trans people, and to follow the trans people from the moment they start with HT, to investigate whether fracture risk changes because of HT. In addition, fracture risk was only studied using clinical fractures, but no vertebral fracture assessment (VFA) was performed. As not all fractures become clinical, using VFA would provide more information.

Future research perspectives

In this thesis, only people who used the standard protocol of hormonal treatment were included. However, the last years more gender variant people who experience all kinds of identity issues may wish to receive different treatments. Examples of these different treatments include gonadectomy without the use of sex hormones, low dosages of sex hormones, or only suppression of the endogenous hormone production. These treatments may result in different effects on bone health. It would be worthwhile to investigate what the minimal amount of sex hormones is that should be used, in order to maintain adequate bone health, but what is also in line with the wishes of the treated individual.

Also in this thesis, only people who started treatment during adulthood (≥18 years of age) were included. Just as the increase in referrals in transgender adults, also a large increase in referrals in transgender adolescents is observed. These adolescents receive a different kind of treatment than adults. Their treatment first starts with gonadotropin-releasing hormone agonists to suppress the development of secondary sex characteristics of the sex assigned at birth. After several months or years of treatment, depending on the age of start with this treatment, sex hormones are added to this treatment. This different treatment approach may have different effects on maintaining adequate bone health, but also on bone development and accrual during puberty. Given the increase in referrals in transgender adolescents, it is necessary to investigate this population as well.

Implications for clinical practice

Trans women

Trans women often experience low bone mass at baseline, but it increases during HT. Therefore, regularly performing DXA scans to measure BMD during HT is not necessary. However, we observed that BMD decreased in those with lower estradiol concentrations (mean 118 pmol/L, range 20-182 pmol/L) and remained stable or increased in those with higher (mean 238 pmol/L and 443 pmol/L, respectively) estradiol concentrations. This indicates that concentrations of 118 pmol/L are too low for bone health in trans women and higher concentrations, at least 180 pmol/L, should be aimed for.

This should be weighed against the possible increased risk for side effects of hormonal treatment, for example cardiovascular events (CVE) or breast cancer. However, a recent study analyzing the CVE risk found that trans women with a CVE used similar dosages of estradiol compared to trans women without a CVE [35], although they do not give any information regarding estradiol concentrations or compliance. A recent study that reported on breast cancer risk in trans women found that trans women who experienced breast cancer had similar mean estradiol concentrations during HT than trans women without breast cancer.[36] These studies indicate that the concentrations of estradiol are not linear related to the increased CVE and breast cancer risk, and therefore increasing the lower limit of estradiol concentrations would not be detrimental regarding these side effects. This is also more in line with the aimed estradiol concentrations as described in the Endocrine Society Guidelines [37], which are between 367 and 734 pmol/L. In addition to this, it should be considered to perform a DXA in trans women with potential therapy incompliance after gonadectomy, as they will have low estradiol concentrations and therefore may experience a decrease in BMD.

We also observed that trans women often are vitamin D deficient at baseline (around 70%). Supplementation with vitamin D supplements increased BMD more than in trans women without supplementation of vitamin D. Therefore, all trans women should be treated with vitamin D supplementation daily to increase or maintain the vitamin D concentrations and to increase BMD. Also giving them life style advises for optimizing bone health, such as quitting smoking and becoming more physically active, seems relevant, as earlier studies found that trans women had lower muscle mass and smoked more often than their control cis peers, which has negative effects on bone health.[38]

Cis women experience decreases in estradiol concentrations because of menopause. No guidelines exist about whether estradiol dosing should be lowered or stopped in trans women using HT. Based on the low initial BMD in trans women, the decrease in BMD in trans women with low estradiol concentrations, and the increased fracture risk of trans women compared with cis men, it may be thought that lowering or stopping estradiol dosing should not be standard in trans women based on their bone health. However, what concentrations should be aimed for is not known yet and should be a topic for further research.

Trans men

We found that at baseline trans men had a BMD as expected for age. BMD did not change during the first ten years of HT. After long-term use of HT, fracture risk was not increased in trans men compared with the control population. Therefore, it does not seem necessary to check BMD in trans men before or during HT. However, if a DXA scan is performed in trans men and low bone mass is found, one might aim for suppressed LH concentrations (<2 U/L) during HT, as we observed an increase in

BMD in trans men with suppressed LH concentrations. This however should be done on a case-by-case base, also taking the possible increased risk for side effects taking into account, such as polycythemia.[39]

Reference values

Different reference values exist for cis men and cis women to calculate T-scores and Z-scores. However, some studies found that fracture risk was similar in cis men and cis women with the same absolute BMD.[40] Therefore, there is discussion about whether sex-specific reference values should be used for both cis men and cis women, or that female reference values should be used for everybody. It is not known what reference values should be used for trans people if sex-specific reference values are used. Most trans people already achieved their peak bone mass before they started with HT, therefore the reference values of the sex-assigned at birth are most commonly used. In the ideal situation to determine what reference values should be used, BMD of the trans population should be compared with the BMD of cis men and cis women with a similar fracture risk. Unfortunately, such study has not been performed yet. Based on the results of this thesis that bone geometry of the hip in trans women are more related to male reference values, and in trans men are more related to female reference values or in between female and male reference values, and did not change during HT, the reference values of the birth-assigned sex should be used. However, the change in TBS in both trans women and trans men might indicate that the reference values of the identified sex should be used in trans people using HT. Furthermore, both trans women and trans men had a mean BMD at baseline that was more closely related to female reference values than male reference values.[41] This might indicate that female reference values should be used for all trans people. Therefore, data about which reference values should be used is not clear yet and more research is needed.

Conclusion

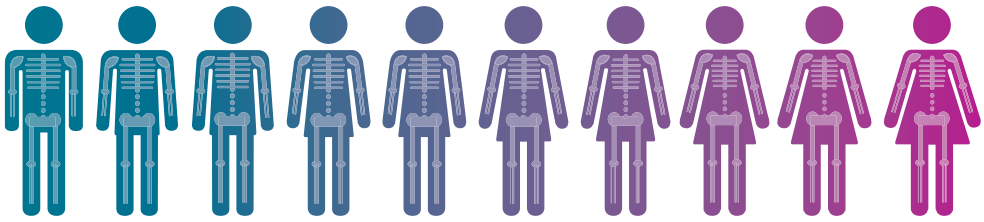
In conclusion, we found in this thesis that bone mineral density did not decrease after short term and long-term hormonal treatment in both trans women and trans men. Fracture risk in trans people did not exceed the fracture risk of cis people. This indicates that hormonal treatment does not have detrimental effects on bone health. However, bone health should be an important health topic in trans people, given that trans women often had low bone mineral density before the start of hormonal treatment, and the high prevalence of vitamin D deficiency. Taken all these results into account, we consider the results of this thesis as reassuring with regard to the safety of hormonal treatment in trans people.

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Addendum

Summary in Dutch – Nederlandse samenvatting

Acknowledgements – Dankwoord

List of publications

Presentations and courses

Awards and grants

About the author

Nederlandse samenvatting (Summary in Dutch)

Transgender personen

Transgender personen zijn mensen die gediagnosticeerd zijn met genderdysforie. De diagnose genderdysforie kan gesteld worden als er een verschil is tussen het geboortegeslacht en met welk geslacht iemand zich identificeert, en als de persoon daar onder lijdt. Iemand die gediagnosticeerd is met genderdysforie kan een medische behandeling ondergaan om het lichaam aan te passen aan het geïdentificeerde geslacht. Deze medische behandeling kan bestaan uit een hormoonbehandeling en operaties.

Transvrouwen zijn mensen aan wie na de geboorte het geslacht man is toegewezen, maar die zich identificeren als vrouw. De hormoonbehandeling bestaat uit het gebruiken van antiandrogenen, om de testosteronproductie (mannelijk geslachtshormoon) te onderdrukken, en het gebruiken van oestrogenen (vrouwelijk geslachtshormoon). Door deze hormoonbehandeling treden er lichamelijke veranderingen op, zoals borstgroei, een vrouwelijke vetverdeling en een afname van de haargroei op het lichaam. Nadat een transvrouw minimaal een jaar hormoonbehandeling heeft gehad, kan zij in aanmerking komen voor operaties. De meest uitgevoerde operatie is een vaginaplastiek, waarbij de penis en testikels worden verwijderd en er een vagina en schaamlippen worden gemaakt. Daarnaast is het mogelijk om een borstvergroting, gezichtsoperatie of adamsappeloperatie te ondergaan.

Transmannen zijn mensen aan wie na de geboorte het geslacht vrouw is toegewezen, maar die zich identificeren als man. Zij kunnen behandeld worden met het hormoon testosteron. Hierdoor ontstaan er verschillende lichamelijke veranderingen, zoals toename van haargroei op het lichaam, meer spiermassa en een lagere stem. Naast de lichamelijke veranderingen die ontstaan door hormonen, is het ook mogelijk om operaties te ondergaan. De meest ondergaande operatie is een borstverwijderende operatie. Ook is het mogelijk om de baarmoeder en eierstokken te laten verwijderen. Ten slot zijn ook genitale operaties mogelijk, zoals het verwijderen van de vagina en het construeren van een penis.

Naast het effect van hormonen op de uiterlijke lichamelijke veranderingen, kunnen deze hormonen ook invloed hebben op de botten. Voordat dit besproken wordt, zal er eerst uitleg gegeven worden over botten in het algemeen.

Botten

Botten in het menselijk lichaam bestaan uit twee onderdelen: corticaal bot en trabeculair bot. Corticaal bot is de buitenzijde van de botten, 80% van de totale botmassa bestaat uit corticaal bot. Dit bot is met name te vinden in de lange botten, zoals de botten in de armen en benen. Trabeculair bot is de binnenzijde van het bot en is met name te vinden in de rugwervels.

In de botten wordt voortdurend nieuw botweefsel gevormd en oud botweefsel afgebroken. Dit gebeurt om te kunnen groeien tijdens de kinderleeftijd en jongvolwassenheid, en om beschadigingen, zoals een breuk, te kunnen herstellen om zo de stevigheid te behouden. Bij deze vernieuwing zijn botcellen actief die botweefsel afbreken, ook wel osteoclasten genoemd, en botcellen die botweefsel opbouwen, ook wel osteoblasten genoemd. Deze cellen scheiden bij hun activiteit stoffen uit die te meten zijn in het bloed. Dit noemen we botmarkers. Deze botmarkers kunnen inzicht geven in de activiteit van de osteoblasten en osteoclasten, om bijvoorbeeld te onderzoeken of een behandeling aanslaat. De balans tussen de activiteit van deze botcellen bepalen of de botmassa toeneemt, gelijk blijft of afneemt. Tijdens de kinderleeftijd, adolescentie en jongvolwassenheid neemt de botmassa toe. Daarna zien we dat de botmassa licht afneemt met het toenemen van de leeftijd. Bij vrouwen na de overgang neemt de botmassa sneller af.

Naast het meten van de botmarkers in het bloed, kunnen botten ook gemeten worden met een botdichtheidsmeting. Bij deze meting wordt op een tweedimensionale manier gemeten hoeveel mineralen er in de botten zitten en wat de oppervlakte van het bot is. Door de hoeveelheid mineralen te delen door de oppervlakte van het bot, wordt de botmineraaldichtheid, ook wel botdichtheid genoemd, gemeten. Deze botdichtheid wordt gebruikt om te onderzoeken of er sprake is van osteoporose, ook wel botontkalking genoemd. Hierbij is er sprake van een lage botdichtheid. Dit geeft een grotere kans om een bot te breken. Ook kan de botdichtheidsmeting gebruikt worden om te onderzoeken of er veranderingen in de botdichtheid optreden, bijvoorbeeld na het starten van een behandeling tegen osteoporose.

Effecten hormonen op de botten

Voordat de puberteit intreedt, is de botmassa gelijk tussen jongens en meisjes. Tijdens de puberteit neemt de hoeveelheid hormonen in het lichaam toe: geboren jongen krijgen meer testosteron, geboren meisjes krijgen meer oestrogenen. Naast het effect van deze hormonen op lichamelijke veranderingen, hebben deze hormonen ook invloed op de botten, zowel op de botopbouw als het onderhouden van (de stevigheid van) de botten. Jongens ontwikkelen bredere botten dan meisjes. De botmassa is daardoor ook hoger bij mannen dan bij vrouwen. Er zijn meerdere mogelijke verklaringen voor het verschil in botbreedte, ook wel botgeometrie genoemd, tussen mannen en vrouwen. Ten eerste zou het kunnen dat testosteron een direct effect heeft op de botten. Het effect kan echter ook indirect zijn. Door testosteron krijgen mannen meer spieren dan vrouwen. Deze spieren zorgen voor een zwaardere belasting op de botten, wat er voor kan zorgen dat de botten breder worden.

Voor het onderhouden van de kwaliteit van de botten spelen hormonen ook een belangrijke rol. Dit wordt met name duidelijk tijdens de overgang in vrouwen. Tijdens deze fase daalt het oestrogeengehalte in het bloed. Het is bekend dat oestrogenen een

positief effect hebben op de botten. Zodra deze oestrogenen wegvallen, zien we dat de botdichtheid afneemt in vrouwen. Bij mannen is het niet goed duidelijk welke hormonen effect hebben op het onderhouden van de kwaliteit van de botten. Het is bekend dat mannen na een castratie, waarbij het testosterongehalte daalt, een achteruitgang in de botdichtheid laten zien. Echter, testosteron wordt door het lichaam ook omgezet in oestrogenen. Dit betekent dat er in deze gecastreerde mannen ook een daling was in het oestrogeengehalte. Sommige studies laten zien dat in mannen het onderhoud van de botdichtheid ook met name geregeld wordt door oestrogenen en minder door testosteron.

Doel van dit proefschrift

Het doel van dit proefschrift is om de korte- en langetermijneffecten van de hormoonbehandeling in transgender personen op de botten te onderzoeken. Dit hebben we gedaan door te kijken naar de kortetermijneffecten op de botdichtheid, botmarkers, vitamine D huishouding en spiermassa. Voor de langetermijneffecten hebben we gekeken naar de botdichtheid, botgeometrie en botbreuken, waarbij het risico op botbreuken werd vergeleken met cisgender mannen en vrouwen (mensen bij wie het geboortegeslacht overeen komt met het geïdentificeerde geslacht).

Hoofdbevindingen

In hoofdstuk 2 beschrijven we de transgender populatie van het VU medisch centrum. Het aantal mensen dat op de polikliniek voor genderdysforie is geweest voor een psychologische of medische behandeling sinds 1972 tot eind 2015 werd in kaart gebracht. We vonden dat er een grote toename was in het aantal nieuwe mensen per jaar. Dit kan mogelijk verklaard worden door een toegenomen aandacht in de media. Hierdoor is er meer bekendheid over het bestaan van genderdysforie en dat er behandeling mogelijk is, maar de aandacht in de media kan ook leiden tot een grotere sociale acceptatie. Slechts een klein deel van de mensen die een operatieve behandeling heeft ondergaan, heeft spijt van deze beslissing.

In de hoofdstukken 3 en 4 hebben we de kortetermijneffecten van de hormoonbehandeling op de botdichtheid en de botmarkers onderzocht. In transvrouwen vonden we dat de botdichtheid toenam en de botmarkers afnamen. Door het toedienen van oestrogenen aan de transvrouwen werd de botafbraak geremd. We zagen dan ook dat transvrouwen met een hogere oestrogeenwaarde in het bloed een grotere afname in botmarkers en een grotere toename in botdichtheid hadden. De daling in de botmarkers hing samen met de stijging in de botdichtheid. Tevens vonden we dat transvrouwen die extra vitamine D kregen tijdens de hormoonbehandeling, een grotere toename in botdichtheid hadden dan de transvrouwen die geen vitamine D gebruikten.

In transmannen zagen we dat de botdichtheid toenam. We zagen ook dat de botmarkers toenamen, maar dat er een relatief grotere toename in de botaanmaakmarkers was dan de botafbraakmarkers, wat kan leiden tot een positief effect op de botdichtheid. We vonden dat de oudere transmannen, boven de 50 jaar, een grotere toename in botdichtheid hadden dan de jongere transmannen en een daling in de botmarkers. Dit kan verklaard worden doordat deze oudere transmannen vanwege de overgang een lage oestrogeenwaarde in het bloed hadden voordat zij gingen starten met de hormoonbehandeling. Vanwege de omzetting van testosteron in oestrogenen hadden deze oudere transmannen een relatief grotere verandering in de oestrogeenwaarden in het bloed dan de jongere transmannen die nog normale oestrogeenwaarden in het bloed hadden bij het starten van de hormoonbehandeling. Omdat de testosteronwaarden even veel veranderden in beide groepen transmannen, ondersteunt deze bevinding de literatuur dat met name oestrogenen belangrijk zijn voor het onderhoud van de botten en testosteron wat minder.

In hoofdstuk 5 hebben we het effect van de hormoonbehandeling op de vitamine D huishouding onderzocht. Vitamine D is belangrijk voor het onderhouden van de botkwaliteit. Het is bekend dat hormonen invloed kunnen hebben op de hoeveelheid actief vitamine D in het lichaam. We vonden dat de hormoonbehandeling in transvrouwen en transmannen slechts minimale veranderingen liet zien in de vitamine D huishouding. Wel vonden we dat het grootste deel van de populatie een tekort aan vitamine D heeft.

In hoofdstuk 6 werd het effect van de hormoonbehandeling op gripkracht en spiermassa onderzocht. In transvrouwen nam de gripkracht en de spiermassa af. Hoewel dit negatieve effecten zou kunnen hebben op de botdichtheid, vonden we in deze transvrouwen een toename van de botdichtheid. In transmannen vonden we dat de gripkracht en spiermassa toe nam. We vonden ook dat deze toename in spiermassa samenhing met een toename in de botdichtheid. Dit kan betekenen dat de toename in botdichtheid in transmannen deels te verklaren is door de toename in spiermassa.

In hoofdstuk 7 worden de langetermijneffecten van de hormoonbehandeling op de botdichtheid beschreven. In transvrouwen werd gevonden dat zij voor de start van de hormoonbehandeling een lagere botdichtheid hebben vergeleken met leeftijdsgenoten. Mogelijk is dit te verklaren doordat zij een minder actieve leefstijl hebben, wat kan leiden tot minder spiermassa, een lagere vitamine D waarde en hierdoor een lagere botdichtheid. Tijdens de eerste tien jaar van de hormoonbehandeling zien we dat de botdichtheid gelijk blijft. Zoals eerder beschreven is het bekend dat de botdichtheid afneemt bij het ouder worden. We zouden dus verwachten dat de botdichtheid in de transvrouwen zou afnemen tijdens deze tien jaar. Het feit dat de botdichtheid gelijk is gebleven, betekent

dat de hormoonbehandeling een positief effect heeft op de botdichtheid. Ook vonden we in transvrouwen dat de verandering in botdichtheid samenhing met de hoogte van de oestrogeenwaarde in het bloed. Transvrouwen met een hogere oestrogeenwaarde hadden een grotere toename van de botdichtheid, terwijl transvrouwen met een lagere oestrogeenwaarde een daling in de botdichtheid lieten zien.

In transmannen vonden we dat zij voor de start van de hormoonbehandeling een normale botdichtheid hadden. Ook in de transmannen vonden we dat de botdichtheid niet veranderde tijdens de tien jaar hormoonbehandeling. Zoals hierboven beschreven zou er een daling in de botdichtheid zijn verwacht vanwege het toenemen van de leeftijd. Dat de botdichtheid niet veranderde, betekent dat de hormoonbehandeling een positief effect heeft op de botdichtheid. Ook hier zagen we dat oudere transmannen een grotere toename in de botdichtheid lieten zien dan de jongere transmannen.

In hoofdstuk 8 worden de veranderingen in botgeometrie beschreven. Hiervoor is in de heup met name naar de botbreedte gekeken, wat voornamelijk bestaat uit corticaal bot. In transvrouwen vonden we dat er geen verschillen zaten in de botbreedte in de heup tussen transvrouwen die nog geen hormonen gebruikten en transvrouwen die wel hormonen gebruikten. In de rugwervels is er naar trabeculair bot gekeken, dit wordt uitgedrukt als een score. Een lage score hangt samen met een verhoogde kans op een botbreuk. In transvrouwen vonden we dat de trabeculaire score hoger was in degene die hormonen gebruikten vergeleken met transvrouwen die geen hormonen gebruikten.

In jongere transmannen vonden we geen verschil in botbreedte tussen de transmannen die nog geen hormonen gebruikten en de transmannen die wel hormonen gebruikten. In oudere transmannen vonden we dat de botbreedte groter was in de transmannen die hormonen gebruikten ten opzichte van de transmannen die nog geen hormonen gebruikten. Dit is mogelijk te verklaren doordat deze laatste groep in de overgang was en daardoor smallere botten hadden dan degene die geen overgang hadden doorgemaakt. In de rugwervels vonden we dat transmannen die hormonen gebruikten een lagere trabeculaire score hadden dan transmannen die geen hormonen gebruikten. Echter zagen we niet dat de score lager was bij het langer gebruiken van hormonen, wat betekent dat de daling niet aanhoudt bij het langer duren van de hormoonbehandeling.

Tot slot wordt in hoofdstuk 9 het risico op botbreuken beschreven. In de algemene populatie hebben op jonge leeftijd cisgender mannen een hoger risico op botbreuken dan cisgender vrouwen, terwijl op latere leeftijd cisgender vrouwen een hoger risico op botbreuken hebben dan cisgender mannen. In onze studie vonden wij dat transvrouwen op jonge leeftijd een risico op botbreuken had dat tussen het risico van cisgender mannen en vrouwen in lag. Op latere leeftijd was het risico van transvrouwen gelijk aan het risico van cisgender vrouwen, maar hoger dan het risico van cisgender mannen. Transmannen hadden op jonge leeftijd een risico op botbreuken dat gelijk was aan het

risico van cisgender vrouwen en lager dan het risico van cisgender mannen. Het risico op latere leeftijd in transmannen kon niet worden bepaald omdat daar te weinig personen voor in de studie zaten.

Betekenissen voor de praktijk

Tot recent werd er in het VUmc regelmatig botdichtheidsmetingen verricht bij transgender personen. In dit proefschrift vinden wij dat de hormoonbehandeling geen negatieve effecten heeft op de botdichtheid op korte en lange termijn. Dit heeft er toe geleid dat het regelmatig verrichten van een botdichtheidsmeting niet meer standaard wordt uitgevoerd. Echter, aangezien er wel werd gevonden dat transvrouwen een lagere botdichtheid hadden voor de start van de behandeling, is uitleg over een goede botkwaliteit wel gewenst. Omdat er werd gevonden dat transvrouwen met een lage oestrogeenwaarde in het bloed een daling hadden van de botdichtheid, moet er naar een hogere oestrogeenwaarde worden gestreefd. Daarnaast vonden wij dat het grootste deel van de transgender populatie een tekort aan vitamine D heeft. Dit houdt in dat er advies gegeven moet worden om extra vitamine D te gebruiken, om zo de botdichtheid te optimaliseren.

Toekomstperspectieven

Dit proefschrift heeft zich geconcentreerd op het effect van de hormoonbehandeling bij transgender personen die de standaard hormoonbehandeling hebben gekregen. De laatste jaren is er steeds meer bekendheid over dat er niet alleen (trans)mannen en (trans)vrouwen bestaan, maar dat er ook personen zijn die zich niet als man en niet als vrouw identificeren. Deze personen hebben vaak ook andere wensen ten aanzien van de hormoonbehandeling en de operaties. Het is nog niet bekend wat dit voor invloed kan hebben op de botten en dit moet nog verder onderzocht worden.

Daarnaast is er in dit proefschrift alleen gekeken naar de effecten van de hormoonbehandeling bij volwassenen. Er is echter ook een toename te zien in het aantal kinderen en adolescenten die zich aanmelden voor een genderaanpassende behandeling. Deze behandeling bestaat meestal eerst uit puberteitsremmers voordat er gestart wordt met hormonen. Ook dit kan effecten hebben op de botdichtheid en moet in toekomstige studies verder onderzocht worden.

Conclusie

In dit proefschrift vonden wij dat de botdichtheid niet af neemt na korte en lange termijn hormoonbehandeling in zowel transvrouwen als transmannen. Het risico op botbreuken was niet hoger dan het risico in de algehele populatie. Dit betekent dat de hormoonbehandeling geen negatieve effecten heeft op de gezondheid van de botten bij transgender personen. Daarom beschouwen wij de resultaten van dit proefschrift als geruststellend.

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Presentations and courses

Oral and poster presentations

Effect of One Year Cross-sex Hormonal Treatment on Bone Mineral Density of the Lumbar Spine in Transgender Patients
Oral presentation, Annual Meeting of the Dutch Society for Calcium and Bone Metabolism 2015, Zeist, the Netherlands

An increase of bone mineral density in male-to-female and female-to-male transgender patients after one year cross-sex hormonal treatment
Poster presentation, Dutch Endocrine Meeting 2016, Noordwijkerhout, the Netherlands

Effect of One Year Cross-sex Hormonal Treatment on Bone Mineral Density of the Lumbar Spine in Transgender Patients
Oral presentation, Endocrine Society 2016. Boston, the United States of America

An increase of bone mineral density in male-to-female and female-to-male transgender persons after one year cross-sex hormonal treatment
Oral presentation. European Congress of Endocrinology 2016. Munich, Germany

An increase of bone mineral density in male-to-female and female-to-male transgender patients after one year cross-sex hormonal treatment
Oral presentation. World Professional Association for Transgender Health 2016. Amsterdam, the Netherlands

The Effects of Cross-sex Hormonal Treatment in Transgender Persons on their Bone Mineral Density: a 1 year Prospective Observational Study
Poster presentation. The American Society for Bone and Mineral Research 2016. Atlanta, the United States of America

Long-term effects of cross-sex hormonal treatment on bone mineral density in transgender persons
Oral presentation. European Professional Association for Transgender Health 2017. Belgrade, Serbia

The ENIGI initiative, the endocrine part
Symposium. European Professional Association for Transgender Health 2017. Belgrade, Serbia

Increase in Lumbar Spine Z-score after 10 Years of Cross-sex Hormonal Treatment in Transwomen and Transmen
Oral presentation, Dutch Endocrine Meeting 2018, Noordwijkerhout, the Netherlands

Increase in Lumbar Spine Z-score after 10 Years of Cross-sex Hormonal Treatment in Transwomen and Transmen
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Change in bone mineral density during the first 10 years of gender affirming hormonal treatment in transwomen and transmen
Oral presentation. European Congress of Endocrinology 2018. Barcelona, Spain

Fracture risk is not increased in transwomen and transmen receiving long-term gender-affirming hormonal treatment: a nationwide cohort study
Oral presentation. Young Dutch Society for Endocrinology 2018. Nijmegen, the Netherlands

Change in bone mineral density in transwomen and transmen during the first ten years of gender-affirming hormonal treatment
Oral presentation. World Professional Association for Transgender Health 2018. Buenos Aires, Argentina.

Fracture risk is not increased in trans women and trans men receiving long-term gender affirming hormonal treatment: a nationwide cohort study
Oral presentation. World Professional Association for Transgender Health 2018. Buenos Aires, Argentina.

Bone health in transgender people
Oral presentation, invited speaker. Dutch Endocrine Meeting 2019. Noordwijkerhout, the Netherlands

Medical follow-up after the initiation of gender affirming hormonal therapy
Symposium. European Professional Association for Transgender Health 2019. Rome, Italy

Courses

2015 Research Integrity Course, VU University Medical Center, Amsterdam

2016 Course Legislation and Organization for Clinical Researchers (BROK), Amsterdam UMC, location VU University Medical Center, Amsterdam

2016 Writing a Scientific Article, VU University, Amsterdam

2015-2018 Post initial Master's Education in Epidemiology, EpidM, Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam

- Epidemiological research: design and interpretations
- Principles of epidemiological data analysis
- Regression techniques
- Clinimetrics: assessing measurement properties of health measurement instruments
- Practical epidemiology: designing a study
- Methodological advising
- Longitudinal data analysis
- Multilevel analysis
- Missing data: consequences and solutions
- Mediation analysis

Awards and grants

Goodlife Healthcare in combination with The Dutch Society for Endocrinology travel grant for presentation at the European Congress of Endocrinology, 2016. Abstract: An increase of bone mineral density in male-to-female and female-to-male transgender persons after one year cross-sex hormonal treatment. Authors: **Chantal M Wiepjes**, Mariska C Vlot, Maartje Klaver, Renate T de Jongh, Paul Lips, Annemieke C Heijboer, Alessandra D Fisher, Thomas Schreiner, Guy T'Sjoen, Martin den Heijer

American Society for Bone and Mineral Research. Fund for Research and Education Young Investigators Award. 2016. Abstract: The Effects of Cross-sex Hormonal Treatment in Transgender Persons on their Bone Mineral Density: a 1 year Prospective Observational Study. Authors: **Chantal M Wiepjes**, Mariska C Vlot, Maartje Klaver, Renate T de Jongh, Paul Lips, Annemieke C Heijboer, Alessandra D Fisher, Thomas Schreiner, Guy T'Sjoen, Martin den Heijer

Amsterdam Reproductive and Development Grant 2018. Project title: Sex hormones, cognition, and the role of cardiovascular and psychological factors: a study in elderly transgender people receiving gender-affirming hormone therapy. Applicants: Baudewijntje PC Kreukels, Koen MA Dreijerink, **Chantal M Wiepjes**, Jason O van Heesewijk

About the author

Chantal Maria Wiepjes was born on the 12th of June 1991 in Amsterdam, the Netherlands and she grew up in Zaandam. From 2002 to 2009 she followed pre-university education at the Pascal College in Zaandam, the Netherlands. In 2009 she started studying medicine at the VU University in Amsterdam, the Netherlands. During her Bachelor's education she followed extra-curricular courses in anatomy. In 2012 she received her Bachelor's degree and started with her Master's education. During her Master's education she was a board member of the Co-Raad and was responsible for evaluating and improving the education for students during their internships. In 2015 she received her Master's degree Cum Laude. From 2014 to 2019, she performed her PhD research at the Center of Expertise on Gender Dysphoria at the Amsterdam UMC, location VU University, supervised by professor Martin den Heijer and doctor Renate de Jongh. In the same period, she worked as a medical doctor in Endocrinology at the Center of Expertise and followed the Master's education Epidemiology, which she completed Cum Laude in 2018. In 2017 she married to Floris Booij and in September 2019 she became mother of Julian and Mia.

