Bone health in transgender people

Wiepjes, C.M.

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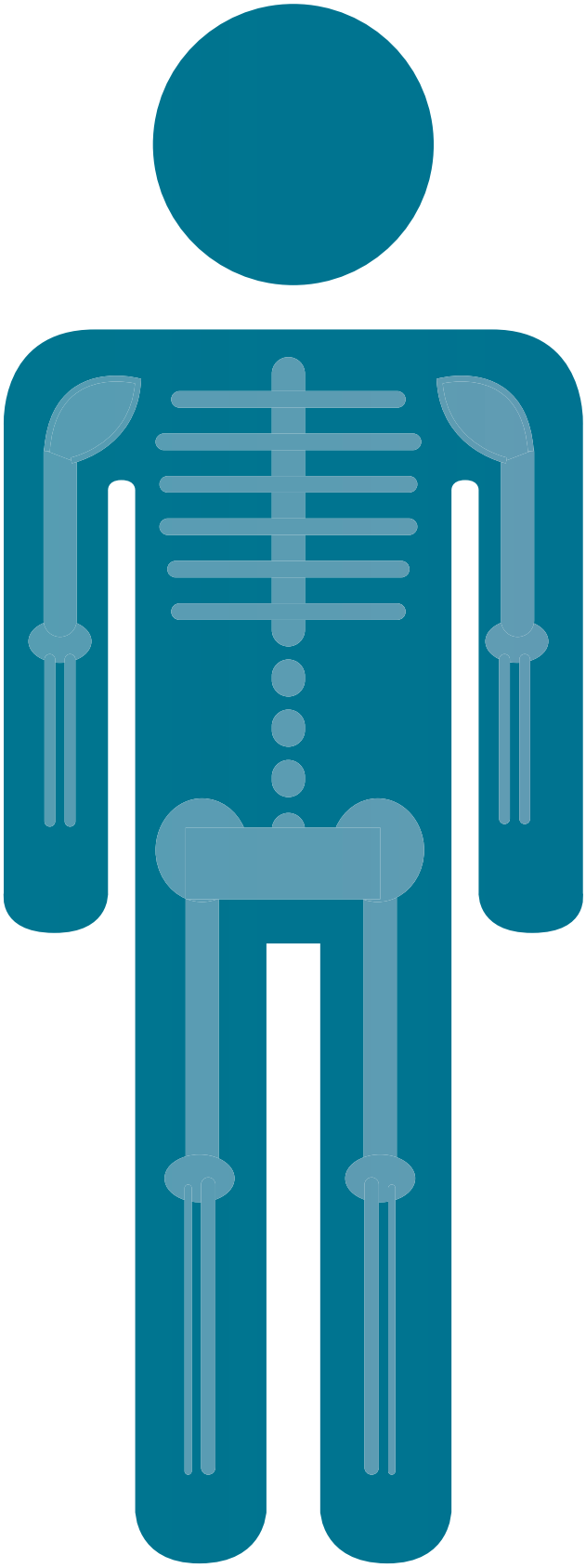

Chapter 1

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.

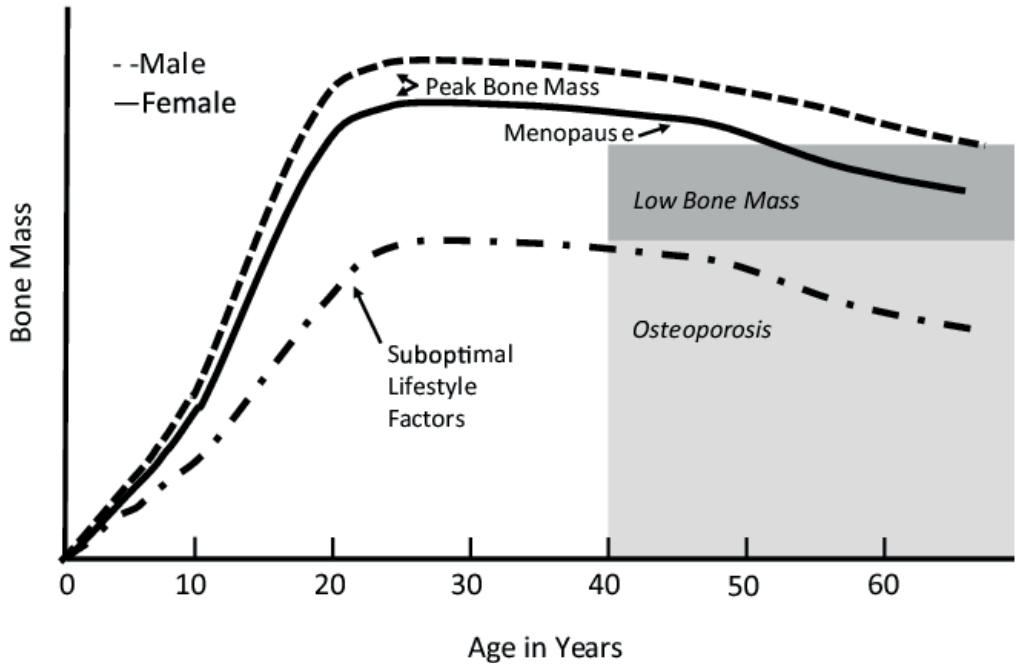

Figure 1.
Changes in bone mass during life in cis males and females. Re-used from Weaver C.M. et al.[10] This article is available under the Creative Commons CC-BY-NC 4.0 license (https://creativecommons.org/licenses/by-nc/4.0/) and permits non-commercial use, distribution and reproduction. No changes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

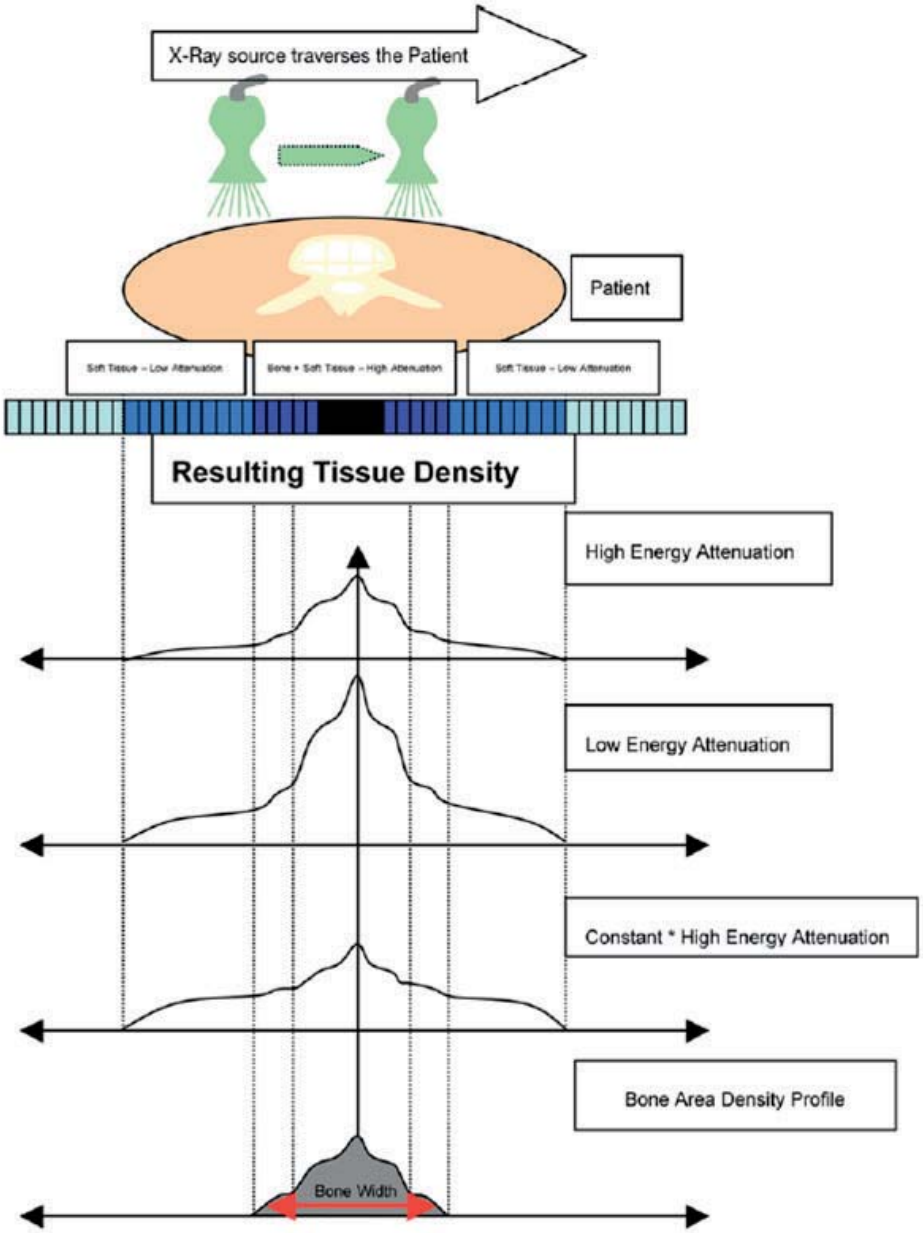

Figure 2.
A schematic explanation of the high-energy and low-energy level beams of the DXA. The bone profile, observed as the x-ray moves linearly across the patient, and the corresponding tissue density profiles. Re-used from Crabtree et al.[12], with permission from Springer Nature.

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.

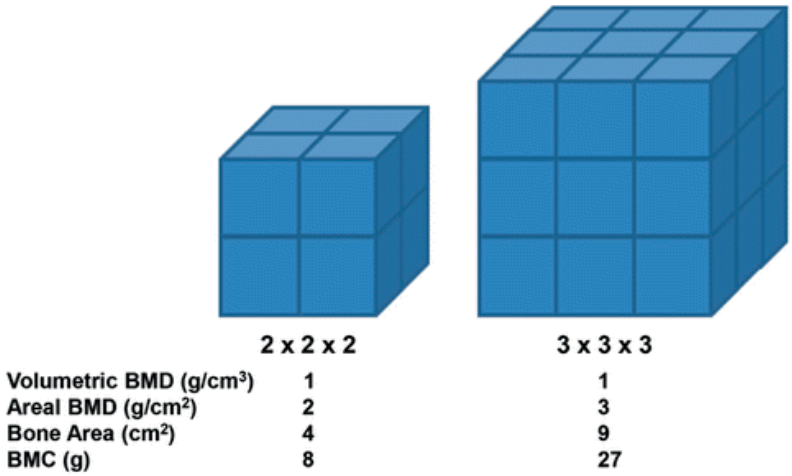

Figure 3.
The volumetric BMD, areal BMD, bone area, and bone mineral content (BMC) are shown for two different cubes, as an example of differences in bone size. It is shown that areal BMD is higher with wider bones, even if volumetric BMD is similar. Re-used from Logan et al.[15] with permission from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

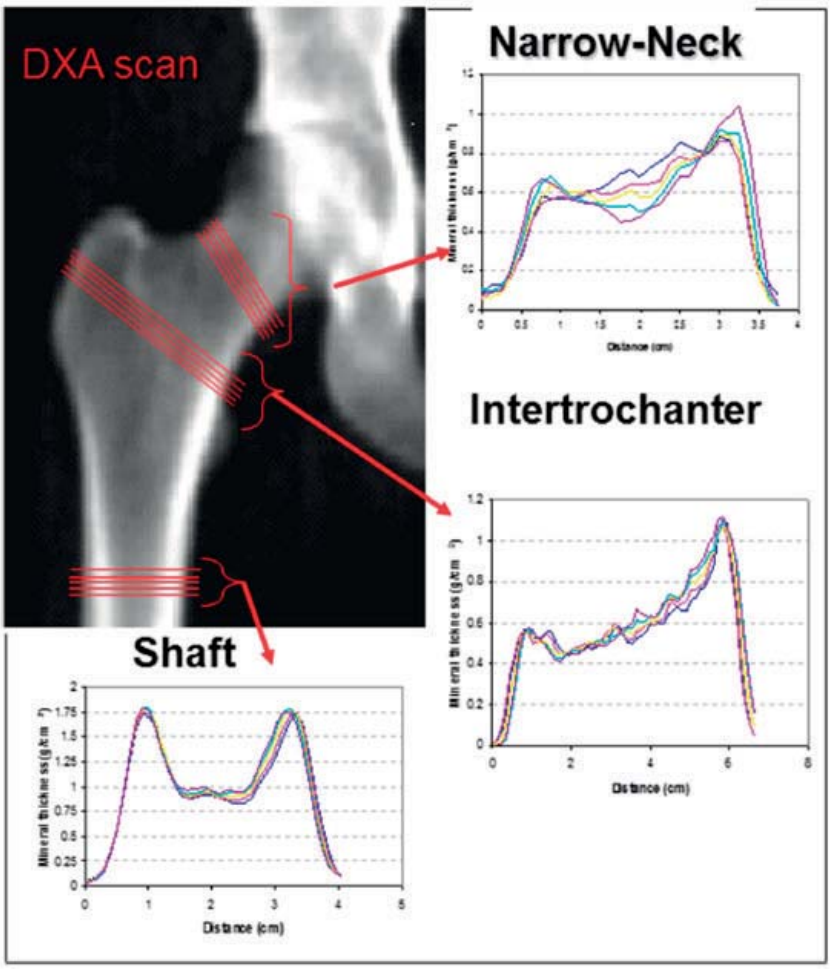

Figure 4.
Method of performing the hip structure analysis by using the DXA hip images, including locations of the cross-sections and the corresponding plots of mass profiles. Re-used from Beck et al.[18] with permission from Elsevier.

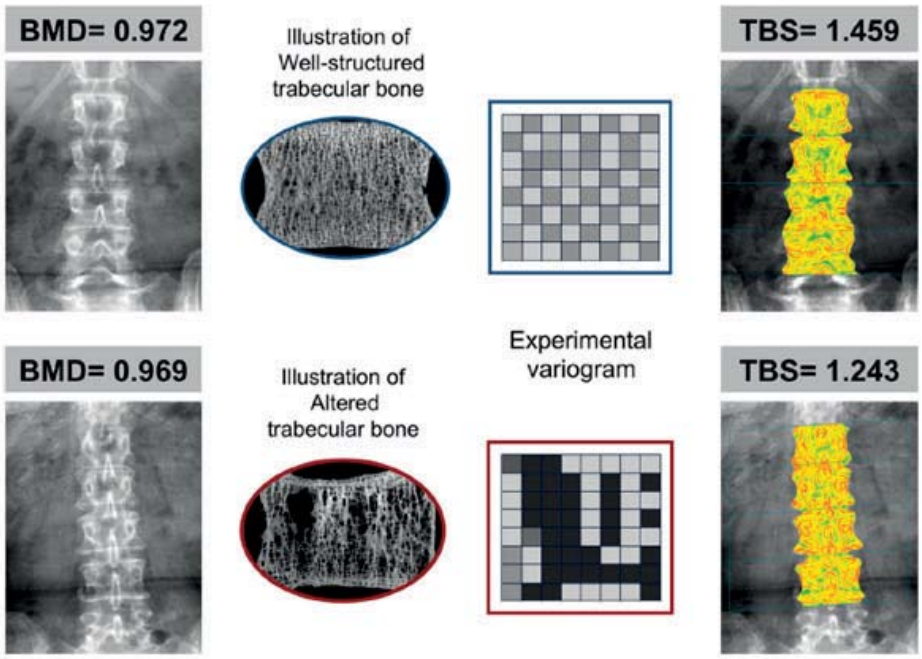

Figure 5.
Method of calculating trabecular bone score based on the DXA lumbar spine images. Representation of the TBS principles and an example where the TBS appears to be independent from BMD. Re-used from Silva et al.[17] with permission from John Wiley and Sons.

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]

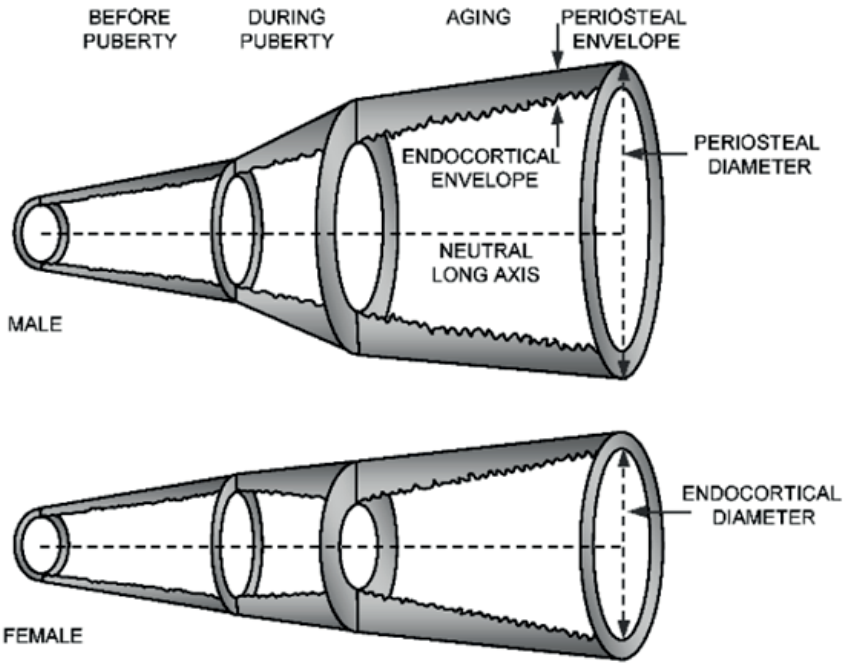

Figure 6.
Changes in periosteal surface (outer surface of cortex) and endocortical (endosteal) surface (inner surface of cortex) during life, different for cis males and cis females. Re-used from Duan et al.[25] with permission from John Wiley and Sons.

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]

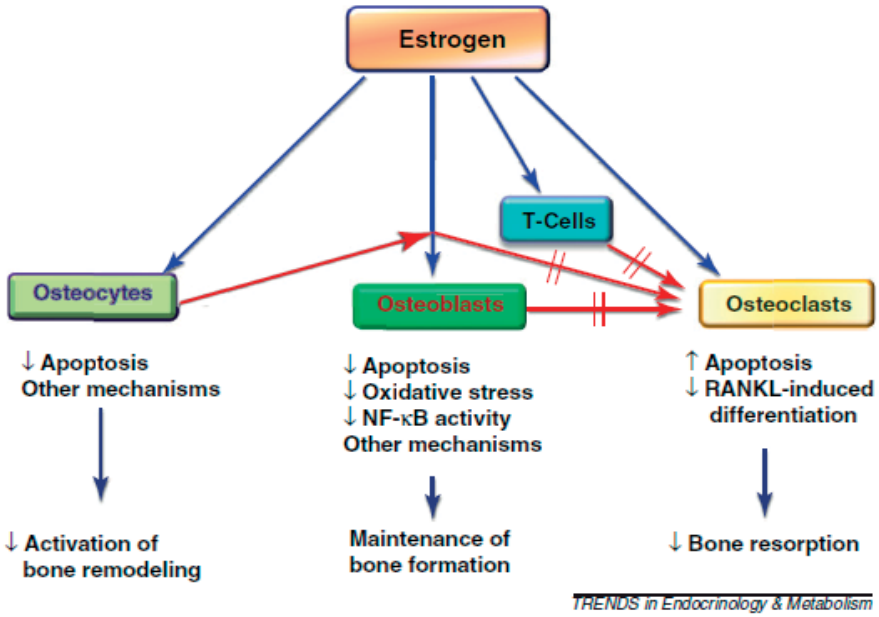

Figure 7.
Working model for estrogen regulation of bone turnover via effects on osteocytes, osteoblasts, and osteoclasts. Re-used from Khosla et al.[34] with permission from Elsevier.

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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