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TERAPIA HORMONAL CRUZADA, DENSIDADE MINERAL ÓSSEA E COMPOSIÇÃO CORPORAL EM INDIVÍDUOS TRANSGÊNEROS

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Esta tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia: Faculdade de Medicina, Universidade Federal do Rio Grande do Sul. Será apresentada na forma de manuscritos sobre o tema da Tese:

- Artigo original: Impact of cross-sex hormone therapy on bone mineral density and body composition in transwomen. Clin Endocrinol 2018, 88 (6): 856-862.
- Artigo original: Bone mass effects of cross-sex hormone therapy in transgender people: updated systematic review and meta-analysis. **Artigo submetido** – jc.2018-02632, em dezembro de 2018.

LISTA DE ABREVIATURAS E SIGLAS

BMD / DMO = bone mineral density / densidade mineral óssea
CSHT = cross-sex hormone therapy / terapia hormonal cruzada
DXA = dual-energy X-ray absorptiometry / absorciometria de raio-X de dupla energia
GAS / CAS = gender affirmation surgery / cirurgia de afirmação sexual
TH = terapia hormonal

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RESUMO

A disforia de gênero é definida pela incongruência entre o sexo de nascimento e o sexo de identidade, com duração superior a 6 meses. Esta condição está associada a sofrimento pessoal e prejuízo em diferentes áreas, e os indivíduos submetidos à terapia hormonal (TH) cruzada podem apresentar melhora da qualidade de vida com o tratamento adequado.

A TH cruzada tem como objetivo principal suprimir os hormônios endógenos e as características sexuais secundárias do sexo biológico e induzir características sexuais compatíveis com o sexo de identidade. Em indivíduos submetidos à cirurgia de afirmação sexual (CAS), a TH cruzada é utilizada como forma de reposição hormonal, uma vez que o procedimento gera uma situação de hipogonadismo persistente. A TH cruzada inadequada ou irregular, neste contexto, poderia acarretar prejuízo à saúde óssea e maior risco de baixa massa óssea e/ou fraturas.

No que se refere às mulheres trans, os estudos mostram aumento ou preservação da densidade mineral óssea (DMO) na coluna lombar quando avaliada a massa óssea antes e após a terapia estrogênica. Quando comparadas aos controles do sexo masculino, a terapia estrogênica parece não afetar significativamente a DMO nos sítios avaliados. No presente estudo, uma elevada prevalência de baixa massa óssea foi observada em mulheres trans quando comparadas a controles de ambos os sexos. A maior parte das mulheres trans avaliada utilizava terapia estrogênica por longo período de forma irregular, e um terço já havia realizado CAS.

No que se refere aos homens trans, não foi observada diferença significativa na DMO considerando a massa óssea antes e após a terapia androgênica, ou quando comparado aos controles do sexo feminino.

As evidências atuais indicam que a TH cruzada não afeta a DMO em homens trans, e em mulheres trans está associada a aumento da DMO na coluna lombar. Contudo, as evidências são de baixa à moderada qualidade, e estudos com maior tempo de acompanhamento e uso regular da TH são necessários para confirmar estes dados.

ABSTRACT

Gender dysphoria is defined by the incongruence between the biological sex and the sex of identity, lasting more than 6 months. This condition may be associated with personal distress and impairment in different areas, and individuals undergoing cross-sex hormone therapy (CSHT) may have improvement in quality of life with appropriate treatment.

The main objective of CSHT is to suppress endogenous hormone secretion and the sex characteristics of the expressed gender and maintain sex hormone levels and sex characteristics consistent with the other gender. In individuals undergoing gender affirmation surgery (GAS), CSHT is used as hormone replacement, since the procedure generates a condition of persistent hypogonadism. Inadequate or irregular CSHT in this context could lead to bone health impairment and increased risk of low bone mass and / or fractures.

Regarding trans women, studies showed a preservation or increase in bone mineral density (BMD) in the lumbar spine when evaluated bone mass before and after estrogen therapy. When compared to male controls, estrogen therapy did not significantly affect BMD at any site evaluated. In our study, higher prevalence of low bone mass was observed in trans women compared to natal men and women. Most of the trans women evaluated used irregular estrogen therapy for long period, and a third had already performed CAS.

Regarding trans men, no significant difference was observed in BMD considering bone mass before and after androgen therapy, or when compared to female controls.

Current evidence indicates that CSHT does not affect BMD in trans men, and in trans women it is associated with increased BMD in lumbar spine. However, the evidence is of low and moderate quality and further studies with regular CSHT and longer follow-up are needed to confirm this data in trans women.

INTRODUÇÃO

Disforia/Incongruência de gênero

A disforia de gênero é definida pelo desconforto persistente e sensação de inadequação no papel do gênero expresso ao nascimento. É uma condição marcada pelo sofrimento psicológico que acompanha a incongruência entre o gênero de nascimento e o gênero desejado (1,2). Epidemiologicamente, a prevalência da disforia de gênero é difícil de ser avaliada. Dados da Holanda e Bélgica mostram que 1:11.900 homens e 1:30.400 mulheres apresentam disforia de gênero com desejo de tratamento hormonal e/ou cirúrgico, com uma razão de aproximadamente 3:1 (3,4). Em uma meta-análise sobre prevalência de transexualismo que incluiu 12 estudos, foi observada prevalência de 4.6 pessoas trans para cada 100.000 indivíduos (6.8 para mulheres trans e 2.6 para homens trans) (5). Com relação à etiologia, os dados ainda são limitados. Estudos com ressonância magnética estrutural e functional do cérebro de indivíduos transgêneros mostram diferenças na espessura cortical, substância branca e ativação de áreas distintas do sistema nervosa central quando comparados a controles (6). É possivel que estas diferenças sejam uma resposta adaptativa à diferentes comportamentos, mais do que uma etiologia para disforia de gênero (7).

O tratamento de afirmação sexual é multidisciplinar, focado na atenuação dos sintomas disfóricos relacionados à imagem corporal, incluindo abordagem psicosocial, TH cruzada e CAS, quando esta for desejada pelo indivíduo (8). Em homens e mulheres trans, a TH cruzada está associada à melhora da qualidade de vida do ponto de vista mental, psicosocial e auto-estima (9,10), redução de sintomas de ansiedade (10) e de sintomas dissociativos (11). As mudanças físicas induzidas pelo tratamento, compatíveis com o gênero de identidade, possivelmente reforçam a afirmação do gênero, melhoram o bem estar e a aceitação pessoal e social do indivíduo (12,13). Apesar das manisfestações clínicas desta condição ocasionalmente iniciarem na infância, apenas uma pequena parte persiste com disforia do gênero até a vida adulta. Por este motivo, o momento adequado para iniciar a transição deve ser individualizado, mas as evidências atuais suportam a indicação de tratamento com esteroides sexuais preferencialmente a partir dos 16 anos (8,14).

Terapia hormonal cruzada

A TH é realizada com o objetivo de suprimir os hormônios sexuais endógenos e as características sexuais secundárias do sexo biológico, e manter os níveis hormonais e características sexuais do sexo de identidade. Em mulheres trans não gonadectomizadas, a terapia estrogênica é utilizada em associação com anti-andrógenos. Não há uma recomendação unânime com relação à escolha do anti-andrógeno, sendo a espironolactona e o acetato de ciproterona os mais utilizados (15). As mudanças físicas podem ser perceptíveis já nos primeiros meses de tratamento. Entre 3 e 12 meses após o início da TH cruzada, pode ocorrer redução das ereções espontâneas, redução dos pelos corporais, aumento do tecido mamário e redistribuição da gordura com predomínio ginoide (16). Nos homens trans, o tratamento é baseado no uso de diferentes formulações de testosterona intramuscular ou transdérmica, com o objetivo de atingir concentrações fisiológicas deste hormônio, compatíveis com o gênero desejado (17). Nos primeiros 6 meses de tratamento ocorre já, com frequência, interrupção dos ciclos menstruais, aumento do desejo sexual e pelos corporais, aumento da massa muscular e redistribuição da gordura corporal com predomínio androide. Em 30% dos indivíduos os ciclos menstruais não cessam com o uso da testosterona e a associação com um progestágeno pode ser necessária. Alterações na voz, clitoromegalia e alopecia podem ocorrer após o primeiro ano de tratamento (16,17).

Para muitos indivíduos, a CAS pode ser uma etapa importante para satisfação plena com o gênero desejado. A remoção das gônadas em homens e mulheres afeta a fertilidade de forma irreversível e a TH cruzada neste contexto é necessária como reposição hormonal em indivíduos com hipogonadismo permanente após a cirurgia. A má aderência ou insatisfação do indivíduo com a TH cruzada durante o processo de transição são parâmetros que contra-indicam o procedimento de redesignação sexual (8).

Esteroides sexuais e a massa óssea

Os esteroides sexuais atuam diretamente na aquisição e preservação da massa óssea em ambos os sexos (18). As diferenças na formação óssea periosteal entre homens e mulheres são consideradas um reflexo da ação estimulatória da

testosterona nos homens, e da ação inibitória do estrogênio nas mulheres. Durante a puberdade, a aquisição de massa óssea é aproximadamente 10% maior em meninos do que em meninas. Esta diferença ocorre principalmente devido à maior expansão periosteal, estimulada pela massa muscular e atividade física, maior tempo de crescimento puberal e exposição direta aos androgênios (19,20). A testosterona pode agir diretamente no tecido ósseo através do receptor androgênico ou indiretamente via aromatização em estradiol (18). Além disso, ao aumentar a massa muscular, age de forma indireta estimulando a formação óssea (21). Contudo, estudos em modelos animais mostram que baixas concentrações de estrogênio são essenciais para a ação androgênica, considerando que a inativação do receptor estrogênico α ou da aromatase resulta em menor crescimento ósseo radial (22,23). Dessa forma, o processo de expansão óssea periosteal tipicamente associado ao fenótipo masculino, pode ser estimulado, em parte, pelo estrogênio. O estrogênio é o principal regulador da homeostase óssea, atuando em todo o processo de remodelamento que inclui osteócitos, osteoblastos e osteoclastos. Favorece a formação óssea por reduzir a apoptose de osteócitos e osteoblastos. Por outro lado, diminui a reabsorção óssea, inibindo a osteoclastogênese e induzindo apoptose e menor diferenciação dos osteoclastos (24).

Em indivíduos transgêneros, a TH cruzada pode influenciar a massa óssea diretamente ou indiretamente através de alterações na composição corporal (25). O uso de terapia estrogênica em mulheres trans parece ter efeito favorável sobre o tecido ósseo (26,27), porém a supressão prolongada da testosterona pode estar associada a redução da massa e da força muscular (28). Alguns trabalhos mostram maior prevalência de baixa massa óssea e menor tamanho de osso cortical em mulheres trans quando comparadas aos controles homens (29-31), sendo alguns destes trabalhos em indivíduos virgens de tratamento (31,32). Estes achados indicam que outros fatores não hormonais podem influenciar a massa óssea destes indivíduos. A terapia androgênica, por outro lado, está associada ao aumento da massa muscular (33-35), o que poderia explicar o discreto aumento do diâmetro de osso cortical (33,36) e da massa óssea observado em alguns estudos (26,37) (Figura 1). Independente do gênero, a idade de início da TH possivelmente é um fator determinante no impacto sobre a massa óssea, assim como o uso regular da reposição hormonal, principalmente em indivíduos submetidos a CAS (20).

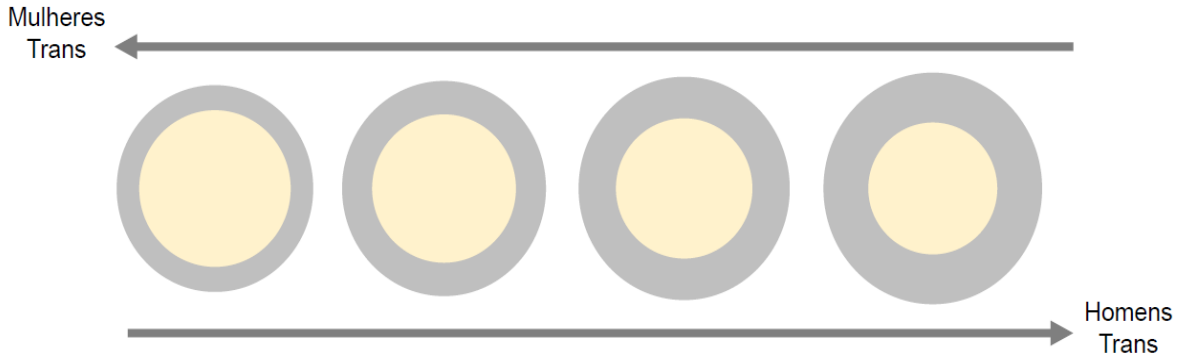

Figura 1: Adaptado de Van Caenegem and T'Sjoen (27)

TH cruzada e parâmetros de composição corporal

A composição corporal realizada por DXA é o método de escolha para avaliar os diferentes compartimentos corporais. A partir da aquisição de uma imagem de corpo total, permite a quantificação da massa magra e massa gorda do corpo inteiro e de regiões pré-definidas por linhas de referência. Além disso, é um método com baixo coeficiente de variação e mínima exposição a radiação (38).

Os esteroides sexuais são determinantes na distribuição de gordura e massa magra em diferentes regiões corporais. Desde a puberdade, as meninas apresentam acúmulo de gordura na região ginoide, enquanto os meninos apresentam maior desenvolvimento da massa muscular e concentração de gordura na região androide (39). Achados similares são observados com a TH cruzada em indivíduos transgêneros. Estas mudanças corporais nos compartimentos de massa gorda e massa magra permitem a melhor aceitação da imagem corporal, compatível com o sexo de identidade. Estudos em mulheres trans mostram que a terapia estrogênica está associada ao aumento da gordura corporal, com predomínio da gordura ginoide, e diminuição da massa magra. Efeito oposto é observado em homens trans durante a terapia androgênica, com redução da gordura ginoide e aumento da massa magra (35,40).

Com base nestas considerações, o objetivo deste estudo foi avaliar a massa óssea e os parâmetros de composição corporal de indivíduos com disforia de gênero submetidos a terapia hormonal cruzada.

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PARTE 1

Impact of cross-sex hormone therapy on bone mineral density and body composition in transwomen

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Impact of Cross-Sex Hormone Therapy on Bone Mineral Density and Body Composition in Transwomen

Running title: Impact of CSHT on BMD in transwomen

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Conflict of Interest Statement

The authors declare no competing interests.

Summary

Objective: Cross-sex hormone therapy (CSHT) has been associated with changes in bone and lean/fat mass. This study assessed BMD, appendicular lean mass (ALM), and total fat mass in transwomen undergoing CSHT.

Patients and Design: We evaluated 142 transwomen (mean age: 33.7±10.3 years; BMI: 25.4±4.6; 86.6% with previous CSHT) during the first three months of regular estrogen treatment (with or without anti-androgens). A reference group including 22 men and 17 cis women was also studied.

Measurements: Clinical and hormonal evaluation and dual energy X-ray absorptiometry (DXA).

Results: BMD was similar in trans and reference women, and lower at all sites in transwomen vs. men. Low bone mass for age was observed in 18% of transwomen at baseline vs. none of the reference women or men. ALM and total fat mass were positively correlated with L1-L4 BMD, explaining 14.9% of the observed variation in lumbar spine BMD and 20.6% of the variation in total femur BMD. ALM was similar in trans and reference women, and lower in transwomen vs. men. Total fat mass was lower in trans vs. reference women. Densitometry was repeated after a mean of 31.3±6.5 months in 46 transwomen. There was a significant increase in total fat mass and a significant decrease in ALM. BMD remained stable over time.

Conclusions: The fairly high prevalence of low bone mass in this sample of transwomen from southern Brazil seems to be related to lower ALM. Non-pharmacological lifestyle-related strategies for preventing bone loss could be beneficial for transgender women receiving long-term CSHT.

Key words

Transsexualism, cross-sex hormone treatment, bone mineral density, lean mass, fat mass, DXA

Introduction

Gender dysphoria is defined as the desire to live and be accepted as a member of the opposite sex, often accompanied by the wish to make the body congruent with the sex of identity through cross-sex hormone therapy (CSHT) and gender-affirming surgery. The two major goals of CSHT are to reduce endogenous sex hormone levels and the secondary characteristics of the individual's assigned sex and to maintain sex hormone levels consistent with the individual's gender identity[1].

Sex steroids are important determinants of bone acquisition and bone homeostasis. In men, testosterone stimulates the process of periosteal apposition during puberty, producing ~~a~~ greater cortical bone size and wider bones as compared to women[2,3]. Male to female transsexual individuals (transwomen) undergo estrogen therapy associated or not

with anti-androgens, reducing endogenous testosterone and inducing feminization. While some studies report that transwomen undergoing testosterone deprivation are at risk of low bone mass[4,5], other studies show preservation of bone mineral density (BMD) in the first years of treatment[6-8]. Loss of bone mass has been reported as more likely after gender-affirming surgery in transwomen who are less compliant with estrogen therapy[9]. However, there are few data regarding long-term CSHT effects on bone in transwomen, with contradictory results from most studies, which are also limited by their small samples[4,6,10,11].

Both lean and fat mass have been positively correlated with BMD. However, the relative contribution of each type of mass remains to be established[12]. The positive relationship between BMD and lean mass[13,14] reported by some studies is usually stronger than that detected for fat mass[15]. Low lean mass has been suggested as a risk factor for fracture in older adults independently of BMD or other risk factors, but the clinical utility of this indicator to predict relevant endpoints, such as fractures, is also unclear[16,17]. In turn, the relationship between fat mass and bone health is more controversial[18,19], but recent evidence suggests a positive association between the two[20].

Taking these aspects into consideration, the aims of this study were to assess BMD and body composition over time in transwomen undergoing CSHT, and to analyze the relationship of bone mass with body fat and muscle mass in this group.

Material and Methods

Subjects and study protocol

Transwomen were recruited from the outpatient endocrine clinic of the Gender Identity Program at the Hospital de Clínicas de Porto Alegre (HCPA), Brazil. Individuals receiving CSHT for at least 3 months and who signed the informed consent form were enrolled. Transwomen receiving CSHT who had already undergone gender-affirming surgery were also eligible.

Considering that participants might have used some CSHT for variable periods of time before the study, the baseline assessment was performed 3 months after the start of the standard Gender Identity Program CSHT. After enrollment, assessments were performed according to the Program's usual protocol: clinical follow-up every 3 months in the first year and twice a year after that, with laboratory tests performed every 6 or 12 months,

depending on individual clinical conditions. BMD and body composition are evaluated by DXA at 3 months. For the present study, a second DXA was performed in all women with at least 1 year of follow-up.

Thirty-nine individuals (22 men and 17 cis women) aged between 18 and 40 years were selected after advertisement in the hospital's home page and served as a reference group. This group underwent the same protocol described for transwomen. Blood samples of cis women were obtained at the follicular phase of the menstrual cycle.

CSHT is provided free of charge by the public health care system to participants of the Gender Identity Program (estradiol valerate 1-4 mg/d or conjugated equine estrogen 0.625-2.500mg/d associated with spironolactone 50-150mg/d or cyproterone acetate 50-100mg/d, depending on availability). A few transgender women received transdermal 17β-estradiol 0.5-2mg/d for individual clinical reasons. Dosages were individualized according to clinical response. Most of the participants who had already been submitted to gender-affirming surgery received estrogen-only treatment.

The study protocol was approved by the Ethics Committee at Hospital de Clínicas de Porto Alegre (University Hospital), and all participants signed an informed consent form before joining the protocol.

Hormone measurements

Venous blood samples were obtained after a 12-hour fast. Blood samples were collected between 8 a.m. and 10 a.m. Total testosterone levels were measured by chemiluminescence immunoassay (CLIA, Siemens Advia, Centaur XP), with sensitivity of 10 ng/mL and intra and interassay coefficient of variation (CV) of 3.3 and 7.5% respectively. Estradiol was measured by electro-chemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany), with assay sensitivity of 5.0 pg/mL and intra- and interassay CV of 5.7 and 6.4% respectively. SHBG was measured by CLIA (Immulite 2000), with sensitivity of 0.02 nmol/L and intra- and interassay CV of 5.3 and 6.6% respectively. Free androgen index (FAI) was estimated by dividing total testosterone (TT) (in nanomoles per liter) by sex hormone binding globulin (SHBG) (in nanomoles per liter)×100.

BMD and body composition

Anthropometric data including weight, height and body mass index were assessed at the first visit. BMD and body composition were assessed by dual-energy X-ray absorptiometry (DXA) using a Lunar Prodigy Primo device (Encore version 14.10, Radiation

Corporation, Madison, WI). BMD was measured in lumbar spine (L1-L4), femoral neck and total femur of the left side and expressed as g/cm^2. In the presence of artifacts, a right femur scan was performed. Z-score for BMD was calculated using age-matched controls from the National Health and Nutrition Examination Survey III study group (NHANES III). Male reference values were used for transgender women and cis men, and female reference values were used for cis women. The coefficient of variation for lumbar spine and femur was 0.022g/cm² (1.8%) and 0.033g/cm² (1.9%) respectively. DXA quality control is performed daily by the same technician, with variation < 2%. Low bone mass was considered as Z-score ≤ -2.0 SD for age. Appendicular lean mass (ALM), obtained by measuring lean mass in both arms and legs, and total fat mass (FM) were expressed as kg and acquired with measurements of the whole body.

Statistical analysis

Data were expressed as means ± standard deviation (SD) for variables with normal distribution and medians and interquartile range for variables with non-Gaussian distribution. Comparisons between groups were performed by one-way ANOVA followed by the Tukey post hoc test and the chi-square (χ^2) test for categorical variables. Variables without normal distribution were log-transformed for statistical analysis and back-transformed into their original units for presentation. Paired-sample t-test was used to compare variables from the same transgender women over time. Correlations were assessed using Pearson's correlation coefficient. Two multiple linear regression models were carried out with age, estradiol, total fat mass and ALM as independent variables and BMD of lumbar spine and total femur as dependent variables. The Statistical Package for the Social Sciences (SPSS version 18) (Chicago, IL, USA) was used for analysis. A p-value < 0.05 was considered to be statistically significant.

Results

We evaluated 142 transwomen receiving CSHT. Regarding skin color, 96% of participants were white and the remaining subjects were of mixed African/European ancestry. Regarding formal education, 54% had completed high school and 10% had a university degree. CSHT had been used previously by 123 (86.6%) participants for variable periods of time. Thirty-three (33%) participants had already undergone gender-affirming surgery. This group was older than those with no surgery (37.1±10.6 years vs. 32.6±9.9 years, p=0.029). Concerning other clinical variables, no differences were found between participants with or without surgery.

Table 1 shows baseline hormone levels, BMD, and body composition in transwomen and reference participants. Age and BMI were similar in both groups. Transwomen had intermediate median estradiol and serum TT levels when compared to reference men and women. SHBG and FAI levels were similar in trans and reference women. However, these levels were significantly lower in transwomen when compared to reference men. ALM was similar in transgender women as compared to reference women, but lower than in reference men. Conversely, total fat mass was lower in transwomen vs. reference women, and similar in transwomen and reference men.

Regarding bone mass, BMD was similar in trans and reference women at the three sites considered, but trans women had significantly lower L1-L4 BMD, femoral neck BMD, femoral neck Z-score, total femur BMD, and total femur Z-score in comparison to reference men. The frequency of low bone mass for age in transwomen was 18.3%, while in the reference groups all individuals had normal bone mass for age (p=0.001). A positive correlation was observed between ALM and L1-L4 BMD (r=0.327, p=0.0001) (Figure 1A), which remained significant after adjustment for TT (r=0.300, p=0.001). The correlation between total fat mass and L1-L4 BMD was also positive (r=0.334, p=0.0001) (Figure 1B), even after adjustment for estradiol (r=0.345, p=0.000).

Multiple linear regression analysis was performed to examine the independent contribution of age, estradiol, total fat mass, and ALM to BMD in transgender women (Table 2). In model 1, fat mass and ALM were independent predictors of L1-L4 BMD, explaining 14.9% of the variance at this site. In model 2, age and fat mass were predictors of total femur BMD, explaining 20.6% of the variance (Table 2).

In order to assess the effect of CSHT over time on BMD and body composition, a sub-sample of 46 transwomen with at least 1 year of follow-up underwent a second densitometry after a mean of 31.3±6.5 months (12 to 40 months). Estradiol and testosterone levels were similar to baseline in this subgroup. BMD also remained stable over time at all sites. A significant increase in total body fat was observed, along with a slight but significant decrease in ALM (Table 3).

Discussion

In the present study, transwomen had lower lumbar spine and femur BMD when compared to men. In addition, 18% of transwomen had low bone mass (z-score < -2 SD). Still, no changes in BMD were observed in a subgroup that was followed up for a mean of 31

months. BMD was also positively correlated with muscle and fat mass, which in turn contributed to variation in bone mass. These results provide novel evidence that bone mass may be, at least in part, influenced by changes in body composition secondary to CSHT.

Data regarding the effect of CSHT on BMD in transwomen are conflicting. While some studies report that estrogen therapy is able to maintain bone mass[21,22], others have observed a significant decrease in BMD despite CSHT[6,23]. In addition, some of the studies reporting an increase in bone mass with estrogen therapy were in fact performed over a short-term period of less than 2 years[2,10,24,25]. Indeed, Wiepjes et al[2] have recently described an increase in lumbar spine (+3.67%), total hip (+0.97%), and femoral neck (+1.86%) BMD in the presence of estrogen and antiandrogen therapy in a multicenter study including 231 transgender women undergoing CSHT for 1 year. Moreover, a systematic review and meta-analysis[26] **including** 392 transwomen revealed an increase in BMD at the lumbar spine, but not at femoral neck, after 12 and 24 months of estrogen therapy. The increased BMD observed in the first years of CSHT in those studies might have resulted from estrogen-mediated filling of the remodeling space, affecting the balance of osteoblast and osteoclast activity and suppressing bone turnover[8,27]. In the present study, bone mass remained stable over time, perhaps because most transwomen had already used CSHT, albeit at irregular doses and for irregular periods of time, before starting a standard treatment at our outpatient clinic.

Some studies with small sample sizes have shown a high prevalence of osteoporosis and osteopenia in transwomen after long-term CSHT. Wierckx et al[28] evaluated 50 transwomen after gender-affirming surgery and 10 years of regular estrogen therapy. In that group, the frequency of osteoporosis was 23.4% at the lumbar spine, 8.7% at femoral neck, 2.1% at the total hip, and 25.5% at the left radius. Another study[29] showed similar results after 15 years of CSHT. A retrospective analysis including 45 transwomen with mean age of 39.5 years reported that 75% were osteopenic. Also, T'Soen et al[4] evaluated 50 transwomen at least 3 years after the start of CSHT and 1 year after gender-affirming surgery. A prevalence of 26% of low bone mass at lumbar spine and 2% at the total hip was found, but no significant differences on hormone values were observed between subjects with a Z-score > or < -2.0. Taken together, data from these studies and the present work suggest that transgender women undergoing CSHT may be at risk for low bone mass, mainly after long-term hormone treatment.

Changes in body composition could exert a role in bone mass variation in transwomen during treatment. In this sense, the reduction of endogenous testosterone in transwomen could lead to loss of muscle and gain of fat mass, as reported by Lapauw et al[6]. Using DXA, those authors examined the body composition of 23 subjects treated with CSHT for 8 years. They found that total lean mass was 20% lower, and total fat mass was 30% higher, as compared to a male control group. Other studies showed similar results[21,25,28]. The main mechanisms underlying the adverse effect of muscle loss on bone status include decreased mechanical stimuli, since muscle mass has been shown to be an important factor in the acquisition of bone geometry in adulthood[17,30]. By promoting deprivation of the anabolic activity of testosterone, CSHT might cause an increase in fat mass and a decrease in muscle mass, leading to less bone surface strain and smaller bone size over time[6].

While in the present study we found a slight but significant decrease in ALM, a positive correlation between lumbar spine BMD and both ALM and fat mass was observed. These changes are expected as part of the female sexual transition, and may explain, at least in part, the impact of CSHT on BMD in transwomen. ALM and fat mass were independent predictors of lumbar spine BMD, explaining approximately 15% of the bone mass variation in this site. Regarding total femur BMD, age and fat mass were also independent predictors, explaining 20% of BMD variation at this site. These results are similar to those found by Ho-Pham et al[20], who observed that age and fat mass are positively associated with BMD in men and women. Considering the prevalence of 18% of low bone mass detected in our group of transwomen, the increase in fat mass observed may not be enough to prevent the bone loss that is possibly related to lower ALM. Another possible explanation for the high prevalence of low bone mass in our group of transwomen might be the presence of this condition prior to the start of CSHT[31], perhaps as a result of a low level of physical activity[32,33].

One strength of our study is the focus on a less well represented ethnic group, transgender women from southern Brazil. Also, all BMD and body composition data were measured using the same equipment and were analyzed by the same researcher (TMF), which increases the reliability of the results. Conversely, a limitation was the impossibility of recording the baseline BMD and body composition of participants before the start of CSHT, since most transwomen were already using some kind of CSHT when they entered the study protocol. Another limitation was the lack of data on physical activity, dietary calcium and vitamin D intake, and daily sun exposure. However, below a latitude of approximately 35° (which is the case of the city where the study was performed), UVB radiation is known to be sufficient for year-round vitamin D synthesis[34].

In conclusion, the prevalence of low bone mass for age was fairly high in this sample of transwomen from southern Brazil (18%). Lumbar spine BMD was lower than in reference men, but similar to that of reference women. These findings may reflect the lower ALM secondary to CSHT-suppressed levels of testosterone. Further long term studies are needed in order to determine the clinical relevance and the progressive nature of CSHT-related bone loss. Until then, monitoring of bone mass by DXA should be considered at any time in individuals who are not compliant with hormone therapy or who develop risks for bone loss. In low-risk individuals, screening can be performed at age 60 years. Intervals between DXA testing should be individualized according to clinical status. Non-pharmacological lifestyle-related strategies for preventing bone loss may benefit transgender women receiving long-term CSHT.

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Table 1. Baseline hormonal features and body composition of transwomen and reference cis women and men

Variable	Transwomen (n=142)	Cis women (n=17)	Men (n=22)	p
Age	33.70 (10.29)	33.29 (5.26)	30.77 (5.89)	0.408
BMI	25.37 (4.62)	26.58 (5.16)	26.32 (3.91)	0.434
TT (nmol/L)	1.17 (0.38-16.01)[a]	0.79 (0.38-1.07)[b]	16.39 (11.54-18.61)[c]	**<0.001**
SHBG (nmol/L)	63.55 (40.37-99.17)[a]	49.55 (32.27-70.87)[a,b]	27.20 (20.82-36.22)[b]	**<0.001**
FAI	2.27 (0.50-36.51)[a]	1.48 (1.08-2.53)[a]	56.88 (49.86-66.76)[b]	**<0.001**
Estradiol (pmol/L)	166.11 (69.09-267.98)[a]	232.74 (154.73-379.58)[a]	70.48 (52.86-104.26)[b]	**<0.001**
ALM	22.32 (3.95)[a]	22.01 (10.10)[a]	27.43 (10.88)[b]	**0.003**
Total fat mass (kg)	20.78 (10.07)[a]	27.42 (10.87)[b]	22.01 (10.10)[a,b]	**0.040**
L1-L4 BMD (g/cm²)	1.150 (0.160)[a]	1.210 (0.110)[a,b]	1.250 (0.130)[b]	**0.022**
L1-L4 Z-score	-0.3 (1.3)	0.2 (0.9)	0.2 (1.1)	**0.025**
Femoral neck BMD (g/cm²)	1.010 (0.170)[a]	1.020 (0.100)[a,b]	1.130 (0.110)[b]	**0.004**
Femoral neck Z-score	-0.2 (1.3)[a]	0.1 (0.8)[a,b]	0.5 (0.8)[b]	**0.027**
Total femur BMD (g/cm²)	1.010 (0.150)[a]	0.990 (0.080)[a]	1.140 (0.110)[b]	**0.001**
Total femur Z-score	-0.4(1.0)[a]	0.0 (0.6)[a,b]	0.3 (0.8)[b]	**0.003**
% Low bone mass (n)	18.3 (26)[a]	0 (0)[b]	0 (0)[b]	**0.001**

Values are expressed as means ± SD or medians and 25–75 inter-quartile range (one-way ANOVA - *post hoc* Tukey test) or percentage (absolute number) (χ^2 test); different superscript letters in each row indicate which groups differ statistically for the specific variable.

BMI: body mass index, TT: total testosterone, SHBG: sex hormone binding globulin, FAI: free androgen index, ALM: appendicular lean mass, BMD: bone mineral density.

Table 2. Multiple linear regression analysis of lumbar spine/total femur BMD *vs.* age, estradiol, total fat mass and appendicular lean mass

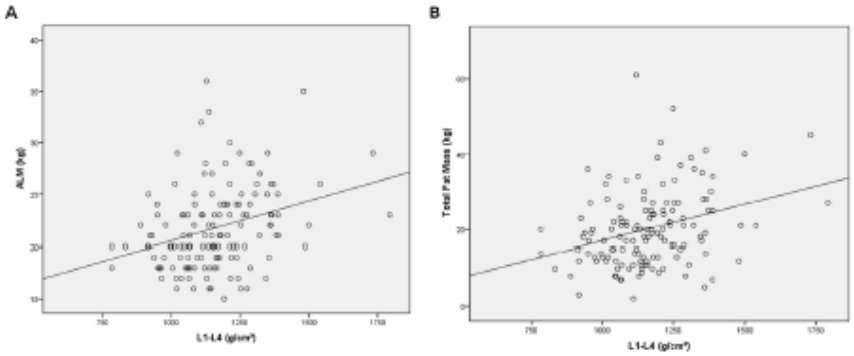
	B (95%CI)	p	R^2	Adjusted R^2
L1-L4 BMD vs.				
Age (years)	-0.002 (-0.005; 0.001)	0.165	0.177	0.149
Estradiol (pmol/L)	0.000 (0.000; 0.000)	0.369		
Total fat mass (kg)	0.005 (0.000; 0.000)	**0.001**		
Appendicular lean mass (kg)	0.009 (0.001; 0.016)	**0.022**		
Total femur BMD vs.				
Age (years)	-0.004 (-0.007; -0.002)	**0.001**	0.233	0.206
Estradiol (pmol/L)	0.083 (0.000; 0.000)	0.356		
Total fat mass (kg)	0.006 (0.000; 0.000)	**0.000**		
Appendicular lean mass (kg)	0.001 (-0.007; 0.009)	0.774		

Table 3. Hormone features and body composition at baseline and after 31 months of follow-up in 46 transwomen

	Baseline	31 months	p
Age (years)	33.70 (10.29)		
BMI	25.66 (4.16)	26.22 (3.96)	0.082
TT (nmol/L)	1.17 (0.48-15.87)	0.86 (0.31-13.31)	0.526
SHBG (nmol/L)	66.4 (39.5 - 95.5)	57.0 (33.5 - 73.6)	0.772
FAI	1.61 (0.51 - 22.3)	5.58 (0.41 - 43.6)	0.407
Estradiol (pmol/L)	209.25 (94.71-358.66)	128.12 (35.61-213.65)	0.082
ALM (kg)	22.3 (3.7)	21.5 (3.3)	**0.004**
Total fat mass (kg)	22.1 (8.7)	24.8 (9.4)	**0.003**
L1-L4 BMD	1.200 (0.190)	1.190 (0.180)	0.106
L1-L4 Z-score	-0.0 (1.5)	-0.2 (1.4)	0.118
Femoral neck BMD	1.030 (0.160)	1.030 (0.170)	0.409
Femoral neck Z-score	-0.1 (1.2)	-0.1 (1.2)	0.938
Total femur BMD	1.040 (0.160)	1.030 (0.160)	0.536
Total femur Z-score	-0.2 (1.0)	-0.3 (1.0)	0.711

Values are expressed as means ± SD or medians and 25–75 interquartile range (paired-sample t-test).

BMI: body mass index, TT: total testosterone, SHBG: sex hormone binding globulin, FAI: free androgen index, ALM: appendicular lean mass, BMD: bone mineral density.



PARTE 2

Bone mass effects of cross-sex hormone therapy in transgender people: updated systematic review and meta-analysis

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Bone mass effects of cross-sex hormone therapy in transgender people: updated systematic review and meta-analysis

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ABSTRACT

Context: The impact of long-term cross-hormone therapy (CSHT) in transgender men and women is still uncertain. **Objective:** To perform a systematic review and metanalysis and update the evidence regarding the effects of CSHT on bone mass density (BMD) in transgender men and women. **Data sources:** MEDLINE, Cochrane Central Register of Controlled Trials, and EMBASE were searched for studies published until August 2018. **Study selection:** Of 10,849 studies, 25 were selected for systematic review. All included individuals aged >16 years receiving CSHT with BMD assessment by dual energy X-ray absorptiometry (DXA). **Data extraction:** Data on BMD, CSHT, and clinical factors affecting bone mass were collected. A National Institutes of Health Scale was used to assess the quality of studies. **Data synthesis:** Nineteen studies were meta-analyzed (487 trans men and 812 trans women). In trans men, mean BMD difference vs. natal women was not significant for any site in both cross-sectional and before-after studies. In trans women, mean BMD difference was not significant vs. natal men at femoral neck, total femur, and lumbar spine in cross-sectional studies; before-after studies reported a slight, but significant increase in lumbar spine BMD after 12 and ≥ 24 months of treatment. **Conclusions:** The available evidence indicates that long-term CSHT does not affect BMD in transgender men. In transgender women, only lumbar spine BMD seemed to be affected after 12 and ≥ 24 months of CSHT. This evidence is of low to moderate quality, and should be confirmed by further studies with longer follow-ups and standardized therapy.

PRÉCIS

The present meta-analysis shows bone mass to be preserved at least in the short term in transgender individuals receiving cross-sex hormone therapy.

INTRODUCTION

Transgender people experience a deep and persistent sense of incongruence between their gender of identity and the sex attributed to them at birth, with significant distress lasting for at least 6 months (1-3). Hormone therapy and gender affirming surgery (GAS) are the main therapeutic strategies for gender transition. Cross-sex hormone therapy (CSHT) suppresses gonadal hormones and secondary sex characteristics of the biological sex while inducing body characteristics of the gender of identity (4). Although gender transition has been associated with improvement in mental health and other areas of functioning (4-6), the full long-term effects of CSHT are still uncertain.

Sex steroids are major determinants of bone homeostasis. In boys, during puberty, testosterone stimulates periosteal apposition, leading to increased bone width and size compared to girls, despite the similar cortical thickness (7). In turn, estrogen plays a main regulatory role in bone metabolism in both women and men, inhibiting bone remodeling. Estradiol acts on the lifespan of osteoblasts, inhibiting apoptosis and increasing the functional capacity of individual osteoblasts. It also induces apoptosis and decreases cellular differentiation by direct effects on osteoclasts (8). Estrogen deficiency is associated with an imbalance between bone resorption and bone formation that is linked to osteoblast apoptosis, oxidative stress, and osteoblastic NF-κB (RANKL) activity.

Not much is known about the effects of CSHT on bone mass in transgender individuals (9). Recent data from transgender men (FtM) and women (MtF) receiving hormone therapy have shown an increase in bone mineral density (BMD) after 12 months of treatment (10). Another study on long-term testosterone therapy reported larger cortical bone size in trans men vs. natal females (11). Conversely, trans women receiving estrogen therapy may lose lean mass in association with androgen deprivation, which over time can lead to smaller bones (12) and higher prevalence of low bone mass (13, 14).

To date, few studies evaluating the impact of CSHT on bone mass have been published, and a definitive conclusion has not been reached. A previous meta-analysis including 13 studies has assessed the relationship between hormone therapy and BMD. The results suggested that BMD was not significantly different in trans men, and that lumbar spine BMD was increased in transwomen with CSHT (15). Since then, however, new evidence has become available. Therefore, the aim of the

present systematic review and meta-analysis was to update the available evidence regarding the effect of CSHT on BMD in transgender men and women.

MATERIALS AND METHODS

This study was performed in accordance with Cochrane Collaboration guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (16).

Eligibility criteria, search strategy and study selection

The research question was developed using the PICOS strategy: the population (P) was defined as transgender individuals; the intervention (I) was defined as CSHT; the comparison group (C) corresponded to natal women and men with no gender incongruence; the outcome (O) was defined as BMD assessed by dual energy X-ray absorptiometry (DXA); and the study design (S) was defined to include non-interventional case control, cross-sectional, or cohort studies with at least 10 participants in each group.

MEDLINE, Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed through Wiley Science) and EMBASE were searched for studies published until August 2018. We also searched http://ClinicalTrials.gov to retrieve RCTs with unpublished results. The following medical subject headings (MeSH) were used in the search: bone AND transsexualism OR “transgender person” OR “person, transgender” OR “persons, transgender” OR “transgender persons” OR “transgender” OR “transgenders” OR “transgendered persons” OR “person, transgendered” OR “persons, transgendered” OR “transgendered person” OR “transsexual persons” OR “person, transsexual” OR “persons, transsexual” OR “transsexual person”. There were no year or language restrictions. Studies with children and adolescents under 16 years of age were not included. Prior GAS was not an exclusion criterion.

In case multiple reports of the same study were identified, the most complete report was chosen. If the abstracts did not provide enough information about inclusion and exclusion criteria, the full text was retrieved for evaluation.

Titles and abstracts of all articles retrieved were independently reviewed by two investigators to assess eligibility of the studies for inclusion in the systematic review and meta-analysis (T.M.F. and T.R.S.). The selected articles were read in full for confirmation of eligibility and data extraction.

Disagreements were resolved by consensus or by consultation to a third reviewer (P.M.S.). If the required data were not located in the published article, authors were contacted to provide the missing information.

Data extraction and quality control assessment

The following data were extracted from each study: name of first author and study group, publication year, country, study design, number of participants, age, body mass index (BMI), smoking, alcohol consumption, physical activity, serum vitamin D levels, calcium intake, use of calcium and vitamin D supplements, duration of CSHT treatment, previous (GAS), duration of follow-up, bone mineral density (g/cm^2), and T-score and Z-score for BMD at various sites and CSHT duration. Exclusion criteria for each study, where available, were also collected. DXA data of the forearm, total femur, femoral neck, and lumbar spine were extracted as well as the type of equipment and manufacturer.

A National Institutes of Health (NIH) scale (retrieved September, 2018, from: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools) was used to assess the quality of before-after (pre-post) and cross-sectional studies included in the meta-analysis. This scale includes items for evaluating potential flaws in the study methods or implementation, covering sources of bias, confounding, study power, the strength of causality in the association between interventions and outcomes, and other factors.

Statistical analysis

Metanalyses were performed separately for each outcome using mean differences (MDs) in order to evaluate CSHT effects. When standard deviations were missing, conservative imputations were made using the biggest standard deviation (SD) observed in the other studies for the same outcome. Mean differences were pooled using random effects models with DerSimonian and Laird variance estimator. The results were stratified by study design (cross-sectional or before-after) and follow-up time (12 and ≥24 months). I^2 statistics and the Cochran Q test were used to assess heterogeneity among studies. All statistical tests were two-tailed and significance was defined as $p< 0.05$. Statistical analyses were performed with R version 3.5.0 (R Foundation for Statistical Computing).

RESULTS

Study Selection

The primary search identified 10,849 articles. After title and abstract screening and exclusion of duplicates, 25 potentially eligible studies were retrieved for full-text analysis. Nineteen were included in the qualitative review (Figure 1) and also in the meta-analyses (10-13, 17-31). Three studies presented both cross-sectional and before-after data (13, 21, 29); one of them was considered in the meta-analyses of both designs (13). The other two were included only in before-after analyses, because participants were not using CSHT at the baseline evaluation (21, 29).

Therefore, six cross-sectional studies analyzing the use of hormone therapy in transgender individuals versus controls (11-13, 17-19), and 14 studies evaluating BMD in transgender individuals before and after CSHT (10, 13, 20-31) were meta-analyzed.

Description of the studies

Table 1 summarizes the characteristics of the six cross-sectional studies and Table 2 describes the 14 before-after CSHT studies.

All 19 studies included trans men receiving CSHT from 12 months to 18 years and/or trans women receiving CSHT from 12 months to 16 years. Eight studies had a control group (11-13,17-21), corresponding to natal men for comparisons with trans women and natal women for comparisons with trans men. Before-after studies were assigned good (10, 21, 27) and fair (13, 20, 22-26, 28-31) NIH quality assessment scores, whereas all cross-sectional studies were scored as fair (11,12,17-19).

Hormone dosages and formulations are described in Tables 1 and 2, stratified by the identity gender. In studies with trans women, the most frequent CSHT was cyproterone acetate with oral estradiol (valerate, conjugated equine estrogen or ethinylestradiol) or transdermal estradiol in both cross-sectional studies (12,13,17,19) (Table 1) and before-after studies (10,21-26,30) (Table 2). Two studies used oral and parenteral contraceptives as well oral estrogens (17,19). Another two studies used spironolactone as antiandrogen therapy added to estrogens (13,25), and four studies used GnRH analogues associated with estrogen therapy (22,23,26,30). Cross-sectional (11,18) (Table 1) and before-after studies (10,20,24,25,27-29,31) (Table 2) with trans men used parenteral testosterone

esters or testosterone undecanoate. Three studies also included patients using transdermal testosterone (11,10,31), and one study included patients with oral testosterone (25).

Six studies included transgender women (12,13,24) and/or men (11,18,24,28) who had previously undergone GAS. Most before-after studies evaluated exclusively transgender women (10,21-26,29,30) or men (10,20,24,25,27,29,31) without prior CSHT. Other exclusion criteria identified in several studies were use of glucocorticoids or bisphosphonates, renal or hepatic disease, alcohol abuse, bone diseases, or severe comorbidities. DXA assessments were performed using equipment manufactured by Hologic, Norland, or GE medical systems.

Additional clinical data, such as calcium intake, serum vitamin D levels, calcium/ vitamin D supplements, smoking habit, alcohol consumption, and physical activity, were reported in some studies (supplemental table 1 and 2). The reported rate of alcohol consumption varied from 4.6% to 75% depending on the criteria used – > 7 drinks/week (10) or casual consumption (26). In four studies, alcohol abuse was an exclusion criterion (11,17,18,29), and one study adjusted the results for alcohol consumption (10). Smoking prevalence varied from 12% (11) to 77% (31), and was higher than 50% in four studies (24,26,28,31). Two studies made adjustments in BMD data for cigarette smoking (10,12). Calcium intake was reported in only three cross-sectional studies (12,17,19), and areal BMD was adjusted for calcium intake in one study (12). Vitamin D levels varied from 11.5 (31) to 38.5 ng/mL (18) in trans men, and from 16 (21) to 23 ng/mL (12) in trans women. Only one study adjusted bone mass for vitamin D status in trans women (21). Regarding physical activity, different criteria were used in trans men and trans women. Among those using Baecke's questionnaire, the scores ranged from 2.68 (12) to 8.9 (20). Some studies presented BMD results already adjusted for weight and/or height (11,17, 19, 20).

Data synthesis and meta-analyses

A total of 812 trans women (MtF) and 487 trans men (FtM) were evaluated in the cross-sectional and before-after studies. Data were analyzed regardless of the dose or route of hormone therapy, since this information was not provided in most studies. Also, because each variable relating to Z-scores and T-scores for different sites and durations of CSHT (11-13,17,19,23,27) was presented

by one single study, we were unable to meta-analyze these data. No study reported data on osteoporotic fractures.

Sixteen studies were performed in European countries. The only study from the U.S. included 15 trans men (28). Two studies from Asia and Latin-America included 28 and 142 trans women respectively (13,19).

MtF

In trans women, the follow-up time in before-after studies varied from 12 to 45.5 months. In cross-sectional studies, CSHT time varied from 5 to 16 years. In at least two studies, mean age was greater than 40 years (12,17). In two studies, mean BMI was slightly higher than 25kg/m² (25.3kg/m² and 26.0kg/m²) (13,17). Figure 2 presents the meta-analysis of BMD changes at different sites in transgender women receiving CSHT of various durations vs. natal men (cross-sectional studies). BMD was not significantly different at femoral neck (MD=0.02; 95% CI: –0.12; 0.16, p=0.753), total femur (MD= –0.08; 95% CI: –0.23; 0.06, p=0.258), or lumbar spine (MD= –0.01; 95% CI: –0.13; 0.10, p=0.806) with ≥24 months of CSHT. Each analysis included three or four studies, with high between-study heterogeneity (I^2=95-97%). In two of these studies, high doses of estrogen or contraceptive pills (up to 4 tablets/day) were used (17, 19). In the other two, around one third (13) or all participants (12) had previously undergone GAS procedures. Regarding before-after studies (Figure 3) BMD values were not significantly different in total femur after 12 (MD=0.01; 95%: –0.01; 0.03, p=0.465) and ≥24 months (MD=0.00; 95% CI: –0.04; 0.04, p=0.950), or in femoral neck after 12 (MD=0.01; 95%CI: 0.00; 0.03, p=0.121) and ≥24 months of CSHT (MD=0.01; 95%CI: -0.01; 0.04, p=0.315), with no heterogeneity between studies (I^2=0% for all analyses). In turn, meta-analysis of lumbar spine BMD showed slightly positive mean differences at 12 months (MD=0.04; 95% CI: 0.02; 0.06, p=0.0001), I^2=0%) and ≥24 months (MD=0.04; 95% CI: 0.00; 0.07, p=0.036) I^2=19%) of hormone therapy (Figure 3).

FtM

In cross-sectional studies with trans men, age varied from 37 to 47 years, and duration of CSHT from 9.9 to 18 years. Compared to cross-sectional studies, participants of before-after studies were younger (24 to 37 years) and had shorter time of CSHT use (12 to 38 months). Mean BMI was normal in most studies, with the highest being 25.67 kg/m² (18). Meta-analysis of cross-sectional studies with trans men (Figure 4) receiving CSHT for ≥24 months showed that BMD was not significantly different vs. natal women at femoral neck (MD=0.05; 95% CI: –0.09; 0.20, p=0.468) or lumbar spine (MD= –0.02; 95% CI: –0.06; 0.03, p=0.460). Only two studies were included in these analyses, with 85 trans men and 85 controls. High (I^2=95%, femoral neck BMD) and low (I^2=19%, lumbar spine BMD) heterogeneity was found between these studies (Figure 4). The meta-analysis of before-after studies (Figure 5) shows that femoral neck BMD did not differ significantly in trans men before or during androgen treatment for 12 (MD= –0.00; 95%CI: –0.02; 0.02, p=0.952) or ≥24 months (MD= 0.03; 95%CI: –0.02; 0.07, p=0.226). Similar results were found at total femur after 12 months of CSHT (MD=0.01; 95% CI: –0.01; 0.03, p=0.342), and at lumbar spine after 12 (MD= –0.01; 95% CI: -0.01; 0.02, p=0.378) or ≥24 months (MD= –0.01; 95%CI: –0.05; 0.04, p=0.759), with no heterogeneity between the studies at ≥24 or 12 months.

A sensitivity analysis excluding before-after studies with transgender women (12,13,17, 19) and men (11,18,28) having previous CSHT did not change the results obtained (data not shown).

DISCUSSION

In this meta-analysis including 19 cross-sectional and before-after studies with a total of 487 trans men and 812 trans women, hormone therapy had a neutral effect on BMD at all sites evaluated, except for the lumbar spine of trans women, where a modest but significant increase in bone mass was detected. Even though all the studies considered were observational, including mostly small samples, the evidence from this meta-analysis indicates that BMD is preserved in transgender individuals during CSHT.

Until now, only one meta-analysis of the effects of CSHT on BMD in transgender individuals has been published (15). That search was conducted for a period ending in April 2015; 13

studies were selected, with 392 MtF aged 14.9 to 43 years and 247 FtM aged 15 to 33.1 years. The authors concluded that hormone therapy did not appear to be associated with significant changes in BMD in FtM individuals, whereas in MtF an increase in BMD was observed in the lumbar spine. Fracture data were not reported. Since then, new articles have been published (10,13,18,26), some of them with larger samples and longer follow-up periods, which were included in the present updated meta-analysis.

Estrogen is considered to be a principal regulator of skeletal homeostasis in both men and women. It is involved in the synthesis of various cytokines and growth factors, affecting the balance of osteoblast and osteoclast and suppressing bone turnover (32). Thus, estrogen therapy in MtF individuals is expected to be associated with increased and/or preserved bone mass, in line with the present results. The present analysis of cross-sectional studies indicated that CSHT does not affect BMD. However, most studies had a small sample, with large variation in mean age (24.1 to 43.0 years). In addition, analysis of the influence of factors that could affect BMD, such as physical activity and vitamin D status, was limited by the lack of this information in many studies, or because definition criteria for these factors were different among studies. However, the two studies reporting an unfavorable impact of estrogen therapy on BMD in trans women did not describe exclusion criteria and may therefore have included individuals with health impairment (12,13). In turn, the studies with favorable results regarding estrogen therapy used high doses in formulations such as oral and injectable contraceptives (17,19).

Interestingly, some observational studies found higher prevalence of low bone mass in trans women. While some of these studies did not present BMD values and were not included in the present meta-analyses, they reported a prevalence of osteoporosis of around 25% in trans women with long-term CSHT (14,33). More recently, the results of another study by our group (13) were in line with these earlier studies, showing a prevalence of 18.3% of low bone mass in MtF individuals after long-term CSHT, while no cases where observed in male or female controls (13). Also, Lapauw et al. (12) found a prevalence of 35% of low bone mass after a mean of 96 months of estrogen therapy. The studies reporting osteoporosis/low bone mass prevalence >25% included trans women with previous

GAS followed for 5 (12,14) to 6.3 years (33) after the procedure. In our experience, hormone therapy is sometimes irregular, involving poor adherence, which may affect BMD, especially after GAS.

It should be noted that studies evaluating bone mass status in trans women used male reference values in DXA analysis, since all individuals experienced normal pubertal development, with the usual effects on bone size and geometry. This may have influenced the results of studies, overestimating the prevalence of low bone mass in transgender women. In this sense, research with trans women and longer follow-ups, with standardized estrogen therapy and larger samples, is needed to obtain more robust evidence on the impact of CSHT on bone mass in the long term.

Regarding the effects of CSHT in FtM individuals, the present results show that testosterone therapy does not impact bone mass, with preservation of BMD up to 3 years after the start of CSHT in before-after studies and after 9.9 to 18 years of testosterone treatment in cross-sectional studies. These data reflect the anabolic effect of testosterone, which acts directly on androgen receptors or indirectly through aromatization to estradiol. In fact, despite testosterone being the predominant sex steroid in natal men, bioavailable estradiol levels are better correlated with male BMD than testosterone (34). In this sense, in a study comparing the addition of an aromatase (letrozole) or 5α-reductase (dutasteride) inhibitor to testosterone therapy, Meriggiola et al. (35) have shown that bone mass was significantly affected by the inhibition of aromatization, while the 5α reductase group produced results that were similar to those of testosterone therapy alone. That study was not included in the present meta-analysis because of the small sample size in each group (n=5). In addition, recently published data (10) showed a larger increase in BMD in trans men at postmenopausal age compared with other age groups, possibly because estradiol levels were low at baseline and increased with testosterone aromatization.

It has been suggested that testosterone also plays an indirect role in bone health, by acting on the maintenance of balance and muscle strength (36). Muscle mass is one of the main triggers of periosteal apposition, leading to larger periosteal circumference (37). Studies in transgender men evaluating bone mass by pQCT, a technique that allows assessment of bone size, showed an increase in volumetric BMD (11,20) and larger endosteal and periosteal bone circumference (11) after androgen therapy. Also, in animal models, estrogens stimulate, rather than inhibit, periosteal apposition. Low estrogen concentrations may decrease the mechanostat set point, which could

indirectly increase bone sensitivity to androgens (36). In transgender men treated with testosterone, studies show an increase in lean body mass (11,20, 27, 31, 38) and strength (11,20), which in turn may be associated with bone mass maintenance. These data may, at least in part, provide a mechanistic basis for the evidence generated by this meta-analysis regarding the impact of CSHT on preserving bone mass in transgender men.

Regarding limitations, the present study included observational studies, most of which had small samples, often using different types and routes of administration of hormone therapy. Also, BMD data were collected with various types of equipment, from different- manufacturers, and were not adjusted for variables known to affect bone tissue, such as vitamin D levels and physical activity. However, until fracture data become available, these studies represent the best available evidence on the impact of CSHT on bone mass.

In conclusion, the available evidence indicates that long-term CSHT does not affect BMD in transgender men. In transgender women, no changes in femoral BMD were found, and an increase in lumbar spine BMD was observed after 12 and ≥ 24 months of CSHT. The evidence produced is of low to moderate quality and further studies with longer follow-up times and standardized CSHT are needed in order to confirm these data in trans women.

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Figure 1

PRISMA flow diagram of the study selection process.

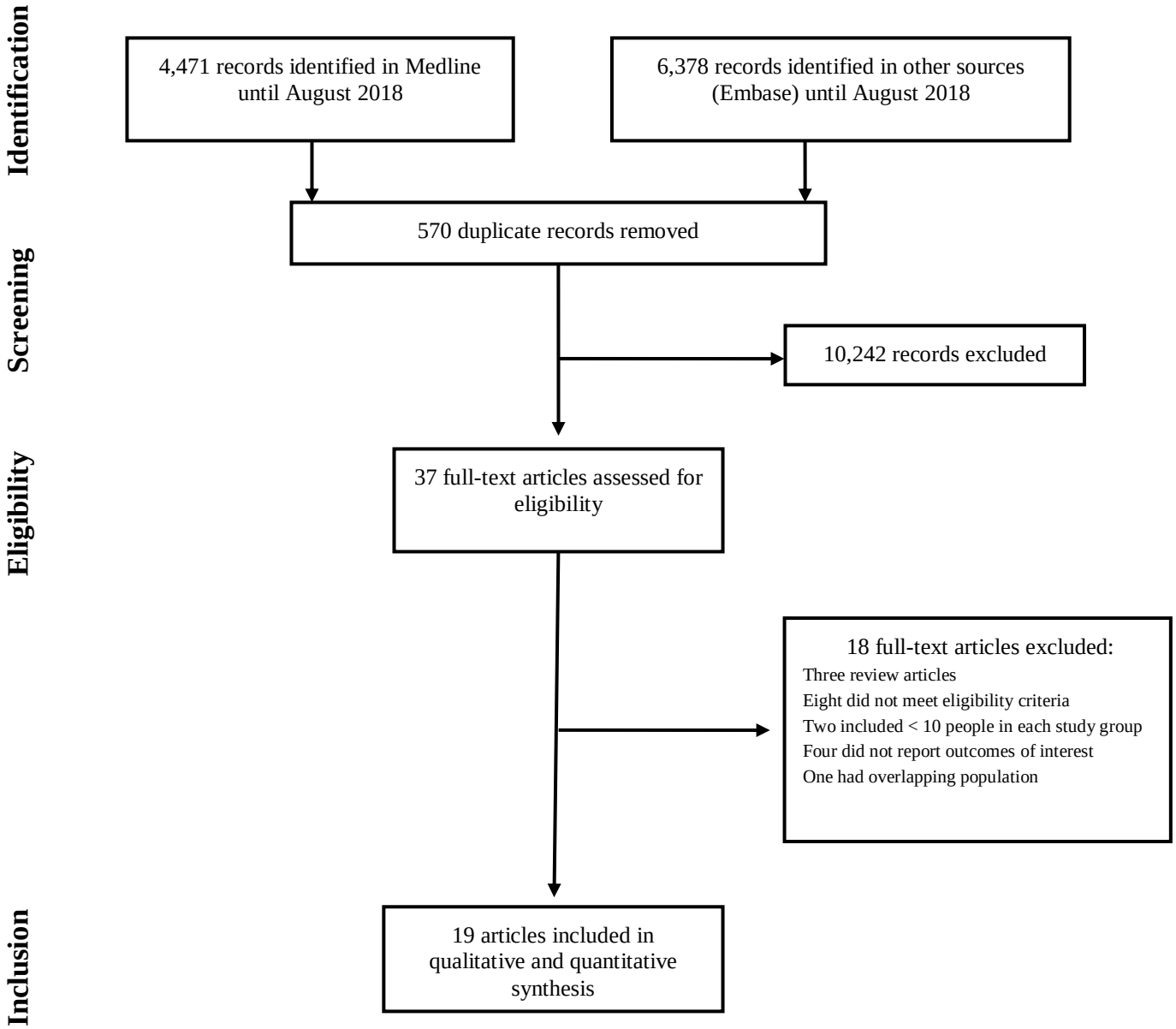

Figure 2

Forest plot showing BMD in cross-sectional studies with transgender women and control natal men

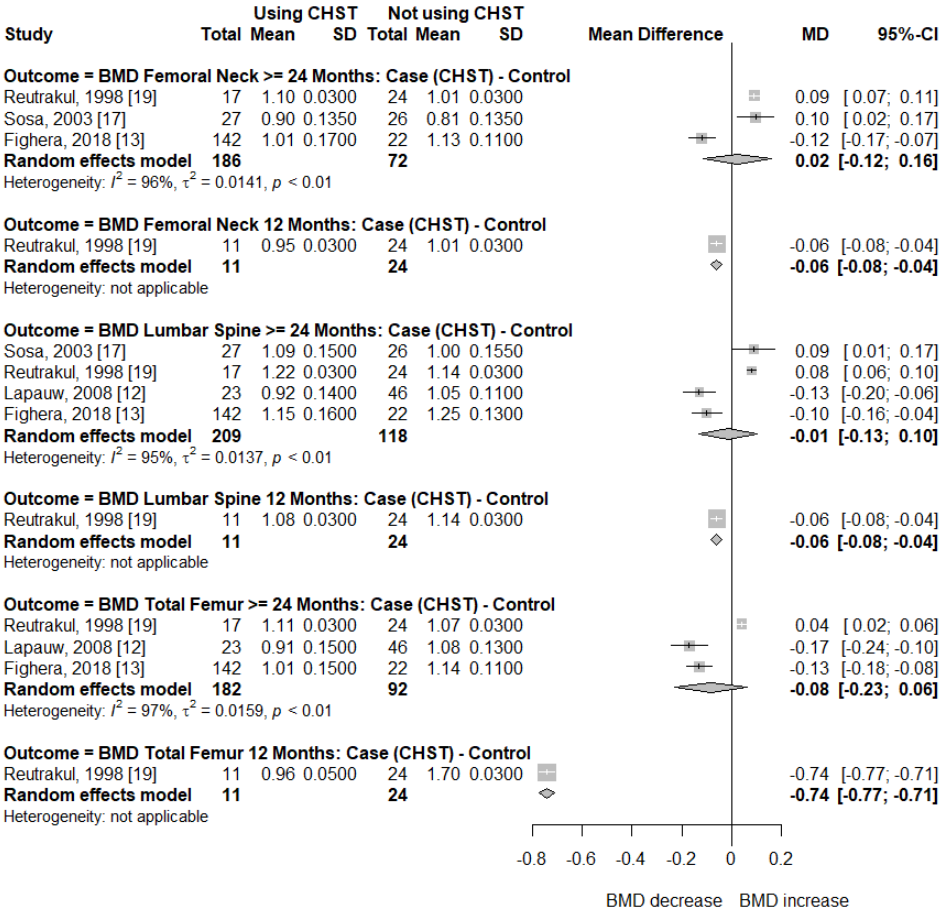

Figure 3

Forest plot showing BMD in before-after CSHT studies with transgender women.

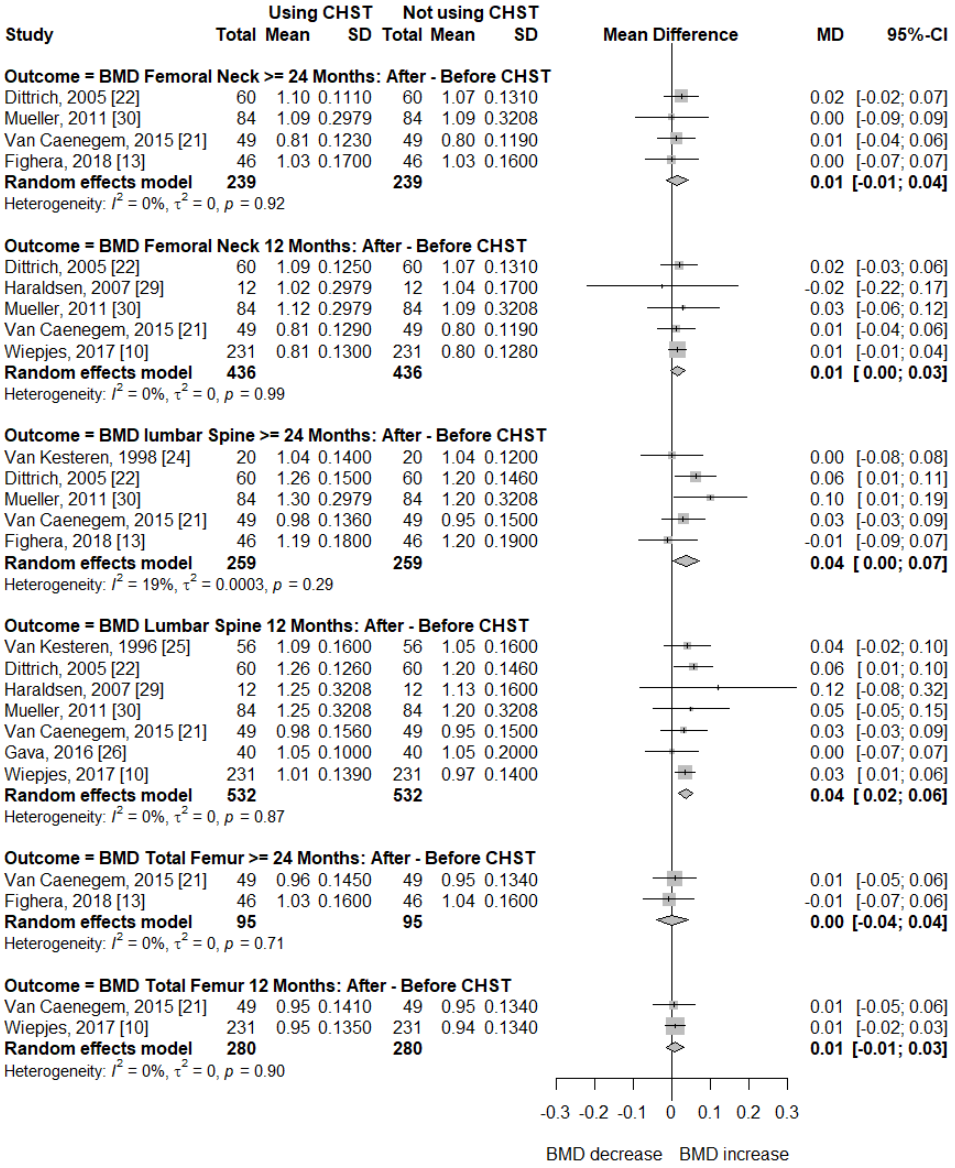

Figure 4

Forest plot showing BMD in cross-sectional studies with transgender men and control natal women.

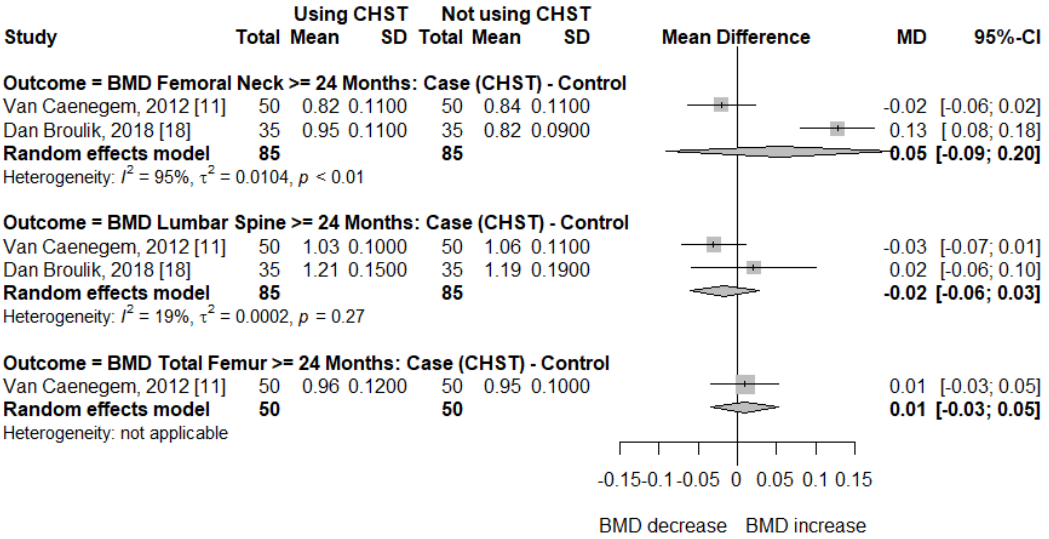

Figure 5

Forest plot showing BMD in before-after CSHT studies with transgender men.

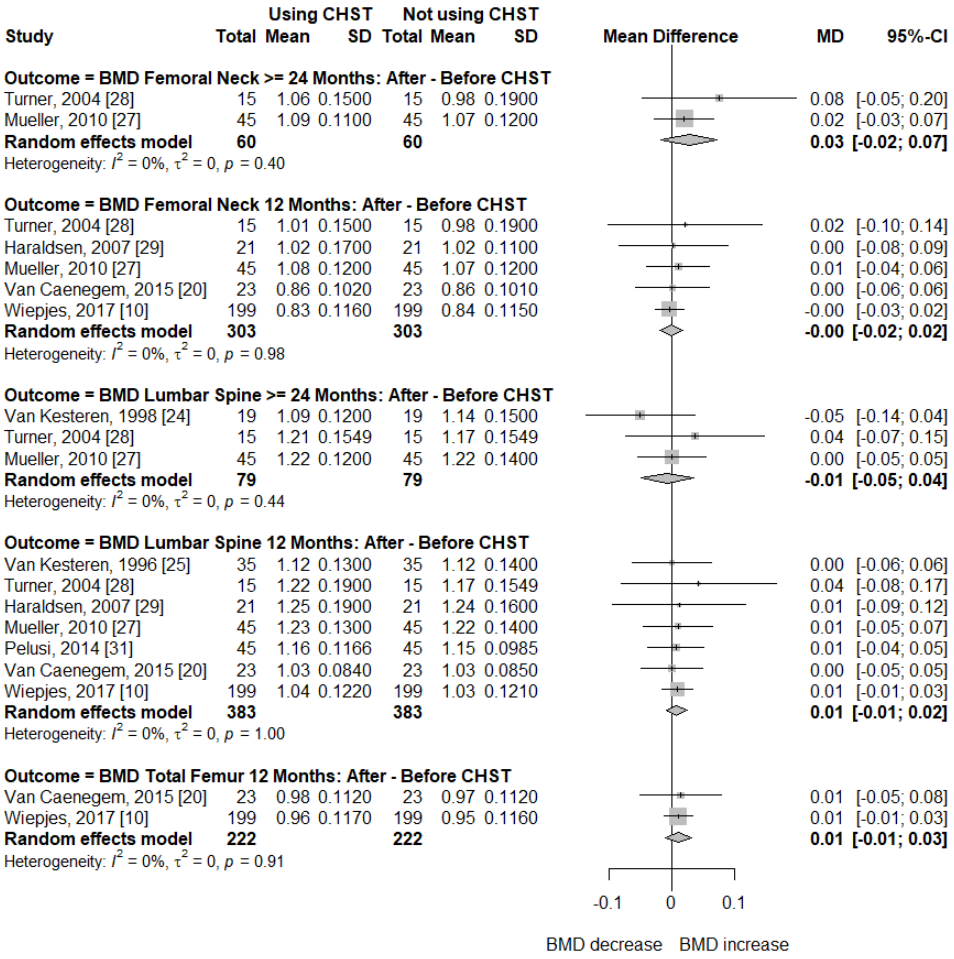

Table 1

Characteristics of the cross-sectional studies included in the systematic review

Study	Country	Comparison group	Duration of exposure (months)	N	Age (years)[a]	BMI[a]	post-GAS (%)	Intervention
FtM								
Van Caenegem et al. 2012 (11)	Belgium	Natal women	119 (9-264)	50	37 ± 8	24.8 ± 3.8	100	Testosterone esters every 2-3 weeks or Testosterone undecanoate 1000mg every 12 weeks or Transdermal testosterone 50mg/day
Broulik et al. 2018 (18)	Czech Republic	Natal women	216 ± 36	35	47.22 ± 4	25.67 ± 3.73	100	Testosterone isobutyrate 25 mg IM every week, or Testosterone propionate 250 mg every third week IM or Testosterone undecanoate 4 × 40 mg daily
MtF								
Reutrakul et al. 1998 (19)	Thailand	Natal men	13.9	11	21.2 ± 1.1	NA	0	Estradiol valerate 10mg/IM 1 to 4/month or Mestranol 0.05mg+norethisterone 1mg/day or
			59.8	17	24.1 ± 0.8	NA	0	Ethinylestradiol and levonorgestrel or cyproterone 1-4 tablets/day or Conjugated equine estrogen 1.25mg 1-2 tablets/day
Sosa et al. 2003 (17)	Spain	Natal men	201 (36-420)	27	43.0 ± 7.7	26.0 ± 4.7	0	Ethinyl estradiol + cyproterone acetate or levonorgestrel or Oral conjugated equine estrogen or Depot estrogens (estradiol valerate or mestranol + norethisterone)
Lapauw et al. 2008 (12)	Belgium	Natal men	96 (48-240)	23	41 ± 7.0	24.4 ± 5.0	100	Cyproterone acetate 50-100mg/day +ethinylestradiol 25-50ug/day. After surgery: ethinylestradiol 25-50ug/day, estradiol valerate 2mg/day, conjugated equine estrogens 1.25mg/day or transdermal estradiol.
Fighera et al. 2018 (13)	Brazil	Natal men		142	33.70 ± 10.29	25.37 ± 4.62	33	Oral estradiol valerate, 1-4 mg/day or Transdermal 17β estradiol 0.5-2.0mg/day or Conjugated equine estrogen 0.625-2.500mg/day with spironolactone 50-100mg/day or cyproterone acetate 50-100 mg/d. After GAS only estradiol was used.

[a] mean and SD; N: number of participants; GAS: gender affirming surgery

Table 2

Characteristics of the before and after CSHT studies included in the systematic review

Study	Country	Duration of exposure	N	Age[a] (years)	BMI[a]	post-GAS (%)	Intervention
FtM							
Van Kesteren et al. 1996 (25)	Netherlands	12 months	35	25 (16-40)	23 (17-32)	0	Testosterone esters 2/ 2 weeks IM (Sustanon 250mg or Testoviron 180mg) or Testosterone uandecanoate 160mg/d (oral)
Van Kesteren et al. 1998 (24)	Netherlands	38.2 months (28-53)	19	25 (16-39)	22.1 ± 2.7	100	Testosterone esters 250 mg every 2 weeks. After GAS testosterone IM every 2-3 weeks
Turner et al. 2004 (28)	USA	24 months	15	37.0 ± 3.0	NA	33.3	Testosterone esters IM (70·7 weekly)
Haraldsen et al. 2007 (29)	Norway	12 months	21	25.1 ± 4.8	NA	0	Testosterone enanthate IM 250mg, every 3 weeks
Mueller et al. 2010 (27)	Germany	24 months	45	30.4 ± 9.1	24.1 ± 4.5	0	Testosterone undecanoate 1000 mg IM every 12 weeks
Pelusi et al. 2014 (31)	Italy	12 months	15	30.9 (27.9-33.9)	22.3 (19.9-24.6)	0	Testosterone enanthate IM 100 mg, every 10 days
			15	29.4 (26.6-32.1)	23.9 (21.1-26.6)	0	Testosterone gel 50 mg/d
			15	28.2 (25.6-30.9)	22.1 (19.5-24.6)	0	Testosterone undecanoate 1.000 mg every 12 weeks
Van Caenegem et al. 2015 (20)	Belgium	12 months	23	27 ± 9	24.5 ± 5.3	0	Testosterone undecanoate 1000mg IM at week 0, 6, 18 and every 12 weeks after
Wiepjes et al. 2017 (10)	Belgium, Norway, Italy, Netherlands	12 months	199	24 (21-31)	23.9 (21.3-28.8)	0	Testosterone gel 50mg/day or testosterone esters IM 250mg every 2 weeks, or testosterone undecanoate IM 1000mg every 12 weeks
MtF							
Van Kesteren et al. 1996 (25)	Netherlands	12 months	56	33 (16-69)	22 (17-28)	0	Ethinylestradiol 100µg/day or Transdermal estradiol twice a week, or estradiol valerate injection or Oral conjugated estrogens with cyproterone acetate 100 mg/d or spironolactone
Van Kesteren et al. 1998 (24)	Netherlands	45.5 months (32-63)	20	25.4 (16-38)	22.1 ± 2.4	100	Ethinylestradiol 100 ug/day with cyproterone acetate 100 mg/d until GAS. After that only estradiol was used.
Dittrich et al. 2005 (22)	Germany	24 months	60	38.37 ± 11.36	24.19 ± 4.34	0	Oral oestradiol-17β valerate/day with 3.8mg goserelin acetate every 4 weeks
Mueller et al. 2005 (23)	Germany	24 months	40	38.39 ± 11.09	24.02 ± 4.00	0	Oral oestradiol-17β valerate/day with 3.8mg goserelin acetate every 4 weeks
Haraldsen et al. 2007 (29)	Norway	12 months	12	29.3 ± 7.8	NA	0	Ethinylestradiol 50ug/d in the first 3 months and 100ug/d thereafter
Mueller et al. 2011 (30)	Germany	24 months	84	36.3 ± 11.3	22.3 (21.7-23.0)	0	Estradiol 17β valerate 10mg IM every 10 days with 3.8 mg goserelin acetate every 4 weeks
Van Caenegem et al. 2015 (21)	Belgium	24 months	49	33 ± 12	NA	0	Oral estradiol valerate, 4mg/day or Transdermal 17β estradiol 100 µg/day (individuals >45 years) combined with oral cyproterone acetate 50 mg/d
Gava et al. 2016 (26)	Italy	12 months	20	32.9 ± 9.4	22.0	0	Transdermal estradiol 1-2mg/day with Cyproterone acetate 50 mg/day
			20	29.4 ± 10.2	21.9	0	Transdermal estradiol 1-2mg/day with leuprolide acetate 3.75 IM every month
Wiepjes et al. 2017 (10)	Belgium, Norway, Italy, Netherlands	12 months	231	28 (23-42)	22.5 (20.5-26.1)	0	Estradiol valerate 2-4mg/day or estradiol patch 50-100mg twice a week with cyproterone acetate 50-100mg/day
Fighera et al. 2018 (13)	Brazil	31 months (12-40)	46	33.70 ± 10.29	25.66 ± 4.16	33	Oral estradiol valerate, 1-4 mg/day or Transdermal 17β estradiol 0.5-2.0mg/day or Conjugated equine estrogen 0.625-2.500mg/day combined with spironolactone 50-100mg/d or cyproterone acetate 50-100 mg/day. After GAS only estradiol was used.

[a] mean and SD; N: number of participants

Supplemental Table 1

Risk of bias in cross-sectional studies designed to assess BMD in transgender people

Study	Calcium/ vitamin D supplements	Calcium intake	Serum vitamin D	Smoking	Alcohol abuse	Physical activity	Adjustments for BMD	Exclusion criteria
FtM								
Van Caenegem et al. 2012 (11)	6%	-	-	28%; 7-12 pack/years	No	8.4±1.8 (Baecke's)	Body weight and height	Illnesses or medications known to affect body composition, hormone levels, or bone metabolism; current or previous use (> 2 years) of glucocorticoids, oral contraception, (anti)androgens (except CSHT in FtM), calcium/vitamin D supplements (allowed for FtM, n=3), insulin, antiepileptic drugs, calcitonin, bisphosphonates, hypogonadism, untreated hyperthyroidism, cystic fibrosis, malabsorption, eating disorders, disorders of collagen or bone metabolism, chronic renal failure, alcohol abuse, autoimmune rheumatoid disease
Control natal women	None	-	-	12%; 3-6 pack/years	No	8.3±1.5 (Baecke's)	-	
Dan Broulik et al. 2018 (19)	-	-	19.95±11 ng/mL	25%	No	-	-	Use of medications known to impact BMD other than calcium, vitamin D or multivitamins, smoking >10 cigarettes daily, alcohol abuse.
Control natal women	-	-	38.5±11.8ng /mL	20%	No	-	-	
MtF								
Reutrakul et al. 1998 (20) **13.9 months**	-	0.7±0.2 Glasses of milk /week	-	2.3±1.6 pack/year	-	1.2±0.5 years of physical activity (>3 times/week)	Body weight	None of the subjects had significant medical history or risk factors for osteoporosis such as hyperparathyroidism, thyroid disorders or glucocorticoid usage.
59.8 months	-	0.5±0.1 Glasses of milk /week	-	4.0±0.9 pack/year	-	4.3±1.1 years of physical activity (>3 times/week)	Body weight	
Sosa et al. 2003 (18)	None	773.9± 257.9 mg/day	-	48%	68%	36% (active)	Body weight and height	Drugs that might affect bone density, hepatic or renal disorders, alcoholism, Paget disease, gonadectomy, hyperparathyroidism, osteoporotic fracture, HIV infection.
Control natal men	None	652.1± 265.6 mg/day	-	40%	72%	48% (active)	Body weight and height	
Lapauw et al. 2008 (12)**	-	528 (431-772) mg/day	23 (14-33) ng/mL	43.5%	1.5 (0.8-12) units/week	2.91±0.71 (Baecke's)	A multivariate analysis explored the contributions of muscle strength, physical activity, age, smoking and calcium intake. Muscle strength predicted cortical bone size. Current smoking was associated with lower BMD at the lumbar spine. Negative association between calcium intake and peri/ endosteal circumference, and positive association between physical activity and cortical BMC and bone area*	-
Control natal men	-	544 (423-804) mg/day	18 (13-25) ng/mL	17.4%	9.0 (3.0-16.5) units/week	2.68±0.79 (Baecke's)	-	-
Fighera et al. 2018 (13)	-	-	-	-	-	-	-	Other treatment protocol.

FtM (female to male), MtF (male to female)
* data not shown **Interquartile range

Supplemental Table 2 Risk of bias in before-after studies designed to assess BMD in transgender people

Study	Serum vitamin D	Smoking	Alcohol abuse	Physical activity	Adjustments for BMD	Exclusion criteria
FtM						
Van Kesteren et al. 1996 (26)	-	45.7%	17.1% (> 3 drinks/ day)	-	-	Subjects with risk factors for osteoporosis (e.g., hyperparathyroidism or thyroid disorders) were excluded. All individuals were CSHT-naïve.
Van Kesteren et al. 1998 (25)	-	52.6%	15.7% (>3drinks/ week)	-	-	All individuals were CSHT-naive.
Turner et al. 2004 (29)	-	53.3%	-	-	-	Use of medications known to impact BMD other than calcium and multivitamins, current pregnancy.
Haraldsen et al. 2007 (30)	-	-	No	-	-	Endocrinological, genetic,, neurological or major psychiatric co-morbidity. All patients were free of any medication, alcohol or drug abuse.
Mueller et al. 2010 (28)	-	-	-	-	-	Prior CSHT, significant abnormalities in the screening laboratory panel.
Pelusi et al. 2014 (32)** Testosterone enanthate IM	11.5 (-0.9-23.9) ng/ml	33%	26% (casual)	-	-	Use of medication for hypertension/ hyperlipidemia/ diabetes/ depression or any psychiatric drugs. All patients were CSHT-naive.
Testosterone gel	14.6 (8-21.2) ng/ml	77%	69% (casual)	-	-	
Testosterone undecanoate IM	23.9 (18.6-29.2) ng/ml	47%	73% (casual)	-	-	
Van Caenegem et al. 2015 (21)	19±11 ng/ml	0 (3-4) pack /year	-	8.9±2.2 (Baecke's)	Height	Prior CSHT, anorexia, cerebral palsy, refusal, other treatment protocol.
Wiepjes et al. 2017 (10)***	54 (31-77) nmol/l	29.3%	4.6% (>7drinks /week)	-	The gain in lumbar spine and femoral neck did not change after adjustment for increase in body weight (+2kg), but an attenuation of total femur BMD change was observed. No significant change in BMD was observed after adjustment for alcohol/cigarette consumption and vitamin D supplement use. After 3 and 12 months, estradiol and testosterone levels were not correlated with BMD change.	Prior CSHT, psychological vulnerability, insufficient knowledge of the protocol language.
MtF						
Van Kesteren et al. 1996 (26)	-	46.4%	14.3% (> 3 drinks /day)	-	-	Subjects with risk factors for osteoporosis (e.g., hyperparathyroidism or thyroid disorders) were excluded. All individuals were CSHT-naive.
Van Kesteren et al. 1998 (25)	-	40%	15% (>3drinks /week)	-	-	All individuals were CSHT-naive.
Dittrich et al 2005 (23)	-	-	-	-	-	-
Mueller et al. 2005 (24)	-	-	-	-	-	Medications known to affect calcium metabolism (glucocorticoids, anticonvulsants, calcium or vitamin D supplements, calcitonin, bisphosphonates), significant abnormalities in laboratory panel, prior estrogen therapy due to self-medication.
Haraldsen et al. 2007 (30)	-	-	No	-	-	Endocrinological, genetic, neurological or major psychiatric co-morbidity. All subjects were free of any medication, alcohol or drug abuse.
Mueller et al. 2011 (31)	-	-	-	-	-	Prior CSHT, longer periods of illness or immobility, subjects requiring treatment change.
Van Caenegem et al. 2015 (22)	16±8 ng/ml	19.1 pack /year	2 (0-7) drinks/week	8.3±1.6 (Baecke's)	The increase in bone mass did not change after adjustment for age, BMI, fat mass, 25 (OH) vitamin D status, PTH or leptin*	Prior CSHT, hypergonadotropic hypogonadism, gastric bypass, unwillingness.
Control natal men	23±7 ng/ml	0 (0-8) Pack /year	10 (3-16) drinks/week	8.7±1.5 (Baecke's)	-	
Gava et al. 2016 (27) CPA + E	56.2±27.8 nmol/l	35%	75% (casual)	-	-	Prior CSHT, use of medications for hypertension/ hyperlipidemia/ diabetes, psychiatric history, drug use.
Leu + E	47.8±28.3 nmol/l	65%	35% (casual)	-	-	
Wiepjes et al. 2017 (10)***	34 (22-52) nmol/l	23.5%	6.1% (>7drinks /week)	-	Gain in lumbar spine BMD did not change after adjustment for weight (+2.4kg), but an attenuation of total femur and femoral neck BMD was observed. Femoral neck BMD increased more in transwomen who used vitamin D supplements compared to those who did not use supplements. No significant change in BMD was observed after adjustment for alcohol and cigarette consumption. After 3 and 12 months, the estradiol levels was correlated with BMD change**	Prior CSHT, psychological vulnerability, insufficient knowledge of protocol language.
Fighera et al. 2018 (13)	-	-	-	-	-	Other treatment protocol.

FtM (female to male), MtF (male to female), CPA: cyproterone acetate, LEU: leuprolide acetate, CSHT: cross-sex hormone therapy
No study reported use of calcium supplements or calcium intake; * data not shown **Median e CI 95% ***Interquartile range

CONSIDERAÇÕES FINAIS

Em nosso estudo com mulheres trans, a DMO e massa magra apendicular foi similar entre mulheres trans e controles femininos, e menor comparando mulheres trans com controles masculinos. Foi observada elevada prevalência de baixa massa óssea quando comparado aos controles homens e mulheres. A maior parte das mulheres trans incluídas apresentava história de uso irregular de terapia estrogênica por longo período, com diferentes tipos e doses de estrogênio. Durante o seguimento por 31 meses, em que um tratamento padronizado foi estabelecido, não houve mudança significativa na DMO, sugerindo que outros fatores podem interferir na avaliação da massa óssea nesta população.

Com base nos achados da revisão sistemática e meta-análise, não foi observada diferença na DMO da coluna lombar e fêmur em homens trans durante a terapia androgênica. Em mulheres trans, não houve diferença significativa no fêmur, mas foi observado discreto aumento da DMO na coluna lombar. Contudo, as evidências são de baixa e moderada qualidade, e estudos com maior tempo de acompanhamento, TH regular e com ajuste para variáveis que podem influenciar a massa óssea são necessários.