Bone Mass, Bone Geometry, and Body Composition in Female-to-Male Transsexual Persons after Long-Term Cross-Sex Hormonal Therapy

E. Van Caenegem, K. Wierckx, Y. Taes, D. Dedecker, F. Van de Peer, K. Toye, J.-M. Kaufman, and G. T'Sjoen

Department of Endocrinology (E.V.C., K.W., Y.T., D.D., F.V.d.P., K.T., J.-M.K., G.T.) and Center for Sexology and Gender Problems (G.T.), Ghent University Hospital, 9000 Ghent, Belgium

Context: Female-to-male transsexual persons (transsexual men) undergo extreme hormonal changes due to ovariectomy and testosterone substitution, allowing studies on sex steroid effects on bone geometry and physiology in the adult.

Objective: The objective of the study was to examine the effects of cross-gender sex steroid exposure on volumetric bone parameters in transsexual men.

Design: This was a cross-sectional study.

Setting: Participants were recruited from the Center for Sexology and Gender Problems at the Ghent University Hospital (Ghent, Belgium).

Participants: Fifty transsexual men after sex reassignment surgery with 50 age-matched control women and an additional 16 transsexual men before testosterone substitution and sex reassignment surgery with 16 control women participated in the study.

Main Outcome Measures: The main outcome measures were areal and volumetric bone parameters using dual-energy X-ray absorptiometry and peripheral quantitative computed tomography, body composition (dual-energy X-ray absorptiometry), sex steroids, markers of bone turnover and grip strength.

Results: Before hormonal treatment, transsexual men had similar body composition and bone geometry as female controls. The transsexual men on long-term testosterone therapy, however, demonstrated a higher lean body mass and muscle mass and a greater grip strength as well as a lower body and subcutaneous fat mass and a larger waist and smaller hip circumference compared with female controls (all $P < 0.001$). We observed a larger radial cortical bone size ($P < 0.001$) and lower cortical volumetric bone mineral density at the radius and tibia ($P < 0.05$) in transsexual men on testosterone therapy.

Conclusions: Transsexual men on testosterone substitution therapy present with a different body composition with more muscle mass and strength and less fat mass as well as an altered bone geometry with larger bones compared with female controls. **(*J Clin Endocrinol Metab* 97: 2503–2511, 2012)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2012 by The Endocrine Society
doi: 10.1210/jc.2012-1187 Received January 23, 2012. Accepted April 11, 2012.
First Published Online May 25, 2012

Abbreviations: aBMD, Areal bone mineral density; BMC, bone mineral content; BMI, body mass index; CSA, cross-sectional area; CTX, C-terminal telopeptides of type I collagen; DXA, dual-energy x-ray absorptiometry; IGFBP3, IGF-binding protein 3; PCOS, polycystic ovary syndrome; P1NP, protocollagen 1 aminoterminal propeptide; pQCT, peripheral quantitative computed tomography; SRS, sex reassignment surgery; vBMD, volumetric bone mineral density.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

Sex steroids are important determinants of bone acquisition during puberty and bone homeostasis in adulthood. During puberty, men develop a larger bone mass and size than women (1, 2). Indeed, testosterone stimulates the process of periosteal apposition, causing a greater cortical bone size in men (3), whereas pubertal girls experience less periosteal expansion and more endocortical apposition compared with boys (4, 5). As female-to-male transsexual persons (will be referred to as transsexual men) undergo ovariectomy and follow life-long testosterone substitution therapy, studies on bone geometry and body composition allow studying sex steroid actions on bone physiology in adults.

Previous studies in transsexual men demonstrated that during the first 2 yr of cross-sex hormonal therapy, androgen administration could prevent possible bone loss due to estrogen deficiency (6–8). Areal bone mineral density (aBMD) was higher at cortical sites (8, 9), and a histomorphometric study showed a significantly larger cortical thickness in transsexual men compared with controls (10). Evaluations after a longer exposure time (respectively 7.6 and 1.5–5.3 yr) to androgens described a preserved aBMD (9, 11). However, a decline in aBMD after a median of 45 months of cross-sex hormonal therapy was reported, possibly due to inadequate substitution therapy because there was an inverse correlation between LH or FSH and aBMD (11).

Mechanical loading and physical activity play an important stimulatory role on the skeleton and were found to be associated with a larger cortical thickness (2, 12, 13). In transsexual men, the mechanical loading on bone increases through the higher muscle mass, induced by androgens (14).

All previous studies on bone mass in transsexual men were conducted using dual-energy x-ray absorptiometry (DXA), which has limitations in assessing bone geometry. Due to the two-dimensional view, bone mineral density of larger bones can be overestimated (1, 15). Peripheral quantitative computed tomography can evaluate accurately the volumetric bone mineral density (vBMD) and differentiate trabecular from cortical bone. In this study, we assessed volumetric bone parameters and body composition in relation to sex steroids and muscle strength in a population of transsexual men before and after a long period of cross-sex hormonal therapy, compared with age-matched healthy control women.

Materials and Methods

Study design and population

All transsexual participants were diagnosed with gender identity disorder (*Diagnostic and Statistical Manual of Mental Disorders*-IV, 302.85; *International Classification of Diseases*, 10th revision, F64.0) and were recruited from the center for Sexology and Gender Problems at the Ghent University Hospital (Ghent, Belgium). Every patient was treated following the World Professional Association for Transgender Health standards of care (16).

Fifty transsexual men, will be referred to as the treatment group and used testosterone substitution therapy and had undergone sex reassignment surgery (SRS; hysterectomy, ovariectomy, and mastectomy) before inclusion in this study. On average, these patients were 8.7 yr after SRS, with a minimum of 9 months and a maximum of 22 yr. Forty-six of them underwent phalloplasty, of whom eight had a previous metaidoioplasty (n = 9), only one person had metaidoiplasty alone, and three transsexual men did not have further genital surgery. Current cross-sex hormonal therapy consisted of intramuscular testosterone treatment with either a mixture of testosterone esters (testosterone decanoate 100 mg, testosterone isocaproate 60 mg, testosterone fenylpropionate 60 mg, testosterone propionate 30 mg/ml) per 2 or 3 wk (n = 35) or testosterone undecanoate 1000 mg per 12 wk (n = 7) or transdermal testosterone 50 mg daily (n = 8). One participant used both oral testosterone undecanoate 40 mg (one daily) and testosterone gel 50 mg per 5 g, 50 mg daily. The type of treatment (testosterone esters IM, testosterone undecanoate IM, or transdermal testosterone gel) was not associated with serum testosterone or LH levels or with body composition or bone parameters. Almost all the participants were Caucasian (48 Belgians and one Dutch), and one participant was Iranian. Additionally, 16 Belgian transsexual men, who will be referred to as the untreated group, were recruited in the diagnostic phase and just before the start of cross-sex hormonal therapy and thus before SRS. The female control population for both groups, matched for age (±2 yr, median 1 yr) was healthy women who responded to posters spread in the Ghent University Hospital and on its web site.

All participants were currently in good physical health and completed questionnaires about previous illness and medication use, current and past smoking habits, and physical activity by recording the weekly frequency of both recreational and/or working activities (using Baecke's questionnaire) (17). Gynecological history and previous fractures were registered. In the treatment group, a history of menstrual irregularities and polycystic ovary syndrome (PCOS) was found to be unreliable due to the long-time course and previous hysterectomy and ovariectomy. In the untreated group, one transsexual man had PCOS. None of the control women had PCOS. Exclusion criteria were defined as illnesses or medication use known to affect body composition, hormone levels, or bone metabolism such as current, prolonged, or previous use (in the last 2 yr) of glucocorticosteroids, (anti)androgens (except for cross-sex hormonal therapy in transsexual men), oral contraception, calcium, and/or vitamin D supplements (was allowed for transsexual men; n = 3), insulin, antiepileptic drugs, calcitonin, bisphophonates; presence of hypogonadism, untreated hyperthyroidism, cystic fibrosis, malabsorption, or current eating disorders or disorders of collagen metabolism or bone development; and chronic renal failure, alcohol abuse, or autoimmune rheumatoid disease. In the untreated group, cerebral palsy was present in one participant who did have an active lifestyle and two participants had an episode of eating disorder in the past. After exclusion of these patients and their age-matched controls, the presented results did not change substantially. Control women aged 45 yr and older with

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

serum levels of LH 20 U/liter or greater were considered to be perimenopausal (n = 5). After exclusion of these women and their matched transsexual men, results remained the same.

The study protocol was approved by the Ethics Review Board of the Ghent University Hospital (study protocol no. EC2009/266), and all participants gave their written informed consent.

Body composition, muscle strength, and aBMD

Body weight and anthropometrics were measured in light indoor clothing without shoes. Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain, Ltd., Crymuch, UK).

Grip strength at the dominant hand was measured using an adjustable handheld standard grip device (JAMAR hand dynamometer; Sammons and Preston, Bolingbrook, IL). The maximum strength of three attempts was assumed to best reflect the current status and history of their musculoskeletal adaptation and was expressed in kilograms.

Body fat and lean mass, bone mineral content (BMC), bone area and aBMD at the lumbar spine, and left proximal femur (total hip and femoral neck region) were measured using DXA with a Hologic QDR-4500A device (software version 11.2.1; Hologic, Inc., Bedford, MA). The coefficient of variation for both spine and whole-body calibration phantoms was less than 1%, as calculated from daily and weekly measurements, respectively.

vBMD and cross-sectional muscle/fat area

A peripheral quantitative computed tomography (pQCT) device (XCT-2000; Stratec Medizintechnik, Pforzheim, Germany) was used to evaluate the cortical volumetric bone parameters at the dominant midradius and tibia (at 66% of bone length) and trabecular bone parameters at the metaphysis (at 4% of bone length) of the dominant radius. Procedure details were as described previously (18).

Biochemical determinations

Venous blood samples were obtained between 0800 and 1000 h after overnight fasting in the treatment group of transsexual men (n = 50). Blood samples in the female control group matched to the treatment group (n = 50) were collected throughout the day. All samples were stored at −80 C until analysis. Commercial immunoassay kits were used to determine serum concentrations of SHBG (Orion Diagnostica, Espoo, Finland), testosterone, LH, estradiol, IGF-I, C-terminal telopeptides of type I collagen (CTX) as a marker of bone resorption, and protocollagen 1 aminoterminal propeptide (P1NP), which reflects bone formation (Modular; Roche Diagnostics, Mannheim, Germany). IGF-binding protein 3 (IGFBP3) was determined by an extraction method (DSL-5600; Diagnostic System Laboratories, Webster, TX). The intra- and interassay coefficients of variation for all assays were less than 10%.

Statistical analysis

Descriptives are expressed as mean and SD or median (first to third quartile) when criteria for normal distribution were not fulfilled. $P < 0.05$ was considered to indicate statistical significance, and all tests were two tailed. Comparison of general, anthropometric, biochemical, and hormonal determinations between groups were made with an independent *t* test or Mann-Whitney *U* test when variables were not normally distributed (Tables 1 and 2). Multiple regression analysis was used to compare bone and body composition in transsexual men of the treatment group compared with controls (Tables 3–5) and used models included height, weight, and a grouping variable (transsexual or control group) as independents. The *P* value of this grouping variable is shown, similarly to other publications of our group (19).

TABLE 1. Descriptives of general and anthropometric determinations in transsexual men before start of cross-gender hormonal treatment and sex reassignment surgery

	Transsexual men (n = 16)	Female controls (n = 16)
Age (yr)	26 ± 7	26 ± 7
Weight (kg)	57.7 (54.3–79.0)	66 (59.1–72.5)
Height (cm)	164 ± 0.05[a]	169 ± 0.06
BMI (kg/m^2)	22.6 (20.7–27.3)	22.8 (21.1–26.1)
Current smoking (%)[b]	31	13
Pack-years (yr)	0 (0–3)	0 (0–1)
Parity	0 (0–0)[a]	0 (0–2)
Physical activity index	9.1 ± 2.1	8.1 ± 1.3
Grip strength (kg)	33 ± 7	32 ± 5

Descriptives are expressed as mean ± SD or as median (first to third quartile) when not normally distributed. Variables were compared between groups using independent *t* tests or Mann-Whitney *U* test when not normally distributed. [a] $0.01 < P \leq 0.05$ [b] using χ^2 test.

We evaluated sex steroids, muscle strength, physical activity, bone turnover markers, and smoking in relation to body composition and volumetric bone parameters in the treatment group and controls using separate multiple regression models.

We evaluated the effect of cross-sex hormonal treatment using a multiple regression model with transsexual men before and on testosterone substitution and their respective control women together (n = 132). Independent variables in this model were age, height, weight, treatment (whether one had used testosterone substitution or not), and the grouping variable (transsexual person or not), and outcome variables were volumetric bone parameters. Data were analyzed using SPSS software, version 19 (SPSS Inc., Chicago, IL).

Results

Transsexual men before the start of cross-sex hormonal therapy

Untreated transsexual men (n = 16) had similar age, weight, body mass index (BMI), smoking and physical activity habits, and muscle strength compared with an age-matched control group of women (n = 16) (Table 1). Control women were taller than transsexual men of this untreated group. No significant differences in body composition were found in the untreated group compared with controls, and bone parameters measured by DXA were comparable with female controls comparable with the exception of lumbar spine area (mean 56.2 *vs.* 60.9, $P = 0.046$) (online Supplemental Tables 1 and 2, pub-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

TABLE 2. Descriptives of general, anthropometric, hormonal, and biochemical determinations of transsexual men on long-term cross-gender hormonal treatment and after sex reassignment surgery

	Transsexual men (n = 50)	Female controls (n = 50)
Age (yr)	37 ± 8	38 ± 8
Weight (kg)	67.5 ± 11.5	69.7 ± 10.9
Height (cm)	165 ± 7	167 ± 6
BMI (kg/m^2)	24.8 ± 3.8	25.1 ± 3.6
Current smoking (%)[a]	28[d]	12
Pack-years	7 ± 12[d]	3 ± 6
Parity	0 (0–0)	2 (0–2)
Physical activity index	8.4 ± 1.8	8.3 ± 1.5
Grip strength (kg)	37 ± 6[b]	29 ± 5
Hematocrit (%)	48.8 ± 2.8[b]	40.1 ± 5.9
Creatinine (mg/dl)	0.93 ± 0.13[b]	0.75 ± 0.1
Cholesterol (mg/dl)	207 ± 35[b]	185 ± 31
LH (mU/ml)	3.7 (0.2–28.5)	6.0 (3.5–10.3)
SHBG (nmol/liter)	32 ± 11[b]	63 ± 27
Estradiol (ng/liter)	34 (25–50)[b]	73 (45–118)
Testosterone (ng/dl)	729 ± 375[b]	21 ± 9
P1NP (ng/ml)	50 ± 24[c]	40 ± 12
CTX (ng/ml)	0.36 ± 0.15[b]	0.20 ± 0.10
IGF-I (ng/ml)	225 ± 65	214 ± 70
IGFBP3 (ng/ml)	3312 ± 352	3420 ± 310

Descriptives are expressed as mean ± SD or as median (first to third quartile) when not normally distributed. Variables were compared between groups using independent *t* tests or Mann-Whitney *U* test when not normally distributed. To convert nanograms per liter to picomoles per liter for estradiol, multiply by 3.671; to convert nanograms per deciliter to nanomoles per liter for testosterone, multiply by 0.0347.

[a] Using χ^2 test; [b] $P \leq 0.001$; [c] $0.001 < P \leq 0.01$; [d] $0.01 < P \leq 0.05$.

lished on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Transsexual men on long-term cross-sex hormonal therapy

General characteristics, anthropometry, and hormone levels

The treatment group of transsexual men (n = 50) had been using cross-sex hormonal therapy for about 10 yr (on average 9.9 yr, range 3.2–27.5 yr). Age, body weight, height, and BMI were comparable between transsexual men and control women (Table 2). There were more smokers in this group compared with controls, and the mean amount of pack-years was significantly higher. As expected, the treatment group had a markedly higher serum testosterone and a lower SHBG and estradiol levels than females, within normal ranges for healthy men (Table 2). Serum estradiol levels were strongly associated with testosterone in those transsexual men ($P < 0.001$), reflecting peripheral aromatization. Hematocrit, creatinine, and total cholesterol levels were all significantly higher in the treatment group *vs.* the female controls. Markers of bone formation and resorption, respectively, P1NP and CTX, were found to be higher in that group.

TABLE 3. Descriptives of measures of body composition of transsexual men on long-term cross-gender hormonal treatment and after sex reassignment surgery

	Transsexual men (n = 50)	Female controls (n = 50)
Waist circumference (cm)	84.2 ± 10.6[a]	77.8 ± 8.1
Hip circumference (cm)	97.7 ± 7.1[a]	104.2 ± 7.9
Waist-hip ratio	0.9 ± 0.1[a]	0.7 ± 0.1
Whole body[b]		
Fat mass (kg)	15.9 ± 5.7[a]	22.5 ± 6.9
Lean body mass (kg)	49.5 ± 6.7[a]	44.9 ± 5.2
Proximal forearm[c]		
Fat CSA (cm^2)	8.6 ± 4.2[a]	14.5 ± 5.1
Muscle CSA (cm^2)	36.3 ± 5.4[a]	26.8 ± 3.8
Proximal tibia[c]		
Fat CSA (cm^2)	18.0 ± 7.8[a]	32.6 ± 10.5
Muscle CSA (cm^2)	74.8 ± 10.8[a]	67.5 ± 9.4

Descriptives are expressed as mean ± SD. All variables were corrected for weight and height.

[a] $P \leq 0.001$.

[b] Measured with DXA.

[c] Measured with pQCT.

Body composition

In transsexual men on testosterone substitution, a different body composition with a significantly larger waist and smaller hip circumference and a higher waist-hip ratio than the female group was found (Table 3). The treatment group had almost 30% less body fat mass and 9% more lean body mass compared with the female controls. More-

TABLE 4. Descriptives of bone parameters as measured by DXA at the lumbar spine and left hip of transsexual men on long-term cross-gender hormonal treatment and after sex reassignment surgery

	Transsexual men (n = 50)	Female controls (n = 50)
Lumbar spine		
Bone area (cm^2)	59.4 ± 6.1	59.5 ± 5.5
BMC (g)	61.3 ± 10.2	63.2 ± 10.8
aBMD (g/cm^2)	1.03 ± 0.10	1.06 ± 0.11
Femoral neck		
Bone area (cm^2)	5.1 ± 0.53[a]	5.0 ± 0.3
BMC (g)	4.1 ± 0.7	4.2 ± 0.5
aBMD (g/cm^2)	0.82 ± 0.11	0.84 ± 0.10
Total hip		
Bone area (cm^2)	35.3 ± 3.0[b]	34.5 ± 2.9
BMC (g)	33.9 ± 5.0[a]	32.9 ± 4.4
aBMD (g/cm^2)	0.96 ± 0.12	0.95 ± 0.10

Descriptives are expressed as mean ± SD. All variables were corrected for weight and height.

[a] $0.01 < P \leq 0.05$ [b] $0.001 < P \leq 0.01$.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

TABLE 5. Descriptives volumetric bone parameters as measured by pQCT at the distal (trabecular parameters) and proximal proximal radius and proximal tibia (cortical parameters) of transsexual men on long-term cross-gender hormonal treatment and after sex reassignment surgery

	Transsexual men (n = 50)	Female controls (n = 50)
Radius		
Trabecular vBMD (mg/cm^3)	221 ± 40[a]	198 ± 41
Trabecular bone area (mm^2)	145 ± 20	144 ± 19
Cortical vBMD (mg/cm^3)	1106 ± 44[b]	1123 ± 33
Cortical bone area (mm^2)	81 ± 11[b]	77 ± 9
Cortical thickness (mm)	2.2 ± 0.3	2.2 ± 0.3
Periostal circumference (mm)	44 ± 4[c]	42 ± 3
Endosteal circumference (mm)	31 ± 5[a]	29 ± 4
Tibia		
Cortical vBMD (mg/cm^3)	1118 ± 28[b]	1126 ± 20
Cortical bone area (mm^2)	291 ± 36	284 ± 28
Cortical thickness (mm)	3.9 ± 0.5	3.8 ± 0.4
Periostal circumference (mm)	87 ± 6	86 ± 6
Endosteal circumference (mm)	62 ± 7	62 ± 8

Descriptives are expressed as mean ± SD. All variables were corrected for weight and height. Logarithmic transformation was used when variables were not normally distributed.

[a] $0.001 < P \leq 0.01$; [b] $0.01 < P \leq 0.05$; [c] $P \leq 0.001$.

over, this group showed a greater muscle cross-sectional area (CSA) and a lower fat CSA, reflecting subcutaneous fat, at both the forearm and lower leg *vs.* female controls. Transsexual men on testosterone substitution therapy also demonstrated a significantly higher grip strength compared with the control subjects (Table 2), whereas in the untreated transsexual men, no difference in grip strength was observed (Table 1).

aBMD using DXA

No significant differences were found in aBMD in the treatment group compared with age-matched control women (Table 4). A significantly higher BMC and bone area of total hip and bone area at the femoral neck were observed in the treatment group compared with the female controls.

Volumetric bone parameters at the upper and lower limb using pQCT

At trabecular sites (distal radius), the treatment group scored significantly higher on vBMD, whereas trabecular area was similar (Table 5). At the proximal radius, a cortical site, the treatment group showed a significantly higher cortical bone area and a larger bone size, with a greater periosteal (+4.5%) and endosteal (+6.7%) diameter at the proximal radius. There was also a trend toward higher cortical bone area in the treatment group at the proximal tibia. Furthermore, at both cortical sites (proximal radius and tibia), a lower cortical vBMD was observed in the treatment group compared with the female controls (Table 5 and Fig. 1).

When evaluating a statistical model that included transsexual men before and during testosterone substitution and their respective female controls (n = 132), treatment with testosterone appeared to be positively associated with bone size [periosteal ($\beta = 3.215$; $P = 0.010$) and endosteal ($\beta = 3.474$; $P = 0.026$) circumference] at the radius and negatively with cortical vBMD ($P = 0.049$). Before treatment, no differences in bone parameters between groups were observed in this model (Fig. 1).

Sex steroids, muscle strength, physical activity, age, and smoking in relation to body composition and volumetric bone parameters

The effect of different variables on volumetric bone parameters and body composition was assessed in the treatment group (n = 50) and the female control group (n = 50) using separate multiple regression models. All analyses were adjusted for age, body weight, and height, and no unadjusted associations were determined. We found that grip strength was independently positively associated with trabecular and cortical bone parameters at the radius in transsexual men and control women (all $P < 0.001$). After adjusting for grip strength, differences between transsexual men and control women remained significant for trabecular and cortical vBMD at the radius, indicative for a direct effect of testosterone on bone ($P = 0.003$). Similarly, muscle mass was positively associated with bone area and periosteal circumference at the radius and tibia in transsexual men and control women (all $P \leq 0.015$).

An inverse relationship was observed between markers of bone turnover (CTX and P1NP) and cortical vBMD at both radius and tibia in transsexual men (all $P \leq 0.022$). Physical activity was positively associated with bone area and periosteal circumference at the tibia in transsexual men (both $P = 0.005$) but not in the control population. No interactions between testosterone and the level of physical activity level were observed in these models (data not shown).

SHBG proved to be an independent positive predictor of bone size (periosteal and endosteal circumference) at the tibia in transsexual men and female controls ($P \leq 0.002$). Volumetric bone parameters were neither associated with testosterone, estradiol, and LH nor with IGF-I, IGFBP3, and smoking (amount of pack-years).

Serum testosterone concentrations were not related to muscle or fat mass, apart from a negative association with fat area at the forearm in transsexual men ($P = 0.010$). We found that LH was positively associated with total body

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

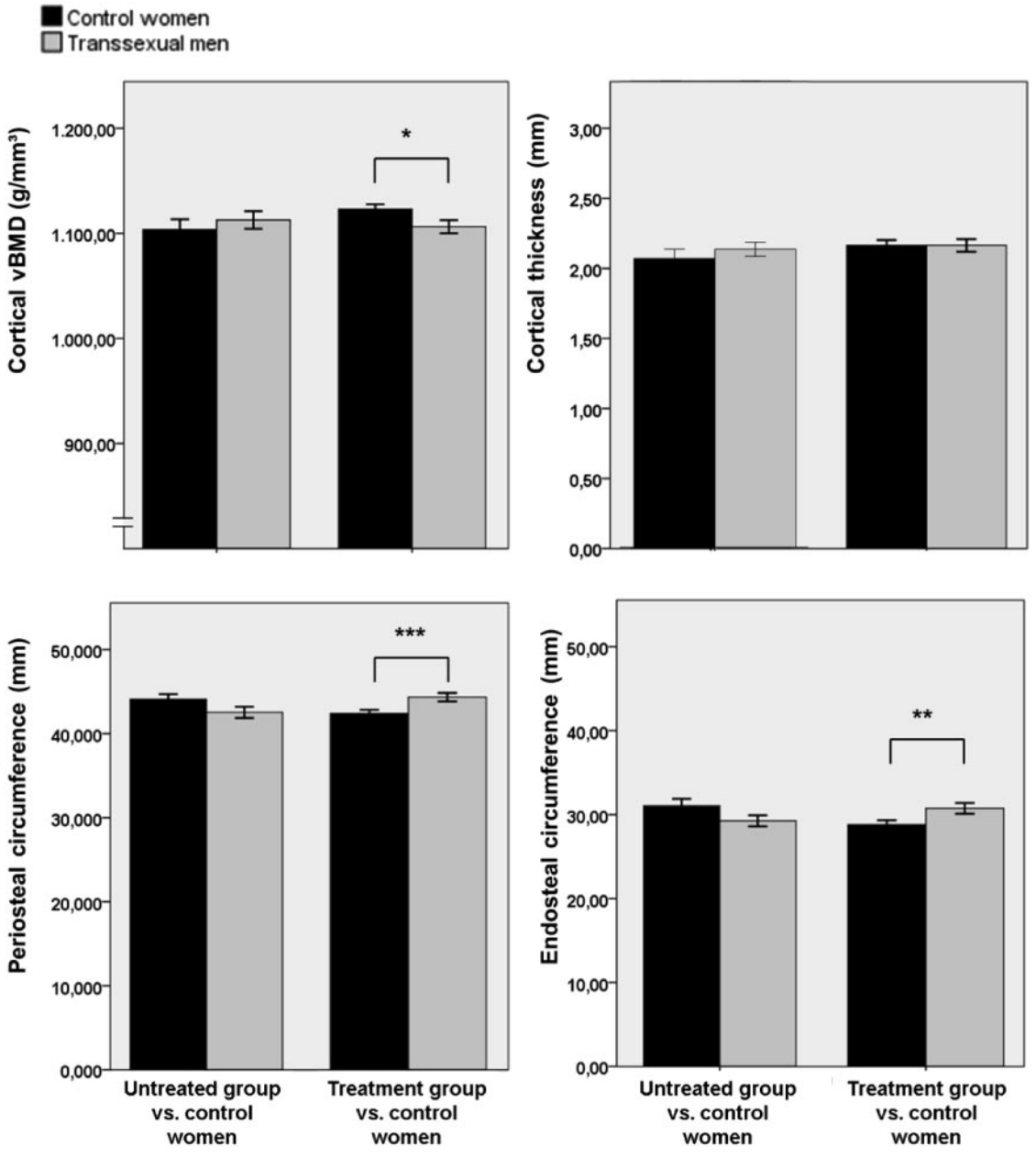

FIG. 1. Cortical bone parameters at the radius. *Bars* represent mean and whiskers 1 SEM. *From left to right*, First cluster: *gray bar* shows the untreated group of transsexual men (n = 16) and age-matched control group in *black bar* (n = 16); second cluster: *gray bar* represents treatment group of transsexual men (n = 50) and age-matched control group (n = 50) in *black bar*. ***, $P \leq 0.001$; **, $0.001 < P \leq 0.01$; *, $0.01 < P \leq 0.05$.

fat mass ($P = 0.010$) and inversely associated with total body lean mass ($P = 0.007$) and muscle area at the forearm and lower leg (respectively, $P = 0.040$ and $P = 0.023$) in transsexual men. The length of time on cross-sex hormonal therapy was not associated with volumetric bone parameters or body composition.

Smoking, expressed as pack-years, was an independent positive predictor of waist-hip ratio in transsexual men ($P = 0.027$) but not in controls. After adjusting for smoking, the differences in waist-hip ratio between transsexual men and control women remained significant ($P < 0.001$).

Discussion

We found that transsexual men, after long-term treatment with testosterone substitution therapy and SRS, had a different body composition with more muscle mass and strength and less fat mass as well as larger bones and lower vBMD compared with age-matched female subjects. To evaluate whether these differences could already be present at baseline, we additionally recruited 16 transsexual men before the start of hormonal therapy and SRS. They had similar bone and body composition compared with an

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

age-matched female control group. To our knowledge, this is the first study to report data on volumetric bone parameters in a sizable group of transsexual men, by use of pQCT. This technique, which is more discriminative than DXA, reveals an increased trabecular vBMD, whereas cortical bone sites are characterized by decreased vBMD and larger endosteal and periosteal bone circumferences in transsexual men on cross-gender hormonal therapy. Thus, these findings indicate that exposure of adult bones to cross-gender hormonal treatment has a significant impact not only on lean and fat mass but also on the adult skeleton.

The expected anabolic effect of testosterone administration on muscle mass is clearly reflected in our data. Although a direct relationship of testosterone with muscle mass or strength was not found, we did observe an independent negative association of lower serum LH with higher total and regional muscle mass of the treatment group, indicating a dose effect relationship between testosterone treatment and gain in muscle mass. The lack of associations with serum testosterone is not unexpected. Considering the differences in types of testosterone administration, the variable timing of sampling in relation to the last testosterone dosing, the pharmacokinetic profile, and the single-point testosterone levels in these men, the measured serum testosterone cannot be expected to reliably reflect testosterone exposure.

The demonstrated lower total and subcutaneous fat mass together with the smaller hip and larger waist circumference in the treatment group of transsexual men are indicative for a more central fat distribution. These findings are consistent with previous studies that showed an android pattern of fat distribution in transsexual men after cross-sex hormonal therapy (14). In fact, men have on average more visceral fat than women (20). Elbers *et al.* (14) already observed an increase of visceral fat depots and a decrease of subcutaneous fat in transsexual men after 1 yr of testosterone administration. After 3 yr of follow-up, the decrease in subcutaneous fat was no longer significant, but the increase in visceral fat remained important (14). Concordant with this finding, the android pattern of fat distribution is also found in women with hyperandrogenism (PCOS) (21, 22) and postmenopausal women undergoing estrogen-testosterone hormonal substitution therapy (23–26). The total cholesterol is higher in transsexual men compared with the control women because of the effects of testosterone. Together with the central pattern of fat distribution, this might mean that the transsexual men have a less favorable metabolic profile compared with the control women.

Our results demonstrated the presence of a higher trabecular vBMD in transsexual men on cross-sex hormonal treatment compared with age-matched women. First, the androgen-induced increased muscle mass and strength appeared to be strong positive predictors of trabecular bone parameters independent of weight and height. This is in line with earlier reports on the positive association of grip strength with trabecular vBMD at forearm sites (27). A second explanation may be found in the altered trabecular microstructure in the treatment group. In their study with high-resolution pQCT, Khosla *et al.* (28) observed thicker trabeculae in young men, which were positively associated with IGF-I but not with sex steroids, indicating the development of thicker trabeculae during youth. Supraphysiological doses of testosterone in transsexual men could induce greater trabecular thickness by interaction with the IGF-I axis. However, in our study with normal-resolution pQCT, volumetric trabecular bone parameters were not associated with IGF-I or IGFBP3. Third, the exogenously administered testosterone may exert a dual favorable action on trabecular bone (29) with a direct effect mediated through the androgen receptor, whereas aromatization to estrogens could contribute to maintenance of trabecular bone despite endogenous estrogen deprivation due to ovariectomy, although no associations between estradiol and trabecular bone parameters were found. The observed higher trabecular vBMD in the treatment group of transsexual men does not confirm results of a histomorphometric study that found similar trabecular bone structure in transsexual men *vs.* a male and postmenopausal controls, although an anabolic effect of testosterone on cortical bone was also observed in this study (10).

The current DXA results confirm earlier reports stating maintained aBMD after cross-sex hormonal therapy in transsexual men. The higher bone area but similar aBMD at the femoral neck and total hip in the treatment group compared with controls are in partial agreement with previous studies, describing an increased aBMD at cortical sites. An increase in aBMD at the femoral neck was observed in 15 transsexual men after a follow-up of 2 yr of testosterone administration (8) and at the whole body and tibia in a cross-sectional study with 15 transsexual men after median duration of 7.6 yr testosterone administration (9). The present findings show that unchanged aBMD in transsexual men is a possibly the result of decreased cortical vBMD and increased bone size.

Several explanations for the observed differences in cortical vBMD and bone size in transsexual males in the treatment group *vs.* healthy females can be put forward. Mechanical loading, through muscle mass and strength, proved to be an independent predictor of volumetric cortical bone parameters and cortical bone size. Mechanical loading can trigger periosteal apposition (mechanostat theory), which could explain the larger periosteal circum-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

ference (13, 30). As previously observed in a group of young healthy men, SHBG was positively associated with bone size (31). Additionally, there could be interplay between the effects of increased mechanical loading and drastic changes in sex hormones on bone size. In the present study, no significant difference was found in physical activity between groups, yet physical activity was positively associated with periosteal circumference in transsexual men of the treatment group but not in control women. This might support the hypothesis formulated from observations in mouse models stating that estrogens, through estrogen receptor-β signaling, may limit the response to mechanical loading in females and low levels of estrogen may decrease the set point of the mechanostat, thereby favoring periosteal apposition in conditions of low estrogens (2, 4). So, in transsexual men, the androgen-induced muscle mass would trigger periosteal apposition by increasing the mechanical loading and, additionally, the mechanosensitivity would be higher due to lower estrogens. This combination could then explain the higher periosteal circumference in transsexual males.

Finally, we found higher markers of bone formation and resorption in transsexual men, presumably related to the larger bone size or higher intracortical remodeling, leading to lower cortical vBMD (5). Previous studies found higher (6, 11) and lower (10) as well as normal markers of bone formation (9), all in combination with preserved aBMD in transsexual men. Bone resorption did not seem to alter with testosterone administration in transsexual men, although blood sampling in our study was performed throughout the day (6, 9, 11).

Our study has a couple of limitations. First of all, the cross-sectional design does not allow establishing causal relationships. Transsexual men of the treatment group could have practiced more and more typically masculine sports during childhood and adolescence, which could have led to larger bone size than same biological sex peers (32). However, the differences observed between treated transsexual men and controls were not seen in the younger group of transsexual men before start of hormonal therapy compared with their controls. Furthermore, we should consider the effects of smoking on body composition and bone because there were more smokers and the mean amount of pack-years was higher in the treatment group of transsexual men. Smoking is independently associated with larger waist-hip ratio, and we could support this with our data, although this was found only in transsexual men (33). However, differences in waist-hip ratio between groups were still significant after adjusting for smoking. Although smoking is a negative factor for bone health (34), it could not explain the variance in volumetric bone parameters in our study, and differences between treatment group and controls remained unchanged after correcting for number of pack-years. As stated before, the different testosterone administration methods and single-point serum testosterone dosages could cloud possible associations between testosterone and body composition or volumetric bone parameters.

Conclusion

We observed that transsexual men with cross-sex hormonal therapy and after SRS had a more masculine bone and body composition compared with age-matched female controls. They had less fat mass and a more central pattern of adiposity and more muscle mass and strength in relation to a larger cortical bone size. These differences were not observed in a group of transsexual men before treatment compared with age-matched female controls. The differences described may result from direct effects of long-term testosterone administration and of diminished estrogen exposure and/or from indirect effects through muscle mass and strength. Our results indicate that transsexual men on long-term hormonal therapy do not have an increased risk of low bone mass, but metabolic changes and associated cardiovascular risk factors are important to address.

Acknowledgments

We are indebted to Griet De Cuypere, M.D., Ph.D.; Gunter Heylens, M.D.; Els Elaut, M.Sc.; Birgit Van Hoorde, M.Sc.; Steven Weyers, M.D., Ph.D.; Piet Hoebeke, M.D., Ph.D.; and Stan Monstrey, M.D., Ph.D. for the referral of participants. We also thank all the volunteers who participated as study subjects.

Address all correspondence and requests for reprints to: Eva Van Caenegem, M.D., Department of Endocrinology, Ghent University Hospital De Pintelaan 185, 9000 Ghent, Belgium. E-mail: eva.vancaenegem@ugent.be.

This work was supported in part by Grant G.0867.11 from the Research Foundation Flanders (FWO Vlaanderen). E.V.C. and Y.T. are holders of a PhD fellowship and a postdoctoral fellowship, respectively, from the Research Foundation Flanders.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Seeman E** 2001 Clinical review 137: sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab 86:4576–4584
2. **Vanderschueren D, Venken K, Ophoff J, Bouillon R, Boonen S** 2006 Clinical Review: sex steroids and the periosteum—reconsidering the roles of androgens and estrogens in periosteal expansion. J Clin Endocrinol Metab 91:378–382
3. **Lorentzon M, Swanson C, Andersson N, Mellström D, Ohlsson C** 2005 Free testosterone is a positive, whereas free estradiol is a neg-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.

ative, predictor of cortical bone size in young Swedish men: the GOOD study. J Bone Miner Res 20:1334–1341
4. **Callewaert F, Sinnesael M, Gielen E, Boonen S, Vanderschueren D** 2010 Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading. J Endocrinol 207:127–134
5. **Riggs BL, Melton Iii 3rd LJ, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, McCollough CH, Bouxsein ML, Khosla S** 2004 Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 19:1945–1954
6. **Haraldsen IR, Haug E, Falch J, Egeland T, Opjordsmoen S** 2007 Cross-sex pattern of bone mineral density in early onset gender identity disorder. Horm Behav 52:334–343
7. **Mueller A, Haeberle L, Zollver H, Claassen T, Kronawitter D, Oppelt PG, Cupisti S, Beckmann MW, Dittrich R** 2010 Effects of intramuscular testosterone undecanoate on body composition and bone mineral density in female-to-male transsexuals. J Sex Med 7:3190–3198
8. **Turner A, Chen TC, Barber TW, Malabanan AO, Holick MF, Tangpricha V** 2004 Testosterone increases bone mineral density in female-to-male transsexuals: a case series of 15 subjects. Clin Endocrinol (Oxf) 61:560–566
9. **Ruetsche AG, Kneubuehl R, Birkhaeuser MH, Lippuner K** 2005 Cortical and trabecular bone mineral density in transsexuals after long-term cross-sex hormonal treatment: a cross-sectional study. Osteoporos Int 16:791–798
10. **Lips P, van Kesteren PJ, Asscheman H, Gooren LJ** 1996 The effect of androgen treatment on bone metabolism in female-to-male transsexuals. J Bone Miner Res 11:1769–1773
11. **van Kesteren P, Lips P, Gooren LJ, Asscheman H, Megens J** 1998 Long-term follow-up of bone mineral density and bone metabolism in transsexuals treated with cross-sex hormones. Clin Endocrinol (Oxf) 48:347–354
12. **Bass SL, Saxon L, Daly RM, Turner CH, Robling AG, Seeman E, Stuckey S** 2002 The effect of mechanical loading on the size and shape of bone in pre-, peri-, and postpubertal girls: a study in tennis players. J Bone Miner Res 17:2274–2280
13. **Frost HM** 1987 Bone "mass" and the "mechanostat": a proposal. Anat Rec 219:1–9
14. **Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ** 1997 Long-term testosterone administration increases visceral fat in female to male transsexuals. J Clin Endocrinol Metab 82:2044–2047
15. **Carter DR, Bouxsein ML, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137–145
16. **Meyer WJ, Bockting W, Cohen-Kettenis P, Coleman E, DiCeglie D, Devor H, Gooren L, Hage JJ, Kirk S, Kuiper B, Laub D, Lawrence A, Menard Y, Monstrey S, Patton J, Schaefer L, Webb A, Wheeler CC**2001 Harry Benjamin International Gender Dysphoria Association's the standards of care for gender identity disorders, 6th version. Int J Transgenderism, vol. 5, no. 1. http://www.wpath.org/documents2/socv6.pdf
17. **Baecke JA, Burema J, Frijters JE** 1982 A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 36:936–942
18. **Lapauw BM, Taes Y, Bogaert V, Vanbillemont G, Goemaere S, Zmierczak HG, De Bacquer D, Kaufman JM** 2009 Serum estradiol is associated with volumetric BMD and modulates the impact of physical activity on bone size at the age of peak bone mass—a study in healthy male siblings. J Bone Miner Res 24:1075–1085
19. **Taes Y, Lapauw B, Griet V, De Bacquer D, Goemaere S, Zmierczak H, Kaufman JM** 2010 Prevalent fractures are related to cortical bone geometry in young healthy men at age of peak bone mass. J Bone Miner Res 25:1433–1440
20. **Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Després JP** 1993 Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. Am J Clin Nutr 58:463–467
21. **Kirchengast S, Huber J** 2001 Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. Hum Reprod 16:1255–1260
22. **Kirchengast S, Huber J** 2004 Body composition characteristics and fat distribution patterns in young infertile women. Fertil Steril 81:539–544
23. **Davis SR, Walker KZ, Strauss BJ** 2000 Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. Menopause 7:395–401
24. **Erdtsieck RJ, Pols HA, van Kuijk C, Birkenhäger-Frenkel DH, Zeelenberg J, Kooy PP, Mulder P, Birkenhäger JC** 1994 Course of bone mass during and after hormonal replacement therapy with and without addition of nandrolone decanoate. J Bone Miner Res 9:277–283
25. **Lovejoy JC, Bray GA, Bourgeois MO, Macchiavelli R, Rood JC, Greeson C, Partington C** 1996 Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women—a clinical research center study. J Clin Endocrinol Metab 81:2198–2203
26. **Notelovitz M** 2002 Androgen effects on bone and muscle. Fertil Steril 77(Suppl 4):S34–S41
27. **Kaji H, Kosaka R, Yamauchi M, Kuno K, Chihara K, Sugimoto T** 2005 Effects of age, grip strength and smoking on forearm volumetric bone mineral density and bone geometry by peripheral quantitative computed tomography: comparisons between female and male. Endocr J 52:659–666
28. **Khosla S, Melton 3rd LJ, Achenbach SJ, Oberg AL, Riggs BL** 2006 Hormonal and biochemical determinants of trabecular microstructure at the ultradistal radius in women and men. J Clin Endocrinol Metab 91:885–891
29. **Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C** 2004 Androgens and bone. Endocr Rev 25:389–425
30. **Haapasalo H, Kontulainen S, Sievänen H, Kannus P, Järvinen M, Vuori I** 2000 Exercise-induced bone gain is due to enlargement in bone size without a change in volumetric bone density: a peripheral quantitative computed tomography study of the upper arms of male tennis players. Bone 27:351–357
31. **Vanbillemont G, Lapauw B, Bogaert V, Goemaere S, Zmierczak HG, Taes Y, Kaufman JM** 2010 Sex hormone-binding globulin as an independent determinant of cortical bone status in men at the age of peak bone mass. J Clin Endocrinol Metab 95:1579–1586
32. **Zucker KJ** 2005 Measurement of psychosexual differentiation. Arch Sex Behav 34:375–388
33. **Stevens J, Katz EG, Huxley RR** 2010 Associations between gender, age and waist circumference. Eur J Clin Nutr 64:6–15
34. **Kanis JA, Johnell O, Oden A, Johansson H, De Laet C, Eisman JA, Fujiwara S, Kroger H, McCloskey EV, Mellstrom D, Melton LJ, Pols H, Reeve J, Silman A, Tenenhouse A** 2005 Smoking and fracture risk: a meta-analysis. Osteoporos Int 16:155–162

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 24 October 2015. at 15:02 For personal use only. No other uses without permission. . All rights reserved.