

## The Effect of Androgen Treatment on Bone Metabolism in Female-to-Male Transsexuals\*

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### ABSTRACT

Histomorphometry was performed in iliac crest biopsies from 15 female-to-male (F-M) transsexuals with a mean age of  $30 \pm 6.1$  (SD) years. At the time of study, they had been treated with parenteral testosterone esters (250 mg/2 weeks) or oral testosterone undecanoate (160 mg/day). The median androgen treatment duration was 39 months. The patients had undergone hysterectomy and bilateral ovariectomy. The bone biopsy was obtained after double tetracycline labeling. Histomorphometric results were compared with data from 8 postmenopausal women and with data from 11 healthy men who had died suddenly. Dynamic parameters were compared with data from the literature. The biochemical picture was characterized by serum testosterone concentrations lower than in eugonadal men and estradiol concentrations lower than in eugonadal women and elevated gonadotrophin levels. The bone mineral density in the F-M transsexuals was as expected for age (Z-score  $-0.31 \pm 1.49$ ). Cortical thickness was significantly higher in F-M transsexuals than in both control groups. Trabecular bone structure was similar in F-M transsexuals and both control groups. The bone formation parameters were generally lower in F-M transsexuals than in the control groups. The eroded surface was lower in F-M transsexuals than in postmenopausal women. The low bone turnover and preservation of trabecular bone is consistent with the assumption that testosterone treatment protects the bone in these F-M transsexuals from the deleterious effects of estrogen deficiency. The increased cortical thickness suggests an anabolic effect of the testosterone treatment. (*J Bone Miner Res* 1996;11:1769-1773)

### INTRODUCTION

**T**RANSSEXUALISM is the condition in which a person with an apparently normal somatic sexual differentiation has the conviction that he or she is actually a member of the opposite sex.<sup>(1,2)</sup> When the condition is confirmed by repeated psychological testing, cross-sex hormonal treatment is started with estrogens and antiandrogens in male-to-female (M-F) and androgens in female-to-male (F-M) transsexuals. This may be followed after 1 or more years of hormone treatment by sex-reassignment surgery including gonadectomy.<sup>(3)</sup> The agonadal patient should be treated with

lifelong hormonal replacement therapy. F-M transsexuals are usually treated with parenteral testosterone. This androgen treatment is associated with a reduction in estrogen levels, although testosterone is partly aromatized to estradiol. The net result is that the estrogen levels in the F-M transsexual are in the range of normal men, even after ovariectomy.

Sex hormones have a major influence on skeletal growth, peak bone mass, and subsequent bone loss.<sup>(4-6)</sup> Deficiency of estrogens or androgens leads to a lower peak bone mass when occurring during pubertal growth and to increased bone loss when occurring later in life.<sup>(6-9)</sup> The study of the effects of cross-sex hormonal treatment on bone metabolism in adult transsexuals may elucidate differences in the effects of sex hormones on the skeleton. Earlier we reported the influence of estrogen and antiandrogen treatment on bone metabolism in M-F transsexuals.<sup>(10)</sup> Bio-

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chemical and histomorphometric data indicated that this treatment suppressed bone turnover and did not lead to bone loss. In this paper, we report histomorphometric data of bone biopsies and biochemical parameters in F-M transsexuals who had been treated with androgens for 1–10 years. The main question was whether the decreased estrogen production in F-M transsexuals following testosterone treatment would lead to bone loss or would influence bone quality in another way.

## MATERIALS AND METHODS

Subjects were 15 F-M transsexuals with a mean age of  $30 \pm 6.1$  (SD) years. They were healthy phenotypical women at the start of treatment. At the moment of study, they had been treated with parenteral testosterone esters 250 mg/2 weeks ( $n = 12$ ) or testosterone undecanoate 160 mg/day orally ( $n = 3$ ). The median androgen treatment period was 39 months. The patients had undergone hysterectomy and bilateral ovariectomy after a minimum of 1 year of hormonal treatment. The median time interval from ovariectomy to bone biopsy was 25 months. The study was approved by the ethical review board of the hospital, and the patients gave their informed consent. Bone biopsies were obtained at the occasion of plastic surgery (phalloplasty). Fasting 2-h urine and a blood sample were obtained between 3 and 4 weeks before surgery when the subjects were still receiving their hormone treatment. The latter was stopped at the moment of surgery to avoid thromboembolic events. The urine and/or serum calcium, phosphate, albumin, creatinine, and alkaline phosphatase concentrations were measured with routine laboratory methods. The urine hydroxyproline concentration was measured by high performance liquid chromatography (HPLC) with an interassay coefficient of variation (CV) below 3.2% and expressed as hydroxyproline/creatinine ratio. The serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone, and osteocalcin concentrations were measured by radioimmunoassay. The serum intact parathyroid hormone concentration (PTH[1–84]) was measured by immunoradiometric assay. Serum 25-hydroxyvitamin D was measured by competitive protein binding assay.

The patients were doubly labeled with tetracycline, 250 mg four times/day according to a 2-10-2 schedule. A transiliac bone biopsy was obtained during operation. The biopsies were fixed in phosphate-buffered formaldehyde, embedded in methyl-methacrylate without decalcification, and cut with a Jung K microtome.<sup>(11)</sup> Sections of 5  $\mu\text{m}$  were stained with Goldner's trichrome, or with solochrome cyanine, or left unstained for tetracycline fluorescence studies. The following parameters were measured with histomorphometric techniques as described before,<sup>(12)</sup> i.e., Zeiss integrating eyepieces I and II (bone mass and turnover) or drawing tube connected with digitizing tablet (fluorescence parameters):

- Cortical thickness (Ct.Th), mean thickness of both cortical plates measured with an eyepiece micrometer ( $\mu\text{m}$ )
- Bone volume (BV/TV), trabecular bone volume as percentage of tissue volume (%)
- Bone surface (BS/TV), trabecular specific surface per unit of tissue volume ( $\text{mm}^2/\text{mm}^3$ )

- Trabecular thickness (Tb.Th), trabecular plate thickness calculated from BV/TV and BS/TV ( $\text{BV}/\text{BS} \times 20$ )
- Osteoid volume (OV/BV), trabecular osteoid volume expressed as percentage of bone volume
- Osteoid surface (OS/BS), trabecular osteoid surface expressed as percentage of bone surface
- Osteoid thickness (O.Th), thickness of osteoid seams calculated with the formula

$$\text{O.Th} = \frac{\Sigma h_o}{\Sigma i_o} \times \frac{d}{2}$$

in which  $\Sigma h_o$  = all hits on osteoid,  $\Sigma i_o$  = all intersections with osteoid, and  $d$  = grid constant

- Eroded surface (ES/BS), total resorption surface as percentage of trabecular bone surface
- Mineral apposition rate (MAR), the mean distance between double tetracycline labels, divided by the labeling interval and multiplied by the correction factor  $\pi/4$ .
- Mineralizing surface (MS/BS), expressed as the total extent of double label plus half the extent of single tetracycline label
- Bone formation rate (BFR/BS), calculated as the product of MAR and MS/BS
- Adjusted apposition rate (Aj.AR), calculated as the product of MAR and MS/BS divided by OS/BS.
- Mineralization lag time (MLT), calculated as osteoid thickness divided by Aj.AR.

The mineralizing surface could be measured in 13 transsexuals (2 were not labeled) and the mineral apposition rate in 12 transsexuals. Definitions and abbreviations are according to the ASBMR Nomenclature Committee.<sup>(13)</sup>

The histomorphometric results were compared with data from bone biopsies of eight healthy postmenopausal women (mean age  $57.5 \pm 5.0$  years) not receiving estrogen therapy. Two of these women had coxarthrosis, one had mandibular surgery, and five had low bone mass but no vertebral deformities. Other diseases influencing the skeleton were excluded in these patients. In addition, histomorphometric results were compared with data from bone samples of 11 healthy men (mean age  $39.6 \pm 9.4$  years) who had died suddenly, following traffic accidents or acute myocardial infarction. These bone samples came from the Department of Pathology in Aarhus (kindly provided by Dr. F. Melsen). In these controls, hepatic and renal diseases, diseases influencing the skeleton, immobility, and alcohol abuse were excluded by history and autopsy. Dynamic parameters of bone formation were compared with data of normal British women and men of similar age.<sup>(14)</sup>

Bone mineral density (BMD) was measured in the lumbar spine with dual-photon absorptiometry (Novo BMC Lab 22A) or dual-energy X-ray absorptiometry (Norland XR 26). Measurements of BMD at the time of biopsy were taken in 11 patients. Values are expressed as Z-scores (compared with healthy age-matched women and men).

Statistical evaluation was done by Student's *t*-test when appropriate. In case of a skewed distribution, log transformation was performed, and if skewness persisted, the Mann-Whitney U-test was performed.

TABLE 1. BIOCHEMICAL AND BMD DATA (MEAN  $\pm$  SD) OF 15 F-M TRANSSEXUALS COMPARED WITH REFERENCE VALUES OF NORMAL ADULTS, MEN (M) AND WOMEN (F)

	<i>F-M</i>	<i>Ref. values</i>
Calcium (mmol/l)	2.40 $\pm$ 0.12	2.15–2.60
Phosphate (mmol/l)	1.10 $\pm$ 0.12	0.80–1.30
Creatinine ( $\mu$ mol/l)	83 $\pm$ 8	60–110
Alkaline phosphatase (U/l)	60 $\pm$ 10	<90
Osteocalcin ( $\mu$ g/l)	3.5 $\pm$ 1.5	1.5–6.5
PTH (pmol/l)	1.6 $\pm$ 0.6	N.D.–4.0
FSH (U/l)	53 $\pm$ 36	(M) < 14 (F) < 12
LH (U/l)	24 $\pm$ 19	(M) < 9 (F) < 20
Testosterone (nmol/l)	6.6 $\pm$ 6.1	(M) 8–24 (F) < 2.5
Oestradiol (pmol/l)	75 $\pm$ 40	(M) 40–200 (F) 110–850
Urine Ca/Cr (mmol/mmol)	0.30 $\pm$ 0.21	<0.45
Urine Hp/cr ( $\mu$ mol/mmol)	21.0 $\pm$ 8.8	<30
BMD lumbar spine (F)	–0.31 $\pm$ 1.49	(min –2.91 max 2.43)
BMD lumbar spine (M)	–0.56 $\pm$ 1.45	(min –2.50 max 2.18)

The BMD was measured in 11 transsexuals and expressed as Z-scores of normal women (F) and normal men (M).

TABLE 2. STATIC HISTOMORPHOMETRIC DATA OF 15 F-M TRANSSEXUALS COMPARED WITH DATA OF 8 POSTMENOPAUSAL WOMEN AND 11 MEN (AUTOPSIES)

	<i>F-M</i>	<i>Postmenopausal women</i>	<i>Men (autopsies)</i>
Cortical thickness ( $\mu$ m)	1272 $\pm$ 263*	943 $\pm$ 320	971 $\pm$ 280
Bone volume, BV/TV (%)	20.3 $\pm$ 5.6	22.9 $\pm$ 4.1	16.7 $\pm$ 5.3
Bone surface, BS/TV (mm <sup>2</sup> /mm <sup>3</sup> )	3.2 $\pm$ 0.6	3.5 $\pm$ 0.5	3.1 $\pm$ 0.7
Trabecular thickness, Tb.Th ( $\mu$ m)	128 $\pm$ 27	132 $\pm$ 21	108 $\pm$ 22
Osteoid volume, OV/BV (%)	1.5 $\pm$ 1.0	2.0 $\pm$ 0.9	2.1 $\pm$ 1.4
Osteoid surface, OS/BS (%)	10.9 $\pm$ 9.9 <sup>†</sup>	9.3 $\pm$ 2.7	21.1 $\pm$ 11.9
Osteoid thickness, O.Th ( $\mu$ m)	12.1 $\pm$ 4.3 <sup>‡</sup>	13.0 $\pm$ 4.6	5.9 $\pm$ 1.6
Eroded surface, ES/BS (%)	2.7 $\pm$ 1.3 <sup>§</sup>	4.1 $\pm$ 1.4	3.7 $\pm$ 1.4

\*Different from both control groups ( $p < 0.02$ ).

<sup>†</sup> Different from men ( $p < 0.05$ ).

<sup>‡</sup> Different from men ( $p < 0.01$ ).

<sup>§</sup> Different from postmenopausal women ( $p < 0.05$ ) and men (borderline,  $p = 0.054$ ).

## RESULTS

Biochemical and BMD data of the F-M transsexuals are presented in Table 1. The biochemical markers of bone turnover in the transsexuals were within reference limits of normal men with a few exceptions. Serum concentrations of PTH were low in general. Serum levels of testosterone were normal or low for men but well above the reference limits for women. Gonadotrophin levels were normal or elevated in most cases. The BMD expressed as Z-score was as expected for age, with some exceptions in the higher and lower range.

The results of static histomorphometric measurements are compared with data of postmenopausal women and male control subjects in Table 2. Ct.Th was higher in F-M transsexuals than in postmenopausal women and men. Trabecular bone structure was similar in F-M transsexuals and both control groups. The OS/BS was lower but O.Th was higher in F-M transsexuals than in male control subjects.

The osteoid values in the transsexuals were similar to those in postmenopausal women. The eroded surface was lower in the F-M transsexuals than in postmenopausal women. The histomorphometric kinetic data are presented in Table 3. The MAR was similar in the transsexuals and the controls. The MS/BS and BFR/BS were lower in F-M transsexuals than in controls.

Significant correlations were observed between Ct.Th and calcium excretion ( $r = -0.65$ ,  $p < 0.05$ ), MS/BS and hydroxyprolin excretion ( $r = 0.71$ ,  $p < 0.01$ ), BFR/BS and hydroxyprolin excretion ( $r = 0.72$ ,  $p < 0.01$ ), BV/TV and osteocalcin ( $r = 0.54$ ,  $p < 0.05$ ).

## DISCUSSION

The androgen treatment in F-M transsexuals leads to a change from a female to a male hormonal environment. The F-M transsexuals in this study had been treated with

TABLE 3. DYNAMIC HISTOMORPHOMETRIC DATA OF F-M TRANSEXUALS COMPARED WITH CONTROL DATA FROM NORMAL BRITISH WOMEN AND MEN<sup>(14)</sup>

	<i>F-M</i>	<i>Normal women</i>	<i>Normal men</i>
Mineral apposition rate, MAR ( $\mu\text{m}/\text{day}$ )	$0.63 \pm 0.21$	$0.59 \pm 0.09$	$0.58 \pm 0.10$
Mineralizing surface, MS/BS (%)	$6.1 \pm 4.5^*$	$11.0 \pm 5.9$	$10.2 \pm 5.0$
Bone formation rate, BFR/BS ( $\mu\text{m}^3/\mu\text{m}^2/\text{day}$ )	$0.040 \pm 0.020^\dagger$	$0.069 \pm 0.037$	$0.058 \pm 0.030$
Adjusted apposition rate, Aj.AR ( $\mu\text{m}/\text{day}$ )	$0.44 \pm 0.39$	$0.42 \pm 0.36$	$0.31 \pm 0.27$
Mineralization lag time, MLT (days)	$48 \pm 30^\ddagger$	$21 \pm 14$	$34 \pm 27$

\*Significantly different from normal women and men ( $p < 0.05$ ).

† Significantly different from normal women and men ( $p < 0.05$ ).

‡ Significantly different from normal women ( $p < 0.01$ ).

androgens for a median time of more than 3 years, and their ovaries had been removed. Their estrogen production was greatly diminished, and their estrogen state resembled that of normal men. However, their histomorphometric picture did not show signs of estrogen deficiency. The eroded surface was significantly lower than in postmenopausal women. In addition, the biopsies did not show a decrease of BV/TV and BS/TV due to a loss of trabeculae as may be seen in late postmenopausal women.<sup>(15-17)</sup> The BMD of the lumbar spine in the transsexuals was similar to age-matched control values. This suggests that the androgen therapy in these ovariectomized women prevented bone loss to a similar degree as estrogen replacement therapy does in postmenopausal women.<sup>(18)</sup> It is known from the literature that the androgen excess in hirsute women may protect the skeleton from bone loss due to their estrogen deficiency.<sup>(19)</sup> Conversely, one may expect in these F-M transsexuals a change of bone metabolism in the male direction. The Ct.Th was higher in these transsexuals than in the postmenopausal women and the male control group. One may argue that the male controls were autopsy controls and thus not perfectly healthy, although diseases influencing the skeleton had been excluded. In addition, the measurement of Ct.Th is not very accurate due to obliquity of the section plane.<sup>(20)</sup> However, the Ct.Th in the F-M transsexuals was also higher than that usually seen in premenopausal women.<sup>(21,22)</sup> This suggests an anabolic effect of the testosterone treatment on cortical bone.<sup>(23)</sup>

It is more difficult to explain the histomorphometric parameters of bone turnover. The interpretation is also hampered by the lack of adequate dynamic control data. Nevertheless, OS/BS, MS/BS, and BFR were significantly lower in the F-M transsexuals than in male controls. The eroded surface was lower in the transsexuals than in postmenopausal women and men (the latter borderline). This suggests a lower bone turnover in these transsexuals during testosterone treatment than in our controls. OS/BS is lower in women than in men in some normal series,<sup>(24,25)</sup> but the BFR is similar in both sexes.<sup>(14)</sup> The lower bone turnover in the transsexuals may be explained by the fact that the androgen levels during parenteral testosterone administration are not very physiological. The serum testosterone concentration is supranormal in the first 2-3 days, decreasing exponentially to subnormal levels until the next injection. On the basis of this pharmacotherapeutical profile, the

mean serum testosterone concentrations in the F-M transsexuals were below the reference limits for healthy men, and the mean serum gonadotrophin concentrations were elevated. This resembles the hormonal picture of men with hypergonadotrophic hypogonadism supplemented with testosterone.<sup>(25,26)</sup>

This is unlike the hormonal profile in M-F transsexuals being more completely changed to the female side. They are treated with supraphysiological doses of estrogen in order to reach an adequate phenotypic change. As shown before, this results in a suppression of bone turnover in M-F transsexuals.<sup>(10)</sup> In general, it must be concluded that continued cross-sex hormonal treatment of transsexuals does not appear to have deleterious effects on the skeleton. In conclusion, this study suggests that testosterone treatment in F-M transsexuals protects the bone from the deleterious effects of estrogen deficiency. It also suggests that testosterone exerts an anabolic effect on cortical bone in these subjects.

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