

Long-term follow-up of bone mineral density and bone metabolism in transsexuals treated with cross-sex hormones

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Summary

OBJECTIVE It is unknown whether long term cross-sex hormone treatment affects the human skeleton. We monitored bone mineral density and biochemical markers of bone turnover for 28–63 months in 20 male-to-female transsexuals (M→F) treated with anti-androgens and oestrogens, and 19 female-to-male transsexuals (F→M) treated with androgens. They underwent gonadectomy 13–35 months after the start of cross-sex hormone administration.

DESIGN Bone mineral density (BMD) and the markers of bone turnover osteocalcin, alkaline phosphatase, fasting urinary calcium/creatinine and hydroxyproline/creatinine, were measured at baseline, after 1 year and after 28–63 months of cross-sex hormone administration.

RESULTS In oestrogen-treated M→F, variables of bone turnover decreased significantly with consecutive measurements. BMD had increased significantly after 1 year, but decreased again to baseline levels after 28–63 months of cross-sex hormones.

In F→M, alkaline phosphatase levels increased during the first year. BMD did not change during the first year but had decreased significantly after 28–63 months following ovariectomy. In both M→F and F→M, the change of BMD correlated inversely with serum LH and FSH levels. Of all biochemical variables LH levels appeared to be the best predictor of loss of BMD; in the long-term LH levels were more elevated in

testosterone-treated F→M than in oestrogen-treated M→F transsexuals.

CONCLUSION In M→F, oestrogen treatment prevented bone loss after testosterone deprivation. In F→M the testosterone dosage used, associated with a decline in serum oestradiol levels, was unable to maintain bone mass fully in all subjects in the longer term. The inverse relationship between BMD and serum LH levels suggests that the dose of hormone replacement has been too low in subjects with a decline in their BMD. Its cause might be underdosing or non-compliance in some patients. We propose that serum LH levels may be used as a measure of the adequacy of replacement with sex steroids.

Sex steroids are important regulators of bone metabolism and bone mineral density (BMD) (Turner *et al.* 1994, Orwoll & Klein, 1995). They play a substantial role in bone growth and the acquisition of the peak bone mass during puberty, and further in the maintenance of bone mass during adulthood. The latter is evidenced by the development of osteoporosis in both women (Shore *et al.*, 1982; Genant *et al.*, 1982; Drinkwater *et al.*, 1984) and men (Francis *et al.*, 1986; Finkelstein *et al.*, 1987; Jackson *et al.*, 1987; Stepan *et al.*, 1989; Murphy *et al.*, 1993) following the decrease of cessation of their own sex steroid production. Sex hormone replacement therapy has been shown to prevent further loss (Recker *et al.*, 1977; Finkelstein *et al.*, 1989; Diamond *et al.*, 1991; Felson *et al.*, 1993; Gulekli *et al.*, 1994; Aitken *et al.*, 1995).

During the process of adaptation to the opposite sex, transsexuals receive cross-sex hormone treatment and therewith undergo a complete change of their hormonal milieu (oestrogens in males and androgens in females). Sex reassignment includes gonadectomy, after which subjects continue their cross-sex hormone treatment analogous to other hypogonadal subjects. Previously, this clinic reported a histomorphometric study showing that continued oestrogen treatment in orchidectomized male-to-female transsexuals was not associated with bone loss (Lips *et al.*, 1989). Subsequently, we observed that bone mass in both male-to-female (M→F) and female-to-male (F→M) transsexuals had not decreased after 1 year of cross-sex hormone administration (Van Kesteren *et al.*, 1996).

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However, it is not known whether longer-term treatment with cross-sex hormones causes skeletal changes. We therefore followed bone mineral density (BMD) and biochemical variables of bone metabolism in both M→F and F→M transsexuals to assess long-term effects of cross-sex hormone administration on bone. In addition, we also collected information on body mass index, smoking habits and alcohol consumption, since these are important co-determinants of BMD.

Subjects and methods

Subjects and sex hormone treatment

Twenty male-to-female transsexuals (M→F) (mean age 25.4 years, range 16–38, body mass index 22.1, range 18.6–27.1) and 19 female-to-male (F→M) transsexuals (mean age 25.0, range 16–39; body mass index 22.1, range 16.9–25.8) were followed for 28–63 months during their sex reassignment treatment. They all gave their informed consent to this study which had been approved by the hospital ethical review board. In our clinic, sex reassignment treatment implies a minimum of 12–18 months of cross-sex hormone treatment (i.e. anti-androgen and oestrogen treatment of males and androgen treatment of females) before sex-reassignment surgery with gonadectomy takes place, after which cross-sex hormone administration is to be continued, generally in dosages comparable to those in hypogonadal subjects. All subjects continued to take their normal diet throughout the study. At the beginning of the study all subjects were eugonadal (both by clinical and biochemical criteria) and had received no earlier hormone administration. All M→F transsexuals were treated with the anti-androgen cyproterone acetate (Schering AG, Germany) 100 mg/day in combination with ethinyl oestradiol (Organon, The Netherlands) 100 µg/day orally until gonadectomy. After surgery, most M→F transsexuals did not require anti-androgen therapy anymore and oestrogen treatment was no longer needed for the induction of a female appearance, but was used to maintain the acquired female features and to prevent osteoporosis. Therefore, oestrogen administration consisted of ethinyl oestradiol 1–2 × 50 µg per day ($n = 15$) or, if the former oestrogen was not well tolerated, other types of oestrogens (conjugated oestrogens, $n = 4$ or transdermal oestradiol, $n = 1$). Eight subjects smoked and 9 were non-smokers, while no information was available for three subjects. Six subjects did not drink alcohol, 9 drank 1–3 and two more than 3 alcoholic drinks per day. No information was available for three subjects.

All F→M transsexuals were initially treated with parenteral testosterone esters (Sustanon, Organon, The Netherlands) 250 mg/2 weeks i.m. Following gonadectomy, most F→M transsexuals continued with parenteral testosterone every 2–3

weeks i.m. Blood samples for hormone determinations were drawn 4–8 days following the last parenteral testosterone administration. Seven subjects disliked further parenteral testosterone administration and changed to testosterone undecanoate (Organon, The Netherlands) 160 mg/day orally. Seven subjects were non-smokers and 10 smoked, while no information was available on 2. Eight subjects did not consume alcohol, 6 drank 1–3, and 3 more than 3 alcoholic drinks per day. No information was available on 2 patients.

Methods

Bone mineral density (BMD), biochemical variables of bone metabolism were determined at baseline (before cross-sex hormone treatment) after 1 year and after 28–63 months (final measurements) of the cross-sex hormone treatment.

BMD of the lumbar spine at the level of L2–4 was measured by dual-energy X-ray absorptiometry (DXA). DXA was performed with a Norland XR-26 bone densitometer. With this bone densitometer, the long-term precision (coefficient of variation) of BMD of the lumbar spine was 2.3%. The drift in daily phantom measurements during 3 years was 0.5%.

Blood samples and fasting 2-hour urine samples to measure variables of bone metabolism and serum hormone levels were collected at between 0900 and 1200 h and included calcium (corrected for serum albumin level: Ca corr.), phosphate, alkaline phosphatase, and osteocalcin. Urinary calcium (Ca) and hydroxyproline (Hypro) excretion were corrected for the glomerular filtration rate with the following equations: $CaE = [Ca]u \times [creat]s/[creat]u$ and $HyproE = [Hypro]u \times [creat]s/[creat]u$ (units in mmol/l). The renal threshold phosphate concentration ($TmPO_4/GFR$) was calculated according to the method described by Walton and Bijvoet (1975). Serum hormone measurements included 17β -oestradiol, testosterone, LH and FSH.

Assays

The serum and/or urine calcium, phosphate, creatinine, albumin and alkaline phosphatase were measured with routine laboratory methods. Serum osteocalcin concentrations were measured by radioimmunoassay (RIA) using a kit of Incstar Corporation (inter-assay CV 13%). Urinary hydroxyproline was measured as described by Teerlink *et al.* (1989).

The hormonal variables LH, FSH, 17β -oestradiol (E_2) were measured by immunoradiometric assays (inter-assay CV (for low serum levels) LH 10%, FSH: 9%; E_2 : 8%) and testosterone (T) was measured by radioimmunoassay (inter-assay CV 13%, for low serum levels). The lower limits of detection for LH, FSH, E_2 and T were 0.3 U/l, 0.5 U/l, 90 pmol/l and 1.0 nmol/l, respectively.

Statistics

All data are reported as the mean \pm SD. If measurements appeared to be under the lower limit of detection, the value of that lower limit was used for statistical calculations. Skewed data were log transformed. To assess significance, ANOVA for repeated measurements was used. Student's *t*-test or Wilcoxon rank sum test was used if appropriate. Correlations were calculated using Pearson's correlation coefficient or, in case of skewed parameters, Spearman's rank correlation coefficient. The relations between LH levels, age, BMI, smoking habits, alcohol consumption and type of current hormonal treatment on changes in BMD were calculated by linear regression analysis. All calculations were performed using the Statistical Package for the Social Sciences (SPSS).

Results

Male-to-female transsexuals

The results for the M \rightarrow F transsexuals are shown in Table 1. Subjects were followed during 45.5 ± 9.7 (32–63) months of cross-sex hormone treatment. Orchiectomy was performed 20.3 ± 4.1 (17–35) months from the start of hormonal treatment. Final measurements were performed 25.2 ± 10.4 (12–44) months after gonadectomy.

Hormone values. The values of serum 17β -oestradiol at 1 year and at the final measurement were low, which is explained by the fact that the majority of the subjects were treated with synthetic ethinyl oestradiol which is not detected by the 17β -oestradiol assay. Clinical evidence (breast growth, skin

changes), however, confirmed compliance with oestrogen use. Testosterone levels were low after 1 year of hormone treatment and no further changes occurred thereafter. LH and FSH levels declined sharply after 1 year of hormone treatment ($P = 0.002$ and $P = 0.0003$, respectively) but increased after orchiectomy ($P = 0.002$ and $P = 0.002$, respectively: 1 year *vs.* final measurements).

BMD. The mean BMD had increased significantly after 1 year of hormone treatment, but at final measurement was significantly lower than after 1 year of hormone treatment (Fig. 1) but not lower than the baseline values. Remarkably large differences between individual patients were seen. The changes in BMD between the 1-year and final measurement correlated inversely with LH levels at the final measurement ($r = -0.46$, $P = 0.04$, illustrated in Fig. 2), and a similar borderline significant correlation was found with regard to FSH levels ($r = -0.40$, $P = 0.08$). No correlations were found between the change of BMD and age, duration and hormone treatment or time period between orchiectomy and final measurements. Four subjects used conjugated oestrogens after orchiectomy, and the changes of BMD were similar in this group to those observed in the group treated with ethinyl oestradiol. Of the 'fast losers' (percentage change of BMD $> 10\%$: $n = 3$), two used ethinyl oestradiol and one used conjugated oestrogens.

Bone turnover. Both the markers of bone formation, alkaline phosphatase and osteocalcin and the markers of bone resorption, urinary calcium and hydroxyproline excretion were significantly lower after 1 year of anti-androgen and oestrogen administration. The final measurements, however, showed no

Table 1 Male-to-female transsexuals: variables of bone mineral density and bone metabolism, and hormone values at baseline, 1 year of hormone treatment and at final measurements. Significance for the consecutive measurements was calculated by ANOVA

Variables	Baseline	1 year	Final measurements	<i>P</i>
BMD spine (g/cm ²)	1.04 \pm 0.12	1.08 \pm 0.13	1.04 \pm 0.14	0.002
Ca corr. (mmol/l)	2.31 \pm 0.12	2.36 \pm 0.1	2.40 \pm 0.24	NS
Phosphate (mmol/l)	1.04 \pm 0.15	1.04 \pm 0.14	1.04 \pm 0.15	NS
Alk. phosph. (U/l)	65 \pm 34	41 \pm 12	51 \pm 15	0.001
Osteocalcin (μ g/l)	4.7 \pm 2.0	1.9 \pm 0.8	2.6 \pm 1.5	0.001
Serum creatinine (μ mol/l)	81 \pm 7	77 \pm 7	78 \pm 7	0.048
Urinary Ca/GFR (mmol/l)	0.0144 \pm 0.008	0.008 \pm 0.007	0.010 \pm 0.007	0.005
Urinary Hypro/GFR (mmol/l)	0.0022 \pm 0.0012	0.0013 \pm 0.0005	0.0013 \pm 0.0005	<0.001
17β -oestradiol (pmol/l)	109 \pm 18*			
LH (U/l)	3.4 \pm 1.4	0.4 \pm 0.3	9.0 \pm 9.8	0.001
FSH (U/l)	4.7 \pm 6.3	0.7 \pm 0.8	13.0 \pm 15.0	0.01
Testosterone (nmol/l)	28.1 \pm 6.6	1.0 \pm 0.1	1.1 \pm 0.2	<0.001

NS: not significant.

*The majority of the male-to-female transsexuals were treated with ethinyl oestradiol which was not detected by the laboratory assay used. Hence, 17β -oestradiol values at 1 year and final measurements and significance are not presented.

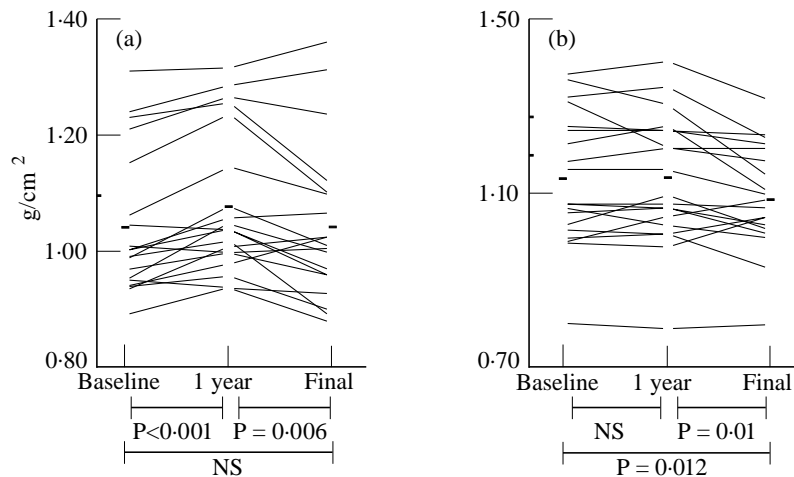


Fig. 1 Bone mineral density at baseline, after 1 year of hormone treatment and at the final measurement in (a) male-to-female (M→F) and (b) female-to-male (F→M) transsexuals (- = mean).

significant changes except for the serum level of alkaline phosphatase. The latter was significantly higher at the final measurement than at 1 year ($P=0.003$), but it was still significantly lower than at baseline ($P=0.008$). With consecutive measurements no correlations were found between markers of bone turnover and the changes in BMD. Serum levels of calcium and phosphate did not change. The data were not complete enough to calculate $TmPO_4/GFR$.

Clinical characteristics. BMI increased during hormone treatment (baseline: 22.1 ± 2.4 , 1 year: 23.0 ± 2.3 , final measurement: 24.2 ± 3.4 kg/m², $P < 0.001$). No correlations were observed between the change in BMD and the change in BMI. Data on tobacco use or alcohol consumption assessed at baseline were insufficient to produce firm conclusions.

Female-to-male transsexuals

The results of the F→M transsexuals are shown in Table 2. Subjects were followed during 38.2 ± 8.6 (28–53) months of hormone treatment. Ovariectomy was performed after on average 15.5 ± 2.1 (13–20) months after the start of the hormonal treatment. Final measurements were performed on average 22.7 ± 9.2 (11–39) months after gonadectomy.

Hormone values

As expected, testosterone levels (measured 4–8 days after the previous injection) had increased significantly after treatment and 17β -oestradiol levels decreased significantly. LH and FSH levels both decreased significantly after 1 year of hormone treatment ($P=0.004$ and $P < 0.001$, respectively) and increased significantly after ovariectomy ($P=0.001$ and $P < 0.001$, respectively: 1 year versus final measurements). At the final measurements, LH and FSH levels were higher in F→M than in M→F transsexuals ($P=0.012$ and $P=0.001$, respectively).

BMD

BMD had not changed after 1 year of testosterone administration (Fig. 1). The final BMD measurements were significantly lower than the values measured after 1 year of hormone treatment and at baseline. The changes in BMD between 1 year

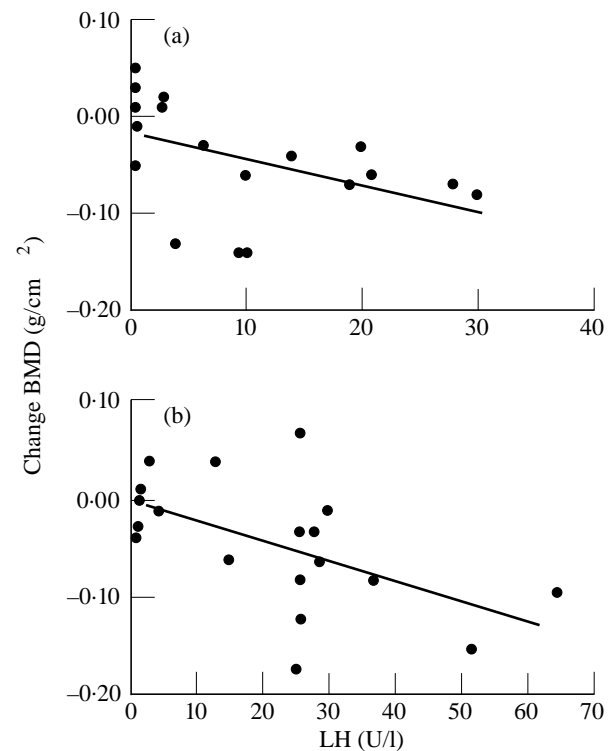


Fig. 2 Relationship between the serum LH level at final measurement and the change in BMD from measurement at 1 year of hormone treatment to the final measurement in (a) male-to-female, $r = -0.46$, $P = 0.04$; and (b) female-to-male, $r = -0.54$, $P = 0.02$, transsexuals.

and final measurements correlated significantly with the serum LH level at the final measurement ($r = -0.54$, $P = 0.02$, illustrated in Fig. 2), and borderline significantly with the FSH level ($r = -0.41$, $P = 0.09$) and with age at the time of the final BMD measurement ($r = -0.45$, $P = 0.05$). The effect of serum LH at the final measurement on the change of BMD after adjustment for age was highly significant ($r = -0.73$, $P = 0.004$). No correlations were found between the decrease in BMD and the time period between ovariectomy and final measurements. The change in BMD was not different between subjects who used oral testosterone undecanoate and those using parenteral testosterone esters as current hormone treatment.

Bone turnover

Serum alkaline phosphatase levels increased significantly with consecutive measurements. Other markers of bone turnover did not change. Changes in serum alkaline phosphatase levels over the first year correlated significantly with changes in serum osteocalcin ($r = 0.76$, $P = 0.010$). Serum phosphate levels decreased significantly. In order to elucidate the decline in serum phosphate levels, the renal threshold phosphate concentration (TmPO₄/GFR) was calculated, which showed a significant decrease (1.24 ± 0.16 , 1.05 ± 0.22 and 0.94 ± 0.20 mmol/l, $P = 0.008$). Both the serum creatinine and the urinary creatinine excretion increased with testosterone administration, although the latter not significantly (serum creatinine: 72 ± 8 , 79 ± 9 and 85 ± 6 μ mol/l, $P < 0.001$; urinary creatinine excretion: 4.2 ± 4.7 , 8.9 ± 8.5 and 8.3 ± 8.0 mmol/l, $P = 0.07$).

Clinical characteristics

There was an increase of BMI upon testosterone treatment (baseline: 22.1 ± 2.7 , 1 year: 22.9 ± 2.6 , final measurement: 23.7 ± 2.4 kg/m², $P = 0.02$). No correlations were found between the change in BMD and the change in BMI, or between the change in BMD and smoking or alcohol consumption.

Discussion

We studied the effects of cross-sex hormones on BMD and on markers of bone metabolism during sex reassignment treatment of transsexuals during a follow-up study of, on average, 3.5 years. As part of their treatment, transsexuals had undergone gonadectomy after approximately 1.5 years of hormonal treatment. BMD measurements were performed only at the level of the lumbar spine, which is a limitation for their general validity.

During the first year of their cross-sex reassignment treatment, M→F transsexuals were treated with ethinyl oestradiol and the antiandrogen cyproterone acetate, which induced a radical change of their hormonal environment: testosterone levels fell to castrate values. This treatment was accompanied by a decrease in bone turnover. Oestrogen treatment has been found to suppress bone turnover in postmenopausal women (Christiansen *et al.*, 1982), and this was also observed in our previous histomorphometric study of M→F transsexuals treated with oestrogens and anti-androgens (Lips *et al.*, 1989). In the present study, the decrease in bone turnover was associated with an increase in lumbar BMD after 1

Table 2 Female-to-male transsexuals variables of bone mineral density and bone metabolism, and hormone values at baseline, one year of hormone treatment and at final measurements. Significance for the consecutive measurements was calculated by ANOVA

Variables	Baseline	1 year	Final measurements	P
BMD spine (g/cm ²)	1.14 ± 0.15	1.14 ± 0.15	1.09 ± 0.12	0.003
Ca corr. (mmol/l)	2.36 ± 0.10	2.29 ± 0.11	2.34 ± 0.08	NS
Phosphate (mmol/l)	1.14 ± 0.15	1.04 ± 0.18	1.02 ± 0.15	0.013
Alk. phosph. (U/l)	49 ± 18	56 ± 20	56 ± 17	0.009
Osteocalcin (μ g/l)	2.8 ± 0.9	3.1 ± 1.0	3.0 ± 1.1	NS
Serum creatinine (μ mol/l)	72 ± 8	79 ± 9	85 ± 6	<0.001
TmPO ₄ /GFR (mmol/l)	1.24 ± 0.16	1.05 ± 0.22	0.94 ± 0.20	0.008
Urinary Ca/GFR (mmol/l)	0.013 ± 0.007	0.016 ± 0.010	0.019 ± 0.014	0.03
Urinary Hypro/GFR (mmol/l)	0.0022 ± 0.0028	0.0019 ± 0.0006	0.0019 ± 0.0004	NS
17 β -oestradiol (pmol/l)	224 ± 207	125 ± 45	105 ± 21	0.012
LH (U/l)	4.1 ± 2.1	1.9 ± 1.7	21.6 ± 17.9	<0.001
FSH (U/l)	5.0 ± 1.8	2.7 ± 1.9	46.2 ± 34.3	<0.001
Testosterone (nmol/l)	1.5 ± 0.5	43.8 ± 44.1	23.5 ± 23.8	0.002

NS: not significant.

year of cross-sex hormones, probably as a result of a decrease in remodelling space (Parfitt, 1980; Eriksen *et al.*, 1993). This was no longer demonstrable at the final measurement, 32–63 months after the start of cross-sex hormones. In our study the BMD measurements may have been influenced by the effects of cross-sex hormones on fat distribution. In male-to-female transsexuals there is an increase in subcutaneous fat following oestrogen administration while in female-to-male transsexuals subcutaneous fat decreases following testosterone administration. However, it could be shown in phantom measurements that even large changes in fat content produce only minimal changes (around 1%) in BMD measurements (Johnson & Dawson-Hughes, 1991).

Hypogonadism is a substantial risk factor for bone loss. During treatment, M→F transsexuals had low testosterone levels ($<1.1 \pm 0.2$ nmol/l), caused initially by treatment with cyproterone acetate and ethinyl oestradiol (first 1.5 years), and by orchiectomy thereafter. However, this did not lead to sizeable bone loss in the subjects; the mean bone mass measured after long-term hypotestosteronaemia was similar to the mean bone mass at baseline. In addition, there was no correlation between the change of BMD and the duration of hypotestosteronaemia. This suggests that oestrogens are able to maintain bone mass in the male skeleton in the virtual absence of testosterone. However, there was a large inter-subject variation and a considerable loss of BMD in some subjects. The change of BMD correlated with LH levels at the final measurements. These findings indicate that the BMD loss in some subjects may have been due to insufficient oestrogen treatment, since adequate oestrogen administration in general would have suppressed LH and FSH levels to a greater degree. Inadequate oestrogen treatment may have been due to the reduction of the dosage and/or change in type of oestrogen therapy after orchidectomy. Its dose may have been insufficient to maintain the initial gain of bone mass acquired after 1 year of hormone treatment or, alternatively, non-compliance of the patients with oestrogen therapy might have played a role. A considerable loss was observed in 2 subjects who used ethinyl oestradiol as well as in 1 who used conjugated oestrogens. The type of oestrogen treatment did not appear to have a relation with loss of BMD. The markers of bone turnover remained suppressed, as became clear from the 1 year and final measurements, although the suppression tended to decrease. This might indicate that hormonal treatment after gonadectomy was suboptimal.

In our study the baseline BMD was higher in F→M transsexuals than in M→F transsexuals. A similar observation was made in a large European population study, where BMD of the lumbar spine was higher in women than in men at the age of around 25 years (Dequeker *et al.*, 1995).

One year of testosterone treatment in F→M transsexuals led

to an increase in the biochemical markers of bone formation, as shown by a significant increase of alkaline phosphatase levels, and by the significant correlation between the 1-year changes of alkaline phosphatase and osteocalcin levels. However, this was not reflected in an increase in BMD. This may be due to the predominant biological effect of testosterone on cortical bone, while we measured BMD of the lumbar spine, which is mainly trabecular bone.

After ovariectomy, LH and FSH levels were significantly higher than before although the mean testosterone level was still within the normal male range. Following ovariectomy, 17β -oestradiol concentrations decreased significantly, the levels being comparable to oestradiol levels in men. Low oestrogen levels in women have a deleterious effect on bone mass. Relatively high androgen levels might protect the female skeleton against the effects of low oestrogen levels by their androgen action on bone formation as was observed in hirsute women (Dixon *et al.*, 1989). However, in our group of biological females who underwent ovariectomy and were continuously treated with testosterone, a significant decrease of BMD was found. This decrease of BMD correlated with the increase of LH levels observed at the last measurement. As in the M→F, this may be due to patient non-compliance with testosterone treatment, although it is our clinical impression that F→M transsexuals are in general more compliant to their hormone treatment after gonadectomy than M→F transsexuals. Seven subjects changed to oral testosterone undecanoate therapy, which might theoretically explain the decrease in BMD since oral testosterone produces lower serum testosterone levels than parenteral testosterone. However, the type of testosterone treatment did not have a relation to the amount of bone loss. The most likely explanation for the decrease in BMD, in view of its correlation to LH levels, is that in most subjects the testosterone dose was not sufficient to preserve bone mass. Serum LH levels were higher than 20 U/l (as an arbitrary division line) in 11 of 19 F→M transsexuals. The above suggests that the adequacy of testosterone replacement with regard to the maintenance of bone mass is better assessed with the measurement of LH than of testosterone itself. A parallel could be drawn with thyroid hormone replacement. The adequacy of the replacement is better assessed by TSH levels rather than T_4 levels. The decrease in BMD correlated (borderline significantly) with age at time of the DXA measurement. Therefore, advanced age may also contribute to the observed bone loss in this group. A strong correlation between serum LH and BMD, adjusted for age, could be established, which is difficult to explain. It is not known whether serum LH levels rise increasingly with ageing due to a decreased negative feedback signal. Alternatively, in this population of F→M transsexuals the older a subject is, the longer ago an ovariectomy has likely to have taken place;

further, once hormonal induction of cross-sex sex characteristics has taken place at the beginning of their sex reassignment, most transsexuals are inclined to dose reductions of cross-sex hormones.

Alkaline phosphatase levels continued to be significantly higher than baseline levels. However, the course of BMD values in the F→M transsexuals did not suggest a net increase of bone formation over bone resorption. Alternatively, the increase of alkaline phosphatase might represent a hepatic effect of the testosterone therapy.

In the F→M transsexuals, serum phosphate levels decreased and this may be explained by the decline in the renal phosphate threshold, as we observed previously (Van Kesteren *et al.*, 1996). With the formula and the nomogram we used to determine $TmPO_4/GFR$, its decline can also be explained partly by the slight increase of serum creatinine.

An intriguing finding in our study is that there was less loss of BMD in oestrogen-treated M→F transsexuals than in testosterone-treated F→M transsexuals, in whom also LH levels were higher. The importance of oestrogens in male bone physiology is suggested by the report of Smith *et al.* (1994) who diagnosed osteoporosis in a young man with a disruption of the oestrogen receptor, and by the observation of marked osteopenia in an aromatase-deficient male (Carani *et al.*, 1997) although the latter cases may have suffered from an insufficient acquisition of peak bone mass during puberty. Oestrogen levels fell in the F→M transsexuals during testosterone administration and they may have been insufficient to maintain bone mass. Since in all subjects (both F→M and M→F transsexuals) serum LH was the best predictor of loss of BMD and serum LH levels were higher in F→M than in M→F transsexuals, it may be argued that testosterone substitution in F→M transsexuals may have been inadequately low. It is well known that parenteral administration of testosterone is associated with strongly fluctuating testosterone levels, which might explain why LH levels were higher in F→M than in M→F transsexuals who take an oestrogen preparation daily. It has been established that parenteral testosterone administration at longer intervals than 2 weeks results in lower-than-normal plasma testosterone levels (Behre *et al.*, 1990). In this light it was interesting to note that the serum levels of bone markers of the oestrogen-treated M→F were similar to those of the F→M before the latter received testosterone and were at that stage biologically indistinguishable from normal women. By contrast, particularly the serum levels of markers of bone formation in the testosterone-treated F→M were lower than in the M→F before the latter received oestrogens and were at that stage comparable to normal men.

In conclusion, cross-sex hormone treatment of transsexuals influenced bone mass and bone metabolism. In M→F transsexuals, there were biochemical signs of lower bone turnover but bone loss was prevented which theoretically might

have followed their testosterone deprivation. In so far as serum LH levels reflect adequacy of oestrogen substitution, there was no concern about severe loss of BMD as long as LH levels were not greatly elevated.

In F→M transsexuals there was no demonstrable loss of bone mass after 1 year of testosterone administration, but over time a loss of BMD occurred. Apparently, androgens in a dose similar to that recommended for treatment of hypogonadal men, were not sufficient to preserve bone mass in the longer term in ovariectomized females. In both M→F and F→M transsexuals, the inverse correlation of BMD with serum LH levels indicated a probably less than adequate sex steroid substitution. Serum LH levels may be a better marker of adequate substitution and prevention of bone loss than serum levels of sex steroids themselves or markers of bone turnover. This issue has hardly been addressed in the literature on testosterone replacement and preservation of bone mass in men.

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