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Changes in body composition during post-menopausal hormone therapy: a 2 year prospective study*

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BACKGROUND: Post-menopausal hormone therapy (pHT) induces changes in both body composition and bone mineral density (BMD). METHODS: In 109 post-menopausal women beginning either tibolone 2.5 mg (n = 29), tibolone 1.25 mg (n = 42) or estradiol 2 mg plus norethisterone acetate 1 mg (E_2 + NETA) (n = 38), we assessed body composition, total and regional BMD by dual energy X-ray absorptiometry, and the serum bone alkaline phosphatase (BAP), osteocalcin and the urinary excretion to type I collagen C-telopeptide (CTX) at baseline and after 2 years. RESULTS: At baseline, BMD at all sites correlated negatively with age and years since menopause, and positively with lean mass and fat mass (r = 0.42, P < 0.001 and r = 0.26, P = 0.006 at the total femur). During treatment, BMD increased at all sites (P < 0.001), and serum BAP, osteocalcin, and urinary CTX decreased in all groups (P < 0.001). Lean mass increased whereas android fat and android obesity index decreased. The increase in BMD at all sites correlated positively with changes of lean mass at 2 years. CONCLUSIONS: Both fat mass and lean mass are related to BMD in post-menopausal women, the relationship being strongest with lean mass; an increase in lean mass and a change in distribution of body fat are observed during treatment with E_2 + NETA and tibolone.

Key words: body composition/body mass index/fat mass/hormone replacement therapy/lean mass

Introduction

Menopause is associated with an accelerated bone loss (Hansen *et al*., 1995; Warming *et al*., 2002), an increase in body weight (Wing *et al*., 1991) with changes in body composition characterized by a decrease in lean mass, and an increase and redistribution of fat mass (Wang *et al*., 1994; Aloia *et al*., 1995; Trémollieres *et al*., 1996). The relative proportion of android fat increases in post-menopausal women; this phenomenon, although it might be beneficial for bone density (Heiss *et al*., 1995), is an independent cardiovascular risk factor (Lapidus *et al*., 1984; Larsson *et al*., 1984).

Several studies attempted to find out which body compartment is the major determinant of bone mineral density (BMD), and, beyond the mechanical loading, what are the other mechanisms by which one compartment or another affects the skeleton. These studies have yielded conflicting results. Some of them found that both fat and lean mass are independently related to BMD (Khosla *et al*., 1996; Pluijm *et al*., 2001), the influence being strongest with fat mass in some studies (Compston *et al*., 1992; Reid *et al*., 1992), whereas BMD is more closely related to lean tissue than to fat mass for others (Salamone *et al*., 1995; Chen *et al*., 1997).

Post-menopausal hormone therapy (pHT) prevents bone loss that follows menopause. Considerable attention has been focused on the effects of pHT on body weight and body composition changes occurring with menopause. Some studies found that changes are not prevented by pHT (Aloia *et al*., 1995), or that its effect on the upper body fat deposition is relatively small (Silverstein and Barret-Connor, 1996); other studies report that pHT blunts the increase in body weight, promotes a gynoid distribution and prevents the central shift of body fat commonly observed after the menopause (Haarbo *et al*., 1991; Reubinoff *et al*., 1995; Troisi *et al*., 1995; Perrone *et al*., 1999; Sorensen *et al*., 2001). However, a recent Cochrane review concluded that there is not enough data to determine whether pHT has a preventive effect on redistribution of body fat which is associated with the menopause (Norman *et al*., 2003).

The objectives of this study were to compare the 2 year effects of tibolone with those of a combined 17β-estradiol and norethisterone acetate (E_2 + NETA) treatment on BMD and body composition in post-menopausal women, and to study the relationship of the changes in each of these parameters to changes in the others.

*Data presented in part at the ASBMR 24th annual meeting at San Antonio, Texas, USA.

Table I. Baseline characteristics of the study population

	Tibolone 2.5 (n = 29)	Tibolone 1.25 (n = 42)	E_2 + NETA (n = 38)
Age (years)	55 (5.2)	53 (4.5)	53 (4.7)
Body mass index (kg/m^2)	23.3 (2.5)	22.9 (2.8)	23.2 (3.8)
Years since menopause	4.4 (3.6)	3.4 (3.2)	3.8 (3.5)
Lean mass (kg)	31.6 (2.9)	32.9 (3.7)	33.1 (3.9)
Fat mass (kg)	21.6 (6.2)	21.7 (6.4)	21.7 (7.8)
Gynoid fat (%)	40.3 (7.0)	42.8 (7.8)	43.8 (8.6)
Android fat (%)	44.9 (7.2)	41.9 (6.3)	41.0 (8.0)
Android index	1.17 (0.34)	1.03 (0.31)	1.01 (0.39)
CTX (μg/mmol creatinine)	375.9 (175.5)	375.7 (190.0)	367.6 (166.3)
Osteocalcin (ng/ml)	26.9 (7.4)	26.8 (7.6)	25.5 (7.4)
Bone alkaline phosphatase (IU/l)	22.8 (5.2)	22.2 (6.8)	21.1 (6.5)
Bone mineral density (g/cm^2)			
Total body	0.899 (0.067)	0.907 (0.072)	0.906 (0.085)
Total femur	0.850 (0.092)	0.836 (0.121)	0.843 (0.104)
Femoral neck	0.721 (0.071)	0.711 (0.122)	0.729 (0.110)
Lumbar spine	0.877 (0.123)	0.879 (0.126)	0.877 (0.132)

E_2 + 17β-estradiol; NETA = norethisterone acetate; CTX = type I collagen C-telopeptide.

Materials and methods

Subjects

A total of 109 healthy post-menopausal women are the basis of this study. They are part of a population of 163 women who completed a prospective randomized, double-blind study of prevention of bone loss (Roux *et al*., 2002), conducted in two centres, using two randomization lists. Subjects included in this part of the study were those referred to one centre, having body composition data available at baseline and at the end of the study. All participants were aged ⩾45 years with intact uterus. Criteria for entry in the study included: menopause for 1–10 years confirmed by serum levels of estrogen ⩽50 pg/ml and FSH ⩾20 IU/l. Exclusion criteria were: bilateral oophorectomy, undiagnosed vaginal bleeding, endometrial hyperplasia, inflammatory disease or any condition likely to require corticosteroid treatment, bone diseases, liver enzyme abnormalities, a body mass index (BMI) <19 or >27 kg/m^2, a history of cancer, or cigarette consumption >10 cigarettes daily. Subjects were also excluded if they had used estrogens within the last 6 months, calcitonin or vitamin D treatment within the last 2 months, and drugs known to interfere with calcium metabolism (Roux *et al*., 2002).

All subjects gave written informed consent to participate in the study, which was carried out in accordance with the Helsinki declaration.

Treatments

Subjects received either tibolone 2.5 mg (n = 29), tibolone 1.25 mg (n = 42), or estradiol 2 mg plus NETA 1 mg (n = 38), daily at bedtime. All women received a daily supplementation of 500 mg of calcium with a meal. Follow-up duration was 2 years.

Assessments

Height, weight and BMI (calculated as the weight in kg per height in m^2) were determined at baseline, and after 2 years of follow-up for each subject. At each time point, BMD (g/cm^2) was measured at the left hip (the femoral neck and the total hip), and the lumbar spine L2–L4 in the antero-posterior scan, by dual energy X-ray absorptiometry (DEXA), using a QDR 2000 device (Hologic, USA). BMD of the whole skeleton, and the body composition, i.e. lean mass and fat mass (in grams) were determined at the same time, on the total body scans.

From body composition data, we calculated the android fat and the gynoid fat percentage, and the android obesity index for each subject. Android fat was the proportion of fat in the trunk region (area between an upper horizontal border below the chin, a lower border formed by the oblique lines passing through the hip joints and vertical borders lateral to the ribs); gynoid fat was the proportion of fat in both legs (area below the upper border formed by the oblique lines passing through the hip joints), both expressed as percentage of subtotal body fat (assuming that brain fat represents 17% of the total body fat, the head region was excluded from our analysis). Android obesity index was the ratio of android fat/gynoid fat (Trémollieres *et al*., 1996).

At the same time points, serum concentrations of bone alkaline phosphatase (using immunoradiometric assay, Ostase; Beckman–Coulter, USA), osteocalcin (using radioimmunoassay, Elsa Osteo; CisBio Internationl, France) and the urinary excretion of type I collagen C-telopeptide (CTX) corrected for creatinine, on the second-void urine sample (using enzyme linked immunosorbent assay, Crosslaps ELISA; Nordic Biosciences, Denmark) were measured in 101 women after an overnight fast.

Statistical analysis

Data are presented as mean and SD. Cross-sectional associations at inclusion between BMD at several sites, BMI, years since menopause, lean mass, fat mass and fat repartition indexes, and associations between relative variations at 2 years of these parameters were assessed using Pearson's correlation coefficient. Multiple linear regression, equivalent to partial correlation coefficients, was used to adjust these correlations for other covariates.

Tests for changes in parameters in the pooled sample used one-sample Student's t-tests. Comparisons of characteristics between the three groups at inclusion and relative variations of the studied parameters were performed using a one-way analysis of variance. Additionally, post-hoc two-by-two comparisons were adjusted for multiple testing using Tukey's correction.

A multiple regression model was built to predict changes in BMD according to treatment and changes in body composition repartition indexes and biochemical markers using analysis of co-variance. The model was selected on the basis of a backward model selection procedure, where all parameters achieving a P-value of 0.20 in the univariable analysis were considered.

All tests were two-sided. For the one-way analysis of variance, type I error rate was fixed at 0.05, except for post-hoc comparisons. To

Table II. Pearson's correlation coefficients (*P*-value) between bone mineral density and other parameters at baseline

Bone mineral density	Body mass index	Years since menopause	Lean mass	Fat mass
Total body	0.27 (0.0043)	–0.40 (< 0.001)	0.47 (< 0.001)	0.18 (0.064)
Lumbar spine	0.32 (0.0006)	–0.35 (0.0002)	0.33 (0.0004)	0.27 (0.0043)
Total femur	0.41 (< 0.001)	–0.26 (0.0063)	0.42 (< 0.001)	0.26 (0.0066)
Femoral neck	0.3 (0.0001)	–0.29 (0.0026)	0.43 (< 0.001)	0.24 (0.013)

Table III. Percentage changes at 2 years from baseline measurements with tibolone 2.5 mg, tibolone 1.25 mg, and E_2 + NETA treatments

	Tibolone 2.5	Tibolone 1.25	E_2 + NETA	P^a	P^b
Body mass index	+2.5 (5.4)	+2.6 (4.3)	+3.5 (6.4)	< 0.001	0.67
Lean mass	+7.3 (4.2)	+3.4 (3.6)	+5.5 (4.3)	< 0.001	0.0004
Fat mass	–1.8 (14.0)	–0.4 (11.7)	+1.2 (18.8)	0.90	0.72
Gynoid fat	–1.1 (7.7)	–1.5 (5.7)	+1.9 (6.4)	0.75	0.057
Android fat	–4.4 (7.0)	–3.3 (4.8)	–5.7 (7.9)	< 0.001	0.29
Android index	–2.4 (13.6)	–1.4 (9.5)	–6.8 (12.4)	0.0023	0.11
CTX	–54.3 (30.2)	–41.6 (44.8)	–56.5 (20.5)	< 0.001	0.23
Osteocalcin	–42.4 (15.8)	–35.2 (19.2)	–32.3 (25.8)	< 0.001	0.32
Bone alkaline phosphatase	–37.1 (18.1)	–23.6 (26.7)	–25.4 (22.6)	< 0.001	0.17
Bone mineral density					
Total body	+2.5 (1.4)	+1.6 (2.1)	+2.9 (2.4)	< 0.001	0.013
Lumbar spine	+3.6 (3.1)	+2.0 (4.4)	+6.9 (4.2)	< 0.001	< 0.001
Total femur	+2.9 (2.7)	+1.4 (2.9)	+3.4 (3.6)	< 0.001	0.011
Femoral neck	+3.4 (4.5)	+0.8 (4.0)	+4.0 (3.4)	< 0.001	0.0009

Values are mean (SD).
[a]Global changes (all groups pooled), one-sample Student's *t*-test.
[b]Test of a group effect, one-way analysis of variance.
E_2 + 17β-estradiol; NETA = norethisterone acetate; CTX = type I collagen C-telopeptide.

account for a relative high number of tests for correlation coefficients, such tests were performed with significance level fixed at 0.01. Analyses were performed using S-Plus 2000 software (MathSoft Inc., USA).

Results

Baseline characteristics of patients according to treatment group are shown in Table I. There was no statistically significant difference in these variables between groups.

At baseline, lean mass correlated negatively with age and years since menopause (YSM), but these correlations did not reach statistical significance: $P = 0.013$ and $P = 0.018$ respectively. Fat mass correlated neither with age nor with YSM. We observed a significant increase in android fat and android obesity index with age: $r = 0.27$, $P = 0.0047$ and $r = 0.25$, $P = 0.0085$ respectively, but not with YSM: $r = 0.22$, $P = 0.020$ and $r = 0.21$, $P = 0.027$. In contrast, there was a decrease in gynoid fat, but this decrease did not reach significance with age $r = –0.21$, $P = 0.027$ or with YSM $r = –0.20$, $P = 0.037$.

The relationships between BMD and different parameters at baseline are shown in Table II. BMD at all sites was related to YSM. Similar results were observed with age (data not shown). Multiple regression analysis showed that BMD was more related to YSM than to age, whereas body composition variables were more related to age than to YSM (data not shown). A positive correlation was found between total and regional BMD and BMI. At all sites, there was a weak correlation between 0.18 and 0.27 with fat mass, and between 0.33 and 0.47 with lean mass.

Changes observed after 2 years of treatment, expressed as a percentage change from the baseline measurements, according to treatment groups, are shown in Table III. In the whole population, BMD increased significantly at all sites. Mean gain was 2.3, 4.1 and 2.5% in the total body, lumbar spine and total hip BMD respectively. These changes were significantly different according to group ($P = 0.013$, $P < 0.001$ and $P = 0.011$ respectively). After adjustment for multiple testing, the increase in lumbar spine BMD was found to be higher in E_2 + NETA group than in tibolone groups, while the analysis only yielded significant differences between E_2 + NETA group and tibolone 1.25 mg at both other sites. Serum bone alkaline phosphatase, osteocalcin and urinary cross-links decreased by 27.4, 35.7 and 50.6%, respectively. There were no significant differences between groups.

The mean increase in BMI was 2.5 ± 5.4, 2.6 ± 4.3 and 3.5 ± 6.4 in tibolone 2.5 mg, tibolone 1.25 mg and E_2 + NETA groups respectively. A mean increase in lean mass equivalent to 7.3 ± 4.2, 3.4 ± 3.6 and 5.5 ± 4.3%, from baseline was observed in tibolone 2.5 mg, tibolone 1.25 mg and E_2 + NETA groups respectively. In the whole cohort, this increase was significant ($P < 0.0001$), and was different according to the group ($P = 0.0004$), but two-by-two comparisons only yielded a significant difference between tibolone 2.5 mg and tibolone 1.25 mg groups. Total body fat decreased in the three groups,

Table IV. Pearson's correlation coefficients (*P*-value) between changes in total and regional bone mineral density with changes in body composition parameters

Δ Bone mineral density	Δ Lean mass	Δ Fat mass	Δ Android fat	Δ Gynoid fat	Δ AOI
Total body	0.30 (0.0016)	–0.23 (0.018)	–0.11 (0.25)	0.11 (0.24)	–0.12 (0.23)
Total hip	0.25 (0.0093)	0.11 (0.24)	–0.02 (0.81)	0.05 (0.59)	–0.03 (0.74)
Femoral neck	0.37 (< 0.001)	0.07 (0.47)	0.0003 (1.00)	–0.012 (0.91)	–0.002 (0.98)
Lumbar spine	0.22 (0.019)	0.10 (0.28)	–0.02 (0.84)	–0.02 (0.83)	–0.011 (0.91)

AOI = android obesity index.

but this was globally not significant. This decline was associated with changes in fat distribution with slight but significant decrease in the percentage of android fat and the android index from baseline. These changes were more pronounced in the E_2 + NETA group than tibolone 2.5 mg and tibolone 1.25 mg groups, although the only significant difference was observed between tibolone 1.25 mg and E_2 + NETA groups for the percentage android fat, when adjusting for multiple testing.

The relationships between the changes in total and regional BMD with changes in body composition are shown in Table IV. There was a significant positive correlation between changes in BMD from baseline at all sites and changes in lean mass at 2 years. In a multiple regression model, the E_2 + NETA treatment ($P = 0.04$), and the changes in lean mass ($P = 0.0008$) at 2 years were selected as independent predictors of changes in femoral BMD from baseline. No interactions between these covariates were found, and the multiple R^2 of the model was 0.18. Similar results were obtained for BMD changes measured at other sites.

Discussion

The present study suggests a link between lean mass increase and the increase in BMD at all sites in post-menopausal women receiving post-menopausal hormone therapy.

At baseline, we observed the expected effects of hormonal deficiency: BMD at all sites was related to YSM. BMD correlated positively with BMI at all sites, as previously reported (Ribot *et al*., 1988; Harris *et al*., 1992). Both total fat mass and lean mass were significantly related to total and regional BMD, but the correlation was stronger with lean mass (~0.4, versus ~0.25 for fat mass), in accordance with others (Salamone *et al*., 1995) who suggested that a low lean mass may be considered as an osteoporotic risk factor. A BMI between 19 and 27 was an inclusion criterion for this study, and this precludes accurate analysis of the role of fat mass in obese post-menopausal women.

The mechanical loading might explain the correlation of fat and lean tissues with BMD, but does not explain the difference in these correlations, as the skeleton cannot distinguish between a pound of lean tissue and a pound of fat (Slemenda, 1995). The correlation of BMD with lean mass may reflect a genetic association between a higher lean tissue mass and a higher peak bone mass (Compston *et al*., 1992; Chen *et al*., 1997). Physical activity has been suggested to play a role in this relationship (Chen *et al*., 1997) as exercise results in greater muscle mass and may increase bone density. However, one may wonder whether the exercise-induced increase in BMD is related to exercise itself or to the increase in lean mass. Another suggested explanation of the link lean mass–BMD is the circulating IGF-I (Salamone *et al*., 1995), which has potent anabolic actions on both skeletal muscles and bone. In our study, lean mass increased significantly in all treatment groups, in contrast to some cross-sectional and longitudinal studies which reported either an acceleration of lean mass loss (O'Sullivan *et al*., 1998) or a failure of pHT to prevent this loss (Hassager and Christiansen, 1989; Haarbo *et al*., 1991). In these studies, pHT used were an estrogen plus either cyproterone acetate or a progestagen other than NETA. In one study (Sorensen *et al*., 2001), E_2 + NETA were used, and results similar to ours were found. In another prospective study where oral E_2 + dydrogesterone, transdermal E_2 + dydrogesterone, or tibolone were used, the loss in lean mass observed with placebo was prevented in tibolone and transdermal E_2 + dydrogesterone, but not with oral E_2 + dydrogesterone (Hänggi *et al*., 1998). NETA is a synthetic progestagen known to have androgenic properties, and tibolone is a synthetic steroid, with a large progestogenic activity, and to a lesser extent androgenic and estrogenic properties. We did not include a placebo group in our study; thus we cannot state that the measured changes are related to treatment. However, because such changes are unexpected in untreated post-menopausal women, we speculate that the gain in lean mass may be related to an anabolic effect of NETA and tibolone on skeletal muscles.

Similarly to our findings, in a large cohort study, including 2016 early post-menopausal women, followed-up for 5 years, the change in lean mass was the best predictor of bone changes in pHT users (Jensen *et al*., 2003). The significant increase in lean mass with treatment and the positive correlation between its changes at 2 years with the changes in BMD at all sites, suggest that lean mass plays a mediating role in the effects of treatment on BMD. This effect may be direct or by the mean of another hormonal factor. Growth hormone has been reported to be a potent mitogen for osteoblasts *in vitro* (Hock *et al*., 1988), in-vivo IGF-I levels correlated positively with BMD at all sites in women (Sugimoto *et al*., 1997), and changes in circulating IGF-I correlated with changes in osteocalcin, PINP and bone alkaline phosphatase in women on pHT, suggesting that some of the effect of E_2 on bone metabolism may be mediated by IGF-I (Garnero *et al*., 1999). However, the estrogen used in our study was an oral form, and, although one study found an increase in IGF-1 with oral estrogen therapy (Posaci *et al*., 2001), some studies (Helle *et al*., 1996; Raudaskoski *et al*.,

1998; Garnero *et al*., 1999) found that oral E_2 led to a decrease in these levels.

The most commonly given explanation for the correlation between BMD and fat mass was aromatization (Compston *et al*., 1992; Lindsay *et al*., 1992), as fat tissue is responsible for this phenomenon which is the principal source of estrogen in post-menopausal women. This theory was supported by the results of Jensen et al., who found that the effect of fat mass on the femoral neck and the lumbar spine BMC was markedly reduced in women receiving pHT as compared with those who were not. The positive relationship found between fat mass and BMD at baseline in this study, and the absence of correlation between fat mass changes and BMD changes under pHT, further supports this assumption. High fat mass is associated with high insulin level in obese people; in addition, insulin has anabolic effects on osteoblasts (Hickman and McElduff, 1989), and serum insulin levels have been reported as related to BMD in post-menopausal women (Reid *et al*., 1993), suggesting that insulin may play a role in this relationship. Although there were no significant changes in total fat mass with pHT in this study, body fat redistribution observed with age and menopause changed during treatment, in accordance with some previous reports (Haarbo *et al*., 1991; Reubinoff *et al*., 1995; Troisi *et al*., 1995; Perrone *et al*., 1999; Sorensen *et al*., 2001). Despite these individual conclusions, a substantive systematic review of randomized studies comparing pHT to placebo or no treatment, failed to find sufficient data to enable a meta-analysis on the effect of pHT on waist:hip ratio (Norman *et al*., 2003). Although android obesity has been reported as beneficial for bone mass because of its association with lower sex hormone binding globulin (SHBG), which results in higher concentrations of the free androgens and estrogens (Heiss *et al*., 1995), a high android obesity index is undesirable because it has been linked to insulin resistance and alteration in lipid profile, both associated with high risk of coronary artery disease (Lapidus *et al*., 1984; Larsson *et al*., 1984).

This study lacks information about previous pHT use, which, if it had happened, might have affected the baseline relationship between body composition and years since menopause. Other limitations to this study are related to the young age of the population and the exclusion of obese women, which cut out a large proportion of post-menopausal women, and make the impact of age and fat mass less easy to determine. Finally, we have no information on physical fitness changes during follow-up.

Despite these limitations, we conclude that bone mass in post-menopausal women is more affected by lean mass than fat mass. During E_2 + NETA and tibolone treatments, a decrease in android fat and an increase in lean mass are observed.

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