Menstrual History as a Determinant of Current Bone Density in Young Hirsute Women

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There is evidence that bone mass is influenced by estrogen, declining in situations characterized by a decrease in the production of this hormone. Usually, amenorrhea and oligomenorrhea are associated with a state of hypoestrogenism, and both situations are frequent in hirsute patients. The aim of the present study was to analyze the relationship between bone mass and menstrual cyclicity in hirsute women. A total of 52 nulliparous women complaining of hirsutism in various degrees with associated oligomenorrhea/amenorrhea (OA) in 27 cases and eumenorrhea in 25 were included in this study. Basal serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β (E2), prolactin (PRL), testosterone (T), androstenedione (A4) dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (OHP), and SHBG were determined, and the area under the curve (AUC) for E2 was plotted. Bone mineral density (BMD) was assessed by dual-energy x-ray absorptiometry (DEXA). The mean age for eumenorrheic patients was 26 years (range, 17 to 31), and for OA patients, 24 (range, 16 to 29). Both groups had similar Ferriman-Gallwey scores. Basal levels of PRL, LH, FSH, E2, T, A4, OHP, and DHEAS were similar for eumenorrheic and OA patients. The AUC for E2 was significantly higher for eumenorrheic patients, and DEXA at the lumbar spine demonstrated a significant difference between eumenorrheic (1.222 ± 0.240 g/cm^2) and OA (1.016 ± 0.108 g/cm^2) hirsute women ($P < .01$). In conclusion, OA, due to a relative hypoestrogenism, may be correlated with osteopenia in young hirsute women.

CHRONIC HYPERANDROGENISM with various degrees of hirsutism in young women is not an uncommon finding, and this situation is usually associated with acne, polycystic ovaries, and menstrual irregularities including amenorrhea.[1,2]

There is evidence that bone mass is influenced by estrogen, declining in situations characterized by a decrease in the production of this hormone.[3] Amenorrhea and oligomenorrhea are commonly associated with a state of hypoestrogenism,[4] and both situations are frequent in hirsute patients. The hypoestrogenemia related to amenorrhea is now believed to contribute to bone loss similar to that occurring at menopause.[5] Moreover, bone mineral content may not be completely replaced upon resumption of menses.[6] This could lead to premature osteoporosis or to an increase in the risk for later osteoporosis. On the other hand, androgens have a well-known anabolic action on bone, and it is not uncommon to find increased androgen levels in women complaining of hirsutism. The role of elevated androgens in the bone mineral density (BMD) in such patients and its effect to compensate for hypoestrogenism has been largely unexplored.

In this study, we applied dual-energy x-ray absorptiometry (DEXA) to determine BMD at the lumbar spine in oligomenorrheic/amenorrheic (OA) and eumenorrheic hirsute women, with the aim of analyzing the relationship between bone mass, steroid plasma levels, and menstrual cyclicity in such women.

SUBJECTS AND METHODS

Subjects

A total of 52 nulliparous women complaining of severe hirsutism in various degrees were included in this study. All patients were referrals from gynecological clinics. The subjects, who had not been on any form of drug therapy for 3 months before the study, were allocated to two groups according to menstrual cyclicity. OA (menstrual interval >35 days) was present in 27 cases and eumenorrhea (24 to 35 days) in 25. Only one subject participated in sports on a weekly basis. Since basal levels of dehydroepiandrosterone sulfate (DHEAS) and 17-hydroxyprogesterone (OHP) were within the normal range, hyperandrogenism of adrenal origin was unlikely. Hypothyroidism, Cushing's syndrome, and prolactinomas were excluded in all subjects. None of the patients recruited reported smoking more than a half-pack per day, and none were vegetarians. Hirsutism was indicated by a score of at least 8 on the Ferriman-Gallwey scale.[7] Patients suffering from diseases or under treatments that may affect bone mass were excluded from the present study. In addition, a dietary history of calcium intake was assessed in each subject to avoid any gross deficiencies or excesses that may affect bone mass.[8] Moreover, in the study of BMD, two normal androgenemic nonhirsute control groups were considered for both eumenorrheics (n = 25) and OA subjects (n = 17). The eumenorrheic control group was obtained from our normal menstruating population, and the OA control group were subjects who complained of stress oligomenorrhea.

Study Design

Before entering the study, all patients underwent a medical history and physical examination, gynecologic examination, pelvic ultrasound, and BMD measurement (by DEXA) in the lumbar spine (Lunar DPX system, Madison, WI). Basal plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β (E2), prolactin (PRL), testosterone (T), androstenedione (A4), DHEAS, OHP, and sex hormone–binding globulin (SHBG) were determined and the area under the curve (AUC) for E2 was plotted using values determined at early, middle, and late follicular and luteal phases, and on the first menstrual day if women had regular menstrual cyclicity or every 4 days for 4 weeks if women were OA. Blood samples were taken in the morning between 8:30 and 10:00 AM, and stored until the time of assay.

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Table 1. Characteristics and BMD of Hirsute and Nonhirsute Patients

	Hirsute		Nonhirsute					
	OA	Eu	OA	Eu	FG	BMD	BMD*	BMD%.
No. of subjects	27	25	17	25				
Age (yr)	24 (16-29)	26 (17-31)	23 (18-28)	24 (18-30)				
FG	19 (14-23)	17 (15-21)	<8	<8				
BMI	21.1 ± 0.8	22.0 ± 1.0	21.1 ± 0.7	21.6 ± 0.9				
Cal	1.8 ± 0.8	1.7 ± 0.8	1.8 ± 0.1	1.6 ± 0.9				
BMD	1.016 ± 0.108	1.222 ± 0.240	0.963 ± 0.201	1.195 ± 0.220				
BMDA	1.03	1.22	0.97	1.19				
BMDP	91.3 ± 3.4	103.5 ± 4.9	89.5 ± 3.5	99.3 ± 5.9				
Hirsutes > nonhirsutes					<0.01			
Hirsute OA > nonhirsute OA						<.05	<.05	<.05
Hirsute Eu > hirsute OA						<.01	<.01	<.05
Hirsute Eu > nonhirsute OA						<.01	<.01	<.01
Nonhirsute Eu > nonhirsute OA						<.01	<.01	<.01
Nonhirsute Eu > hirsute OA						<.05	<.05	<.05

Abbreviations: Eu, eumenorrhea; FG, Ferriman-Gallwey score; Cal, calcium intake; BMDA, BMD adjusted for body weight; BMDP, % of BMD, compared with the reference data in our geographical area (see Del Rio et al[10]).

Hormone Analysis

LH and FSH levels were measured by radioimmunoassay ([RIA] Farmos, Kuopio, Finland). LH and FSH standards were calibrated with IRP 68/40 and IRP 69/104. E2 levels were determined by a coated-tube RIA (Medgenix, Brussels, Belgium). PRL levels were also determined by a coated-tube RIA (Farmos). The PRL standard used was IRP 75/504. T and A4 levels were measured by RIA after extraction with ether and purification by partition chromatography on a chromatolithe A column (bioMérieux, Marcy l'Etoile, France). DHEAS level was measured by a coated-tube RIA (Diagnostic Products, Los Angeles, CA). SHBG level was measured by a two-site fluoroimmunometric assay (Wallac, Turku, Finland). The free T index was calculated with the formula, T × 3.47/SHBG. The coefficients of variation and the cross-reactivity for different steroids have been previously described.[9]

BMD Measurement

BMD in the lumbar spine was determined by DEXA (Lunar DPX system), expressed as grams per centimeter squared, as the mean of the second to fourth lumbar vertebrae. DEXA uses stable k-edge radiation. The typical standard deviation for anterior-posterior lumbar spine in vivo is 0.01 g/cm^2. For a young normal individual with a BMD of 1.2 g/cm^2, the coefficient of variation is 0.8%, and for an osteopenic patient (BMD <0.8 g/cm^2), 1.2%. The precision of the DPX system for the spine is 0.5%, and the patient exposure is low (1.2 to 2.4 mR for spine). All measurements were performed and analyzed by the same operator (F.P.).

Statistical Analysis

Multiple linear regression was used to model bivariate relationships, to test for additive effects, and to control for possible confounding variables. Log transformations were used for AUC data to linearize the relation between E2 versus BMD data. AUC values were calculated for E2 by integrating the E2 AUCs using time (in days) and the concentration of estradiol (picograms per milliliter) at each time interval. Associations between two continuous variables were assessed with Spearman's rank-correlation test; Student's t test for unpaired groups and ANOVA for repeated measures were used for comparison of the means. A p value less than .05 was considered to indicate statistical significance. Results were analyzed using the Statistical Analysis Package (Walonick, Minneapolis, MN).

RESULTS

Physical characteristics were similar for the hirsute groups (Table 1). The mean age for eumenorrheic subjects was 26 years (range, 17 to 31), and for OA subjects, 24 (range, 16 to 29). No differences were detected between both groups of hirsutes in Ferriman-Gallwey scores, estimated calcium intake, and BMI. In such patients, there were also no significant differences in basal plasma gonadotropin or steroid levels (Table 2). Nonhirsutes presented significantly lower androgen levels than hirsutes (Table 2).

Body weight (BW) was correlated with BMD (r = .67, P < .001). BMD results have been presented as observed values (unadjusted for BW) and adjusted values (considering BW as a covariate). Subjects with menstrual irregularities showed significantly lower observed values of BMD compared with eumenorrheic women (Fig 1 and Table 1). Moreover, after adjusting for BW, the difference remains significant. When BMD was expressed as a percent of the

Table 2. Hormone Levels in Hirsute and Nonhirsutes

	Hirsute		Nonhirsute	
	OA	Eu	OA	Eu
No. of subjects	27	25	17	15
LH (mUI/mL)	7.08 ± 3.1	8.53 ± 2.8	5.18 ± 3.5	6.63 ± 1.7
FSH (mUI/mL)	6.02 ± 4.5	6.88 ± 3.9	5.08 ± 3.1	8.77 ± 2.5
PRL (ng/mL)	9.15 ± 2	8.09 ± 1.8	12.55 ± 5.2	10.11 ± 3.2
E2 (pg/mL)	56 ± 15	64 ± 23	50 ± 13	69 ± 19
T (ng/dL)	45 ± 15	40 ± 13	23 ± 09†	35 ± 23†
A4 (ng/dL)	285 ± 49	267 ± 51	111 ± 53‡	153 ± 41‡
SHBG (nmol/L)	35 ± 21	48 ± 32	39 ± 19	56 ± 23
DHEAS (μg/mL)	2.14 ± 0.7	2.04 ± 1.0	2.23 ± 0.3	2.11 ± 1.3
OHP (ng/mL)	1.03 ± 0.3	1.03 ± 0.5	0.97 ± 0.8	1.01 ± 0.6
E2 AUC (pg · d/mL)	14.255 ± 655	22.788 ± 525*	—	—

Abbreviation: Eu, eumenorrhea.

Student's t test for unpaired groups (E2 AUC): *P < .01.

ANOVA for repeated measures (hirsutes have significantly higher androgen levels than nonhirsutes): †P < .05, ‡P < .01.

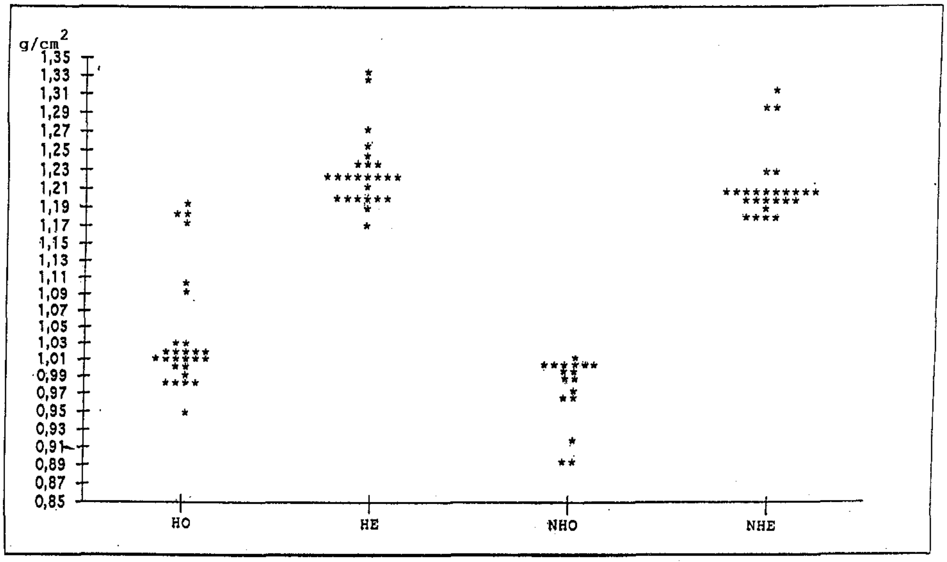

Fig 1. BMD (g/cm²) in hirsute OA subjects (HO), hirsute eumenorrheics (HE), nonhirsute OA subjects (NHO), and nonhirsute eumenorrheics (NHE).

value for young normal healthy women, oligomenorrheics showed significantly lower values than eumenorrheics (Table 1). Reference values are from a previous study.[10] Additionally, hirsute women, both eumenorrheic and OA, presented higher absolute values of BMD than their counterpart controls (Table 1).

Moreover, in hirsute patients we determined the E2 AUC, which was lower in OA subjects than in eumenorrheics (14,255 ± 655 *v* 22,788 ± 525 pg · d/mL, $P < .01$; Fig 2). Significant positive correlations of BMD with E2 AUC were observed in eumenorrheic ($r = .87$, $P < 0.001$) and OA ($r = .59$, $P < .005$) patients. Additionally, after controlling for BW, significant correlations were observed in both groups ($r = .79$, $P < .005$ for eumenorrheics, and $r = .57$, $P < .01$ for OAs).

DISCUSSION

Several studies have found osteoporosis to be a common complication of amenorrhea and oligomenorrhea.[11-14] The purpose of this prospective study was to assess the influence of OA on BMD in severely hirsute women. This study describes lumbar measurements of bone mass in OA and eumenorrheic hirsute women. It shows that OA subjects have significantly lower BMD than eumenorrheics. We hypothesized that different hormonal levels may explain the differences detected on bone mass between eumenorrheic and OA subjects. We comprehensively studied the pituitary-gonadal and adrenal axis and did not observe any difference between eumenorrheics and OA subjects. However, when we plotted AUCs for E2, we detected significant differences between normally menstruating hirsute subjects and OA patients.

Although differences in BMD between hirsute and nonhirsute eumenorrheic subjects were nonsignificant, for eumenorrheic hirsute women the anabolic effect of androgens with adequate estrogen levels resulted in BMD values in the upper-normal range. This androgen action was not so evident in patients with an abnormal menstrual cycle. Thus, in OA subjects, it appeared to be counteracted by the negative effect of hypoestrogenemia, and these patients have a lower BMD than eumenorrheic controls. However,

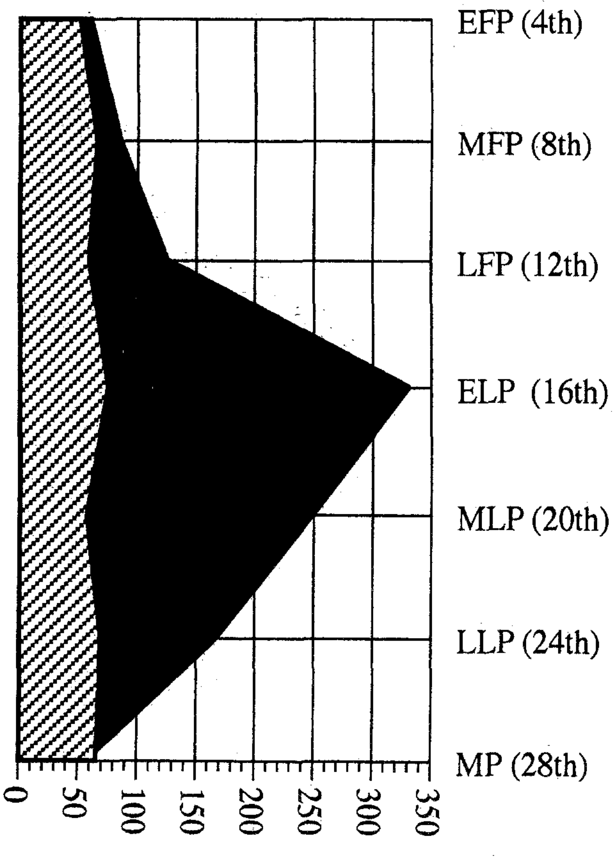

Fig 2. OAUC for E2 (pg · d/mL) in eumenorrheic (■) and OA (▨) hirsute women. EFP, early follicular phase; MFP, midfollicular phase; LFP, late follicular phase; ELP, early luteral phase; MLP, midluteal phase; LLP, late luteal phase; MP, first menstrual day.

OA hirsute women had higher BMD values than nonhirsute OA controls, and this would suggest that hyperandrogenemia may have some protective effect on the bone loss related to hypoestrogenemia.

It is accepted that BW influences BMD,[15,16] and in the present study we expressed BMD as an observed value and as a value adjusted for BW. BW was correlated with BMD ($r = .67$, $P < .001$). Hirsute OA women showed significantly lower observed values of BMD compared with hirsute eumenorrheic women. Moreover, after adjusted for BW by analysis of covariance, the difference remained significant. These results are similar to those observed in eumenorrheic, oligomenorrheic, and amenorrheic athletic women.[6] At this point, the possibility that OA women had lower spinal BMDs than the eumenorrheic subjects before the onset of menses must be taken into consideration, yet is clearly beyond the scope of the present study.

It is well known that estrogens have an antirreabsorptive action,[17] and recent studies suggest that estrogens may play a direct role in the mineralization process.[18] Moreover, no doubts exist about the efficacy of hormone replacement therapy in the prevention of postmenopausal-related bone loss.[19,20] One of the goals of the present study was to evaluate the importance in these patients of chronic low estrogen levels on bone density, and although E2 basal levels were not significantly different between regular menstruating hirsute women and OA women, the mean AUC for this steroid was significantly higher in eumenorrheic hirsute women as compared with OA subjects. Significant positive correlations of BMD with the E2 AUC were observed in both eumenorrheic and OA patients. These correlations were still significant after controlling for BW. These results are in contrast with those reported by Prezelj and Kocijancic,[21] who found that women with hyperandrogenic amenorrhea seem to be spared from osteopenia, and suggest that in OA women it is mandatory, with the independence of ovulation-inducing therapies, to obtain permanent normal E2 levels and to monitor the bone mass, to avoid the deleterious effects of sustained low E2 levels on bone metabolism.

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