Cyclic Medroxyprogesterone Treatment Increases Bone Density: A Controlled Trial in Active Women With Menstrual Cycle Disturbances

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OBJECTIVE: Bone loss occurs in young women who experience amenorrhea or ovulatory disturbances. The purpose of this study was to determine whether bone loss could be prevented by simulating a more normal hormonal pattern, using treatment with cyclic medroxyprogesterone, with or without calcium supplementation, in physically active women with disturbed menstruation.

DESIGN: This study was a 1-year randomized, double-blind, placebo-controlled trial. Women who were stratified by menstrual cycle disturbance were randomized into four groups. The outcome variable was the change in spinal bone density measured by dual energy techniques.

SETTING: A large metropolitan area.

PARTICIPANTS: Sixty-one healthy, normal-weight physically active premenopausal women aged 21 to 45 years who experienced amenorrhea, oligomenorrhea, anovulation, or short luteal phase cycles completed the study.

INTERVENTION: Therapies were cyclic medroxyprogesterone (10 mg/day for 10 days per month) and calcium carbonate (1,000 mg/day of calcium) in four groups: (A) (n = 16) cyclic medroxyprogesterone plus calcium carbonate; (B) (n = 16) cyclic medroxyprogesterone with calcium placebo; (C) (n = 15) placebo medroxyprogesterone with active calcium; or (D) (n = 14) both medroxyprogesterone and calcium placebos.

RESULTS: The initial bone density (mean = 1.12 g/cm^2) did not differ by group (P = 0.85). The 1-year bone density change was strongly related to treatment with medroxyprogesterone (P = 0.0001) and weakly to calcium (P = 0.072) treatment. Bone density increased significantly (+1.7% ± 0.5%, ±SEM, P = 0.004) in the medroxyprogesterone-treated groups (A and B), did not change in the calcium-treated group (C) (-0.7% ± 0.6%, P = 0.28), and decreased on both placebos (D) (-2.0% ± 0.6%, P = 0.005).

CONCLUSIONS: Cyclic medroxyprogesterone increased spinal bone density in physically active women experiencing amenorrhea or ovulatory disturbances.

POTENTIAL CLINICAL SIGNIFICANCE: Amenorrhea, oligomenorrhea, anovulation, and short luteal phase cycles are common in premenopausal women and associated with spinal bone loss occurring at a stage of life when bone density would normally be stable or increasing. This controlled trial shows a significant gain in bone in women in the cyclic medroxyprogesterone intervention group, whereas those subjects in the placebo group lost bone. Calcium supplementation appeared to be helpful but did not reach statistical significance. The implications of these findings for the prevention of osteoporosis warrant further investigation.

Amenorrhea is known to be a risk factor for osteoporosis,[1,2] although abnormally low values for spinal bone mineral density are not inevitable when exercising normal-weight women experience amenorrhea.[3,4] However, few prospective data are available,[5-7] and there are no published controlled, randomized estrogen treatment studies in young women with amenorrhea showing prevention of accelerated bone loss and/or achievement of normal spinal bone densities when they were initially low.

Increased rates of spinal bone loss have been shown to be associated with low serum levels of estrogen[1] as well as with low progesterone levels.[8] Amenorrhea can be understood as a condition in which both estrogen and progesterone deficiencies co-exist. In contrast, ovulatory disturbances such as anovulation and short luteal phase menstrual cycles, which may occur in cycles that are short, normal, or long, can show high, normal, or low estrogen levels. Whereas estrogen levels are variable, the universal characteristic of ovulatory disturbances is some degree of proges-

terone deficiency.[8] These ovulatory disturbances are clinically silent when they occur in cycles that are of normal interval (21 to 36 days apart).[9] Prospective data suggest that anovulation and recurrent short luteal phase cycles are unexpectedly prevalent; they were shown to occur in 41 of 66 initially ovulatory women during 1 year of longitudinal observation.[8]

Methods to prevent spinal bone loss or to reverse low bone density in young, premenopausal women with amenorrhea or ovulatory disturbances have not been developed or proven effective in controlled trials. Although estrogen treatment is known to prevent bone loss in postmenopausal women,[10] one nonrandomized study in premenopausal women with hypothalamic, genetic, and ovarian causes for estrogen deficiency showed no difference in radial bone density between the treated and the untreated young women.[11] It may be that hormonal differences between menopause and amenorrhea, such as cortisol excess that is sometimes present in women with hypothalamic menstrual disturbances,[12-14] interfere with the bone response to estrogen and progestin treatment.

Therapy studies in postmenopausal women and in various animal models indicate that progesterone may act to promote bone formation[15] via specific receptors on the osteoblast,[16] and/or by competition for glucocorticoid receptors on the osteoblast.[17] Regularly menstruating athletic women, in one cross-sectional study, were shown to have 21-day integrated progesterone levels that correlated with spinal bone density.[18]

Furthermore, as increased rates of spinal bone loss in a prospective study of menstruating women have been associated with conditions of decreased progesterone, but not estrogen production,[8] cyclic treatment with the synthetic progesterone, medroxyprogesterone, for 10 days a month to simulate the luteal phase, seemed reasonable. In our pilot study of this approach in sedentary women with hypothalamic amenorrhea, we found that 1-year treatment with cyclic medroxyprogesterone was associated with a dose-related increase in spinal bone density.[19] Physically active women seemed an appropriate population for a more comprehensive, randomized study for two reasons: first, to minimize the potential confounding effect of differing mechanical stimuli on bone, and secondly, because athletes have been reported to be prone to menstrual disturbances.[14]

Calcium supplementation has been recommended to prevent bone loss in amenorrheic athletes,[20] although positive results of intervention trials in this group have not been reported. The effects of calcium supplementation on bone balance are controversial with some studies suggesting benefit[21,22] and other studies not showing any benefit.[23] However, there is evidence to suggest that higher calcium intakes are associated with increased bone gain in children and

young adolescents,[24,25] with prevention of bone loss in sedentary[26] or active young women[27] and with greater increases in spinal bone density in women university students.[28]

This prospective, double-blind, randomized, placebo-controlled trial was designed to document the rates of spinal bone mineral density change in physically active women experiencing menstrual cycle disturbances and the effect of cyclic medroxyprogesterone and supplemental calcium.

PATIENTS AND METHODS
Subject Selection

Physically active premenopausal women aged 21 to 45 years with amenorrhea and abnormal menstrual cycles were recruited through notices on fitness center and park bulletin boards, through neighborhood newspapers, and by word of mouth. The minimal physical activity criterion was a regular program of aerobic activity that raised heart rate to more than 120 beats per minute for more than 1 hour per week.

Abnormal menstrual characteristics were documented in two consecutive cycles before enrollment. Menstrual disturbances were divided into four types as defined by cycle interval (amenorrhea: greater than or equal to 180 days without menstrual flow; and oligomenorrhea: more than 36 days between episodes of flow),[9] or by ovulatory characteristics (anovulation: no significant increase in basal temperature; and short luteal phase cycles: an increase in temperature lasting less than 10 days).[29,30]

The sequence of events leading to randomization is detailed in **Table I**. Volunteers were initially excluded for the following reasons: current or recent

TABLE I
Population Patterns Prior to Randomization

Responded to notices	**285**
Not eligible (contraceptives, glucocorticoids) or declined	-104
Women interviewed and examined	**181**
Ineligible: insufficient exercise, BMI[31] <17 or >26, bone-active drugs, clinical menopause, shift work, age <20 or >45, weight loss	-75
Preliminary eligibility	**106**
With amenorrhea, screened for menopause, pregnancy, androgen or prolactin excess. None excluded.	(33)*
With normal cycle intervals by history, screened by basal temperature monitoring for 2 cycles.	(73)*
Excluded because of normal, ovulatory cycles[29]	-28
Declined	-5
Eligible and enrolled	**73**

*Number of women eligible by menstrual cycle history.

TABLE II		
Two-by-Two Factorial Design of Active and Placebo Medications		
A		**B**
Medroxyprogesterone 10 mg/day, 10 d/month days 16 to 25 of regular cycles **Calcium** 1,000 mg/day		**Medroxyprogesterone** 10 mg/day, 10 d/month days 16 to 25 of regular cycles **Placebo Calcium**
C		**D**
Placebo Medroxyprogesterone, 10 d/month days 16 to 25 of regular cycles **Calcium** 1,000 mg/day		**Placebo Medroxyprogesterone,** 10 d/month days 16 to 25 of regular cycles **Placebo Calcium**

(within 6 months) use of glucocorticoids, other bone-active drugs (fluoride, thiazides, diphosphonates), or oral contraceptives; clinical menopause (amenorrhea with vasomotor symptoms); less than 1 hour per week of aerobic exercise; a body mass index (kg/m^2) of less than 17 or greater than 26[31]; a reported change in weight of more than 2.5 kg in the preceding year; shift work (which would make basal temperature measurements unreliable); and age (less than 20 years or more than 45 years). Potential participants were secondarily excluded if they had normal ovulatory cycles as assessed by basal temperature records kept during two screening cycles. Women with amenorrhea were screened to exclude those with pregnancy, prolactinoma or prolactin elevation, androgen excess, and menopause (Table I). We obtained information about family health, including any history of osteoporosis, and about present and past health and lifestyle. Seventy-three women with abnormal cycles enrolled in the study: 10 with amenorrhea, 21 with oligomenorrhea, 11 with anovulation, and 31 with short luteal phase cycles.

The women gave informed written consent. The study was approved by the Clinical Screening Committee for Research Involving Human Subjects of the University of British Columbia.

Intervention Groups and Randomization

Women were stratified by the four types of menstrual cycle disturbance and randomly assigned to four groups in a two by two factorial design (**Table II**). Medroxyprogesterone or placebo was given on cycle days 16 to 25 in women with regular menstrual intervals or starting on any day and continuing for 10 days each month in those with unpredictable flow. Calcium as calcium carbonate in 500 mg tablets or placebo was given as two chewable tablets per day. The blinded intervention continued for 12 months.

Study Design

After enrollment, the women were seen at approximately 3-month intervals to resupply medications. They were contacted every 2 to 6 weeks by telephone.

Spinal bone density, body morphometric indices, and serum estrogen and progesterone levels were measured during the second screening cycle and the cycle following the completion of the intervention. Menstrual cycle, basal temperature data, and exercise pattern were recorded daily. Dietary intakes were recorded at enrollment and for 3 consecutive days every 3 months.

Exercise Data

All exercise was documented by duration (in minutes), intensity (from mean exercise heart rates), and type (ie, running, aerobics, cycling, cross-country skiing, etc.). Distance (in km) was reported for running and other activities, such as cycling, in which it was appropriate. Exercise heart rates were monitored by 10-second radial pulse counts performed by the trained participants. Three readings taken at the 10-minute, mid-point, and the end of each exercise session were used to calculate the mean exercise heart rate per session. Exercise data were recorded daily and reported as minutes per month.

Menstrual Cycle Data

Morning oral temperatures on wakening were recorded and analyzed using quantitative least squares statistics to determine the onset of the luteal phase.[29] The presence or absence of ovulation and the length of the luteal phase (if ovulation had occurred) were documented.

Dietary and Morphometric Data

Records of each woman's self-reported, self-chosen, and unweighed food and beverage intakes were completed for 3 consecutive days (2 weekdays and 1 weekend day) at baseline and at 3-monthly intervals. Records were analyzed using a computer program based on the Canadian Nutrient File.[32] For each woman, results from a minimum of 12 days were used to calculate group mean intakes.

Height, weight, and skinfold thicknesses at four sites (abdomen, anterior thigh, triceps, and above the iliac crest) using a constant-tension Harpenden

caliper were measured during the follicular phase (or at any time for women with amenorrhea) of the last screening cycle and in the cycle following the end of the study year. Body mass index (BMI, kg/m^2) was also calculated.[31] Percentage of body fat was calculated using a formula validated for athletic women.[33]

Bone Density Measurements

The combined cortical and cancellous bone mineral was measured as areal density in lumbar spinal segments L1 to L4 using a dual energy technique. Dual photon absorptiometry (DPA, Lunar DP4, Lunar Corp, Madison, Wisconsin) measurements were made for the first 33 women enrolled in the study, and dual energy radiographic absorptiometry (DXA, Lunar DPX, Lunar Corp.) measurements were made for the remaining 28 women. The same instrument (whether DPA or DXA) was used for both the initial and the final bone density measurements in each woman.

Data obtained on the DPA machine were converted into DXA-equivalent (DXAe) observations using the following formula: DXAe = DPA + 0.007 (95% confidence limits of the mean = +0.028 to -0.014) g/cm^2. This equation was derived from measurements using both the DPA and the DXA instruments in each of 19 women who were age-, height-, and weight-matched with women in this study. All data from DPA measurements were entered into and analyzed using standard DXA software (Ver. 3.1, Lunar Corp.). The differences between the DPA values as analyzed using DXA software and the bone density data from measurements on the DXA machine in the same women were used to develop the aforementioned formula.

The coefficient of variation for the DXA measurements in L1 to L4 was 1.3% in 16 age- and weight-matched women who had the same spectra of menstrual cycle and bone mineral data as women in this study (1.08 ± .10 g/cm^2). The coefficient of variation for initial DPA measurements (analyzed on DPA software but before conversion into DXA-equivalent units) was 2.1%. No coefficient of variation assessment was available for DXAe data.

The mean interval between the two measurements of bone density was 12.4 ± 0.1 months. For technical and personal reasons, 6 of the 61 women were tested at intervals of less than 11 months or more than 13 months (numbers of individuals in parentheses): 9 months (1), 14 months (4), and 15 months (1). The differences between initial and final bone density measurements were divided by the number of months between measurements. The change in bone density was adjusted to 12 months and expressed as the annual rate of change.

Hormonal and Biochemical Analyses

Blood samples were obtained in the mid-afternoon from rested subjects (no exercise for greater than or equal to 12 hours) during the mid-follicular and premenstrual phases of the second screening cycle and during the mid-follicular and premenstrual phases of the cycle following the 12 months of intervention. Samples were obtained about 14 days apart for oligomenorrheic or amenorrheic women. Four samples were obtained from each woman, and, as previously described,[8] the two from a given cycle were pooled and analyzed as one value. After blood collection and centrifugation of clotted samples, the serum was removed and stored frozen at -70°C until assay. Estradiol and progesterone concentrations were measured in duplicate using single runs of standard radioimmunoassays.[8] In our laboratory, the pooled follicular and luteal phase values (mean ± SEM) for 66 normal premenopausal women during an ovulatory cycle were as follows: estradiol 273.5 ± 17.9 pmol/L; progesterone 15.7 ± 1.1 nmol/L.[8]

Statistical Methods

The annual change in bone density was evaluated by a two-by-two analysis of variance using BMDP statistical software.[34] Linear and multiple regression analyses and Student's t-tests were used for normally distributed data. Chi-square, Kruskal-Wallis, Mann-Whitney, Sign, and Spearman statistical tests were used for categoric variables (such as menstrual cycle type) and for variables such as exercise times that were not normally distributed. All significance levels were based on two-tailed testing. To facilitate statistical analysis, the cycle length for women with amenorrhea was considered to be 180 days. Women with 6 or more consecutive months of no menstrual flow in the past were deemed amenorrheic, and the total number of months without flow was documented by history. An anovulatory cycle was designated as having a "luteal phase" length of 0.0 days. Annual menstrual cycle changes in cycle and luteal phase lengths are reported as the difference between the second screening (the cycle before intervention) and the cycle after the year of intervention because medroxyprogesterone administration may confound both the basal temperature readings and the occurrence or not of withdrawal flow.

The parametric data are expressed as mean ± standard error of the mean (SEM). Non-normally distributed data are reported as medians followed by the range in brackets.

RESULTS

Subjects

Sixty-one physically active premenopausal women with disturbances of the menstrual cycle completed the 1-year study. The age of participants averaged 32.3

TABLE III

Initial Demographic, Morphometric, Dietary and Bone Density Data and Mean Dietary Data

	All	A	B	C	D
Number	61	16	16	15	14
Age (y)	32.3 ± 0.7	34.0 ± 1.2	31.1 ± 1.8	31.3 ± 1.3	32.6 ± 1.4
Ht (cm)	162.4 ± 0.8	162.9 ± 1.6	163.2 ± 1.4	162.4 ± 1.9	160.6 ± 1.7
Wt (kg)	57.1 ± 1.0	57.8 ± 1.4	56.7 ± 1.8	57.1 ± 2.2	56.7 ± 2.8
BMI (kg/m^2)	21.6 ± 0.3	21.8 ± 0.5	21.3 ± 0.6	21.6 ± 0.6	21.9 ± 0.8
% Fat	18.2 ± 0.6	18.3 ± 0.8	17.4 ± 0.9	18.4 ± 1.4	18.6 ± 1.6
Energy (kJ)	7567 ± 482	6941 ± 392	7757 ± 668	8272 ± 406	7280 ± 482
Protein (g/d)	73.6 ± 2.9	66.9 ± 4.1	68.1 ± 5.7	84.7 ± 7.0	72.2 ± 4.9
Carbohydrates (g/d)	238.5 ± 11.2	207.6 ± 13.0	275.2 ± 39.0	264.0 ± 15.7	211.6 ± 17.6
Fat (g/d)	64.6 ± 2.4	64.3 ± 4.9	57.2 ± 3.3	68.3 ± 4.9	66.5 ± 5.1
Calcium (mg/d)	987 ± 49	803 ± 66	1066 ± 132	1107 ± 84	983 ± 106
Initial DXA*(g/cm^2)	1.123 ± 0.02	1.113 ± 0.03	1.105 ± 0.41	1.133 ± 0.03	1.145 ± 0.04

*Includes the DXA-equivalent values (see Methods for the conversion factor) in the 33 women studied on the DPA instrument as well as the DXA values from the 28 women measured on the DXA instrument.

Demographic, morphometric, dietary and bone density data at the outset of a double-blind, placebo-controlled study and mean dietary data (± SEM) in 61 physically-active women with abnormal menstrual cycles. Random assignment to treatment groups A through D in a factorial design assigned women to cyclic medroxyprogesterone and supplemental calcium (group A), to cyclic medroxyprogesterone and placebo calcium (B), to placebo medroxyprogesterone and supplemental calcium (C) and to both placebos (D).

± 0.7 years (range: 21 to 45 years) (**Table III**). All were healthy, and none abused alcohol or were smokers (although 19 had previously smoked). All were white except for one woman of Oriental origin. The study population was similar to North American population averages in height (162 cm) and within the height-adjusted normal weight range of 48 to 62 kg (Metropolitan Life Tables, 1983).

Twelve women withdrew before study completion for 1 of the following reasons: pregnancy (2); job-related transfers (3); side effects ascribed to intervention (3); need to start oral contraceptives (1); and non-study related changes in health, or in family or work commitments (3). The three women with side effects ascribed to intervention experienced bloating (one woman on placebo medroxyprogesterone), depression (one woman on medroxyprogesterone), or facial pigmentation (one woman on medroxyprogesterone). Equal numbers of women (three) from each group discontinued before completion of the study.

The initial demographic, morphometric, and bone density information by groups and the mean dietary intake data averaged during the study year were not different among intervention groups (Table III). The average dietary calcium intake was almost 1,000 mg/day.

The mean exercise and menstrual cycle data, historical information, and initial and final hormone and lipid values were not different among intervention groups (**Table IV**). Most of the women ran as their primary activity; other participants regularly cross-country skied, swam, bicycled, did aerobics, exercised on stationary equipment, or did a combination of several activities. The average total exercise time at a heart rate greater than 120 beats/minute was 26 minutes daily.

Twenty-six of the 61 women gave a history of amenorrhea. More women in group A (medroxyprogesterone and calcium intervention) had a past history of amenorrhea (chi-square = 9.8, $P = 0.02$); the number of months of amenorrhea in the past did not differ among the groups (chi-square: greater than or equal to 18 months versus less than 18 months = 0.424, $P = 0.935$).

The initial serum estradiol levels were similar to those in the ovulatory reference population (227.9 ± 17.3 pmol/L, $P = 0.070$).[8] Initial serum progesterone levels were lower (9.8 ± 1.2, $P = 0.0003$).[8] The four randomized intervention groups did not differ in abnormal menstrual cycle category at screening (Kruskal-Wallis test statistic = 1.04, $P = 0.793$) nor in cycle characteristics: the median cycle length was 31 days (21 to 180 days), $P = 0.777$; the follicular phase length was 24 days (16 to 180 days), $P = 0.800$; and the luteal phase length was 5.0 days (0 to 9 days), $P = 0.432$.

Initial Bone Density Data

The initial spinal bone density in DXA and DXA-equivalent units (Table III) was 1.12 ± 0.02 g/cm^2, with values ranging from 0.75 g/cm^2 to 1.40 g/cm^2. Using the 10th percentile for values in normal race- and weight-matched young women as a lower limit of normal, 14 of the 61 women (23%) had abnormally low values. The women experiencing amenorrhea at screening (n = 10) had a lower mean initial bone density than the remaining 51 women with other menstrual cycle disturbances (0.994 ± .05 versus 1.148 ± .02 g/cm^2, $P = 0.0007$).

The initial bone density was positively correlated with the screening weight (r = .528, $P = 0.001$), BMI (r = .515, $P = 0.001$), the percentage of subcutaneous fat (r = .296, $P = 0.021$), and the initial serum estradiol

TABLE IV

Exercise Data, Historical, Hormonal and Lipid Characteristics

	All	A	B	C	D
Years run*	6.9 ± 0.6	5.8 ± 1.1	7.2 ± 1.4	9.2 ± 1.3	5.4 ± 0.9
Minutes exercise/month	720.9	730.9	725.8	865	632
	(125-2,007)	(149-1,476)	(162-1,907)	(160-1,750)	(125-2,006)
History amenorrhea†	26/61	11/16	4/16	8/15	3/14
Previous months amenorrhea	21.0 (17-36)	24.0 (15-36)	28.5 (-30-117)	19.0 (8-41)	6.0 (-13-38)
Initial Estradiol (pmol/L)‡	227.9 ± 17.3	208.4 ± 36.1	260.8 ± 40.1	187.3 ± 27.1	256.1 ± 31.3
Final Estradiol (pmol/L)§	292.4 ± 26.7	269.3 ± 38.1	286.0 ± 43.5	273.9 ± 57.9	345.9 ± 75.9
Initial Progesterone‡ (nmol/L)	9.8 ± 1.2	9.8 ± 2.4	10.3 ± 2.3	7.9 ± 2.1	11.0 ± 3.0
Final Progesterone (nmol/L)‡	12.9 ± 1.7	11.9 ± 2.9	13.1 ± 3.9	9.9 ± 2.2	17.2 ± 4.2
Initial HDL Cholesterol (mmol/L)	1.45 ± 0.04	1.39 ± 0.07	1.47 ± 0.09	1.42 ± 0.07	1.54 ± 0.06
Final HDL Cholesterol (mmol/L)	1.42 ± 0.04	1.41 ± 0.07	1.39 ± 0.10	1.38 ± 0.06	1.49 ± 0.09

The measured values (estradiol, progesterone and HDL cholesterol) do not differ among intervention groups, and non-parametric characteristics also do not differ except as noted.

*The number of years of running experience prior to entry into the study.

† The number of women in each group with a history of amenorrhea in the past. Groups differ by chi-square = 9.8, P = 0.02.

‡Compared with a reference ovulatory population, n = 66, initial estradiol level not different (P = 0.070), but the initial progesterone level was lower (P = 0.0003).[8]

§Estradiol and progesterone final values do not differ from the ovulatory reference population, n = 66, (P = 0.548 and 0.165, respectively).[8]

Exercise data, historical, hormonal and lipid characteristics during the study in 61 physically-active women as assigned to interventions: A (n = 16), medroxyprogesterone and calcium, B (n = 16), medroxyprogesterone and calcium placebo, C (n = 15), medroxyprogesterone placebo and active calcium, D (n = 14), both placebos. Minutes of exercise during the year and months of past amenorrhea are shown as median values with range in brackets, and other data as mean ± SEM.

level (r = .329, P = 0.01), but not with the initial progesterone concentration. The initial bone density was negatively correlated with the history of past amenorrhea (chi-square: -3.84, n = 26, P = 0.049) and with the total number of months of past amenorrhea experienced (n = 26, Spearman rank correlation r = -.462, P = 0.017). The initial bone density was not significantly related to the screening cycle lengths (greater than or equal to 36 days versus less than 36 days) (chi-square: 2.94, P = 0.086) nor to screening luteal phase lengths (less than 4 days or 5 to 9 days) (chi-square: 1.44, P = 0.229). Stepwise forward and backward regression analysis of the initial bone density showed total body weight and previous months of amenorrhea as the only significant explanatory variables (Multiple R^2 = .446, with a contribution to R^2 of 0.279 for weight and of 0.167 for months of amenorrhea).

Annual Change in Spinal Bone Density

The change in spinal bone density during the year differed significantly by intervention (**Figure**). Cyclic medroxyprogesterone administration was associated with a highly significant increase in spinal bone density (by two-by-two analysis of variance, F = 19.42, P = 0.0001). The effect of calcium supplementation was just less than statistically significant (F = 3.34, P = 0.073), and there was no interaction between cyclic medroxyprogesterone and calcium in their effects on

spinal bone density (F = 0.02, P = 0.878). The results were virtually unchanged when the six women with less than 11 months or more than 13 months between bone density measurements were excluded (F = 19.52, P = 0.0001).

The spinal bone density changes in DXA and DXA-equivalent units during the year in each of the individuals in the four intervention groups are shown in the Figure. There was a significant net bone density increase of 2.2 ± 0.6% (P = 0.003) in group A (medroxyprogesterone and calcium), a nonsignificant increase of 1.2 ± 0.9% (P = 0.203) in group B (medroxyprogesterone alone), no bone density change (-0.70 ± 0.6%, P = 0.280) in group C (calcium alone), and a 2.0 ± 0.6% loss of bone density (P = 0.005) in group D (both placebos).

To ensure that the unavoidable changes in spinal bone density methodology during this study had not influenced the results, we analyzed the data in two additional ways: (1) by treating DPA and DPX data as separate studies, and (2) by using Z-score conversions of the initial data from both the DPA and DPX instruments. Both analyses confirmed the results that used DXAe data. First, medroxyprogesterone influenced the change in bone density significantly in both DPA and DPX sub-studies, despite the smaller numbers of subjects in each: DPA study, n = 33, F = 16.51, P = 0.0003; DXA study, n = 28, F = 5.32, P = 0.030.
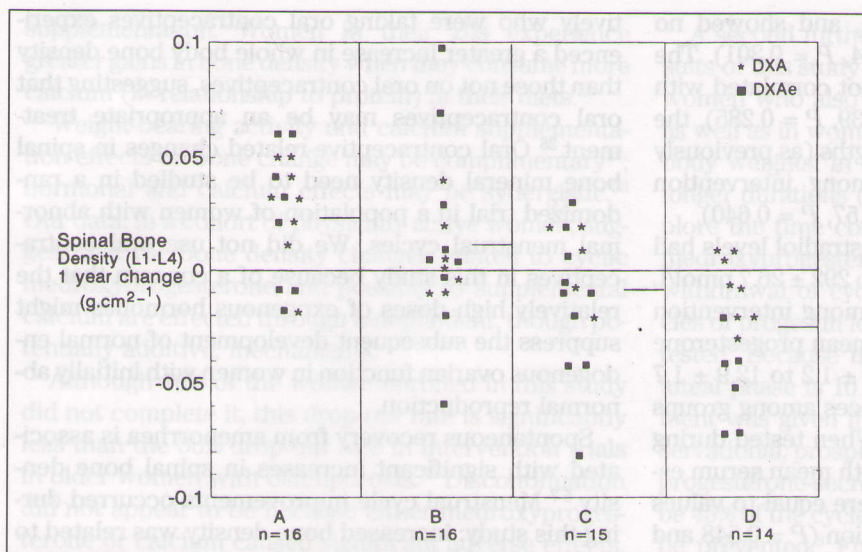
Figure. This graph shows the annual change in spinal (L1-L4) bone mineral density by dual energy radiographic absorptiometry (DXA) and DXA-equivalent values (as derived from dual photon absorptiometry [DPA] measurements) in 61 physically active women with abnormal menstrual cycles. Group mean changes are indicated by small horizontal lines. Intervention groups are: **A** = medroxyprogesterone 10 mg/day for 10 days/month, and calcium carbonate supplement of 1000 mg/day, **B** = cyclic medroxyprogesterone and calcium placebo, **C** = placebo medroxyprogesterone and active calcium, and **D** = both placebos. Analysis by two-by-two analysis of variance shows the F for cyclic medroxyprogesterone = 19.42, P = 0.0001, F for calcium = 3.34, P = 0.073, and F for interaction = 0.02, NS.

Calcium had a significant effect on bone in the DXA study (F = 4.62, P = 0.042), but did not reach significance in the DPA study (F = 2.37, P = 0.135). Second, in analysis using Z-scores related to the mean and standard deviation of the normal age, sex, race, and weight-matched women in the DPA (mean: 1.20 ± 0.13 g/cm^2) and DXA (1.18 ± 0.12 g/cm^2) reference populations, the initial bone density was -0.564 ± 0.14 SEM Z-scores and the change in bone density was 0.011 ± 0.03 SEM Z-scores. Twenty-one women had initial values of less than -1, and 6 women had values of less than -2 Z-scores. Two-by-two analysis of variance using Z-scores confirmed the highly significant effect of medroxyprogesterone (F = 20.01, P = 0.0001) and showed a nearly significant effect of calcium (F = 3.53, P = 0.065).

The major influence on the annual change in spinal bone density was intervention with cyclic medroxyprogesterone. However, bone change was inversely correlated with the initial bone density (r = -.342, P = 0.007). Change in spinal bone density was correlated with luteal phase length but not with cycle length changes: Spearman r = -.045, P = 0.727 and r = .315, P = 0.017 for changes in cycle and luteal phase lengths, respectively. Bone density change did not relate to the increase in serum estrogen (r = .150, P = 0.249) or progesterone levels (r = 0.052, P = 0.688). No other initial values or changes in value including weight, exercise, nutrition, or morphometric parameters influenced the change in spinal bone density. In a stepwise regression model of change in bone density that included medroxyprogesterone and calcium interventions and the change in luteal phase length, medroxyprogesterone contributed 0.228 to R^2, whereas luteal phase length change and calcium made nonsignificant contributions of 0.065 and 0.043, respectively, to the multiple R^2 of 0.336.

Changes in the Menstrual Cycle, Weight, Exercise, Hormones, and Lipids

The mean menstrual cycle changes for the entire population showed a decreased cycle length from a median of 31 days (range: 21 to 180 days) to 28 days (range: 22 to 180 days) (Sign test, P = 0.001), and an increased luteal phase length from 5.0 days (range: 0 to 9 days) to 9.0 days (range: 0 to 15 days) (Sign test, P = 0.0001). Medroxyprogesterone intervention was not significantly related to the menstrual cycle changes that were documented. By Mann-Whitney rank-sum test, those subjects receiving medroxyprogesterone compared with those receiving placebo medroxyprogesterone had no differences in cycle length changes from before to after the intervention [-2.0 days (+33 to -152 days) versus -1.0 days (+19 to -156 days); 0.34, P = 0.562], nor in luteal phase length changes [+1.0 days (+12 to -9 days) versus +1.0 days (+11 to -8 days); 0.02, P = 0.869]. Changes in exercise did not correlate with the increases in cycle and luteal phase lengths (Spearman r = -.229 and .101, respectively). The nonsignificant weight change also did not correlate with the menstrual cycle improvements that were observed (Spearman r = -0.043 and -0.157 for changes in cycle and luteal phase lengths, respectively).

Weight was stable in the group as a whole (mean initial weight was 57.1 ± 1.0 kg and mean final weight was 57.7 ± 1.0 kg, P = 0.08), was not correlated with the change in bone (r = -.031, P = 0.814), nor was it different between those who lost and those who gained bone (P = 0.725). After removal of one outlier who gained 12.7 kg, the change in weight was normally distributed and did not differ by intervention group (analysis of variance F = 1.46, P = 0.233).

The median change in total exercise duration from the last screening to the cycle after intervention was -20.0 minutes/month (range: 1,036 to -1,013 min-

utes/month), normally distributed, and showed no significant change (paired $t = -1.04$, $P = 0.301$). The change in exercise duration was not correlated with the change in bone density ($r = .139$, $P = 0.285$), the change in luteal phase or cycle lengths (as previously shown), nor was it different among intervention groups (analysis of variance $F = 0.57$, $P = 0.640$).

At the end of the study, serum estradiol levels had increased from 227 ± 17.3 pmol/L to 292 ± 26.7 pmol/L ($P = 0.024$) with no differences among intervention groups ($F = 0.28$, $P = 0.839$), and mean progesterone concentrations increased from 9.8 ± 1.2 to 12.9 ± 1.7 nmol/L ($P = 0.09$) with no differences among groups ($F = 0.27$, $P = 0.846$) (Table IV). When tested during the cycle after the intervention, both mean serum estrogen and progesterone levels were equal to values in the ovulatory reference population ($P = 0.548$ and $P = 0.165$, respectively) (Table IV).[8] As described previously, bone density changes were not related to the increases in estradiol or progesterone levels. Serum HDL cholesterol levels (Table IV) were initially normal (1.45 ± 0.04 mmol/L), not different among intervention groups ($F = 0.46$, $P = 0.71$), did not change (final value 1.42 ± 0.04 mmol/L), and showed no medroxyprogesterone-related change ($P = 0.832$).

COMMENTS

We have shown that cyclic medroxyprogesterone given to physically active, premenopausal women with amenorrhea, oligomenorrhea, anovulation, and short luteal phase menstrual cycles not only prevented the bone loss that occurred in the placebo group, but also caused a significant increase in bone density. Although the hormonal changes that occur at menopause are a significant factor in women's increased risk for osteoporosis, we confirm that amenorrhea and other menstrual cycle disturbances occurring prior to menopause are associated with abnormally low spinal bone density[1,2,35] and with accelerated spinal bone mineral loss.[5-7]

Amenorrhea for 6 or more months is reported in 1% to 5% of all reproductive-aged women[36] and in much higher proportions of adolescents[30] or athletes.[14] Subclinical disturbances of ovulation (anovulation and short luteal phase) within normal cycle lengths are also prevalent.[8,37] In our recruited population of 73 recreational athletes with regular menstrual cycles by history, we were surprised to find that only 28 (38%) were excluded because ovulatory menstrual cycles were documented by prospective basal temperature monitoring (Table I).

The common treatments for amenorrhea (such as cyclic hormone therapy[11] and oral contraceptive agents) have not been shown to increase bone density in young women with abnormal menstrual cycles. Twenty-year-old cycling women studied prospectively who were taking oral contraceptives experienced a greater increase in whole body bone density than those not on oral contraceptives, suggesting that oral contraceptives may be an appropriate treatment.[28] Oral contraceptive-related changes in spinal bone mineral density need to be studied in a randomized trial in a population of women with abnormal menstrual cycles. We did not use oral contraceptives in this study because of a concern that the relatively high doses of exogenous hormones might suppress the subsequent development of normal endogenous ovarian function in women with initially abnormal reproduction.

Spontaneous recovery from amenorrhea is associated with significant increases in spinal bone density.[5-7] Menstrual cycle improvements occurred during this study: increased bone density was related to lengthening of the luteal phase but not to shorter cycle lengths. Our data show that intervention with cyclic medroxyprogesterone did not interfere with improved reproductive function. We did not find evidence that the shortened cycle intervals and increased luteal phase lengths we documented were associated with cyclic medroxyprogesterone administration. Thus, cyclic medroxyprogesterone increases spinal bone density and does not interfere with the also-beneficial menstrual cycle recovery.

All of the women in this study were initially deficient in levels of endogenous progesterone. The abnormal cycle types they experienced, including amenorrhea, oligomenorrhea, and anovulation and short luteal phases in normal length cycles, are characterized by low exposure to progesterone and low or normal levels of estrogen; to our surprise, their initial estrogen level of 227.9 ± 17.3 pmol/L was not much lower than in ovulatory women, $P = 0.070$. Our previous prospective 1-year study in ovulatory women showed that short or absent luteal phases are strong predictors of trabecular bone loss by quantitative computed tomography.[8] The hypothesis that progesterone promotes bone formation[15,19] is strengthened by the results of this study showing that cyclic synthetic progesterone treatment increases bone density.

Although the physically active women in this study had intakes of dietary calcium of about 1,000 mg/d, 1000 mg/d of additional calcium appeared to confer some benefit. The significant bone loss that occurred in group D subjects (two placebos) was not present in the group (C) receiving calcium alone (although there was considerable individual overlap in the rates of bone change, Figure). Medroxyprogesterone and calcium likely act independently on bone. The proportional calcium effect can be calculated as a non-significant 0.7% increment in spinal bone density that was associated with the addition of 1,000 mg/d of calcium

supplementation. Women in their 20s experience greater gains in bone density when they consume more calcium (in relationship to protein) in their diets.[28]

Weight-bearing activity and calcium supplementation effects on bone change may be complimentary[27]; hormonal and calcium effects may be synergistic.[38] Our data, in a cohort of physically active women, suggest that the bone density changes related to cyclic medroxyprogesterone and possibly to supplemental calcium are effected through independent, though potentially additive, mechanisms.

Although 16% of the women enrolled in this study did not complete it, this drop-out rate is significantly less than the 36% drop-out rate in intervention trials in older women with osteoporosis.[39] Discontinuation did not appear to be because either medroxyprogesterone or calcium caused significant adverse effects. Even if we ignore the four placebo-controlled trials that did not show depression to be caused by medroxyprogesterone treatment,[40-43] only one woman left the study because of symptoms of depression that some investigators might ascribe to medroxyprogesterone. There is no good evidence that would implicate cyclic medroxyprogesterone in the cause of facial pigmentation (as occurred in one woman on active treatment who discontinued the study). There were no adverse HDL cholesterol level changes related to medroxyprogesterone. Therefore, in this population, cyclical medroxyprogesterone was well tolerated. Whether these results can be generalized to a less active population is not known.

There are several aspects of this study that need further exploration. First, some reproductive improvement occurred for many women in this study, although these positive changes were not shown to be related to any intervention nor to any measured variable or change in variable. Alternative, nonpharmacologic approaches to menstrual disturbances (eg, education, counselling, support) warrant controlled study. Estradiol levels increased significantly even though no estrogen treatment was given. Although the initial bone density level correlated with the screening estrogen level, the change in bone was not related to the change in estradiol level nor to the final estradiol value. Initial bone density was lowest in those subjects with amenorrhea (and low estrogen levels) at entry. However, the bone gain was greatest in those subjects with the lowest bone density, suggesting, paradoxically, that women with long-standing amenorrhea may have a better response to progestins than those with normal cycle intervals. This could occur because prolonged amenorrhea is associated with low rates of bone turnover.[13] Alternately, the increased bone gain in those with the lowest initial bone densities could simply result from the mathematical phenomenon of regression toward the mean.

A second further investigation suggested by the results of this study is the need to repeat it in less active women who also have menstrual cycle disturbances, as well as in women with anorexia or persistently low body weights. In addition, subsequent studies during longer durations (2 or more years) are needed to explore the time course of bone changes during cyclic medroxyprogesterone treatment and following the withdrawal of cyclic medroxyprogesterone. Also, cycles of progestin longer than 10 days/month need to be tested. Because the minimal duration of the normal luteal phase is 10 days,[30] medroxyprogesterone treatment was given for 10 days/month; however, our observational, prospective study suggested that the mean progesterone-secreting portion of the cycle needs to be 45% of the cycle length for bone loss to consistently be prevented.[8] Finally, detailed evaluations of bone turnover using sensitive markers of formation (such as bone alkaline phosphatase, and human osteocalcin) and resorption (such as the excretion rates of cross-links of pyridinoline or n-telopeptide) are needed in studies of menstrual cycle disturbances and during intervention with medroxyprogesterone. A number of studies indicate that the rate of bone turnover is increased for several years after a significant, abrupt hormonal change such as occurs with the onset of amenorrhea[44] or with premenopausal oophorectomy.[45] Investigations are needed to determine whether the initial state of bone remodelling influences the rate of bone change during progestin therapy.

In summary, physically active women with amenorrhea, oligomenorrhea, anovulation, and short luteal phase menstrual cycle disturbances experienced significant increases in spinal bone density related to intervention with cyclic medroxyprogesterone. The results of this trial using cyclic synthetic progesterone support earlier observations that normal cyclic exposure to physiologic progesterone levels plays an important role in preserving a positive bone balance in premenopausal women.[8,18] If the spinal bone mineral density gains documented in this 1-year study persist in longer studies and occur in less-active, more diverse populations, cyclic medroxyprogesterone may be an effective means to prevent subsequent osteoporosis in young women experiencing amenorrhea and other abnormalities in menstrual function.

ACKNOWLEDGMENT

REFERENCES

1. Klibanski A, Neer RM, Beitins IZ, et al. Decreased bone density in hyperprolactinemic women. *NEJM*. 1980;303:1511–1513.

2. Drinkwater BL, Nilson K, Chestnut CH, et al. Bone mineral content of amenorrheic and eumenorrheic athletes. *NEJM*. 1984;311:277–281

3. Rigotti NA, Nussbaum SR, Herzog DB, Neer RM. Osteoporosis in women with anorexia nervosa. *NEJM* 1984;311:1601–1605

4. Snyder AC, Wenderoth MP, Johnston CC Jr, Hui SL. Bone mineral content of elite lightweight amenorrheic oarswomen. *Hum Biol*. 1986;58:863–869

5. Lindberg JS, Powell MR, Hund MM, et al. Increased vertebral bone mineral in response to reduced exercise in amenorrheic runners. *West J Med*. 1987;146:39–42

6. Drinkwater BL, Nilson K, Ott SM, Chestnut CH. Bone mineral density after resumption of menses in amenorrheic athletes. *JAMA*. 1986;256:380–382

7. Bachrach LK, Katzman DK, Litt IF, Guido D, Marcus R. Recovery from osteopenia in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*. 1991;72:602–606

8. Prior JC, Vigna YM, Schechter MT, Burgess AE. Spinal bone loss and ovulatory disturbances. *NEJM*. 1990;323:1221–1227

9. Abraham GE. The normal menstrual cycle. In: Givens JR. *Endocrine Causes of Menstrual Disorders*. Chicago: Year Book Medical Publishers; 1978: 15–44.

10. Lindsay R, Hart DM, Purdee D, et al. Comparative effects of oestrogen and a progestogen on bone loss in post menopausal women. *Clin Sci Mol Med* 1978;54:193–195

11. Emans SJ, Estherann G, Hoffer FA, et al. Estrogen deficiency in adolescents and young adults: impact on bone mineral content and effects of estrogen replacement therapy. *Obstet Gynecol*. 1990;76:585–592

12. Prior JC, Vigna Y, Barr SI, Alojado N. Amenorrhea and anovulation — risk factors for osteoporosis that precede menopause. In: Lorrain J. *Comprehensive Management of Menopause*. New York, NY: Springer Verlag; 1994: 79–96.

13. Biller BM, Coughlin JF, Saxe V, et al. Osteopenia in women with hypothalamic amenorrhea: a prosepctive study. *Obstet Gynecol*. 1991;78:996–1001

14. Loucks AB, Horvath SM. Athletic amenorrhea: a review. *Med Sci Sports Exerc*. 1985;17:56–72

15. Prior JC. Progesterone as a bone–trophic hormone. *Endocr Rev*. 1990;11:386–398

16. Tremollieres FA, Strong DD, Baylink D, Mohan S. Progesterone and promogestone stimulate human bone cell proliferation and insulin-like growth factor 2 production. *Acta Endocrinol* (Copenh). 1992;126:329–337

17. Chen TL, Aronow L, Feldman DL.Glucocorticoid receptors and inhibition of bone cell growth in primary culture. *Endocrinology*. 1977;100:619–628

18. Snead DB, Weltman A, Weltman JY, et al. Reproductive hormones and bone mineral density in women runners. *J Appl Physiol*. 1992;72:2149–2156

19. Prior JC. Progesterone and its role in bone remodelling. In: *Ziegler R. Sex Steroids and Bone*. Berlin: Springer-Verlag; 1993:29-56

20. Committee on sports medicine. Amenorrhea in adolescent athletes. *Pediatrics*. 1989;84:394–395

21. Heaney RP, Recker RR, Saville PD. Menopause changes in calcium balance performance. *J Lab Clin Med*. 1978;92:953–963

22. Dawson-Hughes B, Dallal GE, Krall EA, et al. A controlled trial of calcium supplementation on bone density in postmenopausal women. *NEJM*. 1990;323:878–883

23. Riis BJ, Thomsen K, Christiansen C. Dose calcium supplementation prevent postmenopausal bone loss? *NEJM*. 1987;316:173–177

24. Johnston CC Jr, Miller JC, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. *NEJM*. 1992;327:82–87.

25. Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and bone mineral density in adolescent girls. *JAMA*. 1993;270:841–844

26. Baran D, Sorensen A, Grimes J, et al. Dietary modification with dairy products for preventing vertebra bone loss in premenopausal women: a three–year prospective study. *J Clin Endocrinol Metab*. 1990;70:264–270

27. Kanders B, Dempster DW, Lindsay R. Interaction of calcium nutrition and physical activity on bone mass in young women. *J Bone Min Res*. 1988;3:145–149.

28. Recker RR, Davies KM, Hinders SM, et al. Bone gain in young adult women. *JAMA*. 1992;268:2403–2408.

29. Prior JC, Vigna YM, Schulzer M, et al. Determination of luteal phase length by quantitative basal temperature methods: validation against the midcycle LH peak. *Clin Invest Med*. 1990;13:123–129.

30. Vollman RF. The menstrual cycle. In: Friedman EA. *Major Problems in Obstetrics and Gynecology*. Vol. 7. Toronto: W.B. Saunders Company; 1977:11–193.

31. Thomas AE, McKay DA, Cutlip MB. A nomograph method for assessing body weight. *Am J Clin Nutr*. 1976;29:302–304.

32. Verdier P, Beare-Rogers JL. The Canadian nutrient file. *J Can Diet Assoc*. 1984;45:52–55.

33. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. *Med Sci Sports Exerc*. 1980;12:175–182.

34. Brown MB, Engelman L, Frane JW, et al. *BMDP Statistical Software*. Berkeley, California: University of California Press, 1983: 1–733.

35. Lindberg JS, Fears WB, Hunt MM, et al. Exercise-induced amenorrhea and bone density. *Ann Intern Med*. 1984;101:647–648.

36. Pettersson F, Fries H, Nillius SJ. Epidemiology of secondary amenorrhea. *Am J Obstet Gynecol*. 1973;117:80–86.

37. Nagata I, Kato K, Seki K, Furuya K. Ovulatory disturbances. Causative factors among Japanese student nurses in a dormitory. *J Adolesc Health Care*. 1986;7:1–5

38. Ettinger B, Genant HK, Cann CE. Postmenopausal bone loss is prevented by treatment with low-dose estrogen with calcium. *Ann Intern Med*. 1987;106:40–45.

39. Tilley B, Peterson E, Kleerekoper M, et al. Designing clinical trials of treatment for osteoporosis. Recruitment and follow-up. *Calcif Tissue Int*. 1990;47:327–331.

40. Bullock JL, Massey FM, Gambrell RD Jr. Use of medroxyprogesterone acetate to prevent menopausal symptoms. *Obstet Gynecol*. 1975; 46:165–168.

41. Kirkham C, Hahn PM, Van Vugt DA, et al. A randomized, double-blind, placebo-controlled, cross-over trial to assess the side effects of medroxyprogesterone acetate in hormone replacement therapy. *Obstet Gynecol*. 1991;78:93–97.

42. Morrison JC, Martin DC, Blair RA, et al. The use of medroxyprogesterone acetate for relief of climacteric symptoms. *Am J Obstet Gynecol*. 1980;138:99–104

43. Prior JC, Alojado N, McKay DW, Vigna YM. No adverse effects of medroxyprogesterone treatment without estrogen in postmenopausal women: double-blind, placebo-controlled, cross-over trial. *Obstet Gynecol*. 1994;83:24–28.

44. Katzman DK, Bachrach LK, Carter DR, Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *J Clin Endocrinol Metab*. 1991;73:1332–1339.

45. Stepan JJ, Pospichal J, Vratislav S, et al. The application of plasma tartrate-resistent acid phosphatase to assess changes in bone resorption in respone to artifical menopause and its treatment with estrogen or norethisterone. *Calcif Tissue Int*. 1989;45:273–280.