Effects of an Oral Contraceptive Containing Drospirenone on Bone Turnover and Bone Mineral Density

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OBJECTIVE: To compare the effects of a new 21-day combined oral contraceptive containing 30 μg ethinyl/estradiol plus 3 mg drospirenone with a 21-day preparation containing 30 μg ethinyl/estradiol plus 75 μg gestodene on bone turnover and bone mineral density in young fertile women.

METHODS: A randomized, controlled trial was conducted with healthy fertile women treated with 30 μg ethinyl/estradiol plus 3 mg drospirenone (group A; n = 24), 30 μg ethinyl/estradiol plus 75 μg gestodene (group B; n = 24) and healthy controls (group C, n = 23). At 3, 6, 9, and 12 months of the study, serum and urinary calcium, osteocalcin, urinary pyridinoline, and deoxypyridinoline were measured. At baseline and after 12 months, lumbar bone mineral density was determined by dual-energy X-ray absorptiometry.

RESULTS: In groups A and B, urinary pyridinoline and deoxypyridinoline at 6, 9, and 12 months were significantly reduced in comparison with basal values and group C ($P < .05$). Pyridinoline and deoxypyridinoline levels were lower in group A than in group B throughout the study, but not significantly. In group A serum calcium levels were significantly increased after 6 months. At 12 months, no significant difference was detected in lumbar bone mineral density values among the 3 groups and in comparison with basal values.

CONCLUSION: Both combined oral contraceptives exert a similar positive influence on bone turnover and bone-sparing effect in young postadolescent women. (Obstet Gynecol 2005;105:53–60. © 2005 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: II-1

Bone mineral density achieved during the premenopausal years is one of the major determinants of osteoporosis risk in elderly women. Hormone concentrations, alterations in bone loading, and alterations in nutritional or lifestyle factors are considered to be the 3 major factors that influence peak or premenopausal bone mass.[1]

Contrasting effects of treatment with combined oral contraceptives on bone mineral density of pre-, peri-, and postmenopausal women have been reported.[2–13] The estrogen dose and the type of progestogen are thought to be the main contributory factors for these contrasting results.

Given the well-known actions of estrogens on bone metabolism,[14] in the last few years most clinical and experimental studies have focused on the mechanism exerted by the estrogen component of combined oral contraceptives on the skeleton and on identifying the minimal estrogen dose necessary for maintaining such beneficial actions on the bone.[6,15,16] For the past 20 years, the main goal in the development of oral contraceptives has been to improve their safety and tolerability without compromising their efficacy. Pharmaceutical research has produced phasic regimens, lower doses of the estrogen components, and new progestogens.

In a previous study,[16] we have shown that both a low-dose, 21-day combined oral contraceptive containing 20 μg of ethinylestradiol and 75 μg of gestodene and an ultra–low-dose, 24-day combined oral contraceptive containing 15 μg of ethinylestradiol and 60 μg of gestodene exert a similar positive effect on bone turnover in young postadolescent women, without any significant modification of bone mineral density.

Only a few studies have addressed the effects of different progestogens on bone metabolism[17–19] and bone mass[19–24] in fertile women using oral contraceptives, producing conflicting results. In this respect, the new progestogen drospirenone, a 17-α-spironolactone derivative, is particularly interesting because of its unique pharmacological profile, which closely resembles that of endogenous progesterone.[25]

Unlike other currently available synthetic progestogens, drospirenone possesses both antiandrogenic and

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antimineralocorticoid activity, while it is devoid of any androgenic, estrogenic, glucocorticoid, and antiglucocorticoid activities.[26] As a result of these properties, a combined oral contraceptive containing drospirenone and ethinylestradiol may minimize fluid retention and other adverse effects, which can occur with conventional combined oral contraceptives. Moreover, similarly to its precursor, drospirenone exhibits a much higher affinity for the mineralocorticoid receptor than aldosterone. Therefore, it may exert a bone-protective effect similar to that of spironolactone.[27]

The combination of 3 mg drospirenone with 30 μg ethinylestradiol was recently approved for marketing as an oral contraceptive in Europe and the United States. The preparation is characterized by a high contraceptive efficacy in combination with excellent cycle control, good tolerability, and a favorable impact on lipid and glucose metabolism.[25]

The aim of this study was to compare the effect of a 21-day regime of the combined oral contraceptives, 30 μg ethinylestradiol and 3 mg drospirenone, with 30 μg ethinylestradiol and 75 μg gestodene on bone turnover and bone mineral density in young, healthy, fertile women.

MATERIALS AND METHODS

Between November 2002 and March 2003, all women referring to the Family Planning Clinic of our department were asked to participate in a study on the effects of oral contraceptives on bone metabolism and bone density. The study protocol was approved by our Institutional Review Board and the study was conducted according to the guidelines of the Helsinki Declaration on Human Experimentation; a fully informed signed consent was obtained from all subjects participating in the study.

The participants were between 22 and 34 years of age (because peak total body bone mineral density in most women is achieved by age 20), had reached the age of menarche between 12 and 14 years, showed demonstrable ovulation during a pretreatment cycle, and had no abnormal menstrual cycles nor abnormal dietary requirements. Definitive exclusion criteria were confirmed pregnancy or suspicion thereof, pregnancy or breastfeeding in the previous year, acute, chronic, or progressive liver disease or disturbed biliary secretion, evidence of vascular or metabolic disorders, bone disease or disorders of bone metabolism (Paget's disease, hyperparathyroidism, renal osteodystrophy), smoking 10 cigarettes per day, history of migraine with aura, use of drugs known to affect bone metabolism (bisphosphonates, sodium fluoride, calcitonin, estroprogestins or anabolic steroids, corticosteroids, calcium or vitamin D, phosphate [P], thiazidic diuretics), use of drugs known to interfere with contraceptive steroids, hysterectomy or oophorectomy, and all other clinically relevant contraindications for the use of combined oral contraceptives.

Before inclusion, patients underwent general and gynecological history, Pap test, bimanual pelvic examination, evaluation of systolic and diastolic blood pressure, calculation of body mass index, and a complete hematochemical evaluation. In a pretreatment control cycle, each women underwent 3–4 ultrasound scans between days 10 and 16; follicles with a diameter 15 mm or greater were followed until rupture or disappearance to assess the presence of normal ovulatory cycles and the absence of pelvic and adnexal disease.

From a pool of 125 women who were short-listed for oral contraceptive treatment, 85 agreed to participate (participation rate 68%). Of these, 62 met the inclusion criteria and were asked if they were willing to accept a random assignment into 2 groups of treatment. Fourteen women refused the randomization process, leaving a total of 48 women. These women were divided into 2 groups of 24 members each according to a computer-generated randomization sequence performed by an administrative staff member. The randomization sequence was concealed both to researchers and patients until treatments were assigned. After treatments were assigned, neither researchers nor patients were blinded to group assignment.

Each of the women in group A was given the oral contraceptive pill containing 30 μg ethinylestradiol plus 3 mg drospirenone (Yasmin; Schering, Milan, Italy), and each woman in group B was given the oral contraceptive pill containing 30 μg ethinylestradiol plus 75 μg gestodene (Ginoden; Schering). After confirming the inclusion criteria, 23 women who did not ask for hormonal contraception participated in the study as controls (group C; Fig. 1).

No additional treatment was given. Patients in both groups A and B were instructed to take the pill for 21 days, starting with the first day of the next spontaneous menses, with a 7-day pill-free interval. The treatment period lasted for 12 months.

All women participating to the study were asked to keep calendars of their vaginal bleeding and side effects. Changes over time in bone metabolism were assessed qualitatively by biochemical serum and urinary analysis of bone resorption indices (levels of serum and urinary calcium, pyridinoline, and deoxypyridinoline) and formation (osteocalcin) (*primary outcome measures*).

Pyridinoline and deoxypyridinoline are the 2 major cross-link molecules involved in collagen stabilization. Bone collagen undergoes a higher rate of turnover than

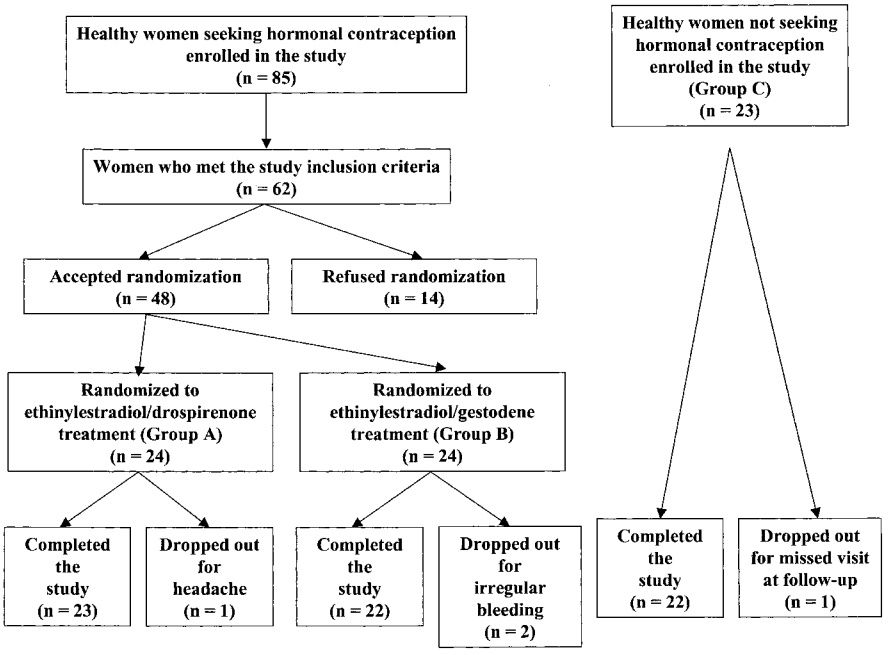

Fig. 1. Patient enrollment and randomization. Ethinylestradiol/drospirenone = 30 μg ethinylestradiol plus 3 mg drospirenone; ethinylestradiol/gestodene = 30 μg ethinylestradiol plus 75 μg gestodene.

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other sources of collagen. Thus, the measurement of these molecules provides a highly specific and sensitive marker for bone resorption.

Osteocalcin is the most abundant noncollagenous protein in bone and is produced almost exclusively by osteoblasts. Serum osteocalcin concentration, therefore, is a sensitive marker of bone formation that correlates with histomorphometric measurements of bone formation in bone biopsy specimens.

Blood and urine samples were collected in the morning, between 8:00 and 9:00 AM, after a 12-hour fast. Blood samples were collected in tubes with clot-activating factor and immediately centrifugated in a refrigerated centrifuge. Sera were stored at −80°C until assayed. Urine samples were stored at −20°C until biochemical analysis was performed. All samples were analyzed in the same assay and were analyzed in a laboratory blinded to the treatment. Baseline urinary and serum samples were collected on one of the first days (days 3–7) of pretreatment menstrual cycle. In each subject, blood and urine samplings were repeated every 3 months throughout a 12-month period, during the third to seventh day after the onset of spontaneous or pill-induced menstrual bleeding.

Serum and urinary levels of calcium were analyzed as a part of the biochemical routine evaluation (complete blood count, aspartate aminotransaminase, alanine aminotransferase, creatininemia, glycemia, blood urea nitrogen, urinalysis, fibrinogen, prothrombin time, and activated partial thromboplastin time). The values of pyridinoline and deoxypyridinoline were measured with specific monoclonal antibodies (Metra Biosystem; Mountain View, CA) and were expressed as values over the urinary creatinine. Osteocalcin was measured by radioimmunoassay (Nichols Institute Diagnostic, San Clemente, CA).

Quantitative longitudinal changes of bone mineral density (*secondary outcome measure*) were determined by dual X-ray absorptiometry (DEXA QDR 1000; Hologic, Waltham, MA) of the posterior-anterior lumbar spine (L1–L4). The precision of the measurements, expressed as coefficients of variation (CV) in vitro for repeated bone mineral density determinations in 2 standard phantoms, was 0.42%. The CV in vivo, evaluated by comparing 2 measurements performed at 7-day intervals in 33 volunteers, was 1.2% for the lumbar spine. The reference population adopted in this study was the international pooled sample provided by the manufacturer. Their data, however, did not differ significantly from those obtained on a local sample in a study performed when the instrument was set up.[28] Bone mineral content (g/cm) was divided by bone width (cm) to give an index (g/cm^2) that was used to standardize the findings for bone size. The absorptiometry was performed by the same observer (G.A.T.), who was blinded to the different treatment regimens. Absorptiometric findings are expressed as percentage of change of baseline values.

Baseline scans were performed during the third to seventh day of the pretreatment cycle. Follow-up scans were made on one of the first days after the onset of pill-induced menstrual bleeding after 12 months of treatment.

On the basis of a previous study[16] performed in our department, we calculated that, to observe a reduction of

Table 1. Baseline Characteristics of the Patients by Study Group

	Group A (n = 23)	Group B (n = 22)	Group C (n = 22)
Age (y)	27.2 ± 5.3	26.9 ± 5.5	28.1 ± 6.1
Body mass index (kg/m^2)	22.2 ± 0.3	21.8 ± 0.7	22.6 ± 0.4
Systolic pressure (mm Hg)	121.2 ± 8.6	120.8 ± 8.9	119.3 ± 9.0
Diastolic pressure (mm Hg)	71.3 ± 5.6	73.0 ± 5.3	72.7 ± 6.0
Menarche (y)	13.0 ± 1.3	12.8 ± 0.6	12.6 ± 0.9
Serum calcium (mmol/L)	2.38 ± 0.02	2.36 ± 0.02	2.36 ± 0.04
Urinary calcium (mmol/mmol Cr)	289 ± 32	279 ± 35	284 ± 31
Serum osteocalcin (ng/mL)	7.3 ± 0.6	7.2 ± 0.5	7.2 ± 0.5
Urinary deoxypyridinoline (nmol/mmol Cr)	6.8 ± 0.9	7.0 ± 0.8	7.2 ± 0.7
Urinary pyridinoline (nmol/mmol Cr)	36.4 ± 3.3	36.2 ± 4.0	36.8 ± 3.5
Spinal bone mineral density (g/cm^2)	1.039 ± 0.08	1.041 ± 0.09	1.042 ± 0.16

Cr, creatinine.
Data are expressed as mean ± standard deviation.

5 mmol of pyridinoline levels, a bone resorption index with very high specificity and sensibility, with a power of 95% and $P < .05$, a sample of 20 patients in each arm would be needed.

The Shapiro-Wilk test showed that all continuous variables were normally distributed in our study groups. One-way analysis of variance followed by Newman-Keuls multiple-range test was used to compare age, age of menarche, body mass index, and systolic and diastolic blood pressure among the 3 groups at the beginning of the study. Variations in bone mineral density and biochemical data among groups and in the same group at different times were statistically evaluated by 2-way analysis of variance followed by the Newman-Keuls multiple-range test. Interaction between factors (treatment and time) was also evaluated. Statistical analysis was performed with SPSS 9.0 (SPSS Inc, Chicago, IL). Statistical significance was set at $P < .05$. Data were expressed as mean ± standard deviation.

RESULTS

Of the 71 women selected for the study, 67 completed the study. One patient from group A dropped out owing to complaints of headache, 2 patients from group B discontinued the treatment because of irregular bleeding, and 1 patient from group C was excluded for missing the follow-up visit after 6 months (Fig. 1). Table 1 delineates the baseline characteristics of the subjects studied. The 3 groups were comparable with regard to clinical characteristics and basal values of bone metabolism indices and bone mineral density.

In groups A and B, urinary levels of pyridinoline and deoxypyridinoline at 6, 9, and 12 months were significantly reduced in comparison with basal values and with the control group ($P < .05$) (Fig. 2A-B). Although not statistically significant, pyridinoline and deoxypyridinoline reduction was observed as early as 3 months in the treated groups. Both pyridinoline and deoxypyridinoline levels showed a greater reduction in group A than in group B throughout the observation period, but this difference never reached statistical significance. No significant changes occurred in bone resorption indices values during the 12-month period of observation in group C (Fig. 2A-B).

In group A, serum calcium levels showed an increasing trend, which reached statistical significance after 6 months in comparison with basal values and groups B and C (Fig. 3A). Urinary calcium excretion decreased in both groups A and B, with a greater reduction observed in group A. These changes were, however, not statistically significant (Fig. 3B). No significant variations of serum calcium and urinary excretion were observed in group C (Fig. 3A-B).

Throughout the 12 months of study, although serum osteocalcin was slightly reduced in groups A and B, it was not statistically significant. No significant trend in osteocalcin values were observed during the period of observation in group C. At 12 months, no significant differences were detected either in spinal bone mineral density values among the 3 groups or in comparison with basal values (Table 2). No significant interaction between time and treatment has been observed for all the dependent variables analyzed. No significant changes in body mass index were observed over the 12-month study period in any group (group A, 22.2 ± 0.3 versus 22.7 ± 0.6; group B, 21.8 ± 0.7 versus 21.6 ± 0.5; group C, 22.6 ± 0.4 versus 22.9 ± 0.6).

DISCUSSION

The progestogen drospirenone is a 17-α-spironolactone derivative with a unique pharmacological profile. It combines potent progestogenic with antiandrogenic and antimineralocorticoid activity.[25–26] Although its antiandrogenic activities may have a negative impact on bone

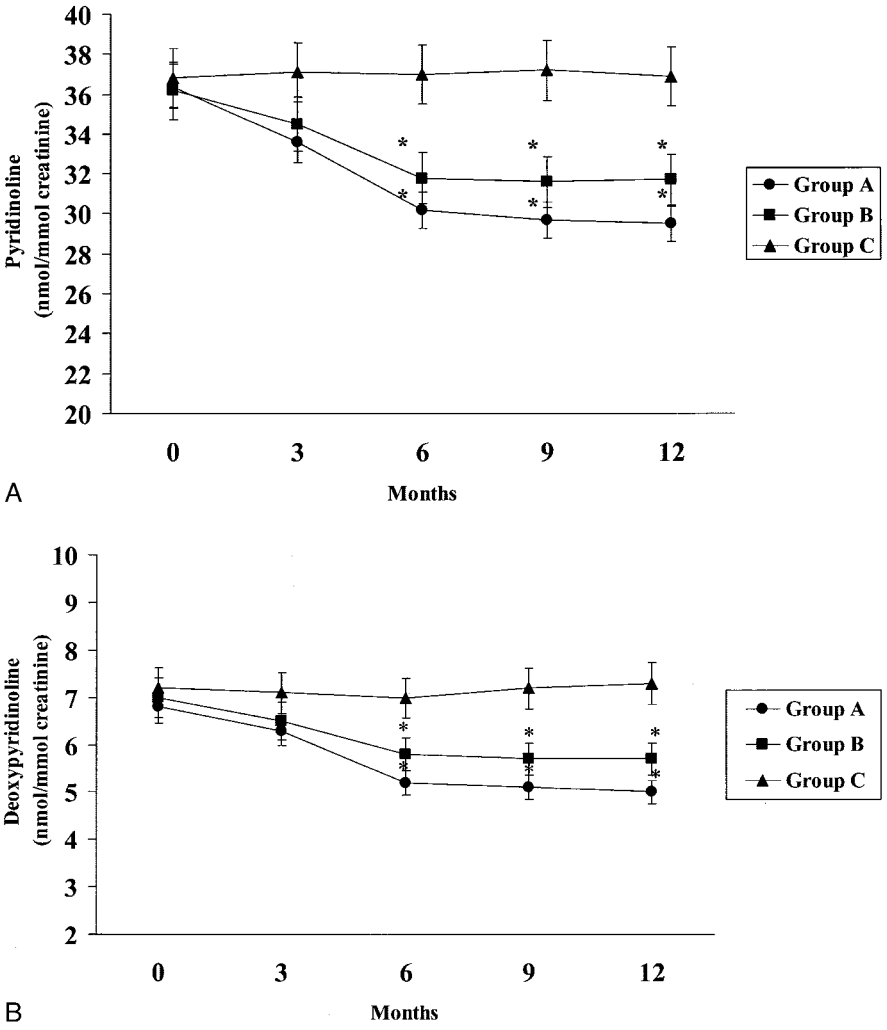

Fig. 2. Trends in urinary pyridinoline **(A)** and deoxypyridinoline **(B)** levels in group A *(dark circles)*, group B *(dark squares)*, and group C *(dark triangles)* during the 12-month study period. * $P < .05$ versus group C and baseline. Group A: patients were treated with a pill containing 30 μg ethinylestradiol plus 3 mg drospirenone; group B: patients were treated with a pill containing 30 μg ethinylestradiol plus 75 μg gestodene; group C: controls. Values are presented as mean ± standard deviation.

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metabolism, its antimineralocorticoid effects may counteract this impact.

We investigated the influence of a new combined oral contraceptive containing 30 μg ethinylestradiol and 3 mg drospirenone on bone metabolism indices and bone mineral density during 12 cycles of treatment in healthy, fertile women in comparison with a reference 21-day combined oral contraceptive containing an equal dose of ethinylestradiol combined with 75 μg gestodene. Biochemical markers of bone resorption evaluated in our study included urinary pyridinoline and deoxypyridinoline and serum and urinary calcium. In fact, an increased bone resorption uncoupled with bone formation is associated with an increased calcium passage from the solid phase into the extracellular fluid. This results in a rise of serum calcium and calcium excretion. Serum osteocalcin has been measured as a marker of bone formation.

Our results showed a positive effect of both ethinylestradiol/drospirenone and ethinylestradiol/gestodene combined oral contraceptives on bone turnover, with a significant reduction of pyridinoline and deoxypyridinoline and a slight, but not significant, decrease of osteocalcin. Thus, the prevalent reduction of pyridinoline and deoxypyridinoline indicates a decreased bone resorption.

Although not statistically significant, a greater reduction of both pyridinoline and deoxypyridinoline in the group treated with ethinylestradiol/drospirenone was observed. A longer period of observation is needed to confirm whether drospirenone may significantly reduce bone resorption in comparison with gestodene.

An interesting finding was related to the calcium homeostasis. While women treated with ethinylestradiol/gestodene showed a slight decrease in serum calcium levels, those treated with ethinylestradiol/drospirenone showed increased serum calcium levels, reaching statistically significant levels after 6 months of treatment. Urinary calcium excretion decreased in both treatment

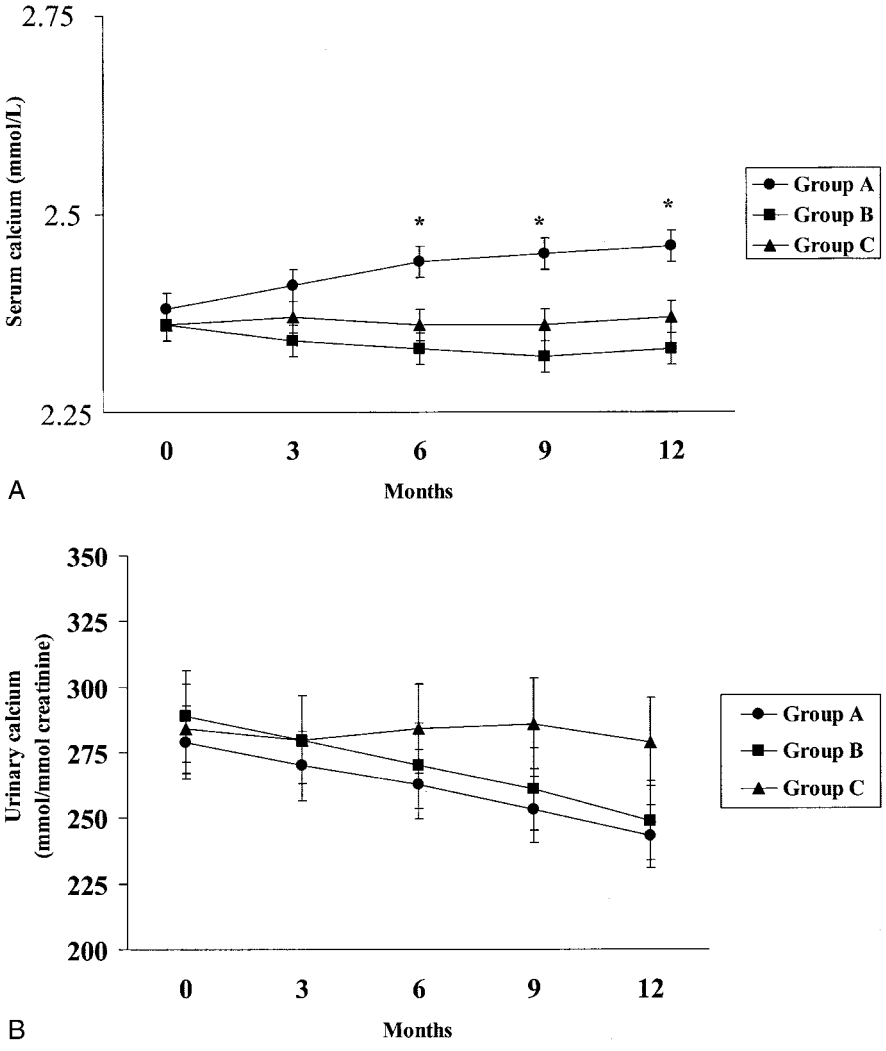

Fig. 3. Trends in serum **(A)** and urinary **(B)** calcium levels in group A *(dark circles)*, group B *(dark squares)*, and group C *(dark triangles)* during the 12-month study period. * $P < .05$ versus other groups and baseline. Group A: patients were treated with a pill containing 30 μg ethinylestradiol plus 3 mg drospirenone; group B: patients were treated with a pill containing 30 μg ethinylestradiol plus 75 μg gestodene; group C: controls. Values are presented as mean ± standard deviation.

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groups, with a greater reduction observed in those treated with ethinylestradiol/drospirenone, but these changes were not significant.

To evaluate whether this positive effect on bone turnover could significantly affect bone mineral density, spine bone mineral density was evaluated by dual X-ray absorptiometry in all groups at baseline and after 12 cycles of treatment. The spine is generally the favorite site for measurements of bone mineral density, and examinations are routinely performed in the posterior-anterior projection. Owing to its nature as a projectional technique, the posterior-anterior scan of the lumbar spine includes, not only the metabolically active trabecular bone of the vertebral body, but also a substantial amount of cortical bone, particularly in the posterior elements.

Table 2. Bone Mineral Density in the Three Groups at Baseline and After 12 Months

Spinal Bone Mineral Density (g/cm^2)	Group A (n = 23)	Group B (n = 22)	Group C (n = 22)
Baseline	1.039 ± 0.08	1.041 ± 0.09	1.042 ± 0.16
After 12 months	1.065 ± 0.11	1.047 ± 0.10	1.039 ± 0.09

Group A: patients were treated with a pill containing 30 μg ethinylestradiol plus 3 mg drospirenone; group B: patients were treated with a pill containing 30 μg ethinylestradiol plus 75 μg gestodene; group C: controls.

Values are presented as mean ± standard deviation.

Although no significant difference was detected in spinal bone mineral density values among the 3 groups and in comparison with basal values at 12 months, a trend toward higher values of bone mineral density in the group treated with ethinylestradiol/drospirenone was observed. Again, because an observation period of

12 months is a very limited time to evaluate changes in bone mineral density, a longer period of observation could indicate a different impact of drospirenone or gestodene on bone mineral density.

However, data seem to support the hypothesis of a specific contribution of drospirenone to the beneficial effect on bone of the novel ethinylestradiol/drospirenone combined oral contraceptive. Indeed, the addition of drospirenone to ethinylestradiol was associated with a significant reduction of bone resorption and with a slight, although not significant, increase of bone mineral density.

Although our results do not allow a conclusive identification of the mechanism of action of drospirenone on bone metabolism, they suggest some intriguing hypotheses. Drospirenone is a derivative of 17-α-spironolactone and an analogue of spironolactone. Consequently, as its precursor, in addition to its progestogenic propriety, it has antimineralocorticoid and antiandrogenic activities. Neither of these activities, individually nor in combination, have been described for the currently available synthetic progestogens (including gestodene) and, thus, may provide an explanation for the bone-sparing effect of drospirenone.

Given that androgens have been demonstrated to exert a positive influence on bone mass in several studies,[14,29–31] it is more likely that the bone-protective action of drospirenone is linked to its renal effect as an antagonist of mineralocorticoid hormones. Indeed, the lack of mineralocorticoid activity is associated with enhanced tubular reabsorption of calcium, increases of serum calcium, and inhibition of parathyroid hormone secretion.[32] The higher serum calcium levels with only marginal changes in calcium excretion in the group treated with ethinylestradiol/drospirenone seem to support this hypothesis. The higher reduction of pyridinoline and deoxypyridinoline in the group treated with ethinylestradiol/drospirenone and the similarly slight reduction of osteocalcin values in both treated groups could explain the greater suppression of bone resorption rather than a greater stimulation of bone formation by drospirenone. As previously reported for spironolactone,[27] drospirenone might exert an effect on the renal handling of calcium similar to that of thiazide diuretics, which have been shown to suppress bone turnover and prevent postmenopausal bone loss.[33–34]

In conclusion, the present study shows that the oral contraceptive containing 30 μg ethinylestradiol and 3 mg drospirenone and the oral contraceptive containing 30 μg ethinylestradiol and 75 μg gestodene exert a beneficial effect on bone turnover in young fertile women. A trend toward a higher bone-sparing effect of the combined oral contraceptive containing drospirenone was observed. If this specific contribution of drospirenone to such bone-sparing effect is supported by further long-term studies, the combined oral contraceptive containing this new progestogen could be specifically prescribed in women with risk factors for osteopenia and osteoporosis who require oral contraception.

REFERENCES

1. Sowers M. Lower peak bone mass and its decline. Baillieres Best Pract Res Clin Endocrinol Metab 2000;14: 317–29.
2. Recker RR, Davies KM, Hinders SM, Heaney RP, Stegman MR, Kimmel DB. Bone gain in young adult women. JAMA 1992;268:2403–8.
3. Shargil AA. Hormone replacement therapy in perimenopausal women with a triphasic contraceptive compound: a three year prospective study. Int J Fertil 1985;30:18–28.
4. Lindsay R, Tohme J, Kanders B. The effect of oral contraceptive use on vertebral bone mass in pre- and postmenopausal women. Contraception 1986;34:333–40.
5. Kleerekoper M, Brienza RS, Schultz LR, Johnson CC. Oral contraceptive use may protect against low bone mass. Henry Ford Hospital Osteoporosis Cooperative Research Group. Arch Intern Med 1991;151:1971–6.
6. Mais V, Fruzzetti F, Ajossa S, Paoletti AM, Guerriero S, Melis GB. Bone metabolism in young women taking a monophasic pill containing 20 μg ethinylestradiol: a prospective study. Contraception 1993;48:445–52.
7. Gambacciani M, Spinetti A, Taponeco F, Cappagli B, Piaggesi L, Fioretti P. Longitudinal evaluation of perimenopausal vertebral bone loss: effects of a low-dose oral contraceptive preparation on bone mineral density and metabolism. Obstet Gynecol 1994;83:392–6.
8. Volpe A, Amram A, Cagnacci A, Battaglia C. Biochemical aspects of hormonal contraception: effects on bone metabolism. Eur J Contracept Reprod Health Care 1997;2: 123–6.
9. Mazess RB, Barden HS. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. Am J Clin Nutr 1991;53: 132–42.
10. Rodin A, Chapman M, Fogelman I. Bone density in users of combined oral contraception: preliminary reports of a pilot study. Br J Fam Plann 1991;16:125–9.
11. Murphy S, Khaw KT, Compston JE. Lack of relationship between hip and spine bone mineral density and oral contraceptive use. Eur J Clin Invest 1993;23:108–11.
12. Lloyd T, Buchanan JR, Ursino GR, Myers C, Woodward G, Halbert DR. Long-term oral contraceptive use does not affect trabecular bone mineral density. Am J Obstet Gynecol 1989;160:402–4.
13. Tuppurainen M, Kroger H, Saarikoski S, Honkanen R, Alhava E. The effect of previous oral contraceptive use on bone mineral density in perimenopausal women. Osteoporos Int 1994;4:93–8.

14. Compston JE. Sex steroids and bone. Physiol Rev 2001; 81:419–47.
15. Kuohung W, Borgatta L, Stubblefield P. Low-dose oral contraceptives and bone mineral density: an evidence-based analysis. Contraception 2000;61:77–82.
16. Nappi C, Di Spiezio Sardo A, Acunzo G, Bifulco G, Tommaselli GA, Guida M, et al. Effects of a low-dose and ultra-low-dose combined oral contraceptive on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. Contraception 2003;67:355–9.
17. Fletcher CD, Farish E, Dagen MM, Allam BF, Hart DM. Effects of conjugated equine oestrogens with and without the addition of cyclical norgestrel on serum and urine electrolytes, and the biochemical indices of bone metabolism and liver function. Maturitas 1988;9:347–57.
18. Gallagher JC, Kable WT, Goldgar D. Effect of progestin therapy on cortical and trabecular bone: comparison with estrogen. Am J Med 1991;90:171–8.
19. Ott SM, Scholes D, Lacroix AZ, Ichikawa LE, Yoshida CK, Barlow WE. Effects of contraceptive use on bone biochemical markers in young women. J Clin Endocrinol Metab 2001;86:179–85.
20. Prior JC, Vigna YM, Schechter MT, Burgess AE. Spinal bone loss and ovulatory disturbances. N Engl J Med 1990;323:1221–7.
21. Naessen T, Olsson SE, Gudmundson J. Differential effects on bone density of progestogen-only methods for contraception in premenopausal women. Contraception 1995; 52:35–9.
22. Abdalla HI, Hart DM, Lindsay R, Leggate I, Hooke A. Prevention of bone mineral loss in postmenopausal women by norethisterone. Obstet Gynecol 1985;66: 789–92.
23. Prior JC, Vigna YM, Barr SI, Rexworthy C, Lentle BC. Cyclic medroxyprogesterone treatment increases bone density: a controlled trial in active women with menstrual disturbances. Am J Med 1994;96:521–30.
24. Adachi JD, Sargeant EJ, Sagle MA, Lamont D, Fawcett PD, Bensen WG, et al. A double-blind randomised controlled trial of the effects of medroxyprogesterone acetate on bone density of women taking estrogen replacement therapy. Br J Obstet Gynaecol 1997;104:64–70.
25. Parsey KS, Pong A. An open-label, multicenter study to evaluate Jasmin, a low-dose combination oral contraceptive containing drospirenone, a new progestogen. Contraception 2000;61:105–11.
26. Krattenmacher R. Drospirenone: pharmacology and pharmacokinetics of a unique progestogen. Contraception 2000;62:29–38.
27. Moghetti P, Castello R, Zamberlan N, Rossini M, Gatti D, Negri C, et al. Spironolactone, but not flutamide, administration prevents bone loss in hyperandrogenic women treated with gonadotropin-releasing hormone agonist. J Clin Endocrinol Metab 1999;84:1250–4.
28. del Puente A, Heyse SP, Mandes MG, Mantova D, Carpinelli A, Nutile G, et al. Epidemiology of osteoporosis in women in southern Italy. Aging (Milano) 1998;10: 53–8.
29. Adami S, Zamberlan N, Castello R, Tosi F, Gatti D, Moghetti P. Effect of hyperandrogenism and menstrual cycle abnormalities on bone mass and bone turnover in young women. Clin Endocrinol (Oxf) 1998;48:169–73.
30. Dagogo-Jack S, Al-Ali N, Qurttom M. Augmentation of bone mineral density in hirsute women. J Clin Endocrinol Metab 1997;82:2821–5.
31. Vanderschueren D, Bouillon R. Androgens and bone. Calcif Tissue Int 1995;56:341–6.
32. Muls E, Bouillon R, Boelaert J, Lamberigts G, Van Imschoot S, Daneels R, et al. Etiology of hypercalcemia in a patient with Addison's disease. Calcif Tissue Int 1982; 34:523–6.
33. Reid IR, Ames RW, Orr-Walker BJ, Clearwater JM, Horne AM, Evans MC, et al. Hydrochlorothiazide reduces loss of cortical bone in normal postmenopausal women: a randomized controlled trial. Am J Med 2000; 109:362–70.
34. Rejnmark L, Vestergaard P, Heickendorff L, Andreasen F, Mosekilde L. Effects of thiazide- and loop-diuretics, alone or in combination, on calcitropic hormones and biochemical bone markers: a randomized controlled study. J Intern Med 2001;250:144–53.

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