ORIGINAL ARTICLE

Effects of Combination Oral Contraceptives on Bone Mineral Density and Metabolism in Perimenopausal Korean Women

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Objectives: A retrospective cohort study was conducted to evaluate the effects of combination oral contraceptives (COCs) on bone mineral density (BMD) and metabolism in perimenopausal Korean women.

Methods: The study subjects comprised two groups. The COC group included 55 women who took low-dose COC for at least one year to control vasomotor symptoms. Another 55 women who had annual checkups without history of COC use served as controls. BMD and bone turnover markers were assessed periodically.

Results: In the control group, 12-month BMD values at the lumbar spine (LS) and total hip (TH) significantly decreased with a greater magnitude at LS, and bone resorption (BR) and formation (BF) markers increased concurrently with a larger change in BR. COCs increased BMD at LS after 12 months and prevented BMD decline at TH. Multivariable linear regression revealed a significant difference in LS BMD between groups at 12 months. In the COC group, there were significant negative correlations between baseline BMD and Z-score at LS and corresponding changes at 12 months. COCs did not alter BR markers, whereas BF markers were significantly decreased at 3 months. Group comparison at 12 months, as tested with adjusted linear regression, disclosed significant differences in both BR and BF makers.

Conclusions: Bone loss associated with activated bone turnover is evident during the menopausal transition, and COCs might prevent BMD decrease and suppress bone turnover markers in perimenopausal Korean women. Significant increase in LS BMD and decreases in BF makers suggest underlying mechanisms of greater impact on BF.

Key Words: Bone density, Bone fractures, Bone remodeling, Oral contraceptives, Perimenopause

INTRODUCTION

Osteoporosis is the most common metabolic bone disease, and estrogen deficiency plays a key role in the pathogenesis of postmenopausal osteoporosis [1]. Estrogen therapy prevents bone loss and fragility fractures in postmenopausal women [2].

In 1986, Riggs et al. [3] reported different rates of bone loss in the appendicular and axial skeletons of women during their lifetimes, as assessed by photon absorptiometry. Bone mineral density (BMD) at midradius did not change before menopause and decreased after menopause. In contrast, substantial bone loss at the lumbar spine (LS) occurred before menopause, strongly suggesting etiological factors other than estrogen deficiency, including progesterone insufficiency [4]. The Study of Women's Health Across the Nation (SWAN) is a prospective cohort study of middle-aged American women of multiple ethnicities to detail bone loss across menopause during the five years before and

the five years after the final menstrual period (FMP) [5].

Contraception is needed in perimenopausal women to prevent unwanted pregnancy. Combination oral contraceptives (COCs) provide highly effective contraception and are used most commonly among women younger than 30 years. COC use in women during later reproductive years is increasing in the U.S. [6], with improved safety due to decreases in estrogen dose and introduction of newer progestins. In addition to contraception, COCs alleviate climacteric symptoms and might have a positive effect on BMD [7] Of note, progestin is the dominant component over estrogen in COCs.

Research into perimenopausal bone loss and potential roles of COCs is lacking in Korea. This study was designed to evaluate the effects of COCs on BMD and metabolism in perimenopausal Korean women.

MATERIALS AND METHODS

Subjects and treatments

We conducted a retrospective cohort study including 110 perimenopausal women older than 40 years who visited Samsung Medical Center in Seoul, Korea between January 1997 and June 2017. Women were considered perimenopausal if their menstrual cycle was irregular in cycle or flow and their duration of amenorrhea was shorter than 12 months with serum follicle-stimulating hormone (FSH) level lower than 40 IU/L. The study subjects comprised two groups. The COC group included 55 women who took low-dose COC (daily ethinyl estradiol dose of 30 µg or less or estradiol valerate dose of 3 mg or less) at our Menopause Clinic for at least one year to control vasomotor symptoms. Another 55 women who were registered at the Center for Health Promotion, participated in annual checkups, and did not take COC served as controls. Subjects were not eligible if they underwent surgical menopause or had BMD below the expected range for age (Z-score < 2.0) at spine or hip. Women were excluded if they had a history of certain diseases including hyperthyroidism or hyperparathyroidism, were taking medications that could affect bone metabolism, or were current or recent user of female hormone (within six months before study entry) or osteoporosis medications (within one year). Women with contraindications for COC including smoking, acutely impaired liver function, breast cancer, and venous thrombosis were excluded. The study protocol was approved by the Institutional Re-

view Board of Samsung Medical Center (IRB No. 2019-08-083) and the informed consent was waived by the IRB.

Bone mineral density

Baseline BMD measurements were evaluated via dual-energy X-ray absorptiometry (DXA) at the second to fourth vertebrae of the LS and at the total hip (TH) (GE Healthcare, Madison, WI, USA in the control group and Hologic, Bedford, MA, USA in the COC group). Follow-up BMD was measured at 12 months in the control group, and at six and 12 months in the COC groups.

Biochemical markers of bone turnover

Urinary deoxypyridinoline (DPD [reference value 3.0–7.4 nM DPD/mM creatinine]; Quidel, San Diego, CA, USA) was assessed as a bone resorption (BR) marker by enzyme immunoassay and corrected measurements for creatinine level. Serum N-telopeptide of type I collagen (NTX [reference value 6.2–19 nM of bone collagen equivalents]; Alere Scarborough, Scarborough, ME, USA) and C-telopeptides (CTX [reference value 0.025–0.573 ng/mL]; Roche Diagnostics, Mannheim, Germany) were measured via enzyme-linked immunosorbent assay and electrochemiluminescence immunoassay, respectively. DPD or CTX was used in the control group, and DPD or NTX was used in the COC group.

As bone formation (BF) markers, serum osteocalcin (OC [reference value 11–43 ng/mL]; Roche Diagnostics, Mannheim, Germany) and bone-specific alkaline phosphatase (BSAP [reference value 5.4–14.3 µg/L]; Beckman Coulter, Chaska, MN, USA) were analyzed by electrochemiluminescence immunoassay and chemiluminescent immunoassay, respectively. OC was used in the control group, and OC or BSAP was used for the COC group.

Bone markers were measured at baseline and 12 months in the control group, and at baseline, three, six and 12 months in the COC group.

Hormone assay

Blood concentrations of FSH (DIASource, Ottignies-Louvain-la-Neuve, Belgium) and estradiol (Beckman Coulter, Prague, Czech Republic) were measured at baseline, using radioimmunoassay.

Statistical analysis

Statistical analyses were performed using SAS (ver. 9.4; SAS Institute, Cary, NC, USA). Data are expressed as mean ± SD for continuous variables or number (%) for categorical variables. Baseline clinical characteristics were compared between the control and COC groups by Student's t-tests for continuous variables or Wilcoxon rank-sum test after checking normality via Shapiro–Wilks test, and by chi-square test or Fisher's exact test for categorical variables, as appropriate. To ascertain percentage changes in BMD and bone turnover markers, one-sample t-test was applied for within-group comparison. Multivariable linear regression was used to investigate group differences in BMD, Z-scores, and bone turnover markers with adjustment of baseline characteristics. Time trends and differences at each time point of Z-score and bone markers in the COC group were analyzed using a generalized estimating equation (GEE). Pearson correlation analysis was performed in the COC group to determine the relationships between basal BMD and percentage change at 12 months and between baseline Z-score and differences measured at 12 months. Two-tailed P value less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

The clinical characteristics of study subjects are summarized in Table 1. Compared with the control group, women in the COC group were significantly younger and had shorter duration of exercise and more frequent history of fall. After controlling for age, body mass index (BMI), duration of exercise, and history of fall, regression analysis revealed no significant difference in bone density Z-scores at LS and TH between the two groups (Table 1). However, the distribution of bone turnover markers differed significantly, as examined by chi-square test adjusted for age, BMI, duration of exercise, and history of fall. Progestins used in COC preparations are shown in Table 2. Levonorgestrel and desogestrel were the major progestin component.

Bone mineral density

Figure 1A displays mean percentage changes from baseline in BMD. In the control group, BMD significantly decreased at both LS (–1.6%) and TH (–0.7%), according to one-sample t-test. In the COC group, however, spine BMD at 12 months was significantly increased (2.2%). Multivariable linear regression adjusted for age, BMI, duration of exercise, and history of fall disclosed a significant difference at 12 months between

Table 1. Baseline characteristics, bone density Z-scores, and bone turnover markers

Variable	Control (n = 55)	COCs (n = 55)	P value
Age (y)	48.4 ± 2.9	46.8 ± 3.4	0.007
Age at menarche (y)	14.4 ± 1.3	14.5 ± 1.7	0.888
Parity	2.0 ± 0.6	1.7 ± 0.9	0.068
BMI (kg/m²)	22.4 ± 3.1	22.4 ± 2.5	0.943
FSH (mIU/mL)	13.7 ± 10.5	13.5 ± 12.2	0.660
Estradiol (pg/mL)	120.4 ± 105.6	119.3 ± 117.6	0.964
Alcohol		n = 43	
No	42 (76.4)	35 (81.4)	0.547
Yes	13 (23.6)	8 (18.6)	
Duration of exercise (h/wk)	2.7 ± 1.8	2.0 ± 1.4	0.046
Activity		n = 43	
Below average	3 (5.5)	2 (4.7)	0.092
Average	45 (81.8)	28 (65.1)	
Above average	7 (12.7)	13 (30.2)	
History of fall		n = 43	
No	55 (100)	39 (90.7)	0.034
Yes	0 (0)	4 (9.3)	
Bone density Z-score			
Lumbar spine, 2–4	0.855 ± 1.458	0.202 ± 1.074	0.246[a]
Total hip	0.124 ± 0.964	0.148 ± 0.835	0.307[a]
Bone turnover marker			
Resorption	n = 54	n = 48	0.012[b]
Increased	3 (5.6)	9 (18.8)	
Normal	46 (85.2)	32 (66.7)	
Decreased	5 (9.3)	7 (14.6)	
Formation	n = 54	n = 28	0.033[b]
Increased	1 (1.9)	8 (28.6)	
Normal	53 (98.1)	14 (50.0)	
Decreased	0 (0)	6 (21.4)	

Data are presented as mean ± standard deviation or number (%).
Unadjusted P values in baseline characteristics according to Wilcoxon rank sum test, Fisher's exact test, chi-square test, or two-sample t-test, as appropriate.
COCs: combination oral contraceptives, BMI: body mass index, FSH: follicle-stimulating hormone.
[a]P value by linear regression adjusted for age, BMI, duration of exercise, and history of fall.
[b]P value by chi-square test controlling for age, BMI, duration of exercise, and history of fall.

the two groups (P = 0.013). Further, there was a significant negative correlation (r = −0.275, P = 0.042) between baseline BMD at LS and percentage change at 12 months in the COC group by Pearson's correlation test (Fig. 1B). Hip BMD did not change during the study in the COC group. No group difference was observed in TH BMD at 12 months.

Z-score for bone density (Fig. 2A) did not change

Table 2. Progestins contained in low-dose combination oral contraceptives (COCs)

Progestin	Number
Levonorgestrel	30
Desogestrel	31
Gestodene	6
Drospirenone	5
Dienogest	3

Duplicate counts were recorded if COC preparation changed during the study.

at either LS or TH in the control group. In contrast, GEE demonstrated that Z-score at LS was significantly increased with time in the COC groups (P = 0.008). Compared with baseline, the lumbar Z-score also showed significant increase at both six and 12 months. Moreover, Pearson's correlation analysis (Fig. 2B) showed a significant negative correlation between spine Z-score at baseline and change at 12 months in the COC group (r = −0.396, P = 0.003). Z-score at TH was not altered in the COC group and there was no difference at either LS or TS between the two groups at 12 months.

Biochemical marker of bone turnover

In the control group, percentage changes in both BR (Fig. 3A) and BF (Fig. 3B) makers significantly increased at 12 months. Conversely, no significant time trend in bone markers was observed in the COC group. Time trends, however, showed different patterns for the two markers: BR markers did not change over time, whereas BF markers at three months declined signifi-
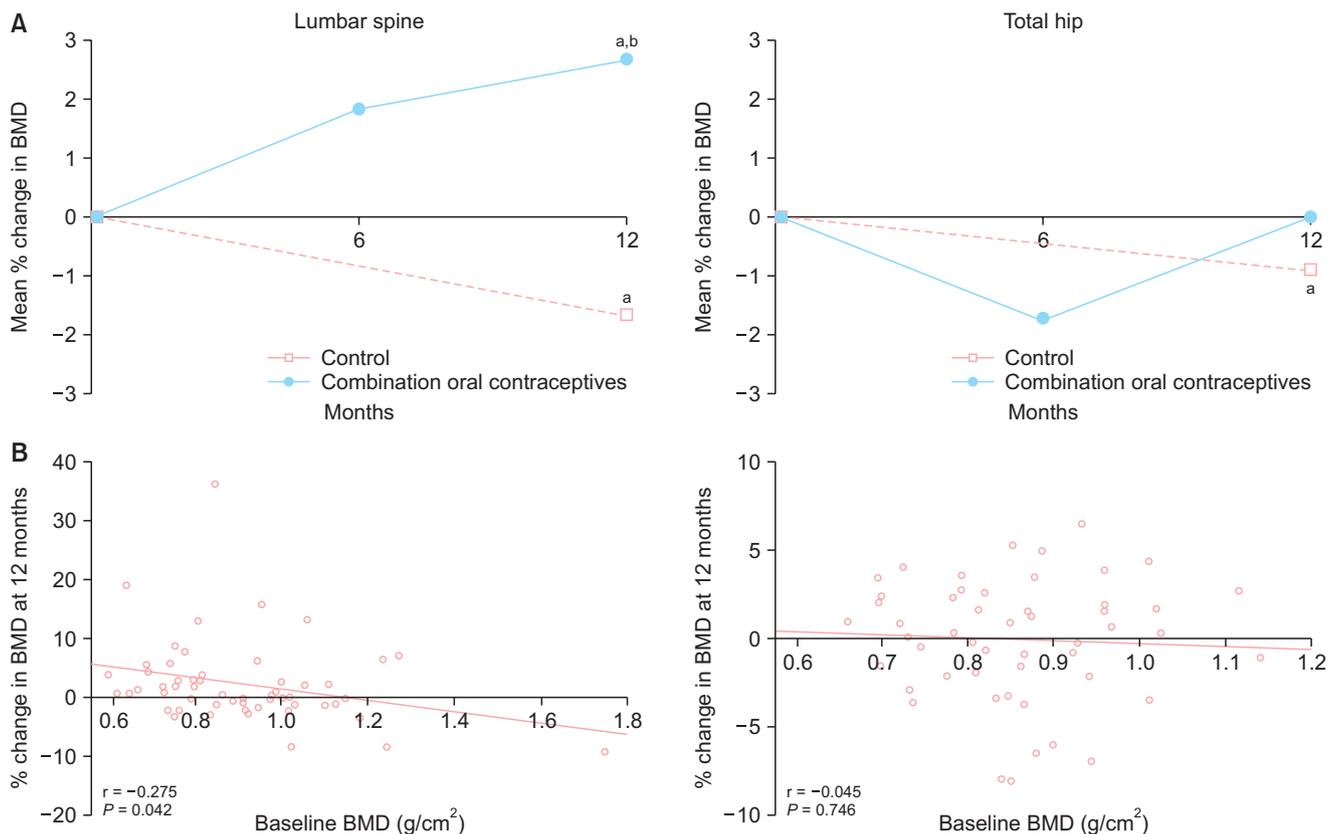
Fig. 1. (A) Mean percentage change in bone mineral density (BMD) during the study period. (B) Correlation analysis between basal BMD and percentage change from baseline at 12 months. [a]P < 0.05 versus corresponding basal values by one-sample t-test; [b]P < 0.05 versus control by linear regression after controlling for age, body mass index, duration of exercise, and history of fall.
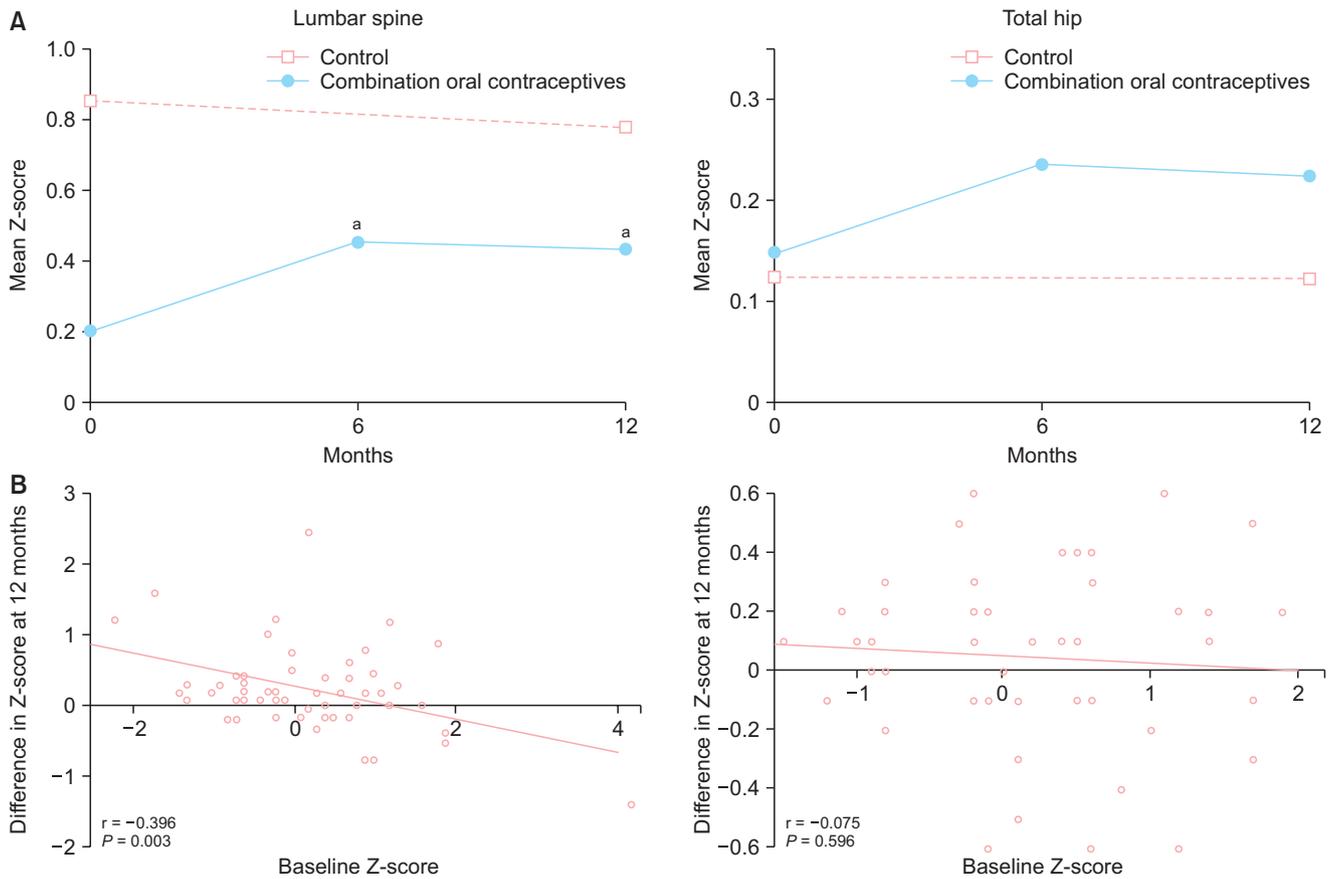
Fig. 2. (A) Mean Z-score for bone density during the study period. (B) Correlation analysis between basal Z-score and change from baseline at 12 months. There was a significant time trend in Z-score at the lumbar spine in the combination oral contraceptive group according to the generalized estimating equation ($P = 0.008$). [a]$P < 0.05$ versus baseline values.
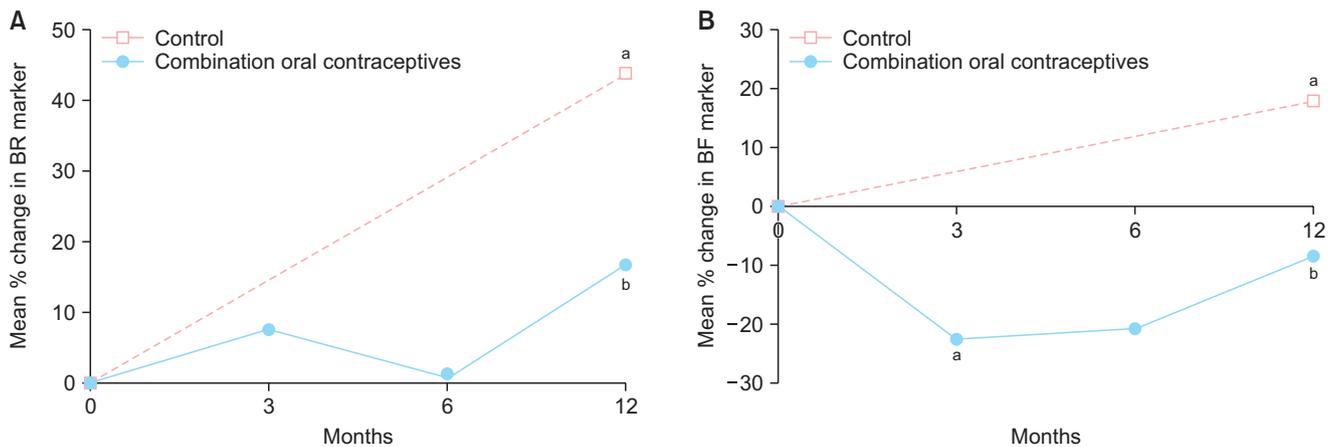
Fig. 3. Mean percentage change in bone turnover markers during the study period. (A) Bone resorption (BR) markers and (B) bone formation (BF) markers. [a]$P < 0.05$ versus corresponding basal values by one-sample t-test; [b]$P < 0.05$ versus control by linear regression adjusted for age, body mass index, duration of exercise, and history of fall.

cantly with COC. After controlling for age, BMI, duration of exercise, and history of fall, linear regression showed significant differences at 12 months between the two groups in both BR ($P < 0.001$) and BF ($P = 0.005$) markers.

DISCUSSTION

Different patterns of bone loss had been reported in women between the midradius with 99% cortical in bone composition and LS with >70% trabecular [3]. Because areal BMD measured by DXA cannot differentiate trabecular from cortical bone, volumetric BMD was assessed by quantitative computed tomography to delineate compartment-specific bone loss [8,9]. Cortical bone at the distal radius remained stable until midlife, but slightly decreased over age 50, just before menopause. In marked contrast, decline in trabecular bone at the vertebral body was observed in young adulthood and was accelerated at perimenopause.

In the SWAN study [5], BMD was measured annually using DXA at the femoral neck (FN) with 75% cortical as well as LS. Apparent BMD decreases were observed at one year before FMP at both LS and FN. Precipitous bone losses continued through the two years after FMP, and the rate of loss decelerated thereafter. Patterns of bone loss were similar at the two sites, but magnitude of early decline was greater at LS than FN, probably due to the higher proportion of metabolically active trabecular bone. In addition, serum FSH level was associated with bone loss rate at both sites in the transmenopausal phase [10].

Various kinds of menstrual disturbance associated with shortened follicular phase, luteal phase defect, or anovulation occur after reproductive age [11]. FSH increases during early follicular phase are the first laboratory sign of perimenopause. Typical changes in ovarian hormones include the progesterone insufficiency due to the ovulatory dysfunctions mentioned above. Of interest, serum estradiol level fluctuates widely but does not decrease until the final stage of the menopausal transition, about one year prior to the FMP.

Prospective studies further addressed changes in bone metabolism during the menopause transition. When measured during mid luteal phase of the menstrual cycle, at the peak of progesterone level, OC began to rise about two years before FMP, predating the decline in estradiol [12]. Moreover, markers of BF but not BR were correlated with perimenopausal loss of trabecular bone [13]. In addition, cyclic therapy with medroxyprogesterone acetate increased spinal BMD in younger women (mean age, 32 years) with ovulatory disturbance [14] and progesterone stimulates osteoblast proliferation in vitro [15]. These findings suggest that progesterone insufficiency plays a causative role in trabecular bone loss across the menopausal transition by adversely affecting BF more than BR. Cortical bone loss seems to be closely tied to estrogen deficiency. The reason for the cortical bone loss observed just before menopause is not known. Although not severe enough to result in deficiency, abrupt decreases in estrogen might be related.

The current study showed that BMD in the control group was significantly decreased after one year at both LS and TH compared with corresponding baseline measurements. Greater bone loss was found at LS compared with TH, in line with the SWAN reports [5]. In addition, parallel increases in biochemical markers for both BR and BF were indicative of activated bone turnover. Greater elevation of BR than BF suggests remodeling imbalance. These findings support the hypothesis that significant loss of axial bones associated with increased bone turnover occurs before menopause in Korean women.

COCs provide progestin as well as potent estrogen and stabilize ovarian hormones by inhibiting follicular activity. Therefore, COCs have been proposed to reverse BMD and metabolic changes at perimenopause. Indeed, we found that COCs increased BMD at LS and decreased bone loss at TH measured with DXA, probably by preventing decrease in cortical as well as trabecular bones. COCs also suppressed bone turnover. These findings are consistent with those of previous prospective Italian studies [16-18], in which low-dose COCs increased LS BMD after one year of therapy [18] and increased FN BMD after two years [17]. They also reported that progestin type did not influence increase in LS BMD with COCs [18]. In the present study, several kinds of progestin were used, and COC preparation changed during follow-up if necessary. Hence, statistical analysis did not consider progestin. Interestingly, the present study also observed that COCs did not alter BR markers, compared with baseline. In contrast, COCs significantly decreased BF markers at three months of treatment. Mechanistically, COCs are assumed to exert greater impact on BF mainly mediated by progestin, the dominant hormone component of COC.

At baseline, Z-score at LS was higher (but not significantly) and increases in bone turnover markers were apparent in the COC group, which included individuals complaining of vasomotor symptoms. These findings are in accordance with those of the SWAN study, which reported an association between vasomotor symptoms

and lower BMD [19]. Erratic changes in estradiol level during the menopausal transition might be attributable to changes in BMD and metabolism as well as vasomotor symptoms.

Ethnic differences were also noted in the SWAN study with greater bone loss observed among women of East Asian ancestry [5]. Oral contraceptive use in women of reproductive age is low in Asia (estimated prevalence < 10%, contraceptive use by method 2019, United Nations). Furthermore, COC use after the reproductive years is expected to be rare. To our knowledge, this is the first study from Asia to assess the effects of COCs on BMD and metabolism in perimenopausal women. Number of women who took COC was greater in the present study (n = 55) than the Italian studies (n < 30) [16-18].

Low spine BMD in premenopausal or early perimenopausal women is associated with higher fracture risk across the menopausal transition [20]. Currently, no osteoporosis medicine is approved in premenopausal women. Recent retrospective studies [6,21] reported that oral contraceptive use did not reduce fracture risk around menopause. Because oral contraceptive use in the late reproductive years has not been popular, the numbers of recent and current users with long-term use was very low in these studies. Of note, the fracture risk in younger women aged 18 to 35 years was significantly reduced by COC in a duration-dependent manner [21]. Due to these limitations, we cannot draw definitive conclusions regarding the effect of COC on fracture risk in perimenopausal women. This study showed significant negative correlations between baseline BMD and Z-score at LS and corresponding changes with COC at 12 months. These results indicate that COCs might be a therapeutic option in perimenopausal women with lower spine BMD.

This study is limited by its retrospective design and short duration of COC therapy. Moreover, several kinds of progestin and assay method for bone turnover were used because of the long study period. Further studies are warranted to elucidate the effects of COC on BMD, metabolism, and fracture in women around menopause.

In conclusion, BMD decreases at both LS and TH associated with activated bone turnover are evident during the menopausal transition in Korean women. COCs might prevent bone loss and suppress bone turnover in perimenopausal Korean women. Significant increase in BMD at LS and decreases in BF makers suggest under-

lying mechanisms of greater impact on BF than BR in COCs.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Cauley JA. Estrogen and bone health in men and women. Steroids 2015; 99(Pt A): 11-5.

2. Jackson RD, Wactawski-Wende J, LaCroix AZ, Pettinger M, Yood RA, Watts NB, et al. Effects of conjugated equine estrogen on risk of fractures and BMD in postmenopausal women with hysterectomy: results from the women's health initiative randomized trial. J Bone Miner Res 2006; 21: 817-28.

3. Riggs BL, Wahner HW, Melton LJ 3rd, Richelson LS, Judd HL, Offord KP. Rates of bone loss in the appendicular and axial skeletons of women. Evidence of substantial vertebral bone loss before menopause. J Clin Invest 1986; 77: 1487-91.

4. Prior JC. Progesterone for the prevention and treatment of osteoporosis in women. Climacteric 2018; 21: 366-74.

5. Greendale GA, Sowers M, Han W, Huang MH, Finkelstein JS, Crandall CJ, et al. Bone mineral density loss in relation to the final menstrual period in a multiethnic cohort: results from the Study of Women's Health Across the Nation (SWAN). J Bone Miner Res 2012; 27: 111-8.

6. Scholes D, LaCroix AZ, Hubbard RA, Ichikawa LE, Spangler L, Operskalski BH, et al. Oral contraceptive use and fracture risk around the menopausal transition. Menopause 2016; 23: 166-74.

7. Liu SL, Lebrun CM. Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. Br J Sports Med 2006; 40: 11-24.

8. Riggs BL, Melton LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, et al. Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 2004; 19: 1945-54.

9. Riggs BL, Melton LJ 3rd, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, et al. A population-based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss

in young adult women and men. J Bone Miner Res 2008; 23: 205-14.

10. Crandall CJ, Tseng CH, Karlamangla AS, Finkelstein JS, Randolph JF Jr, Thurston RC, et al. Serum sex steroid levels and longitudinal changes in bone density in relation to the final menstrual period. J Clin Endocrinol Metab 2013; 98: E654-63.

11. Menopause transition and menopause hormone therapy. In: Taylor HS, Pal L, Seli E, editors. Speroff's clinical gynecologic endocrinology and infertility. 9th ed. Philadelphia: Wolters Kluwer; 2020. pp. 580-751.

12. Recker R, Lappe J, Davies K, Heaney R. Characterization of perimenopausal bone loss: a prospective study. J Bone Miner Res 2000; 15: 1965-73.

13. Seifert-Klauss V, Fillenberg S, Schneider H, Luppa P, Mueller D, Kiechle M. Bone loss in premenopausal, perimenopausal and postmenopausal women: results of a prospective observational study over 9 years. Climacteric 2012; 15: 433-40.

14. Prior JC, Vigna YM, Barr SI, Rexworthy C, Lentle BC. Cyclic medroxyprogesterone treatment increases bone density: a controlled trial in active women with menstrual cycle disturbances. Am J Med 1994; 96: 521-30.

15. Tremollieres FA, Strong DD, Baylink DJ, Mohan S. Progesterone and promegestone stimulate human bone cell proliferation and insulin-like growth factor-2 production. Acta Endocrinol (Copenh) 1992; 126: 329-37.

16. 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.

17. Gambacciani M, Ciaponi M, Cappagli B, Benussi C, Genazzani AR. Longitudinal evaluation of perimenopausal femoral bone loss: effects of a low-dose oral contraceptive preparation on bone mineral density and metabolism. Osteoporos Int 2000; 11: 544-8.

18. Gambacciani M, Cappagli B, Lazzarini V, Ciaponi M, Fruzzetti F, Genazzani AR. Longitudinal evaluation of perimenopausal bone loss: effects of different low dose oral contraceptive preparations on bone mineral density. Maturitas 2006; 54: 176-80.

19. Crandall CJ, Zheng Y, Crawford SL, Thurston RC, Gold EB, Johnston JM, et al. Presence of vasomotor symptoms is associated with lower bone mineral density: a longitudinal analysis. Menopause 2009; 16: 239-46.

20. Cauley JA, Danielson ME, Greendale GA, Finkelstein JS, Chang YF, Lo JC, et al. Bone resorption and fracture across the menopausal transition: the Study of Women's Health Across the Nation. Menopause 2012; 19: 1200-7. Erratum in: Menopause 2013; 20: 115.

21. Dombrowski S, Jacob L, Hadji P, Kostev K. Oral contraceptive use and fracture risk-a retrospective study of 12,970 women in the UK. Osteoporos Int 2017; 28: 2349-55.