Decreased Urinary Calcium Loss and Lower Bone Turnover in Young Oral Contraceptive Users

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The effect of ethinyl estradiol–containing oral contraceptives (OCs) on bone health is still not completely understood. This study was therefore performed to investigate the effect of OC use on biochemical parameters of calcium (Ca) and bone metabolism in young women. Twelve OC users ([OC+ group] age, 24.8 ± 0.6 years) and 19 eumenorrheic nonusers ([OC− group] age, 25.5 ± 0.8 years) were studied. Three individual urine samples (fasting 2-hour and 24-hour specimen) and 3 blood samples collected at intervals of 28 days were pooled for data evaluation. Energy, nutrient intake (7-day food record), the body mass index, and serum 25-hydroxyvitamin D levels of the 2 groups were comparable. Serum levels of estradiol (E_2) and sex hormone–binding globulin (SHBG) in the 2 groups mirrored the use and nonuse of OCs. Fasting 2-hour renal Ca excretion was markedly lower in the OC+ group compared with the OC− group (80.2 ± 14.7 *v* 185.1 ± 18.8 μmol/mmol creatinine [Cr], $P < .001$), indicating a decrease in the urinary loss of endogenous, bone-derived Ca. Moreover, 24-hour renal Ca excretion was reduced in the OC+ group (3.65 ± 1.46 *v* 5.03 ± 1.90 mmol/d, respectively, $P < .01$). In addition, serum Ca levels were lower in the OC+ group versus the OC− group (2.19 ± 0.07 *v* 2.29 ± 0.02 mmol/L; $P < .05$). The OC+ group had lower serum levels of carboxy-terminal propeptide of type I procollagen ([PICP] a biomarker of bone formation) compared with the OC− group (78.3 ± 3.6 ng/mL in the OC+ group *v* 96.9 ± 5.5 ng/mL in the OC− group, $P < .001$) and lower renal hydroxyproline (OHPr) excretion (a biomarker of bone resorption, 8.3 ± 1.0 *v* 11.3 ± 1.0 μmol/mmol, Cr, $P < .001$). In summary, OCs reduce urinary Ca loss and slow bone turnover in young women. The results may help to explain the OC effect on bone mass in young women.

THE BONE-PRESERVING EFFECT of endogenous estradiol (E_2) is widely accepted. In postmenopausal E_2-depleted women, hormone replacement therapy reduces bone resorption processes, stops bone loss, and decreases the risk of osteoporotic fracture.[1-4] In amenorrheic E_2-depleted young women, enhanced bone turnover paralleled by a loss of bone mass occurs,[5] whereas correction of an estrogen deficiency can increase spinal bone mineral density.[6] Even in eumenorrheic women, the high E_2 levels at midcycle are associated with a reduction in bone resorption processes compared with the low E_2 levels in the early follicular phase.[7]

In young women, there is widespread use of ethinyl estradiol–containing oral contraceptives (OCs). The mechanism of action of these agents includes suppression of the peak levels of E_2 and luteinizing hormone at midcycle, resulting in a constant low E_2 level throughout the menstrual cycle.

The effect of OCs on bone health is still inconclusive. Two retrospective studies indicate that OC use can increase bone mass,[8,9] while other studies found no effect of OC use on bone mineral density.[10-13] In a prospective investigation, OC administration to young women with long-standing amenorrhea resulted in a significant increase in spinal bone mineral density.[14] However, in healthy young OC users, bone mass remained unchanged after an observation period of 1 year[15] and 5 years,[16] while a group of nonusers had a significant increase in bone mass.[16]

The effect of OCs on calcium (Ca) and bone metabolism can be assessed sensitively by using different biochemical parameters. Reliable markers such as serum levels of carboxy-terminal propeptide of type I procollagen (PICP) and renal hydroxyproline (OHPr) are available for the measurement of bone formation and resorption processes.[17,18] These parameters undergo a rapid turnover, and significant changes can be observed within a few days.[17,19] The present study thus aimed to evaluate the effect of OC use on different indices of skeletal metabolism in young women.

SUBJECTS AND METHODS

Subjects

Thirty-one young women were recruited. Inclusion criteria for participation were a minimum of 3 eumenorrheic cycles (cycle length of 25 to 35 days with a duration of 3 to 4 days, by questionnaire) and a body mass index between 18 and 25 kg/m^2 (measured without shoes and with indoor clothing before the study began). Twelve women who used OCs comprised the OC+ group. The ethinyl estradiol dose of OC preparations was 20 to 40 μg. Eleven of the 12 OCs were monophasic, and 1 was a triphasic preparation. OC use included the intake of a gestagen formulation of different types and doses. The 19 OC nonusers comprised the OC− group. Except for OCs, none of the subjects were on medications known to influence Ca metabolism or had chronic diseases affecting bone metabolism. Subject characteristics are listed in Table 1. All subjects provided written informed consent for the examinations, which were in accordance with the Helsinki Declaration.

Study Protocol

The study period was 8 weeks. The first examination of the subjects was performed randomly with regard to the time of the menstrual cycle. Two additional examinations were then performed 28 days and 56 days later according to the rhythm of OC intake and the mean length of the menstrual cycle. Twenty-four-hour urine specimens were collected before the study began (day 0), on study day 28, and on study day 56. Samples were collected from 7 AM until 7 AM of the following day. On that following morning (days 1, 29, and 57), blood was collected from the antecubital vein after an overnight fast (serum monovettes). A 2-hour fasting urine sample (second spontaneous urine) was collected at 9 AM (before breakfast). Aliquots of the samples were frozen consecutively at −20°C until analysis.

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Table 1. Characteristics (mean ± SEM) of the OC+ and OC−

Characteristic	OC− Group (n = 19)	OC+ Group (n = 12)
Age (yr)	25.5 ± 0.8	24.8 ± 0.6
Height (cm)	168 ± 1.7	168 ± 1.6
Weight (kg)	58.5 ± 1.7	59.0 ± 1.9
Body mass index (kg/m²)	20.6 ± 0.3	20.9 ± 0.6
No. of smokers (>5 cigarettes/d)	1	3

Each subject completed two 7-day food records[20] on study days 8 to 14 and 35 to 41, respectively.

Biochemical Analysis

All samples were measured in duplicate. Serum levels of E_2, sex hormone–binding globulin (SHBG), insulin, and PICP were measured by enzyme-linked immunosorbent assays supplied by IBL (Hamburg, Germany; E_2, SHBG, and insulin) and by Biermann (Bad Nauheim Germany; PICP), respectively. The cross-reactivity of the E_2 assay with ethinyl estradiol was less than 0.01%. The coefficient of variation (CV) for E_2, SHBG, and insulin was 7.2%, 7.6%, and 5.7%. Serum 25-hydroxyvitamin D was analyzed using a radioimmunoassay[21] with a CV of 6.7%. Alkaline phosphatase (AP) activity was determined using a kinetic test[22] with an imprecision of 4%. The total serum cholesterol level was measured with a colorimetric test kit supplied by Boehringer (Mannheim, Germany) with an imprecision of 3.6%. The Ca level was measured by atomic absorption spectrometry (model 420; Perkin Elmer, Ueberlingen, Germany)[23] with a CV of 2.2%. Fasting renal hydroxyproline (OHPr) was determined by a colorimetric reaction with dimethylaminobenzaldehyde after chloramine-T oxidation and after 16-hour resin-catalyzed hydrolysis of peptide-bound OHPr.[24] The CV was 6.2%. The renal creatinine (Cr) level was measured by the Jaffé reaction. Results for renal Ca and OHPr are expressed in relation to renal Cr excretion.

Statistics

Statistical analysis was performed with the package SPSS/PC+ (SPSS, Chicago, IL). For evaluation, individual data for Ca and bone parameters of the 3 sampling points were pooled. The data were tested for homogeneity of variance using the Kolmogorov-Smirnov test. For comparative analyses, the 2-tailed Student's *t* test for unpaired values was used. *P* values less than .05 were considered significant. Data are expressed as the mean ± SEM.

RESULTS

Characteristics of the Study Groups

Energy, macronutrient, and dietary fiber intake were comparable in both groups, with the exception of a lower carbohydrate intake in the OC− group (Table 2). The OC+ group had markedly lower serum E_2 and higher serum SHBG levels compared with the OC− group during all 3 examinations (Table 3). Serum insulin levels were significantly higher and serum cholesterol tended to be higher in the OC+ group compared with the OC− group (Table 4).

Table 2. Energy and Nutrient Intake of OC+ and OC− Groups

Variable	OC− Group (n = 19)	OC+ Group (n = 12)
Energy (kJ/d)	7,444 ± 306	8,210 ± 435
Carbohydrate (g/d)	189 ± 7.5	218 ± 12*
Protein (g/d)	63 ± 2	70 ± 5
Fat (g/d)	75 ± 4	79 ± 6
Ca (mg/d)	1,032 ± 72	1,172 ± 105
Vitamin D (μg/d)	1.9 ± 0.2	1.9 ± 0.2
Fiber (g/d)	23.6 ± 1.2	25.0 ± 2.2

NOTE. Results are the mean ± SEM of two 7-day recording periods.
*$P < .05$.

Table 3. Serum E_2 and SHBG Levels in OC+ and OC− Groups (mean ± SEM)

Parameter	OC− Group (n = 19)	OC+ Group (n = 12)
E_2 (pmol/L)		
Day 1	359 ± 152	55 ± 21
Day 29	412 ± 157	69 ± 41
Day 57	490 ± 117	69 ± 55
SHBG (nmol/L)		
Day 1	77 ± 10	203 ± 19
Day 29	77 ± 13	190 ± 17
Day 57	74 ± 11	194 ± 21

Parameters of Ca and Bone Metabolism

Dietary Ca intake was similar in both groups, covering the actual recommendations.[25] Vitamin D intake and serum 25-hydroxyvitamin D status did not differ between the 2 groups. However, 2-hour fasting renal Ca excretion in the OC+ group was only 43% of that in the OC− group. Moreover, 24-hour renal Ca excretion was lower in the OC+ group (Fig 1). The serum Ca concentration was also lower in the OC+ group versus OC− group (Table 4).

The magnitude of the decrease in 2-hour fasting renal Ca excretion in the OC+ group compared with OC− subjects was similar to the magnitude of the decrease in 24-hour renal Ca excretion (Fig 1). The excess daily Ca excretion in the OC− group compared with the OC+ group was 1.38 mmol (55.2 mg; Table 4).

The OC− group had a 19.2% higher PICP concentration and a 26.6% higher OHPr excretion rate (Fig 2). AP levels were comparable in both groups (Table 4).

DISCUSSION

This study demonstrates a lower 2-hour fasting renal Ca excretion and a lower bone turnover of young OC users compared with E_2-repleted age-matched nonusers. The markedly lower serum E_2 and the higher serum SHBG levels of the OC users compared with the nonusers (Table 3) are in line with the effects of OCs on the endogenous sex hormone status.[26,27]

Table 4. Serum Insulin and Cholesterol and Biochemical Parameters of Ca and Bone Metabolism in OC+ and OC− Groups (mean ± SEM)

Parameter	OC− Group*	OC+ Group†
Insulin (μUI/L)	7.78 ± 0.40	9.15 ± 0.48§
Cholesterol (mmol/L)	5.28 ± 0.11	5.66 ± 0.17‡
25-Hydroxyvitamin D (nmol/L)	56.25 ± 7.2	63.0 ± 12.0
Serum Ca (mmol/L)	2.29 ± 0.02	2.19 ± 0.07§
24 h renal Ca (mmol/d)	5.03 ± 1.90	3.65 ± 1.46‖
Serum AP (U/L)	80.0 ± 2.7	83.7 ± 3.0

*Fifty-seven samples from 19 subjects.
†Thirty-six samples from 12 subjects.
‡$P < .1$.
§$P < .05$.
‖$P < .01$.

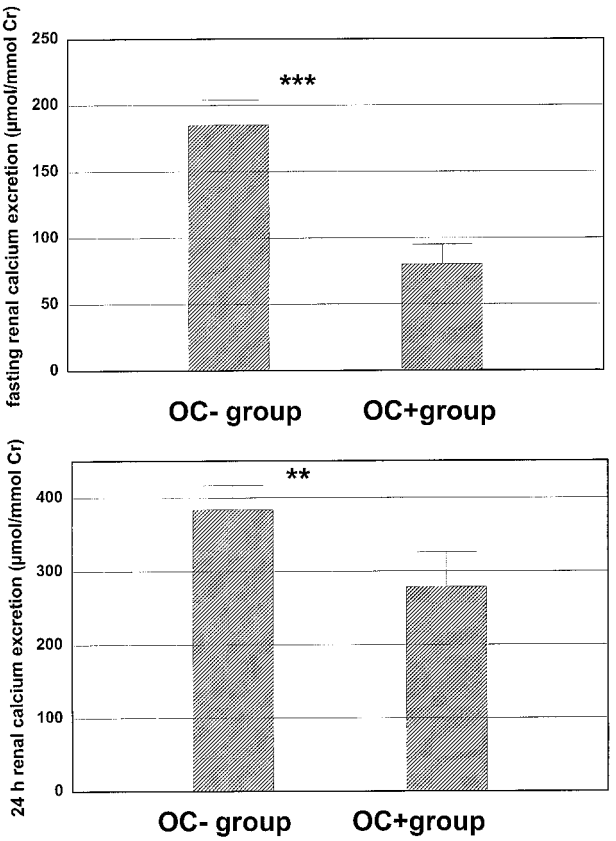

Fig 1. Fasting renal calcium excretion and 24-hour renal calcium excretion (mean ± SEM) in OC+ and OC− groups. Data are based on 36 samples from 12 OC+ and 57 samples from 19 OC− subjects. **$P < .01$, ***$P < .001$.

Since nutrient intake and vitamin D status were comparable between the 2 groups (Tables 2 and 4), the data strongly indicate a direct OC effect on Ca metabolism and bone turnover.

The 2-hour fasting renal Ca excretion reflects obligatory bone-derived Ca loss, whereas 24-hour renal Ca excretion is the sum of both dietary-absorbed Ca and endogenous bone-derived Ca.[18] The similar difference in 2-hour renal Ca excretion and 24-hour renal Ca excretion in the OC+ group compared with the OC− group (Fig 1) therefore indicates a reduced Ca mobilization from the bone without affecting intestinal Ca absorption. The low urinary Ca loss might be explained by the slightly lower serum Ca levels of the OC+ group compared with the OC− group (Table 2), thereby resulting in a decrease of the filtered Ca load.[28] The exact mechanism of these alterations in Ca metabolism is still unclear, since ethinyl estradiol has no effect on circulating parathyroid hormone and free calcitriol levels.[29]

In a study with postmenopausal women,[30] Ca balance became less negative after administration of 25 µg ethinyl estradiol, mostly due to a lower fasting renal Ca excretion. However, fasting renal Ca excretion in the postmenopausal group was approximately 230% higher compared with the value in the OC− group of the present study. Nevertheless, the lower daily renal Ca excretion of 55 mg in the young OC users compared with nonusers (Table 2) may beneficially influence bone mineral content if a similar improvement of Ca balance is induced.

The present data of low serum PICP and renal OHPr concentrations in OC users compared with OC nonusers (Table 2) suggest a depression of bone formation and resorption processes. A decrease of these two biomarkers of osteoblastic and osteoclastic activity has also been observed in postmenopausal women after conventional hormone replacement therapy.[31] Moreover, lower bone turnover has been found in premenopausal OC users in the fourth and fifth decade of life[32] compared with age-matched controls.

Changes in osteoblastic and osteoclastic activity are the basis for building, maintaining, or losing bone matrix. The suppressive effect of OC use on bone turnover might be positive in middle-aged and postmenopausal women. In this age group, bone resorption normally exceeds bone formation, resulting in a reduction of bone mineral content.[33] The suggestion of a positive effect of OCs in middle-aged women is supported by the observation that ethinyl estradiol decreases bone loss in perimenopausal and postmenopausal women.[34,35]

In young women, bone formation exceeds bone resorption.[36] Studies in young adult female monkeys treated with OCs have shown a reduction in several biomarkers of bone metabolism associated with a smaller gain in bone mineral content com-

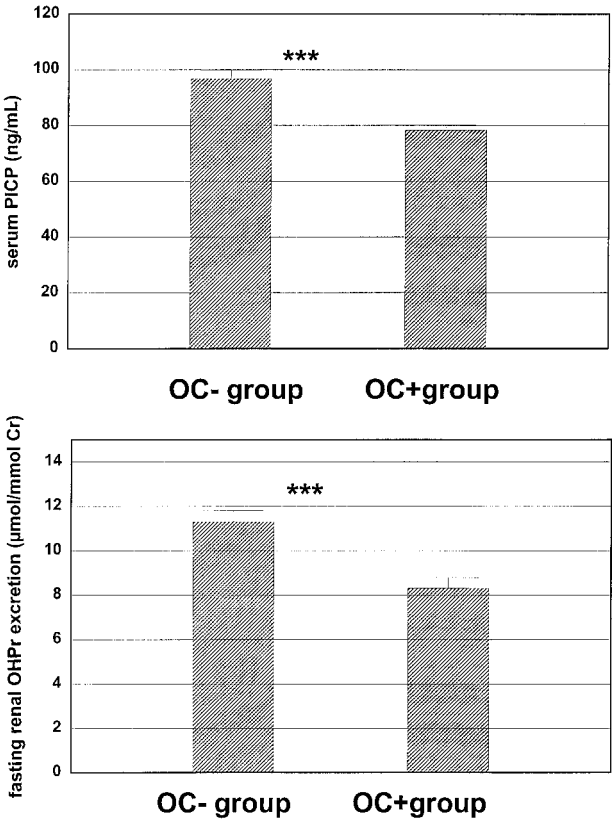

Fig 2. Serum PICP and fasting renal OHPr excretion (mean ± SEM) in OC+ and OC− groups. Data are based on 36 samples from 12 OC+ and 57 samples from 19 OC− subjects. ***$P < .001$.

pared with a control group.[37] Even in females in the third decade of life, some bone accumulation normally occurs.[38] The lower bone turnover of the OC users (Fig 2) might thus adversely affect bone mass accretion if the individual peak skeletal mass is not yet achieved. Such a mechanism might explain the lack of change in bone mass in a group of OC users aged 19 to 22 years during an observation period of 5 years while the bone mass of an age-matched group of nonusers significantly increased.[16]

The magnitude of the lower bone resorption in OC users compared with OC nonusers in the present study slightly exceeded the magnitude of the lower bone formation marker (Fig 2). However, the two markers may differ in their sensitivity to adequately mirror bone formation and resorption processes. Additionally, the imbalance between formation and resorption resulting in bone gain is likely small compared with the overall rate of human bone remodeling. It might also be that OC use increases endogenous fecal Ca loss more than it reduces urinary Ca loss such that Ca balance was lower in the OC users. For this reason, no final conclusions can be drawn about the net effect of OC use on bone mass. Nevertheless, the present data help to explain the mechanism of action of OCs. Comparative prospective studies are mandatory to elucidate the long-term effect of OC use on bone turnover and bone mass in eumenorrheic women from puberty to menopause. These investigations should consider the relative estrogenicity of the preparations, such as OCs based only on gestagen components, monophasic OCs with different doses of ethinyl estradiol (30 to 50 μg/d) and with mestranol, as well as diphasic and triphasic preparations with their different concentrations of ethinyl estradiol on different days of the menstrual cycle.

In summary, this study demonstrates that OC use decreases urinary Ca loss and slows the rate of bone turnover in young women.

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