Low-dose estrogen combined oral contraceptives may negatively influence physiological bone mineral density acquisition during adolescence

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Abstract

Background: The aim was to evaluate changes of bone mineral density (BMD) and markers of bone turnover in healthy adolescents, and in adolescent users of combined oral contraceptives (COCs) with different ethinylestradiol (EE) contents.

Methods: In this prospective crossover study, 56 healthy females (15–19.5 years) with desire to use hormonal contraception were randomized to COC with either 30 or 15 µg of EE in crossover design of 9-month intervention each in reverse order. Nonusers of the same age (n = 28) served as controls. BMD at lumbar spine (LS), total femur, femoral neck, distal radius, and total body, and serum markers (N-propeptide of type I procollagen, and type I collagen C-telopeptide) were measured at baseline and after 9 and 18 months.

Results: In COC nonusers, BMD significantly increased at LS and radius, while markers decreased. In COC users, BMD did not increase, with the exception of LS BMD in the 30 µg COC group (P < 0.05). In the crossover design, a difference between the low- and very low-dose COC users was found in LS BMD changes (P < 0.05), where increase in BMD was more impaired in the 15 µg COC users. The skeletal effects of COC remained significant after adjustments for age and smoking status. Markers declined faster in COC users during the first period, while they remained stable or even increased during the second 9 months.

Conclusion: Physiological acquisition of LS BMD during adolescent age may be prevented by use of COC, especially those containing very low dose of EE.

Introduction

Steroid hormones – both exogenous and endogenous – profoundly influence bone metabolism. It is well recognized that hypo-estrogenic states, such as menopause or a premature ovarian failure, lead to bone mineral density (BMD) decrease (1) and, vice versa. The BMD decrease in such situations can be prevented (2) or reversed by combined oral contraceptives (COCs) use (3, 4, 5). COC use does not seem to significantly influence BMD in fertile age (6, 7, 8, 9); however, a significant lumbar spine (LS) and proximal femur BMD increase was observed in perimenopausal COC users vs nonusers (10, 11).

Adolescent age is specific in regard to BMD development, showing a rapid physiological acquisition (12). Although most prospective studies seem to support the hypothesis that COC use could interfere with the achievement of peak bone mass in adolescent females (13, 14, 15), the results remain inconclusive, and several other studies did not confirm such tendency (16, 17, 18).

Limited data are available concerning the effect of COC with different estrogen doses on BMD and most of them did not find a significant difference (7, 19). However, all these studies included post-adolescent women, in whom physiological BMD growth is minimal.

The aim of our prospective study was to test our hypothesis that different estrogen dose (15 vs 30 µg ethinylestradiol (EE)/day) COCs may have different effects on BMD development in adolescent females. The crossover design, which was applied in COC users, allows for maximal sensitivity of therapeutic effect assessment in a limited number of subjects. At the same time, considering that each subject acts as her own control, interindividual variability is eliminated and the effect of many confounding factors that may significantly influence BMD development is minimized.

Subjects and methods

Subjects and study design

Adolescent females, aged 15–19.5 years, with BMI 20–27 kg/m², and a regular menstrual cycle
(25–35 days) were included in the study. The following criteria constituted a reason for exclusion from the study: recent or past COC use; systemic or chronic diseases including endocrine disorders; currently used medication that could influence bone metabolism or the reliability of hormonal contraception (e.g. corticosteroids, antiepileptics, thyroid hormones); drug use or smoking of more than ten cigarettes per day; daily dietary calcium intake below population average (<600 mg/day); endurance physical activity (competitive or peak sports); immobilization or invalidity; and COC contraindication (for the user group).

Volunteers were recruited amongst clients of an adolescent gynecological unit. Those who requested hormonal contraception were randomized for either 30 µg EE and 75 µg gestodene (Femoden; Schering, Berlin, Germany; further referred to as ‘30’), or 15 µg EE and 60 µg gestodene (Mireelle; Schering; further referred to as ‘15’). The randomization was accomplished using the method of random numbers generated by Statistical Software STATGRAPHICS Centurion (version 15; StatPoint, Inc., Herndon, VA, USA). After 9 months, the two COCs were switched among these two groups and used for another 9 months – the whole study duration was, therefore, 18 months. Those subjects who planned either not to be sexually active or to use a barrier method of contraception for the study duration were assigned to a control group of COC nonusers.

In all study candidates, detailed past medical history was obtained with special regard to possible exclusion criteria, including the risk factors for osteoporosis (e.g. low BMD, fracture in personal history, hip fractures in parents, smoking, alcohol or glucocorticoid use, rheumatoid arthritis, and factors leading to secondary osteoporosis), and blood samples were taken for biochemical examination. The subjects were instructed to maintain a normal hydration and to fast from 0000 h overnight. On the next day at 0800 h, samples of venous blood were collected from fasting subjects. Immediately after the sampling, the blood was centrifuged, routine biochemical analyses were performed with fresh samples; other aliquots were stored at −70 °C before being analyzed.

Daily dietary calcium intake was assessed using a short food frequency questionnaire (20). All enrolled subjects with daily intake of calcium below 1200 mg were supplemented with 500 mg elemental calcium; all subjects were also given 400 IU vitamin D3 daily supplements during the study.

BMD was measured at the beginning of the study, at 9 months, and after 18 months (the study termination); concurrently, blood samples were taken for the assessment of serum levels of estradiol (E2), 25-hydroxyvitamin D3 and biochemical markers of bone metabolic turnover. In the COC nonusers, blood levels of E2 were measured in the second half of the menstrual cycle, while in the COC users between pills no. 14 and 21 of the cycle.

The study protocol was approved by the hospital’s ethics committee. In all cases, an informed consent was signed by both the study subject and her parent.

Biochemical examination

The serum concentrations of E2 were assessed using electrochemiluminescence-based immunoanalysis (the Elecsys 1010 Analyzer; Roche Diagnostics GmbH), the Elecsys Estradiol II kit. The within-run imprecision was <3%, and between-run imprecision was <5% at concentrations between 18.4 and 15 781 pmol/l. The assay for E2 does not detect the EE concentrations. Serum concentration of 25-hydroxyvitamin D was assessed by enzyme immunoassay (OCTEIA, Immuno-diagnostic Systems, UK). The assay is specific to 25-hydroxyvitamin D3 (100%) and 25-hydroxyvitamin D2 (75%). The within-run imprecision was below 6%, and between-run imprecision was below 7% at concentrations between 39 and 72 nmol/l.

The serum concentration of intact aminoterminal propeptide of type I procollagen (PINP; bone formation marker) was assessed by RIA (Procollagen Intact PINP; Orion Diagnostica, Espoo, Finland). The assay is not sensitive to the small molecular weight degradation products of the propeptide. The within-run imprecision was below 5%, and between-run imprecision was below 7% at concentrations between 20 and 90 µg/l.

The serum concentration of type 1 collagen cross-linked C-telopeptide (βCTX1, bone resorption marker) was assessed using electrochemiluminescence-based immunoanalysis (the Elecsys 1010 Analyzer; Roche Diagnostics GmbH). The within-run imprecision of the βCTX1 was below 5% for samples >500 ng/l, below 7% for samples between 200 and 500 ng/l and below 10% for very low βCTX1 concentration samples. The between-run imprecision results were below 7% for samples >500 ng/l and below 9% for samples between 200 and 500 ng/l. The detection limit was <10 ng/l.

Bone densitometry

BMD measurements were performed at baseline, and after 9 and 18 months using the Hologic, Inc. QDR 4500A Bone Densitometer (Hologic, Inc., Waltham, MA, USA) at the LS L1–L4, total proximal femur, femoral neck and distal radius, and total body (total body mineral content, TB BMC). Normative values provided by Hologic, Inc. were used for the determination of Z-scores (comparison with an age- and gender-matched reference population). The short-term in vivo precision errors for LS, total femur, femoral neck, and distal radius BMD, and TB BMC were 0.7, 0.9, 1.9, 0.9, and 1.5% respectively; the long-term precision error
using the Hologic phantom was 0.31%. Daily scanning of a phantom showed the absence of machine drift during the study.

**Statistical analysis**

The treatment effect was evaluated using two ANOVA models. The statistical model was based on crossover design and included the within-subject effects of period and treatment, the between-subject effect of sequence, and the subject effect. The period effect evaluated the time changes from basal values (period 2, basal and period 3, basal) adjusted for the remaining effects. Similarly, the treatment effect evaluated the difference between two treatment modalities adjusted for the remaining effects. The sequence effect evaluated whether there was any difference between groups ‘30’ and ‘15’ (this effect should be insignificant). Finally, the subject effect evaluated the interindividual variance.

In addition to the aforementioned factors, we have tested the effect of age (age above/below median value), the effect of values of parameters at the beginning of the study (initial values above/below median), and effect of light smoking (smoker/nonsmoker).

The second ANOVA model tested the changes of selected markers in controls and sequences 15/30 and 30/15 (from the baseline period) in the periods 1 and 2. No additional covariates were included in this case.

In addition to the ANOVA models, we have applied the robust Wilcoxon signed-rank test for median difference to evaluate unadjusted differences between stages of the trial.

Besides the crossover ANOVA model, we have applied the ANCOVA model testing the same parameters as in the crossover ANOVA model with exception of continuous covariates, which were, however, not categorized in contrast to crossover ANOVA. Nevertheless, the results from the ANCOVA model were consistent with those from crossover ANOVA. The age relationships were more pronounced in the ANCOVA than in crossover ANOVA. For the crossover ANOVA model, we have also completed post hoc power analysis using the data obtained.

The differences between basal values were evaluated by robust Kruskal–Wallis ANOVA followed by Dunn’s multiple comparisons. The dichotomous and categorical data consisting of more than two levels were evaluated using Fisher’s exact test and $\chi^2$-test respectively. The relationship between BMD and $E_2$ levels was evaluated using Spearman’s correlations.

To obtain data symmetry and homoscedasticity, all continuous variables were transformed by a power transformation to maximum agreement with Gaussian distribution. The nonhomogeneities (at maximum two experimental points) were detected using residual analysis and regression diagnostics as described elsewhere (21, 22, 23). When illustrating the results in the figures, the predictions (mean values, regression curves) computed for transformed data were retransformed to the original scale.

**Results**

Altogether, 56 COC users (Fig. 1) and 28 controls were included in the study. Three subjects in the control group and 11 subjects in the COC group discontinued the study prematurely for the following reasons: in the COC group: irregular bleeding (2), pregnancy (1), incompliance with the protocol (8); in the control group: pregnancy (1), request for contraception (1), and incompliance with the protocol (1).

Characteristics of the three studied groups at baseline are shown in Table 1. At baseline, there were no significant differences in the age of menarche, weight, height, BMI, LS BMD, total proximal femur BMD, distal radius BMD, and TB BMC, serum $E_2$ concentration, or 25-hydroxyvitamin D3. Also, the groups did not differ significantly in the level of physical activity, average calcium intake, or in the risk factors for osteoporosis; no subjects had a history of femoral fractures in parents. The control group subjects were a year younger than those in the ‘30/15’ group (16.5 vs 17.5 and 17.2 years) and were less likely light smokers (<10 cigarettes/day; 7%) than the ‘30/15’ group (19%) and the ‘15/30’ group subjects (26%; $P=0.04$).

Percent changes of BMD and serum $E_2$ in both periods are summarized in Table 2. At the LS, in nonusers (controls) BMD increased significantly within the whole study period. Results presented as percent changes are shown in Fig. 2. In COC users during the initial 9-month period, those initially assigned to the ‘30’ showed a 1% increase in BMD, but BMD returned to baseline levels when they switched to the ‘15’ ($P<0.005$). Participants first receiving the ‘15’ showed virtually no increase in spinal BMD, while after

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**Figure 1** Flow chart of subjects randomized to COC containing 30 or 15 µg of EE.

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[Flow chart image]
switching to the higher dosage, spinal BMD accumulation paralleled that of controls. At the end of the study, the average LS BMD increase in healthy controls reached 2%, while in the COC users no significant BMD increase occurred.

At the femoral neck and total femur, no statistically significant changes were observed in percent changes of BMD between the COC users and controls. At the distal forearm, BMD significantly increased by time only in controls (Table 2).

The TB BMC did not accumulate as quickly in COC users as in the control group (Fig. 2). Those starting on ‘15’ and switching to ‘30’ ended up with about half the level of increase seen in the control group (1.7 vs 3.7%, P<0.05), while those taking the reverse order (30/15) had even less increase in the whole body bone mineral content (0.4%, P<0.05 vs both of the other groups).

Serum biochemical a markers of bone turnover declined in all three groups during the first 9 months of the study, and was significantly faster in COC users (Fig. 2). In the control group, serum PINP, a marker of bone formation, and βCTX1, a marker of bone resorption, decreased by 40 and 30% respectively. In COC users in the second period, there was no change in PINP among those switching from the ‘15’ to ‘30’, but a significant increase was observed in the group ‘30/15’.

The randomization was performed only in the COC users. Therefore, adjustments for crossover protocol are reported only in these two groups. The full ANOVA models for crossover protocol allows for inclusion of...
Bone in adolescent users of oral contraceptives

In healthy adolescent females in this study, a rapid and significant LS BMD and TB BMC increase with age was observed by 1 and 2% respectively during each 9 months. The age-dependent increase in BMD and BMC, as well as decline in serum PINP and βCTX1 observed in our control group (nonusers), is in agreement with the decline in bone biomarkers during late adolescence (24), and confirms that while the girls in the present study were sexually mature, they were not skeletally mature (25).

Discussion

In healthy adolescent females in this study, a rapid and significant LS BMD and TB BMC increase with age was observed by 1 and 2% respectively during each 9 months. The age-dependent increase in BMD and BMC, as well as decline in serum PINP and βCTX1 observed in our control group (nonusers), is in agreement with the decline in bone biomarkers during late adolescence (24), and confirms that while the girls in the present study were sexually mature, they were not skeletally mature (25).

Figure 2 (Top) Percent changes of BMD at the LS (left; sequence, subject, period and sequence × period, \( P = 0.571, 0.000, 0.000, \) and 0.001 respectively) and total body BMD (right; sequence, subject, period and sequence × period, \( P = 0.682, 0.000, 0.000, \) and 0.000 respectively) as evaluated using repeated measures ANOVA model. (Bottom) Percent changes in serum PINP (left; sequence, subject, period and sequence × period, \( P = 0.0218, 0.0000, 0.0000, \) and 0.0000 respectively) and βCTX1 (right; sequence, subject, period and sequence × period, \( P = 0.2711, 0.0000, 0.0000, \) and 0.0029 respectively). Filled circle, control group of COC nonusers; filled square, 30/15 users; filled triangle, 15/30 users. (a and b) differences from month 0 and from month 9 respectively were evaluated using Bonferroni multiple comparisons; \( P < 0.002. \) The error bars represent 95% confidence intervals with Bonferroni correction. The nonoverlapping confidence intervals between two subgroups denote statistical significance between them.

Figure 3 The full ANOVA models for crossover design evaluating the effects of dose on changes in absolute values of the variables during the study in the COC users and taking into consideration the interindividual variability (factor subject), sequence, period of the trial, initial values of LS BMD, age, and smoking. (Top) Changes of LS BMD, (middle) serum PINP, and (bottom) βCTX1. Significances are given in Table 3.
In COC users in this study, a significant reduction of BMD acquisition was observed as compared with continuing BMD increase in healthy adolescent female controls. The decreased endogenous estrogen production and a significant decline of plasma E2 level were associated with delayed BMD acquisition at LS, distal radius, and TB BMC and with accelerated decline in markers of bone formation and bone resorption. The COC effect was significantly more pronounced in users of very low-dose COC containing 15 mg EE. Accordingly, in subjects with decreased estroagen production, switching from 15 to 30 mg EE was associated with a larger increase in serum PINP as compared with the 30/15 users. It can be hypothesized that COC with higher (30 mg) E2 dose may more effectively substitute endogenous E2 in comparison with very low-dose COC with 15 μg EE and stimulate bone formation in adolescent girls. It should be noted, however, that the EE is much more potent at activating the estrogen receptors than E2, so that with COCs the overall activation of estrogen receptors is not measured by serum levels of 17-beta E2.

Dynamic changes in bone mass during puberty are mediated namely by interaction of GH and the gonadal steroid hormones. Studies in individuals with panhypopituitarism, in whom adequate replacement with both hormones had not been achieved, and in individuals with estrogen receptor defects or mutations of the aromatase gene, suggest a facilitative role of estrogen receptor-mediated processes on GH secretion and somatomedins production (26, 27). Thus, estrogen action may be rather indirect through modulation of the GH–insulin-like growth factor 1 (IGF1) axis (28, 29). However, exogenous estrogen produces a relative decrease in responsiveness to GH in similar populations, possibly through the achievement of sex steroid concentrations exceeding physiological ranges for age (27). Estrogens stimulate proliferation and differentiation of osteoblasts and inhibit osteoblast apoptosis (30, 31), and are involved in bone mineral accumulation namely at the endocortical bone surface (32). Higher estrogen concentrations have been associated with smaller medullary cavities, greater cortical thickness, and increased total volumetric BMD in pubertal girls (33). In this study, for the first time, important differences were found between COC with different EE doses, with an accentuated negative effect on LS BMD in users of very low-dose COC (15 μg EE/day). The crossover design used in the study allowed for attenuation of interindividual variability and the effect of several confounding factors that can significantly influence BMD development (e.g. age, weight, height, age of menarche, physical activity, endogenous production of hormones, diet, genetic background,  

### Table 3 ANCOVA model for crossover protocol comparing trends for absolute values of BMD at LS and biochemical markers of bone remodeling below and above median.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Independent</th>
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<th>Dependent: βCTX1</th>
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<td>P value</td>
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<td>–</td>
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</table>

Figure 4 Changes in serum estradiol (E2) levels in the control group of COC nonusers (filled circle), 30/15 users (filled square), and 15/30 users (filled triangle). Sequence, subject, period and sequence × period, P = 0.000, 0.000, 0.000, and 0.000 respectively. (a and b) as evaluated using repeated measures ANOVA model. Differences from month 0 and from month 9 respectively were evaluated using Bonferroni multiple comparisons. The error bars represent 95% confidence intervals with Bonferroni correction. The nonoverlapping confidence intervals between two subgroups denote statistical significance between them.
calcium, and vitamin intake). This study confirmed that early age at initiation of COC use may be an important risk factor for low peak bone mass in young women (34). In this study, smoking further exacerbated the negative effect of COC on BMD, confirming that smoking is another major determinant of the development of vertebral bone mass (35, 36).

The negative effect of COC containing EE on BMD development was shown in experimental studies (14) as well as by clinical investigation. One of the most diligent was a prospective cohort study that used DXA to examine BMD in young women between 19 and 22 years of age, with an exceptionally long follow-up period of 5 years (13). Data from 76 COC users and 71 nonusers were compared; in the nonusers, a significant LS BMD increase was observed, while in the COC users, no significant BMD growth was found during the 5-year period. A suppression of bone mineral acquisition in adolescent COC users was also confirmed by a recent prospective study that followed 122 women aged 12–19 years for a period of 4 years (37). Adjusted mean LS and femoral neck BMC was significantly lower in those who used COC for more than 2 years vs shorter term users or nonusers. The main limitation of this study was the large variability in the spectrum of used COCs and in the length of their use.

Adolescent COC users 11.9 ± 0.5 years of age were studied for 7.5 years in a prospective observational study (16). The authors have shown identical total body BMD increase in both COC users and nonusers. However, the results were not adjusted for potential confounding factors, and, most importantly, only a 6-month total duration of COC use was deemed satisfactory for inclusion in the group of COC users.

A large observational study of BMD changes in adolescent girls between 15 and 19 years of age (17) reported an increase in radius BMD during the follow-up period of 18 months to 3 years. No difference was found between COC users, nonusers, or depot medroxyprogesterone acetate users; it was only significantly lower in norethisterone enanthate users. In this study, however, one subject could contribute to different groups. Another two prospective studies evaluated a single group of subjects with different follow-up periods (12 and 24 months) (15, 18). While after 12 months a significantly smaller increase in both LS and femoral neck BMD was found in COC users vs nonusers, in the later analysis with 24 months, use this difference was no longer observed. One of the possible reasons for this discrepancy is a high dropout rate in the later study (almost 50% controls and 70% COC users). So far, only limited attention was paid to the comparison of the effects on BMD of varying estrogen doses in the COCs; however, these involved older women past the age of peak BMD acquisition (7, 8, 19, 38).

The authors are aware of several limitations of this study. Although there are some benefits to the crossover design, this does confuse the results because the effects of estrogen on the skeleton may take several months to be obvious, and thus the results in the second period may be influenced by the first period. However, it was not possible to include a ‘washout’ period between the two periods of the study, as it would be unethical in COC users, with high risk of unwanted pregnancy in adolescent subjects. Another limitation is the small size of the cohort, which is, however, sufficiently corrected by the use of crossover design that allowed for the achievement of statistically significant results. Also disputed could be the rather short (9 month) duration of each intervention; this was designed with the aim to reduce the study dropout rate in a longer study period, which is usually very high when subjects of this particular age group are involved. From the results, however, it is obvious that the length of the intervention period was satisfactory to document significant changes resulting from the rapid BMD changes at this age. Furthermore, the COCs have major effects on sex hormone-binding globulin. However, in this study the bioavailable estrogen was not measured. Also, we did not measure serum IGF1 which may be helpful for the early identification of young women at risk for developing low bone mass and osteoporosis (39).

Any conclusions regarding long-term persistence of lower BMD, osteopenia, or fracture risk could be made based on this study; however, the importance of peak bone mass achievement for the risk of osteoporosis is obvious. Bone mass accumulation during puberty determines peak bone mass as well as fracture risk at older age (24, 40). Every 10% increase in bone mass in adolescence decreases the risk of future fractures by 50%, and theoretically delayed the onset of osteoporosis by 13 years (41). In healthy COC users in this study, the BMD development was prevented significantly. During the 18-month study, the average LS BMD increase in healthy controls reached 2%, while in the COC users no significant BMD increase occurred. In conclusion, despite some limitations, the results of this study serve as an important argument confirming the slowdown of BMD development in adolescent COC users. A significantly more pronounced BMD growth reduction was observed with very low-dose COC. Our study and other publications showing similar results should stimulate further research concerning impact of early start and long-term use of COC on long-term BMD and risk of fractures.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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