


# Effect of two combinations of low-dose oral contraceptives on adolescent bone mass

## A clinical trial with 2 years follow-up

Talita Domingues Caldeirão, PhD<sup>a,b</sup>, Lilian Rodrigues Orsolini, MD, MSc<sup>a,b</sup>, Carla Cristiane da Silva, PhD<sup>c</sup>, Anapaula da Conceição Bisi Rizzo, MD, PhD<sup>a</sup>, Altamir Santos Teixeira, MD, MSc<sup>d</sup>, Hélio Rubens de Carvalho Nunes, PhD<sup>e</sup>, Tamara Beres Lederer Goldberg, MD, PhD<sup>a</sup> 

**Introduction:** Most contraceptive combinations can interfere with the processes of bone formation and resorption.

**Aim:** The aim of this study was to evaluate the effect of 2 combinations of low-dose oral hormonal contraceptives (20 µg ethinyl estradiol [EE]/150 mg desogestrel [COC1] or 30 µg EE/3 mg drospirenone [COC2]) on bone mass acquisition in adolescents over 2 years by means of bone densitometry and measurement of biomarkers of bone remodeling.

**Methods:** Parallel-group, non-randomized controlled clinical trial of 127 adolescents divided into a control group and 2 groups receiving either COC1 or COC2. The participants were submitted to anthropometric assessment and evaluation of secondary sexual characteristics (Tanner criteria) and bone age. Bone densitometry by dual-energy X-ray absorptiometry and measurement of bone biomarkers (bone alkaline phosphatase, osteocalcin, and C-terminal telopeptide) were performed at baseline and after 24 months.

**Results:** No significant differences in the variables analyzed were observed between COC1 or COC2 users and the control group at baseline. After 24 months, non-users had incorporated more bone mass (content and density) than either group of contraceptive users. This negative impact was more pronounced in the COC2 group than in the COC1 group. A significant reduction in the percentage values of bone alkaline phosphatase and osteocalcin was observed in users of oral contraceptives.

**Conclusion:** Bone mass acquisition was compromised in adolescent users of combined hormonal contraceptives. The negative impact was more pronounced in adolescents using contraceptives that contain 30 µg EE/3 mg drospirenone.

**Abbreviations:** BAP = alkaline phosphatase, BMC = bone mineral content, BMD = bone mineral density, BMI = body mass index, COC1 = 20 µg ethinyl estradiol (EE)/150 mg desogestrel, COC2 = 30 µg EE/3 mg drospirenone, CV = coefficient of variation, DXA = dual-energy X-ray absorptiometry, EE = ethinyl estradiol, OC = osteocalcin, PHV = peak height velocity, S-CTX = C-terminal telopeptide.

**Keywords:** adolescents, bone density, bone remodeling, bone resorption, hormonal contraceptives, peak height velocity (PHV)

### 1. Introduction

Osteoporosis is a metabolic bone disease characterized by the deterioration of bone microarchitecture and bone fragility. This disease is responsible for significant health services spending as a result of its main outcome, that is, fractures.<sup>[1]</sup>

Bone mass starts to decrease in women after 30 years of age, by 1% to 2% per year; from 50 years onwards, 30% of women will probably have some type of bone mass deficiency.<sup>[2]</sup> In addition, bone health in adulthood is a reflection of bone mass

acquisition in childhood and, especially, during adolescence, which is one of the main factors that protects against chronic diseases such as osteopenia/osteoporosis and subsequent fractures.<sup>[3–5]</sup> Thus, reaching peak bone mass during adolescence is important.

Bone health is influenced by endogenous and exogenous factors. Exogenous factors include the use of medications such as hormonal contraceptives in their different formulations (injectable, oral, and other routes of administration). Ethinyl estradiol (EE), a synthetic hormone derived from endogenous 17-β

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo [Grants 2007/07731-0, 2011/05991-0, and 2015/04040-2]; Pro-Rector for Research at UNESP and UNIMED ASSIS).

Clinical registration: Registry Number, RBR-5h9b3c.

<sup>a</sup> Postgraduate Program in Tocogynecology, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, <sup>b</sup> Medical School, Assis Municipality Educational Foundation (FEMA), Assis, SP, Brazil, <sup>c</sup> State University of Londrina – UEL, Londrina, Parana, Brazil, <sup>d</sup> Department of Tropical Diseases and Diagnostic Imaging, Botucatu Medical School, São Paulo University (UNESP), Botucatu, São Paulo, Brazil, <sup>e</sup> Statistical Consultant, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

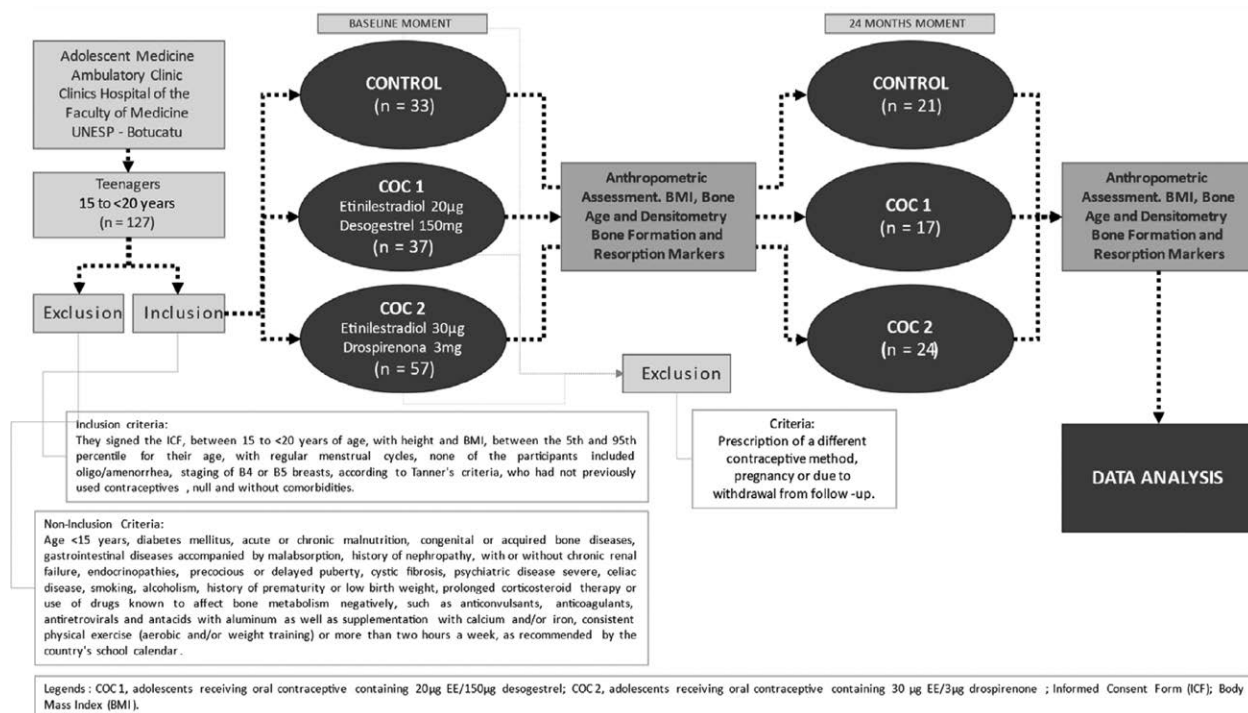
\*Correspondence: Tamara Beres Lederer Goldberg, Postgraduate Program of Tocogynecology, Botucatu Medical School, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil (e-mail: tamara.goldberg@unesp.br).

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Caldeirão TD, Orsolini LR, da Silva CC, Bisi Rizzo AdC, Teixeira AS, Nunes HRdC, Goldberg TBL. Effect of two combinations of low-dose oral contraceptives on adolescent bone mass: A clinical trial with 2 years follow-up. *Medicine* 2022;101:37(e30680).

Received: 16 February 2022 / Received in final form: 19 August 2022 / Accepted: 22 August 2022

<http://dx.doi.org/10.1097/MD.00000000000030680>



**Figure 1.** Flowchart for derivation showing the number of participants and the reasons for loss to follow-up.

estradiol, is the estrogen found in most contraceptive combinations that can interfere with the processes of bone formation and resorption. Contraceptives are being used at increasingly earlier ages because of the early initiation of sexual activity among adolescents as a method of protection of their sexual and reproductive health and for non-contraceptive benefits such as dysmenorrhea, acne, and irregular menstruation.<sup>[3,6]</sup>

Estrogen plays an important role in the regulation of bone metabolism by positively affecting the formation and proliferation of osteoblasts while simultaneously inhibiting the apoptosis of osteoclasts responsible for bone resorption.<sup>[7]</sup> However, the activities described appear to be maturity dependent.<sup>[8,9]</sup>

Most oral hormonal contraceptives contain estrogen and progestin, with recognition of the greater potency of EE on target tissues.<sup>[10]</sup> However, the potent estrogenic activity of EE is not reflected in positive evidence when the bone health of adolescents is evaluated and the results reported in the literature are still conflicting.<sup>[5,10,11]</sup> Therefore, this study aimed to evaluate the effect of 2 combinations of low-dose oral hormonal contraceptives (20 µg EE/150 mg desogestrel or 30 µg EE/3 mg drospirenone) on bone mass acquisition in adolescents over 2 years by means of bone densitometry and measurement of serum biomarkers of bone remodeling.

## 2. Subjects and Methods

### 2.1. Study design and participants

This is a parallel-group, non-randomized controlled clinical trial in which the volunteers were followed up for 2 years. The sample was selected at the Adolescent Medicine Outpatient Clinic of the University Hospital, Botucatu Medical School, UNESP. The project was approved by the Ethics Committee of the institution (Ethical Clearance Certificate: 52928416.6.0000.5411). All participants and their parents or legal guardian signed the free informed consent form. The criteria for inclusion, exclusion, or withdrawal from the study are shown in Figure 1.

The adolescents were divided into 3 groups. Two groups had a prescription of contraceptives, with the COC1 group receiving

20 µg EE/150 mg desogestrel and the COC2 group receiving 30 µg EE/3 mg drospirenone. The control adolescents did not require to use a contraceptive. All participants in the COC groups were advised and encouraged to use dual protection.

### 2.2. Anthropometric assessment

Body weight and height were obtained and the body mass index (BMI) was calculated.<sup>[12]</sup> Sexual maturation was evaluated by visual inspection of the breast by a trained professional according to the Tanner criteria.<sup>[13]</sup>

### 2.3. Bone age and densitometry

For the assessment of skeletal maturation, bone age was obtained by hand and wrist radiography using the Greulich and Pyle method.<sup>[14]</sup> The report was issued by an evaluator who was unaware to which group the participants belonged.

Bone densitometry (dual-energy X-ray absorptiometry) was performed with a Hologic QDR 4500 apparatus by a single experienced and trained evaluator according to the guidelines of the International Society for Clinical Densitometry.<sup>[1,15]</sup> Lumbar spine (L1 to L4), total body and subtotal measurements, excluding the head segment, were obtained. The coefficient of variation (CV) was estimated based on repeated measurements (twice) obtained from 30 patients representative of the population in the regions studied. The results showed a CV of 0.6% and 1.3% for lumbar spine and total body. The bone mineral density (BMD) results were expressed in g/cm<sup>2</sup> and bone mineral content (BMC) in grams.

### 2.4. Markers of bone formation and resorption

Blood samples were collected in the morning after a 10-hour fast by venipuncture and centrifuged for 15 minutes at 1500g for the separation of serum. The samples were stored at -80°C until the analysis of the biomarkers bone alkaline phosphatase (BAP), osteocalcin (OC), and C-terminal telopeptide (S-CTX).

**Table 1**

**Comparison of anthropometric and densitometric variables and bone formation and resorption markers expressed in absolute values between the Control, COC1 and COC2 groups at baseline.**

Variables	Control (n = 33)				COC1 (n = 37)				COC2 (n = 57)				P
	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	
Age (yr) <sup>†</sup>	16.00	1.04	15.00	19.92	16.17	1.40	15.00	20.17	16.42	1.11	15.00	18.75	.270
Bone age (yr) <sup>†</sup>	17.00	0.83	15.00	18.00	17.00	0.89	15.00	18.00	17.00	0.88	15.00	19.00	.390
Weight (kg) <sup>†</sup>	53.00	8.22	42.60	71.00	54.00	8.50	40.50	73.40	54.50	7.49	41.00	72.20	.700
Height (cm) <sup>†</sup>	161.00	–	151.00	173.00	159.66	–	149.00	171.00	159.34	–	140.00	172.00	.450
BMI (kg/m <sup>2</sup> ) <sup>†</sup>	20.48	2.63	16.72	27.39	20.88	2.83	16.63	26.78	21.64	2.67	17.75	29.99	.320
BMI (percentile) <sup>†</sup>	55.96	25.21	9.80	92.21	55.61	28.45	8.18	95.00	63.25	23.67	8.18	95.00	.480
Lumbar BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.917	–	0.690	1.120	0.953	–	0.773	1.094	0.960	–	0.710	1.180	.149
Lumbar BMC (g) <sup>†</sup>	49.98	9.04	28.09	71.63	50.83	8.00	37.70	74.55	49.62	8.15	37.01	79.39	.820
Lumbar Z-score <sup>†</sup>	–0.43	–	–1.90	1.00	–0.27	–	–1.80	1.10	–0.11	–	–1.90	1.60	.370
Total body BMD (g/cm <sup>2</sup> ) <sup>†</sup>	1.090 <sup>A</sup>	–	0.860	1.320	1.000 <sup>B</sup>	–	0.909	1.130	1.020 <sup>B</sup>	–	0.880	1.240	<b>.001</b>
Total body BMC (g) <sup>†</sup>	1975.20	314.73	1260.69	2754.08	1850.52	183.67	1401.96	2456.11	1803.33	250.52	1434.14	2719.86	.070
Total body Z-score <sup>†</sup>	1.15 <sup>A</sup>	–	–1.60	3.20	–0.91 <sup>B</sup>	–	–1.90	0.50	–0.61 <sup>B</sup>	–	–1.80	1.80	<b>.001</b>
Subtotal BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.910	0.070	0.750	1.040	0.870	0.050	0.790	1.000	0.890	0.080	0.800	1.160	.123
Subtotal BMC (g) <sup>†</sup>	1375.57	248.82	891.25	1987.54	1420.76	163.65	1047.63	1995.15	1373.94	212.68	1109.76	2254.86	.750
Fat mass (kg) <sup>†</sup>	18.150	4.169	10.672	27.394	16.551	5.831	8.594	36.443	17.764	4.896	10.151	30.170	.315
Lean mass (kg) <sup>†</sup>	33.489	5.382	27.719	44.072	37.398	4.456	30.858	48.669	35.509	6.885	2.659	51.819	.267
Total body fat % <sup>†</sup>	33.18 <sup>A</sup>	3.69	23.90	39.40	29.17 <sup>B</sup>	4.85	21.00	38.50	31.77 <sup>A</sup>	5.15	19.60	41.40	<b>.010</b>
BAP (U/L) <sup>†</sup>	30.71	12.15	13.29	621.00	36.10	16.40	15.76	84.82	35.27	12.04	19.31	66.47	.208
Osteocalcin (ng/mL) <sup>†</sup>	5.44	5.99	1.01	18.13	7.41	3.90	1.71	21.17	10.32	5.98	0.27	24.35	.077
S-CTx (ng/mL) <sup>†</sup>	0.674	0.217	0.271	1.171	0.549	0.357	0.220	1.760	0.605	0.314	0.220	1.710	.690

Note: Mean\* or Median<sup>†</sup>. Different uppercase letters indicate significant differences between the 3 groups ( $P < .05$ )

BMD = bone mineral density, BMC = bone mineral content, BMI = body mass index, BAP = bone alkaline phosphatase, COC1 = adolescents receiving oral contraceptive containing 20µg EE/150µg desogestrel, COC2 = adolescents receiving oral contraceptive containing 30 µg EE/3µg drospirenone, S-CTx = C-terminal telopeptide, SD = standard deviation.

\*ANOVA for comparison of means between the 3 groups and Bonferroni test for multiple comparisons (Mean).

†Kruskal–Wallis test for comparison between the 3 groups and Dunn multiple comparison (Median).

BAP and OC were measured in an ELISA microplate reader at 405 nm. For OC, the intra-assay CV ranged from 5% to 10% and the interassay CV from 5% to 8%. For BAP, the intra- and interassay CV ranged from 3.9% to 5.8% and from 5% to 7.6%, respectively. S-CTx was measured by electrochemiluminescence immunoassay (ECLIA) using the Elecsys β-Cross-Laps serum assay in an automated Elecsys analyzer (Roche™, Indianapolis, IN). The interassay CV was 5%.

### 2.5. Statistical analysis

The data were analyzed using the statistical package for social science 21 software. Homogeneity between groups was verified. The assumptions of homogeneity of variances and normality were evaluated using Levene test and the Shapiro–Wilk test, respectively. For descriptive analysis, median, extreme values, means and standard deviations were calculated. The 3 groups studied (control, COC1 and COC2) were compared at the different time points (baseline and 24 months) by ANOVA, followed by Bonferroni test for multiple comparisons, when the variables showed a normal distribution. The Kruskal–Wallis test and Dunn test were used when the data were not normally distributed. A level of significance of 5% was adopted for all analyses.

### 3. Results

One hundred twenty-seven participants were included in the study; of these, 62 (48.8%) continued the follow-up until the end of the study, 24 months after adherence to the protocol. Figure 1 shows the number of participants included in each group.

All variables shown in Table 1 were homogenous, except for median total body BMD, which was higher in the control group compared to the COC1 and COC2 groups ( $P = .001$ ), and median total fat percentage, which was lower in the COC1 group compared to the other groups ( $P = .01$ ).

There was a significant difference in BMI percentiles, with a higher median BMI in the control group compared to the COC1 group ( $P = .029$ ) (Table 2). Despite these results, 90% of the adolescents were classified as eutrophic after 24 months. The median lumbar BMD Z-score was closer to zero in the control group when compared to the COC1 and COC2 groups. An intermediate result was observed in the COC2 group ( $P = .039$ ) (Table 2). The median total body BMD was higher among controls compared to the COC2 group, with intermediate results in the COC1 group ( $P = .001$ ). The median total body BMC was higher in the control group than in the COC1 and COC2 groups ( $P = .016$ ). The same trend was observed for body fat percentage ( $P = .012$ ), with higher values in the control group compared to the COC1 and COC2 groups and no differences between contraceptive users (Table 2). The median total BMD Z-score was higher in the control group than in the COC1 and COC2 groups, which did not differ from one another ( $P = .027$ ). The lowest median subtotal BMD was observed in the COC1 group compared to the control and COC2 groups ( $P = .003$ ) (Table 2).

The mean absolute variation in lumbar BMD (Table 3) was higher in the control group than in the COC2 group, with an increase of 0.03 g/cm<sup>2</sup> in the former and a negative variation of –0.02 g/cm<sup>2</sup> in the latter ( $P = .013$ ). The mean absolute variation in lumbar BMC was lower in the COC2 group than in the control group, with an increase of 1.92 g in the control and a reduction of –0.26 g in COC2. The median variation in lumbar Z-score was positive in the control group, indicating an increase in bone mass, while negative results were observed in the COC1 and COC2 groups ( $P = .005$ ). There was a higher median absolute variation in total body BMD in the control group (increase of 0.073 g/cm<sup>2</sup>) compared to the other groups (increase of 0.013 g/cm<sup>2</sup> in COC1 and of 0.02 g/cm<sup>2</sup> in COC2). Regarding the median absolute variation in total body BMC, there was an increase of 98.85 g in the control group, while a reduction of –2.20 g was observed in the COC1 group and an increase of 15.08 g in the COC2 group. The mean absolute variation in total body Z-score was positive in

**Table 2****Comparison of anthropometric and densitometric variables and bone formation and resorption markers expressed in absolute values between the Control, COC1, and COC2 groups 2 years follow-up.**

Variables	Control (n = 21)				COC1 (n = 17)				COC2 (n = 24)				P
	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	
Age (yr) <sup>†</sup>	18.00	1.13	17.00	21.83	18.00	1.30	16.50	21.50	18.00	0.94	16.92	20.25	.980
Bone age (yr) <sup>†</sup>	18.00	0.60	16.00	18.00	18.00	0.41	17.00	18.00	17.00	0.51	17.00	18.00	.280
Weight (kg) <sup>†</sup>	54.70	9.20	45.00	77.00	53	12.17	40.10	90.00	55.95	9.04	42.00	72.50	.380
Height (cm) <sup>†</sup>	162.17	–	153.00	173.00	160.74	6.23	151.00	170.00	158.92	8.06	140.00	173.00	.280
BMI (kg/m <sup>2</sup> ) <sup>†</sup>	21.64	2.93	18.18	29.33	20.23	4.04	16.56	31.89	22.05	2.76	18.16	30.17	.199
BMI (percentile) <sup>†</sup>	65.32 <sup>A</sup>	–	23.89	93.44	39.60 <sup>B</sup>	–	1.45	96.20	55.81 <sup>AB</sup>	–	10.56	84.61	<b>.029</b>
Lumbar BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.963	0.143	0.690	1.120	0.891	0.069	0.820	1.120	0.927	0.107	0.790	1.130	.330
Lumbar BMC (g) <sup>†</sup>	51.99	9.30	30.00	73.57	47.92	5.70	41.02	62.67	50.78	7.22	41.32	69.34	.280
Lumbar Z-score <sup>2</sup>	–0.18 <sup>A</sup>	–	–1.10	1.10	–0.97 <sup>B</sup>	–	–1.80	1.00	–0.37 <sup>AB</sup>	–	–1.70	1.20	<b>.039</b>
Total body BMD (g/cm <sup>2</sup> ) <sup>†</sup>	1.130 <sup>A</sup>	–	0.900	1.350	1.000 <sup>AB</sup>	–	0.927	1.225	1.040 <sup>B</sup>	–	0.940	1.210	<b>.001</b>
Total body BMC (g) <sup>†</sup>	2063.10 <sup>A</sup>	–	1290.32	2876.67	1824.81 <sup>B</sup>	–	1562.33	2153.10	1868.42 <sup>B</sup>	–	1514.59	2570.34	<b>.016</b>
Total body Z = score <sup>†</sup>	1.90 <sup>A</sup>	1.15	–0.70	3.30	–1.50 <sup>B</sup>	1.19	–2.00	1.70	–0.90 <sup>B</sup>	0.93	–1.40	3.10	<b>.027</b>
Subtotal BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.930 <sup>A</sup>	–	0.780	1.060	0.860 <sup>B</sup>	–	0.798	0.929	0.920 <sup>A</sup>	–	0.890	1.120	<b>.003</b>
Subtotal BMC (g) <sup>†</sup>	1508.09	–	1006.32	1944.02	1363.72	–	1169.30	1642.05	1401.66	–	1032.05	1792.41	.058
Fat mass (kg) <sup>†</sup>	22.898	5.267	14.963	30.298	18.281	7.222	9.120	34.651	19.987	5.768	8.571	31.029	.138
Lean mass (kg) <sup>†</sup>	31.676	6.251	28.714	47.304	35.481	6.810	28.490	53.000	35.969	5.099	26.680	43.231	.620
Total body fat % <sup>†</sup>	38.44 <sup>A</sup>	4.99	27.10	46.50	31.49 <sup>B</sup>	5.68	22.70	38.60	33.10 <sup>B</sup>	7.30	16.80	48.20	<b>.012</b>
BAP (U/L) <sup>†</sup>	33.48	12.12	14.30	64.33	30.83	10.20	18.61	48.60	27.00	9.45	13.29	49.24	.170
Osteocalcin (ng/mL) <sup>†</sup>	5.18	7.85	0.88	28.41	4.44	4.68	0.22	16.78	6.22	5.63	2.09	2121.00	.313
S-CTx (ng/mL) <sup>†</sup>	0.511	0.200	0.200	0.960	0.630	0.200	0.309	0.874	0.567	0.240	0.150	1.010	.343

Note: Mean<sup>\*</sup> or Median<sup>†</sup>. Different uppercase letters indicate significant differences between the 3 groups ( $P < .05$ ).

BMD = bone mineral density, BMC = bone mineral content, BMI = body mass index, BAP = bone alkaline phosphatase, COC1 = adolescents receiving oral contraceptive containing 20 µg EE/150 µg desogestrel, COC2 = adolescents receiving oral contraceptive containing 30 µg EE/3 µg drospirenone, S-CTx = C-terminal telopeptide, SD = standard deviation.

\*ANOVA for comparison of means between the 3 groups and Bonferroni test for multiple comparisons (Mean).

†Kruskal–Wallis test for comparison between the 3 groups and Dunn multiple comparison (Median).

**Table 3****Comparison of the variation of densitometric variables and bone formation and resorption markers expressed in absolute values between the Control, COC1 and COC2 groups between baseline and 2 years follow-up.**

Variables	Control (n = 21)				COC1 (n = 17)				COC2 (n = 24)				P
	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	Med/Mean	SD	Min	Max	
Lumbar BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.030 <sup>A</sup>	0.084	–0.240	0.340	–0.009 <sup>AB</sup>	0.055	–0.070	0.095	–0.020 <sup>B</sup>	0.062	–0.16	0.10	<b>.013</b>
Lumbar BMC (g) <sup>†</sup>	1.92 <sup>A</sup>	4.97	–16.36	11.43	0.22 <sup>AB</sup>	3.37	–3.89	6.97	–0.26 <sup>B</sup>	3.68	–9.76	46.00	<b>.005</b>
Lumbar Z-score <sup>†</sup>	0.19 <sup>A</sup>	–	–0.30	1.00	–0.27 <sup>B</sup>	–	–1.00	0.80	–0.47 <sup>B</sup>	–	–1.70	0.50	<b>.005</b>
Total body BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.073 <sup>A</sup>	–	–0.200	0.160	0.013 <sup>B</sup>	–	–0.034	0.220	0.020 <sup>B</sup>	–	–0.030	0.150	<b>.005</b>
Total body BMC (g) <sup>†</sup>	98.85 <sup>A</sup>	–	–268.32	349.85	–2.20 <sup>B</sup>	–	–39.10	12.83	15.08 <sup>B</sup>	–	–90.62	509.50	<b>.005</b>
Total body Z-score <sup>†</sup>	0.80 <sup>A</sup>	0.42	–0.30	1.20	–0.05 <sup>B</sup>	0.84	–0.70	2.40	–0.20 <sup>B</sup>	0.95	–1.20	2.80	<b>.002</b>
Subtotal BMD (g/cm <sup>2</sup> ) <sup>†</sup>	0.022	–	–0.040	0.170	0.005	–	–0.033	0.039	0.019	–	–0.030	0.140	.147
Subtotal BMC (g) <sup>†</sup>	76.63 <sup>A</sup>	–	–59.92	636.85	–18.66 <sup>B</sup>	–	–59.66	270.12	5.72 <sup>B</sup>	–	–77.71	155.60	<b>.001</b>
BAP (U/L) <sup>†</sup>	1.03 <sup>A</sup>	9.45	–30.43	23.70	–4.72 <sup>AB</sup>	12.98	–25.80	11.02	–7.02 <sup>B</sup>	10.61	–39.68	3.26	<b>.014</b>
Osteocalcin (ng/mL) <sup>†</sup>	0.16	6.07	–12.59	11.82	–3.52	6.34	–13.61	11.54	–2.07	5.19	–10.61	5.80	.081
S-CTx (ng/mL) <sup>†</sup>	0.025	0.258	–0.750	0.140	–0.047	0.376	–0.840	0.302	–0.091	0.269	–0.880	0.310	.692

Note: Mean<sup>\*</sup> or Median<sup>†</sup>. Different uppercase letters indicate significant differences between the 3 groups ( $P < .05$ ).

BMD = bone mineral density, BMC = bone mineral content, BAP = bone alkaline phosphatase, COC1 = adolescents receiving oral contraceptive containing 20 µg EE/150 µg desogestrel, COC2 = adolescents receiving oral contraceptive containing 30 µg EE/3 µg drospirenone, S-CTx = C-terminal telopeptide, SD = standard deviation.

COC1, adolescents receiving oral contraceptive containing 20 µg EE/150 µg desogestrel; COC2, adolescents receiving oral contraceptive containing 30 µg EE/3 µg drospirenone.

\*ANOVA for comparison of means between the 3 groups and Bonferroni test for multiple comparisons (Mean).

†Kruskal–Wallis test for comparison between the 3 groups and Dunn multiple comparison (Median).

the control group (0.80) but negative in the COC1 (–0.05) and COC2 (–0.20) groups ( $P = .002$ ) (Table 3).

Regarding subtotal BMC, the median absolute variation indicated that the control group incorporated 76.63 g, while a negative result was observed in the COC1 group (–18.66 g) and a slight increase (5.72 g) in the COC2 group ( $P = .001$ ). BAP showed a positive mean absolute variation in the control group, while a negative variation was observed in the COC2 and COC1 groups, with an intermediate result in the latter ( $P = .014$ ) (Table 3). The Figures 2 and 3 indicates the comparison of the variation of densitometric variables and the variation of bone formation and resorption markers, respectively. The

values are expressed in percentage between the Control, COC1 and COC2 groups between baseline and 2 years follow-up. A significant reduction in the percentage values of all variables was observed in users of oral contraceptives, exception in subtotal BMD and S-CTx. The results demonstrated that the impact on lumbar spine densitometry was stronger with COC2.

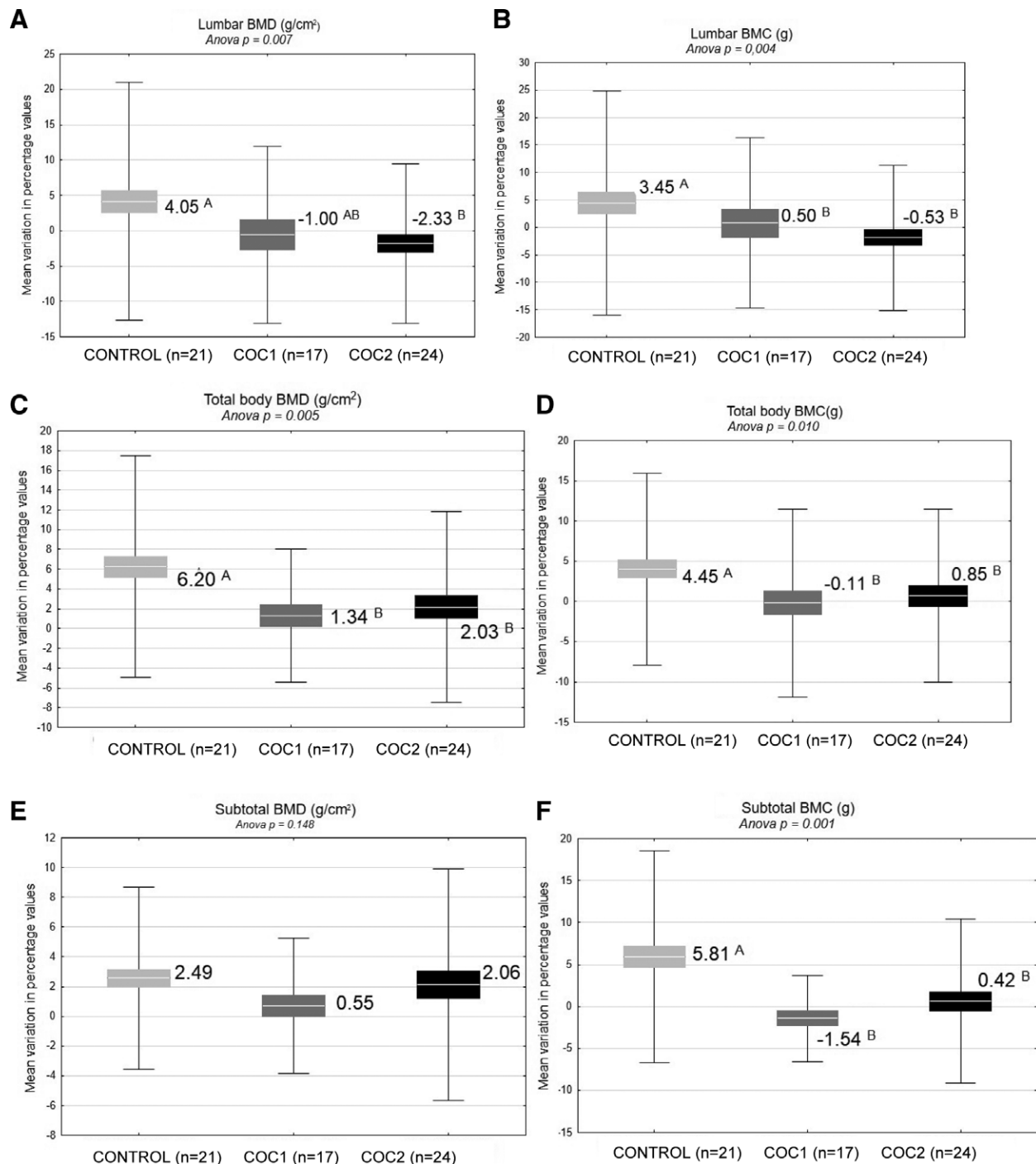
#### 4. Discussion

In the present 2-year follow-up study, bone densitometry demonstrated significantly higher bone mass acquisition at the skeletal sites evaluated (lumbar spine, total body and subtotal)

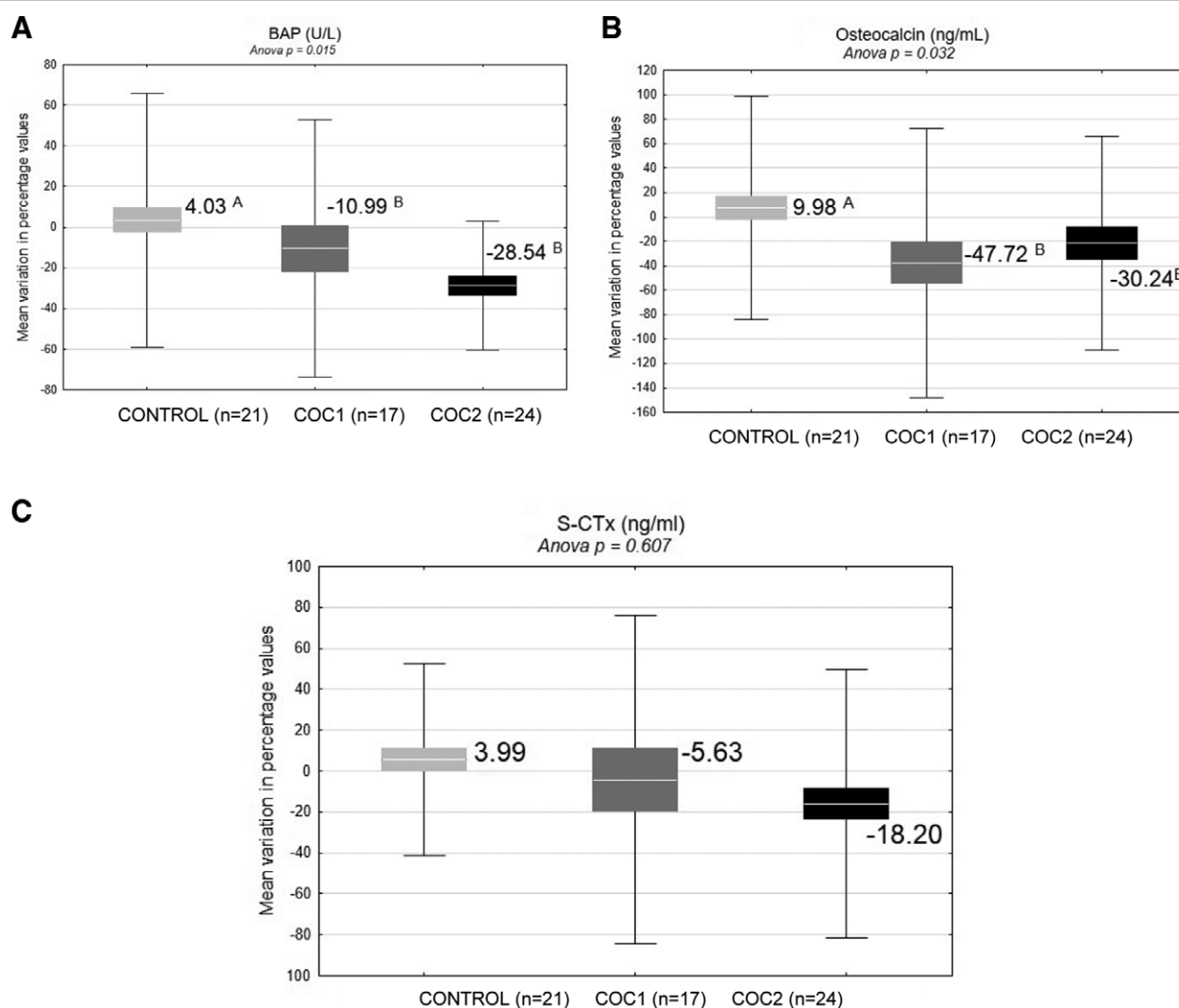
in adolescents who did not use oral hormonal contraceptives compared to adolescents of the COC2 group (30 µg EE/3mg drospirenone). In the latter group, bone mass acquisition was compromised at most of the sites evaluated, with more intense impairment in the lumbar spine. Users of COC1 (20 µg EE/150mg desogestrel) also exhibited a reduction in bone mass acquisition but the lumbar region was less affected. These findings indicate a more negative impact on the bone mass of adolescents that used the COC2 combination (Table 3 and Fig. 2). In addition, the bone formation biomarkers OC and BAP remained stable among adolescents of the control group since their age limit at the time of inclusion in the study ranged

from 15 complete to 20 incomplete years. On the other hand, in oral contraceptive users, the reductions in the concentrations of bone formation markers suggested a decrease in bone metabolic activity whose impact was more intense among COC2 users (Table 3 and Fig. 3).

In a prospective longitudinal study of adolescent girls and young adult women exposed to different contraceptive formulations, Jackowisk et al<sup>[8]</sup> showed a negative impact on the development of BMC and BMD when COCs were introduced immediately after peak height velocity (PHV). However, the intensity was not the same when contraceptives were introduced 5 years after PHV, suggesting an effect dependent on level of bone



**Figure 2.** Comparison of the variation of densitometric variables expressed in percentage values between the Control, COC1 and COC2 groups between baseline and 2 years follow-up. COC1 = 20 µg ethinyl estradiol (EE)/150mg desogestrel, COC2 = 30 µg EE/3mg drospirenone.



**Figure 3.** Comparison of the variation of bone formation and resorption markers expressed in percentage values between the Control, COC1 and COC2 groups between baseline and 2 years follow-up. COC1 = 20 µg ethinyl estradiol (EE)/150mg desogestrel, COC2 = 30 µg EE/3mg drospirenone.

maturity.<sup>[8]</sup> The present results differ from those of Jackowisk et al as they demonstrated impaired bone mass acquisition, although COCs were introduced within an average interval of 3 years after PHV. We emphasize that the girls participating in our study were in late puberty and therefore had already undergone the growth spurt, and were in a stage of growth deceleration when COC was introduced (the median time interval between menarche and starting COC use (gynecological age) was 36 months). However, they should still have been incorporating bone mass, an event that occurs after peak height velocity.<sup>[16]</sup> In addition, a 5-year follow-up study conducted in Italy demonstrated an increase of 7.8% in the lumbar BMD among non-users of contraceptives and found no significant change in the group that used 20 µg EE/150 µg desogestrel.<sup>[17]</sup> The mean age of the participants was 20 years, thus confirming the effect of oral contraceptives on bone mass even when used much later after PHV, in agreement with our findings.

All adolescents were classified as Tanner breast stages 4 and 5, a period characterized by peak bone mass acquisition during adolescence.<sup>[18]</sup> The results of a Brazilian study, which evaluated 101 female adolescents, showed a correlation of bone mass acquisition with advances in chronological age, bone age and pubertal stage.<sup>[3]</sup> Considering that 92% of the total bone mass has already been incorporated in late adolescence,<sup>[11]</sup> the results obtained for the groups of contraceptive users, particularly the

COC2 combination, are relevant and indicate that the combination of oral contraceptives should be chosen carefully when prescribed for this age group.

Cibula et al<sup>[19]</sup> conducted a cross-over study that included a control group and 2 contraceptive groups containing the same progestin, gestodeno, and 2 different concentrations of EE (30 and 15 µg). The authors observed an increase of BMD in the control group but not in the groups of contraceptive users.<sup>[19]</sup> Since adolescents were sequentially exposed to 2 EE concentrations, the interpretation of the results is difficult because the effect of estrogen on bone mass can take several months to be detected and the results obtained in the second period may therefore have been influenced by those of the first period.

A systematic literature review demonstrated the negative influence of the use of oral hormonal contraceptives for 1 and 2 years on lumbar spine bone density in adolescents.<sup>[20]</sup> The results showed a reduction of 0.02 g/cm<sup>2</sup> in bone density in the lumbar spine both after 1 and after 2 years of contraceptive use. Five articles were evaluated in the 24-month analysis and the heterogeneity between studies was 96% and 85% for the 1-year and 2-year analysis, respectively. This small number of included studies reflects the difficulties of the authors in selecting good quality articles and the lack of studies that analyzed other bone sites for densitometry, as well as the adversities encountered by several researchers in monitoring adolescents who use

contraceptive methods, the prescription of contraceptives with different doses and compositions, and the high dropout rates in this age group, especially after 12 months of the use of this method.<sup>[20,21]</sup> We emphasize that the average reduction in lumbar BMD found in the present study was 0.02 g/cm<sup>2</sup>, similar to the cited meta-analysis.<sup>[21]</sup>

Regarding EE dose, in a systematic review of a series of articles that selected adolescent users of different hormonal compositions to evaluate the effect of contraceptives on bone mass, the authors concluded that EE doses between 20 and 30 µg can affect peak bone mass acquisition.<sup>[22]</sup> The same outcome was observed in our study. The 2 groups of contraceptive users exhibited impairment of densitometric parameters, with a greater impact in users of the combination with 30 µg EE (Table 3 and Fig. 2).

A prospective multicenter study conducted on Canadian adolescents aged 15 to 19.5 years found no differences in lumbar spine densities between users of COC and non-users after 2 years of use,<sup>[23]</sup> in contrast to the present study in which bone density was lower at this site in COC users. Based on the analysis of both lumbar BMD and BMC, this effect was more pronounced for the use of 30 µg EE/3 mg drospirenone, with controls incorporating 0.030 g/cm<sup>2</sup> and 1.92 g, respectively, while negative results were obtained for COC2 users (−0.020 g/cm<sup>2</sup> and −0.26 g, respectively). When total body densitometry was analyzed, the control group exhibited an increase of 98.85 g and COC2 users of only 15.08 g over 24 months, a finding reinforcing the interference of oral contraceptives with bone mass deposition in adolescents. Similar observations have been published by Bisi Rizzo et al<sup>[5]</sup> who followed up adolescents using the same COC formulations for a period of 12 months, although the authors performed linear regression analysis to determine the evolution among controls. We can therefore state that the negative impact on the bone mass of adolescents observed after 1 year of follow-up persists when the period of use is extended to 2 years. Possibly, the effect observed on bone mass is due to the fact that estradiol and EE act on estrogen receptors through the same biological mechanisms, emphasizing that EE, a non-physiological form, has been recognized to exert a more potent effect on target tissues.<sup>[10]</sup> However, the oral route of EE administration implies the hepatic first-pass and a consequent reduction in Insulin-like growth factor 1, a hormone that is also essential in the acquisition of bone mass in adolescence. EE also results in an increase in the sex hormone-binding globulin, decreasing the bioavailability of estradiol.<sup>[24]</sup> These effects possibly collaborate in the reduction in bone mass deposit in adolescents using COC.

The literature has demonstrated significant negative correlations between the concentrations of bone remodeling biomarkers and chronological age, bone age, breast development and BMD during the second decade of life, indicating that bone mass deposition still occurs in late adolescence and that the concentrations of markers of bone formation and resorption are lower.<sup>[3]</sup> Callegari et al<sup>[25]</sup> reported reference values for bone markers in young people aged 16 to 25 years and observed a reduction in the concentrations of S-CTX and PINP (propeptide of type I collagen) with increasing age. It is noteworthy that the results obtained by us for all markers analyzed showed an evolution that corroborates the results of Callegari et al; however, among contraceptive users, especially those taking COC2, the decline in BAP and OC levels was significant (Fig. 3). As demonstrated by the same authors in a subsequent study COC users exhibited a 22% reduction in the concentrations of bone metabolism markers compared to those not using hormonal contraceptives.<sup>[26]</sup> Similarly, there was a reduction of −28.54% in BAP concentrations, of −30.24% in OC concentrations, and of −18.28% in S-CTX concentrations among COC2 users compared to baseline.

In a literature review, Herrmann and Seibel<sup>[2]</sup> observed a significant reduction in the concentrations of markers of bone formation and resorption in users of hormonal contraceptives.

However, despite this observation, the authors were unable to establish the influence of these findings on the future fracture risk of contraceptive users. We found no fractures among adolescent users over the 2 years of follow-up; however, bone remodeling might be compromised.

The progestins present in the composition of the COCs used, drospirenone and desogestrel, are known to act on androgen receptors by competitive inhibition, with this mechanism resulting in important antiandrogenic effects on bone metabolism since testosterone plays a key role in bone mass acquisition.<sup>[27]</sup> Hadji et al<sup>[28]</sup> found no effect on the fracture risk of users of contraceptives containing only progestin, demonstrating the preservation of bone mass. Thus, the effect of progestin on bone metabolism may be associated with its combination with the estrogen component, either 17β-estradiol or EE, and with the route of administration, either oral or transdermal.<sup>[28]</sup> A study comparing the use of different progestins observed a greater reduction in the concentrations of bone markers in the drospirenone group compared to the gestodene group, a progestin of the same generation as desogestrel used in our study.<sup>[29]</sup> A meta-analysis concluded that progesterone combined with estrogen has an antiresorptive effect on bone. In vitro, progesterone exerts a stimulatory effect on osteoblast differentiation at physiological concentrations and an inhibitory effect at pharmacological concentrations.<sup>[30]</sup>

The paucity of studies on the influence of progestins present in COC formulations makes it difficult to distinguish which effects on bone metabolism would be arising from progesterone and which from estrogen levels. In addition, the heterogeneity of study designs impairs comparisons of the hormonal effect of oral contraceptives on bone remodeling in adolescents. The studies on this topic identified in the databases differed in terms of the type of contraceptives, EE concentrations, and time of onset of contraceptive use during bone mass acquisition. If bone mass acquisition during puberty could be optimized, it is likely that adults and older adults would be less susceptible to the complications of osteoporosis because a 10% increase in peak bone mass results in a delay of 13 years in the onset of this condition.<sup>[28]</sup>

The present study aimed to reduce uncertainties regarding the debate in question; however, although strict selection criteria were adopted to moderate them, some limitations of this study must be highlighted, including the small sample size and the loss to follow-up of practically 50% of the sample at the end of the 2 years of follow-up. Retaining adolescent participants in research is a challenge because of their geographic mobility, major life transitions, and their choices and goals, including the preservation of their reproductive health.

The present results suggest that only long-term evaluation will permit to identify the repercussions of the use of oral hormonal contraceptives by this age group on bone mass, when the adolescents become adults or reach menopause, prospectively monitoring the evolution of their densitometric parameters at all stages of life.

## 5. Conclusion

The present study demonstrated lower bone mass acquisition among COC users after 24 months. This deleterious effect was more pronounced in adolescents using 30 µg EE/3 mg drospirenone. Significant differences were found in the evolution of densitometric parameters between healthy adolescents who did not use contraceptives (control group) and users of low-dose oral hormonal contraceptives (20 µg EE/150 mg desogestrel or 30 µg EE/3 mg drospirenone) of the same age group. A negative impact on bone health was demonstrated both by bone densitometry and by serum biomarkers of bone formation. Understanding the process of bone acquisition that occurs during childhood and adolescence will allow to develop strategies for the early prevention of osteoporosis.

## Author contributions

**Conceptualization:** Carla Cristiane da Silva, Anapaula da Conceição Bisi Rizzo, Tamara Beres Lederer Goldberg.

**Data curation:** Anapaula da Conceição Bisi Rizzo, Altamir Santos Teixeira.

**Formal analysis:** Altamir Santos Teixeira, Hélio Rubens de Carvalho Nunes.

**Funding acquisition:** Tamara Beres Lederer Goldberg.

**Investigation:** Talita Domingues Caldeirão, Lilian Rodrigues Orsolini, Anapaula da Conceição Bisi Rizzo, Altamir Santos Teixeira, Tamara Beres Lederer Goldberg.

**Methodology:** Talita Domingues Caldeirão, Anapaula da Conceição Bisi Rizzo.

**Project administration:** Talita Domingues Caldeirão.

**Software:** Altamir Santos Teixeira, Hélio Rubens de Carvalho Nunes.

**Supervision:** Altamir Santos Teixeira, Tamara Beres Lederer Goldberg.

**Validation:** Carla Cristiane da Silva.

**Writing – original draft:** Talita Domingues Caldeirão, Lilian Rodrigues Orsolini, Carla Cristiane da Silva, Hélio Rubens de Carvalho Nunes, Tamara Beres Lederer Goldberg.

**Writing – review & editing:** Talita Domingues Caldeirão, Lilian Rodrigues Orsolini, Carla Cristiane da Silva, Hélio Rubens de Carvalho Nunes, Tamara Beres Lederer Goldberg.

## References

- Weaver CM, Gordon CM, Janz KF, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int*. 2016;27:1281–386.
- Herrmann M, Seibel MJ. The effects of hormonal contraceptives on bone turnover markers and bone health. *Clin Endocrinol (Oxf)* [Internet]. 2010;72:571–83.
- Fortes CMT, Goldberg TBL, Kurokawa CS, et al. Relationship between chronological and bone ages and pubertal stage of breasts with bone biomarkers and bone mineral density in adolescents. *J Pediatr (Rio J)* [Internet]. 2014;90:624–31.
- Lattakova M, Borovsky M, Payer J, et al. Oral contraception usage in relation to bone mineral density and bone turnover in adolescent girls. *Eur J Contracept Reprod Heal Care*. 2009;14:207–14.
- Rizzo A da CB, Goldberg TBL, BIASON TP, et al. One-year adolescent bone mineral density and bone formation marker changes through the use or lack of use of combined hormonal contraceptives. *J Pediatr (Rio J)* [Internet]. 2019;95:567–74.
- Lopez LM, Grimes DA, Schulz KF, et al. Steroidal contraceptives: effect on bone fractures in women. *Cochrane Database Syst Rev*. 2014;CD006033.
- Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis\*. *Endocr Rev* [Internet]. 2000;21:115–37.
- Jackowski SA, Baxter-Jones ADG, McLardy AJ, et al. The associations of exposure to combined hormonal contraceptive use on bone mineral content and areal bone mineral density accrual from adolescence to young adulthood: a longitudinal study. *Bone Reports* [Internet]. 2016;5:e333–41.
- Yilmaz D, Ersoy B, Bilgin E, et al. Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. *J Bone Miner Metab* [Internet]. 2005;23:476–82.
- Stanczyk FZ, Archer DF, Bhavnani BR. Ethinyl estradiol and 17 $\beta$ -estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. *Contraception* [Internet]. 2013;87:706–27.
- Creatas G. Estradiol – containing contraceptives. *Maturitas* [Internet]. 2015;81:119–20.
- Jelliffe EFP, Jelliffe DB. Anthropometry in action. I. Dental second year malnutrition. (Practical age-grouping in young children in areas without birth verification.). *J Trop Pediatr* [Internet]. 1968;14:71–4.
- Tanner JM. Growth at adolescence: with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity [Internet]. 2nd ed. Oxford: Blackwell Scientific Publications, Oxford. 1962.
- Reynolds E. Radiographic atlas of skeletal development of the hand and wrist. *Am J Phys Anthropol* [Internet] 1950;8:518–20
- Shuhart CR, Yeap SS, Anderson PA, et al. Executive summary of the 2019 ISCD position development conference on monitoring treatment, DXA cross-calibration and least significant change, spinal cord injury, peri-prosthetic and orthopedic bone health, transgender medicine, and pediatrics. *J Clin Densitom*. 2019;22:453–71.
- Vatanparast H & Whiting SJ. Adolescence, the optimum time to maximize bone mass through calcium & vitamin D. University of Saskatchewan, College of Pharmacy and Nutrition. The Whitehall-Robins Report 2005; 14.
- Polatti F, Perotti F, Filippa N, et al. Bone mass and long-term monophasic oral contraceptive treatment in young women. *Contraception* [Internet]. 1995;51:221–4.
- Tanner JM, Whitehouse RH, Marshall WA, , , et al. Prediction of adult height from height, bone age, and occurrence of menarche, at ages 4 to 16 with allowance for midparent height. *Arch Dis Child*. 1975;50:14–26.
- Cibula D, Skrenkova J, Hill M, et al. Low-dose estrogen combined oral contraceptives may negatively influence physiological bone mineral density acquisition during adolescence. *Eur J Endocrinol* [Internet]. 2012;166:1003–11.
- Goshgasebi A, Subotic Brajic T, Scholes D, et al. Adolescent use of combined hormonal contraception and peak bone mineral density accrual: a meta-analysis of international prospective controlled studies. *Clin Endocrinol (Oxf)*. 2019;90:517–24.
- Gersten J, Hsieh J, Weiss H, et al. Effect of extended 30  $\mu$ g ethinyl estradiol with continuous low-dose ethinyl estradiol and cyclic 20  $\mu$ g ethinyl estradiol oral contraception on adolescent bone density: a randomized trial. *J Pediatr Adolesc Gynecol* [Internet]. 2016;29:635–42.
- Ziglar S, Hunter TS. The effect of hormonal oral contraception on acquisition of peak bone mineral density of adolescents and young women. *J Pharm Pract* [Internet]. 2012;25:331–40.
- Brajic TS, Berger C, Schlammerl K, et al. Combined hormonal contraceptives use and bone mineral density changes in adolescent and young women in a prospective population-based Canada-wide observational study. *J Musculoskelet Neuronal Interact*. 2018;18:227–36.
- Ackerman KE, Singhal V, Baskaran C, et al. Oestrogen replacement improves bone mineral density in oligo-amenorrhoeic athletes: a randomised clinical trial. *Br J Sports Med*. 2019;53:229–36.
- Callegari ET, Gorelik A, Garland SM, et al. Bone turnover marker reference intervals in young females. *Ann Clin Biochem*. 2017;54:438–47.
- Callegari ET, Garland SM, Gorelik A, et al. Bone turnover marker determinants in young women: results from the Safe-D study. *Ann Clin Biochem*. 2018;55:328–40.
- Wolff RB, Teixeira Gomes RC, Verna C, et al. Aspectos moleculares dos esteroides sexuais sobre a cartilagem e os ossos. *Rev Assoc Med Bras* [Internet]. 2012;58:493–7.
- Hadji P, Colli E, Regidor P-A. Bone health in estrogen-free contraception. *Osteoporos Int* [Internet]. 2019;30:2391–400.
- Nappi C, Di Spiezo Sardo A, Greco E, et al. Effects of an oral contraceptive containing drospirenone on bone turnover and bone mineral density. *Obstet Gynecol*. 2005;105:53–60.
- Seifert-Klauss V, Prior JC. Progesterone and bone: actions promoting bone health in women. *J Osteoporos* [Internet]. 2010;2010:1–18.