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Oral contraceptives partially protect from bone loss in young women with anorexia nervosa

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Objective: To evaluate the potentially protective effects of oral contraceptives (OC) on bone loss in a large population of young women with anorexia nervosa (AN).

Design: Cross-sectional study.

Setting: University hospital.

Patient(s): Three hundred and five patients with AN (99 of them using OC) and 121 age-matched controls.

Intervention(s): None.

Main Outcome Measure(s): Areal bone mineral density (aBMD) evaluated by dual-energy X-ray absorptiometry and bone turnover markers, with leptin evaluated concomitantly.

Result(s): Although the AN patients taking OC presented lower aBMD compared with the controls at all bone sites, the whole body excepted, their aBMD values were systematically higher than those of AN patients who were not taking OC for the whole body and the lumbar spine, femoral neck, hip, and radius. These differences persisted after multiple adjustments. Preservation of aBMD improved with longer durations of OC use and shorter delays between disease onset and the start of OC. Moreover, patients with the lowest body mass index showed the best bone tissue responses to OC. Bone formation markers were systematically lower in the two groups of patients with AN compared with the controls. The markers of bone resorption were normalized in AN patients using OC.

Conclusion(s): Although OC use does not provide total protection of aBMD, our data suggest that OC might be prescribed for young women with AN to limit their bone loss.

Key Words: Anorexia nervosa, bone loss, bone remodeling markers, oral contraceptives

The chronic undernutrition observed in patients with anorexia nervosa (AN) is often associated with functional hypogonadotropic hypogonadism and subsequent primary or secondary amenorrhea (1–3) and low

estrogen levels (1, 3–5). A low body mass index (BMI) also contributes to estrogen deficiency because under the threshold of 15 kg/m² plasma estradiol is not detected (6). The alteration in the hypothalamopituitary–gonadal axis may nevertheless be transitory because weight regain in these patients is generally associated with a return of normal menstruation and increased estrogen levels (7).

In addition to gynecologic disorders, patients with AN also show notable bone loss with very specific disease-related kinetics characterized

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by early and intense demineralization at both cancellous bone such as the lumbar spine and cortical bone such as the proximal femur (2, 8–10). The changes can be detected in adolescent girls after only 6 to 12 months of disease (11), and osteoporosis can be diagnosed fewer than 24 months later (9, 12). Bone loss predisposes both adult and adolescent patients to an increased risk of fractures (13–16).

Various factors potentially implicated in bone loss in patients with AN have been described. They include endocrine and metabolic disturbances such as hypothyroidism–sick euthyroid syndrome (17), hypercortisolism (3, 18), and insulin-like growth factor I (IGF-I) deficiency (2, 9, 10) among others—as well as undernutrition effects due to calcium and vitamin D deficiency (2). In addition, a possible mechanism for this bone loss is hypogonadism, as hypogonadal states are characterized by low mineralization in young non-AN women (19), and the severity of the bone demineralization in these patients is positively correlated with the duration of the amenorrhea and negatively correlated with the duration of regular menses before amenorrhea (7, 9, 20). An alteration in normal estrogen secretion in the adolescent period may be particularly detrimental for bone mass acquisition, as demonstrated by the systematically greater areal bone mineral density (aBMD) deficiency in patients with primary amenorrhea than in patients with secondary amenorrhea and later disease onset (20). Moreover, adult women who develop AN during adolescence have lower aBMD than those who develop the condition in adulthood, even when the duration of amenorrhea is comparable (11).

Most clinical guidelines recommend that the investigations of functional hypothalamic amenorrhea should include an assessment of systemic and endocrine etiologies and that a multidisciplinary treatment approach is necessary, including medical, dietary, and mental health support (21). Weight gain and restoration of menstrual function appear to be the optimal strategy to recover bone mass (7, 22), but it is often insufficient in the context of a psychiatric illness.

The apparently central role of estrogen deficiency in bone demineralization has rightly oriented clinicians toward oral contraceptives (OC), notably because AN occurs within the first two decades of life, a period of rapid skeletal growth and aBMD acquisition that is estrogen dependent (19, 23). Disappointingly, however, both retrospective and prospective studies have shown that OC have no protective effect (4, 12, 18, 24, 25) or only a limited effect at specific bone sites, principally the lumbar spine (20, 26, 27). These divergent results have several explanations, including the duration, type, and administration mode of OC (12) and the disease severity, which can play an essential role in the response to OC (4). In addition, a survey reported that practitioners caring for adolescent females with AN commonly prescribe OC for the treatment of osteopenia independent of the evaluation of their bone mineral status (28). This strategy nevertheless remains controversial; the recent British national guidelines (29), for example, suggest hormone replacement therapy with 17 β -estradiol (with cyclic progesterone) rather than OC and only for young women with AN (13–17 years) with long-term low body

weight and low aBMD. This restriction is partially explained by the need for a better assessment of the benefit–risk ratio.

In this context, a more in-depth evaluation of this practice may improve the medical care of these young patients. Our study evaluated the potentially protective effects of OC on bone loss in a large population of young patients with AN.

MATERIALS AND METHODS

Study approval was obtained from the Regional Research Ethics Committee (Comité de Protection des Personnes Sud-Méditerranée IV, Montpellier, France; reference: 11 02 03), and permission for the clinical trials was granted by the French Agency for the Safety of Health Products (Agence Française de Sécurité Sanitaire des Produits de Santé; AFSSAPS; reference: 2011-A00108-33). Written informed consent was obtained from all participants and from their parents when the volunteers were minors.

Patients

A total of 426 adolescents and young women with ages ranging from 14.5 to 34.9 years were enrolled in this study, of whom 305 had a diagnosis of AN. The patients were consecutively recruited from the Endocrinology Department of Montpellier University Hospital in France from 2009 to 2016. They fulfilled the criteria for the diagnosis of restrictive AN as defined by the *DSM-IV: Diagnostic and Statistical Manual of Mental Disorders*: amenorrhea, BMI <18 kg/m², fear of gaining weight, and altered body size perception (30). A full description of the diagnostic procedure can be found elsewhere (31).

The control group was recruited from the community by advertisement. It consisted of 121 healthy normal-weight adolescents and young women with 18 <BMI<25 kg/m². None had a history of eating disorders or other psychiatric illness as determined by the SCOFF questionnaire (32) and the Mini International Neuropsychiatric Interview (33). All of the control group had normal menstrual cycles and no lifetime history of eating disorders. None of the participants was taking a medication known to affect bone metabolism, and none of them presented with primary amenorrhea.

Methods

This study followed a case-control design, which has been described in detail elsewhere (8–10). Briefly, standing height was measured with a stadiometer to the nearest 0.1 cm. Weight was determined using a weight scale with a precision of 0.1 kg. The BMI was calculated as weight (kg) divided by the square of height (m). The height standard deviation score (height SDS) and weight standard deviation score (weight SDS) were calculated according to the French standard curves.

Medical and Menstrual Histories

Each participant or her parents responded to a medical questionnaire designed to assess the general medical and menstrual history, with questions on the age of menarche and

the presence of menstrual disorders. Age of menarche, duration of the eating disorder, and menstrual function were determined for each participant. Moreover, data concerning OC use, age of disease onset, and duration of OC use were also collected. The time between AN onset and the start of OC use was calculated.

Assays

Blood samples (25 mL) were collected in the morning (08:30–10:00) in sterile, chilled tubes by the standard venipuncture technique. The samples were allowed to clot at room temperature and were then centrifuged at 2,500 rpm for 10 minutes at 4°C. Plasma samples were stored at –80°C until analysis. All samples were run in duplicate; to reduce interassay variation, all the plasma samples were analyzed in a single session. In the controls, the date of the last menses was not recorded, so hormone values were obtained at an unsynchronized menstrual stage.

Concerning bone metabolism, plasma samples were assayed by Cobas 6000 (Roche Diagnostic) for osteocalcin, procollagen type I N-terminal propeptide (PINP), and type I-C telopeptide breakdown products (CTX). The interassay and intra-assay coefficients of variation (CVs) for the latter three parameters were lower than 7%. The intra-assay and interassay CVs for leptin were, respectively, <5% and <7.6% (Mediagnost GmbH). For all the biological parameters, the CVs for the intra-assay and interassay variations were given by the manufacturer.

Bone Mineral Density, Body Fat, and Fat-free Soft Tissues

We used dual-energy X-ray absorptiometry (DXA, Hologic QDR-4500A; Hologic) to measure the areal bone mineral density (aBMD; g/cm²) of the whole body and at specific bone sites: the antero-posterior lumbar spine (L1–L4), the dominant arm radius, and the proximal part of the left femur (TPF). The soft tissue body composition (fat mass: FM, in kg), percentage of body fat mass (% FM) and fat-free soft tissue (FFST, in kg) was derived from the whole-body scan. All scanning and analyses were performed by the same operator to ensure consistency, after following standard quality control procedures.

Quality control for the DXA was checked daily by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (DPA/QDR-1; Hologic X-Caliber Anthropometrical Spine Phantom). The CVs provided by the manufacturer were 0.8% for spine and radius, 1.1% at the total proximal femur, and <1% for FFST and FM.

Statistical Analysis

The study population data are described with mean and standard deviation (SD) for quantitative variables and frequencies for qualitative variables. The continuous variable distributions were tested with the Shapiro-Wilk statistic. Quantitative variables were compared with the parametric Student's *t*-test when the distribution was Gaussian, and with the Mann-

Whitney test otherwise. For qualitative variables, groups were compared with the chi square or Fisher test.

To determine the potential effect of OC on the biochemical parameters and aBMD of patients, the patients taking OC (AN with OC) were compared with those who were not (AN without OC). To take into account the parameters known to influence aBMD (age, weight, FFST, and age of AN onset), linear regression adjusted on these factors was performed. In addition, patients using estrogen progestin and progestin alone were compared to determine the specific effect of OC types.

The effect of OC according to peripheral bone site (femoral neck, hip, lumbar spine, and radius) was assessed by linear modeling on a normalized centered value of aBMD adjusted on age, weight, FFST, and age of AN onset. The equality of the regression coefficients of contraceptive status was tested between the bone sites using the Wald test. For multiple comparisons, Holm's correction was applied.

Seeman et al. (20) suggested that the duration of OC use during AN may be more informative than the total duration of use. We thus divided the duration of OC use during AN into three periods according to its tertiles (>3 years, [1;3] years, [0;1] year and ≥ 3 years, [0.5;3] years, and ≤ 0.5 year) to determine the impact on each aBMD site using linear regression adjusted on age, weight, and age of AN onset. The same adjustments were used to assess the relationship between each aBMD site and the time between AN onset and the start of OC use. This time was divided into three classes (≥ 3 years, [0.5;3] years, and ≤ 0.5 year). An adjusted trend test determined whether the aBMD increased with the duration of OC use during AN and with the length of time between AN onset and the start of OC use.

To assess the relationship between each aBMD site and undernutrition as defined by BMI, depending on OC use, a linear model was used. As the BMI-OC status interaction was significant, the slope of aBMD (adjusted by age and age of AN onset) was estimated in each OC group. These results are illustrated with adjusted regression slopes (with 95% confidence intervals) according to BMI in each OC group. For pairwise comparisons between controls, AN with OC, and AN without OC, Holm's correction was used. Statistical significance was set at 0.05, and analyses were performed using SAS version 9.1 (SAS Institute).

RESULTS

When the control group ($n = 121$) and AN group ($n = 305$) were compared (data not shown), no difference in age was observed; however, as expected, the AN patients presented lower anthropometric characteristics than the controls in weight, body fat mass, body FFST, and aBMD at all sites evaluated (all $P < .001$). Moreover, the patients presented a higher value of CTX ($P < .001$), a marker of bone resorption, and lower values of osteocalcin and PINP ($P < .001$), two markers of bone formation, as well as leptin levels ($P < .001$).

The anthropometric characteristics of the controls taking OC ($n = 68$, 56%) were very close to those not taking OC ($n = 53$, 44%, data not shown), and the two groups differed only for age (22.3 ± 3.4 vs. 19.5 ± 4.6 years; $P < .001$) and bone turnover markers, with values systematically lower

TABLE 1

Clinical profiles of anorexia nervosa and control participants.

| Parameters | Controls | AN without OC | AN with OC | Difference between AN with OC and AN without OC |
|--|-------------|-------------------------|-------------------------|---|
| No. of patients | 121 | 206 | 99 | |
| Age, y | 21.1 ± 4.2 | 20.9 ± 5.3 | 22.4 ± 4.6 | < .01 |
| Anthropometric data | | | | |
| Weight, kg | 59.2 ± 7.6 | 42.5 ± 5.9 ^a | 44.6 ± 4.6 ^a | < .01 |
| Weight, SDS | 0.9 ± 1.2 | -1.8 ± 1.0 ^a | -1.4 ± 0.8 ^a | < .05 |
| Height, cm | 165.2 ± 6.0 | 164.2 ± 6.3 | 165.3 ± 5.5 | NS |
| Height, SDS | 0.5 ± 1.1 | 0.3 ± 1.2 | 0.5 ± 1.0 | NS |
| BMI, kg/m ² | 21.6 ± 2.3 | 15.8 ± 1.7 ^a | 16.3 ± 1.4 ^a | < .01 |
| WB fat mass, % | 27.8 ± 4.9 | 15.8 ± 5.2 ^a | 17.2 ± 5.8 ^a | < .05 |
| WB fat mass, kg | 16.6 ± 4.7 | 7.1 ± 3.3 ^a | 7.9 ± 3.1 ^a | < .05 |
| WB fat-free soft tissue, kg | 40.5 ± 4.4 | 34.3 ± 4.3 ^a | 35.3 ± 4.8 ^a | NS |
| Characteristics of AN | | | | |
| Age of AN onset, y | — | 16.9 ± 3.3 | 18.4 ± 4.1 | < .001 |
| Duration of AN, y | — | 4.0 ± 4.5 | 4.0 ± 3.9 | NS |
| Hyperactivities, n (%) | — | 58 (28.3) | 27 (27.3) | NS |
| Gynecologic profile | | | | |
| Age of menarche, y | 12.7 ± 1.4 | 12.9 ± 1.4 | 12.9 ± 1.8 | NS |
| Menstrual disorders, n (%) | — | 190 (88.8) | — | — |
| Duration of amenorrhea, mo | — | 25.3 ± 45.4 | — | — |
| Age of contraceptive start, y | 19.3 ± 3.3 | — | 19.0 ± 3.6 | — |
| Delay between AN onset and use of contraceptive, y | — | — | 0.6 ± 4.2 | — |
| Duration of contraceptive, y | 3.7 ± 2.8 | — | 3.7 ± 3.9 | — |

Note: Data are presented as mean ± standard deviation. AN = anorexia nervosa; NS = not statistically significant; OC = oral contraceptives; SDS = standard deviation score; WB = whole body. ^a Statistically significant difference between controls and either AN without OC or AN with OC for $P < .001$.

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($P < .001$) in those taking OC: 27.6 ± 9.5 vs. 41.5 ± 20.9 ng/mL for osteocalcin; 55.9 ± 27.9 vs. 124.1 ± 111.6 ng/mL for PINP; and 0.44 ± 0.15 vs. 0.63 ± 0.26 ng/mL for CTX. No effect of OC use on aBMD was demonstrated in the controls, thus justifying pooling the OC and without OC groups.

Effect of Oral Contraceptives

The subgroup analysis was conducted in the patients to compare AN with OC (n = 99) and AN without OC (n = 206) to determine the potential effect of OC use on bone tissue (Table 1 and Table 2). The AN patients taking OC were older and presented higher weight, BMI, and fat mass (% and kg) than those not taking OC. The duration of AN, the age of menarche, and the percentage of hyperactivities were comparable between the two AN groups, but the age of AN onset was older in patients taking OC (see Table 1).

The two patient groups systematically presented lower aBMD values in all bone sites, compared with the controls (see Table 2). Only the mean whole-body aBMD value did not differ between the AN with OC group and the controls. Age adjustment did not modify the difference between the groups. When an analysis was performed according to OC status (see Table 2), the AN with OC group presented systematically higher aBMD values for whole body, lumbar spine, femoral neck, hip, and radius than the AN without OC group. Except at the radius, all aBMD differences between the patient groups based on OC status persisted after age, weight, FFST, and age of AN onset adjustment (Table 3).

Concerning bone remodeling (see Table 2), the two patient groups presented lower mean values for bone formation

markers (osteocalcin and PINP), whereas the mean values for the bone resorption marker (CTX) were only higher in the AN patients without OC compared with the controls. The AN patient CTX and osteocalcin levels were statistically significantly lower for AN with OC than for AN without OC. Leptin levels were statistically significantly lower in both patient groups, with no difference based on OC status (see Table 2). Age (see Table 2) or age and BMI adjustment (not shown) did not modify the difference between the AN groups for these parameters (OC, CTX, and leptin), except for PINP for which the difference was no longer statistically significant after adjustment.

Effect of the Type of Contraceptive

Among the AN patients taking OC, 84 used estrogen-progestin and 15 used progestin alone. The seven patients who were using a hormone intrauterine device were not analyzed. No statistically significant differences were observed between estrogen-progestin and progestin-alone for age, age of disease onset, the duration of OC, anthropometric or gynecologic parameters, disease specificities, or aBMD, which allowed us to pool the two OC groups. Only the PINP ($P = .07$) and CTX ($P = .10$) values tended to differ between the OC groups.

Different Modality Effects (aBMD Site, Duration of AN and OC Use, Time Between AN Onset and Start of OC Use, and Severity of AN) of OC on aBMD

After adjusting for age, weight, FFST, and age of AN onset, OC had the same effect on all peripheral bone sites (see Table 3).

TABLE 2

Areal bone mineral density and biochemical parameters in controls and anorexia nervosa patients according to contraceptive use adjusted or not by age.

| Parameters | Controls | AN without OC | AN with OC | Difference between AN with OC and AN without OC |
|------------------------------------|---------------|----------------------------|----------------------------|---|
| aBMD (g/cm ²) | | | | |
| Whole body | 1.070 ± 0.074 | 1.023 ± 0.087 ^a | 1.065 ± 0.081 | < .001 |
| Lumbar spine | 0.983 ± 0.106 | 0.853 ± 0.118 ^a | 0.908 ± 0.108 ^a | < .001 |
| Femoral neck | 0.846 ± 0.108 | 0.731 ± 0.134 ^a | 0.777 ± 0.106 ^a | < .01 |
| Hip | 0.944 ± 0.143 | 0.792 ± 0.139 ^a | 0.844 ± 0.106 ^a | < .01 |
| Radius | 0.547 ± 0.035 | 0.520 ± 0.047 ^a | 0.533 ± 0.042 ^b | < .05 |
| Adjusted aBMD (g/cm ²) | | | | |
| Whole body | 1.070 ± 0.007 | 1.023 ± 0.006 ^a | 1.067 ± 0.008 | < .001 |
| Lumbar spine | 0.982 ± 0.010 | 0.852 ± 0.008 ^a | 0.911 ± 0.011 ^a | < .001 |
| Femoral neck | 0.844 ± 0.011 | 0.729 ± 0.008 ^a | 0.785 ± 0.012 ^a | < .001 |
| Hip | 0.943 ± 0.012 | 0.790 ± 0.009 ^a | 0.852 ± 0.013 ^a | < .001 |
| Radius | 0.547 ± 0.004 | 0.520 ± 0.003 ^a | 0.532 ± 0.004 ^b | < .05 |
| Biological parameters | | | | |
| CTX, ng/mL | 0.530 ± 0.228 | 0.881 ± 0.540 ^a | 0.576 ± 0.267 | < .001 |
| PINP, ng/mL | 88.9 ± 86.8 | 61.9 ± 34.9 ^b | 48.2 ± 38.7 ^a | < .001 |
| OC, ng/mL | 34.4 ± 17.4 | 24.2 ± 10.9 ^a | 20.0 ± 12.1 ^a | < .001 |
| Leptin, ng/mL | 9.9 ± 5.7 | 1.4 ± 1.7 ^a | 1.7 ± 2.2 ^a | NS |
| Adjusted biological parameters | | | | |
| CTX, ng/mL | 0.518 ± 0.041 | 0.874 ± 0.030 ^a | 0.604 ± 0.043 | < .001 |
| PINP, ng/mL | 87.7 ± 5.4 | 61.3 ± 4.0 ^a | 50.9 ± 5.8 ^a | NS |
| OC, ng/mL | 34.1 ± 1.3 | 24.0 ± 0.9 ^a | 20.6 ± 1.4 ^a | < .05 |
| Leptin, ng/mL | 9.8 ± 0.4 | 1.4 ± 0.4 ^a | 1.8 ± 0.6 ^a | NS |

Note: Data are presented as mean ± standard deviation and mean ± standard error of the mean when data are adjusted by age. aBMD = areal bone mineral density; AN = anorexia nervosa; CTX = type I-C telopeptide breakdown products; OC = oral contraceptives; PINP = procollagen type I N-terminal propeptide.

^a Statistically significant difference between controls and either AN without OC or AN with OC for $P < .001$.

^b Statistically significant difference between controls and either AN without OC or AN with OC for $P < .05$.

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Figure 1 shows that, after adjustment for age, weight and age of AN onset, aBMD increased with the lengthening duration of OC use during AN (subdivided in tertiles) at whole body, lumbar spine, femoral neck, and hip. Moreover, aBMD adjusted for age, weight, and age of AN onset increased at whole body, femoral neck, and hip with shorter times between AN onset and the start of OC use. A similar tendency was observed at the lumbar spine ($P = .08$) but not at the radius (Supplemental Fig. 1, available online).

Supplemental Figure 2 (available online) shows the relationship between each aBMD site and BMI adjusted for age

and age of AN onset in patients with and without OC. In the AN without OC group, aBMD at whole body and femoral neck (not shown) was positively and strongly associated with BMI ($P < .01$ and $P < .001$, respectively); in the AN with OC group, aBMD remained stable whatever the BMI ($P = .70$ and $P = .52$, respectively). The same observation was made at the hip (without OC, $P < .01$; with OC, $P = .29$), although the aBMD variability was greater and the difference between the two slopes of the linear regression was less marked between patients with or without OC (test of interaction, $P = .16$). At the lumbar spine, aBMD decreased with BMI

TABLE 3

Effect of oral contraceptives on areal bone mineral density according to the contraceptive status and bone site.

| Bone sites | AN without OC ^a | AN with OC ^a | Contraceptive effect ^b | Comparison of contraceptive effect between each bone site ^c |
|--------------|----------------------------|-------------------------|-----------------------------------|--|
| Whole body | 1.029 ± 0.005 | 1.05 ± 0.008 | 0.027 ± 0.010 ^d | — |
| Lumbar spine | 0.859 ± 0.007 | 0.896 ± 0.011 | 0.037 ± 0.013 ^d | NS |
| Femoral neck | 0.736 ± 0.008 | 0.768 ± 0.011 | 0.032 ± 0.014 ^e | NS |
| Hip | 0.797 ± 0.008 | 0.836 ± 0.011 | 0.038 ± 0.014 ^d | NS |
| Radius | 0.523 ± 0.003 | 0.528 ± 0.004 | 0.005 ± 0.005 | NS |

Note: AN = anorexia nervosa; OC = oral contraceptives; NS = not statistically significant.

^a Data are presented as adjusted mean (on age, weight, fat-free soft tissue, and age of AN onset) ± standard error of the mean.

^b Data are presented as mean difference (mean adjusted AN with OC – mean adjusted AN without OC) ± SD_{Mean difference}.

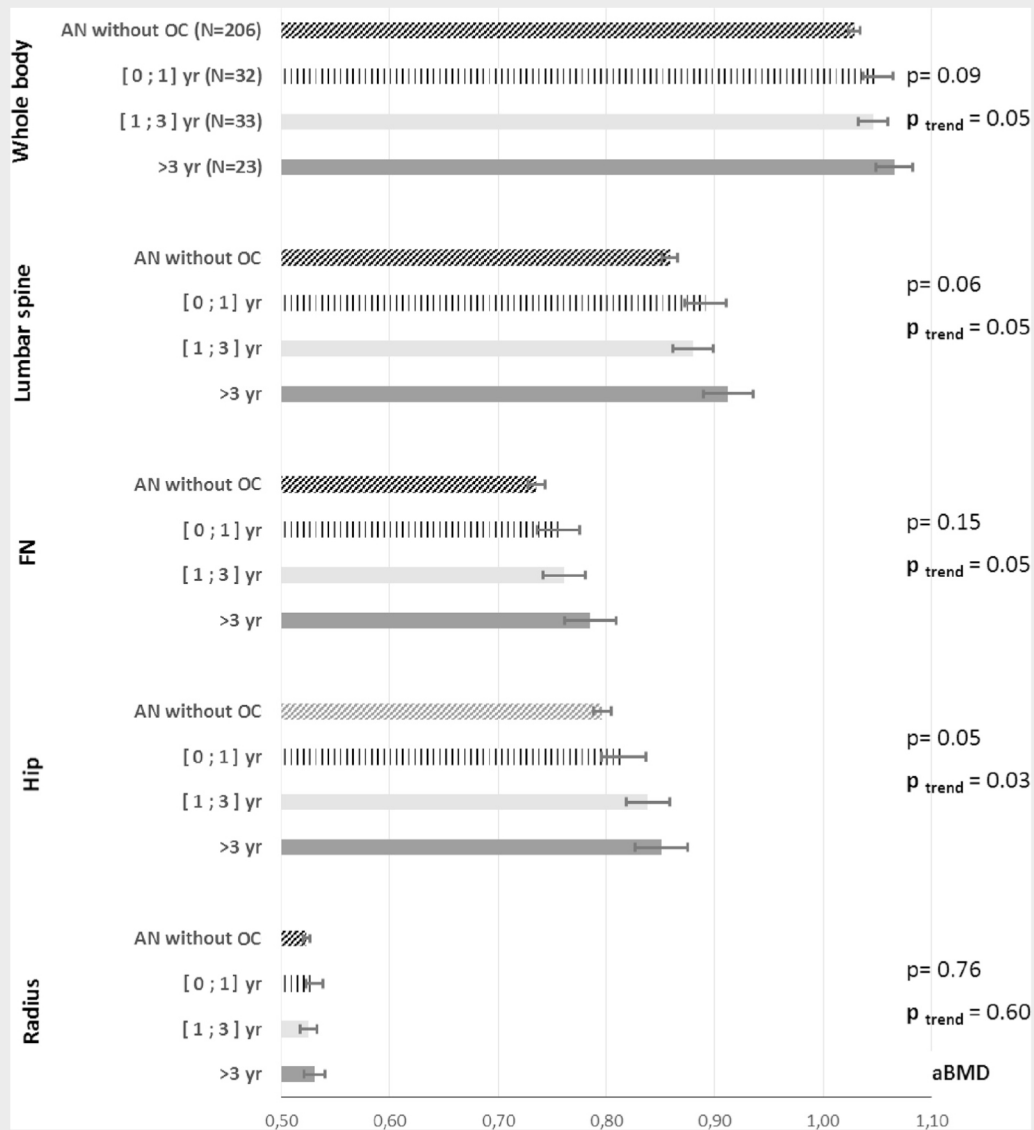
^c Pairwise comparisons of regression coefficients between each peripheral bone site using the Wald test (Holm's correction). Contraceptive adjusted regression coefficient evaluated by modeling normalized centered value of areal bone mineral density.

^d Statistically significant mean difference for $P < .01$.

^e Statistically significant mean difference for $P < .05$.

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FIGURE 1



In patients, areal bone mineral density adjusted on age, weight, and age of anorexia nervosa (AN) onset according to the duration of contraceptive use during anorexia nervosa. Data are presented as mean areal bone mineral density (aBMD, g/cm²) adjusted \pm standard error of the mean. P trend = trend test adjusted; FN = femoral neck; OC = oral contraceptives.

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similarly in both groups (no difference between the two slopes, test of interaction $P = .41$), but the AN with OC group had a higher mean aBMD than the AN without OC group ($P < .01$). At the radius, a negative association between BMI and aBMD was observed only for the AN without OC group ($P < .01$).

DISCUSSION

This study revealed for the first time that the AN patients in routine clinical practice who used OC had higher aBMD compared with the patients who were not using OC. Our data further showed that aBMD preservation improved with longer durations of OC use and shorter delays between disease

onset and the start of OC. We also observed that patients with the more severe forms of AN, defined by a lower BMI, showed the best bone tissue responses.

The initial acute stage of AN and its protracted course usually occur during puberty and late adolescence, covering the most important period for bone mass acquisition (23). In this study, patients presented with reduced aBMD values, ranging from -4.2% at the radius to -14.3% at the hip compared with the controls. This deficient bone mass acquisition is quite worrisome because peak bone mass is known to be an important prognostic factor of osteoporosis in aged subjects (34). It therefore requires medical care specifically adapted to the life period of these patients, who are still growing and of reproductive age.

We focused on the potential effects of OC on bone metabolism in these patients, as this type of treatment has become fairly widespread but its effects are a source of considerable debate (4, 12, 18, 20, 24–27). Moreover, when a favorable effect of OC on bone tissue has been found, the effects were limited and were evaluated in a small number of patients (20, 26, 27). A strength of our study is therefore the large sample size of patients treated with OC ($n = 99$), in addition to the extensive analysis of bone metabolism, with measurements of localized aBMD and bone remodeling markers. We found that OC had a protective effect on bone tissue in routine clinical practice. This was demonstrated by the higher values of aBMD in patients with AN using OC compared with the nonusing patients. This favorable effect persisted after multiple adjustments for confounding variables that influence bone mass such as age, weight, FFST, and age of AN onset.

The difference in aBMD between the two AN groups may have been due to the various mechanisms of action on bone cell activities, but this aspect of contraceptives has been poorly investigated (18, 25, 27, 35–37). Our results demonstrated that although AN causes substantial modifications in bone remodeling, defined by higher resorption activity and lower formation activity, the patients using OC presented normal resorption activity but an accentuated reduction in formation activity, thus displaying a specific bone remodeling profile tending toward a suppression of bone turnover. Our results confirmed that the effects of estrogens on bone metabolism are inhibitory for resorption while decreasing bone formation (38–40). In fact, the bone biochemical markers in AN patients appear more sensitive than DXA for detecting an OC effect on the skeleton (25, 35), and analyzing them may be useful for evaluating treatment compliance.

In this context, it should be noted that observational (35) and randomized clinical trials using different molecules (4, 24, 25) have all failed to show any significant benefit of contraceptives on aBMD in patients with AN. However, the number of studied patients was relatively limited, resulting in low statistical power. Moreover, it is likely that the treatment duration was too limited (12–18 months) because we found that, although aBMD increased with the duration of contraceptive use during AN, the highest aBMD gain was observed in patients with the longest duration of use (i.e., >3 years). Similarly, Karlsson et al. (26) reported that although no normalization was observed, the volumetric BMD in patients using OC for over 2 years was greater than that in women treated for 1 to 2 years, and their values were similar to women recovered from AN. In physiologic conditions as well, although the recovery of the menstrual cycle and thus normal estrogen secretion was found to induce a significant gain in bone mass at the lumbar spine and hip, it did not allow normalization after 10 to 24 months (7, 18, 22). These data suggest a delayed bone tissue response to estrogen, but it is interesting to note that these specific sex hormone effects appear to be independent of the effects of nutritional factors (41).

It is also likely that treatment efficacy is dependent on the degree of disease severity, such as the weight at initiation of

substitution (4). Klibanski et al. (4) reported that treated patients with the lowest percentage of ideal body weight (<70% of their ideal weight) presented an increase in aBMD ($4.0 \pm 8.8\%$), whereas nontreated patients presented a significant loss ($20.1 \pm 16.2\%$). However, only the lumbar spine was analyzed, and the authors did not propose any explanation for this discrepancy in response according to BMI.

Also, we included a very wide spectrum of BMIs our study, including very underweight AN patients with BMI $\sim 9.3 \text{ kg/m}^2$, and we evaluated several bone sites. Thus, we report for the first time that, in contrast to our observations in patients not using OC, the aBMD for the whole body, femoral neck, and hip in patients using OC was not influenced by BMI. This suggests that the protective effect of contraceptives on the skeleton may be greatest in the most severe patients at greatest risk of developing low bone mass. This encouraging result was not observed at the lumbar spine, as patients using contraceptives always presented higher aBMD at this bone site, whatever their BMI. The response of bone tissue to contraceptives based on disease severity has been incompletely evaluated, and no effects based on BMI were found in a study including a limited number of AN patients (24).

The favorable effect of contraceptives does not seem to totally offset the effect of AN on bone tissue, however, because patients taking contraceptives systematically showed a lower aBMD than the control participants of similar age. There are several explanations. First, the duration of OC use was shorter than the disease duration, suggesting a period when bone tissue was not protected. Our study suggests that the shorter the time span between the onset of AN and the start of OC use, the greater the aBMD preservation will be, which favors early treatment prescription. In addition, a better protective effect might be achieved if the OC prescription were started right after diagnosis, rather than after the occurrence of secondary amenorrhea, which is the case in routine clinical practice. The problem here is that secondary amenorrhea is a clinical sign that reflects a certain degree of disease severity and probably bone loss that is underway. In this context, Audi et al. (7) reported osteopenia at the lumbar spine after relatively brief durations of amenorrhea in some adolescent patients. Misra et al. (27) demonstrated a similar bone mass gain in AN patients using OC compared with controls, but a normalized aBMD over time was not observed, suggesting that the initial bone loss was not compensated.

A second explanation is that the mode of administration may also affect the drug efficacy. Therefore, although encouraging, the favorable effects of OC observed in this case-control study will need to be confirmed in future randomized controlled trials. In particular, a study that prospectively enrolls adolescent patients with AN to receive OC or transdermal estradiol is needed, as the latter has been found to be a more physiologic form of estrogen replacement and more promising than oral administration in managing osteopenia in adolescents with AN (27, 42).

Third, as osteopenia is much more severe in AN patients compared with normal-weight women with hypothalamic amenorrhea and an equivalent degree of estrogen deficiency (43), it is probable that its genesis in AN is multifactorial and

that other bone mineralization regulators—including a bone mass peak before disease onset, physical activity, and genetic background—should be considered (7, 20). Moreover, a low estrogen level may be one endocrine alteration among other nutritionally mediated changes, including the dramatic alteration in the growth hormone–IGF-I system that results in lower IGF-I levels, as observed previously elsewhere (1, 2, 7, 9, 10, 35). Insulin-like growth factor I has been described as one of the principal regulators of bone metabolism in both normal-weight women (44) and AN patients (35). The OC effect of decreasing the bone loss due to estrogen deficiency may therefore not be sufficient to compensate the bone loss due to other hormonal factors, such as low IGF-I or high cortisol levels (3). Studies have also suggested that OC may have an indirect negative action on bone metabolism by suppressing the liver production of IGF-I and reducing IGF-I bioavailability by altering hepatic IGF-binding proteins (45, 46) in the normal-weight population. These data may partially explain the accentuated reduction in bone-formation markers (osteocalcin and PINP) in the patients with OC compared with the patients without OC observed in our study.

Finally, Karlsson et al. (26) suggested that malnutrition may account for reduced bone size, whereas estrogen deficiency may account for reduced volumetric BMD (a measurement of BMD independent of bone size). A final point is that the OC dose may have been too low for young women just attaining their peak bone mass, who may require the higher daily doses that are used in postmenopausal replacement therapy to protect bone tissue (24, 47).

Our results should not be taken as an encouragement to clinicians to rely solely on this treatment to protect bone, but they should encourage continued vigilance in monitoring these patients for variations in bone mass. Moreover, because estrogen seems to reduce bone resorption activity, additional treatment should target the increase in bone formation activity. This might include nutritional supplementation (i.e., proteins) or mechanical approaches (i.e., physical activity), possibly combined with drugs such as recombinant human IGF-I (1) to fully preserve bone tissue in a period of intense bone mass acquisition.

Dehydroepiandrosterone (DHEA) may be another efficient agent in treating adolescents with AN. According to the findings of DiVasta et al. (48), the combined administration of adrenal (DHEA: 50 mg/day) and gonadal (20 µg ethinyl estradiol/0.1 mg levonorgestrel) hormone replacement improves bone density and bone strength. As previously suggested elsewhere (24, 28), however, the decision to prescribe OC is not without drawbacks. For example, the return of menstrual function that indicates adequate weight restoration is masked by the withdrawal bleeding induced by the contraceptives. Also, OC use may provide a sense of reassurance that patients are protected against osteopenia, which can reduce efforts at weight rehabilitation.

Our study presents some limitations. In particular, the inherent biases of the case-control study design and the different treatments and doses received by the patients limit the conclusions that can be drawn. Nor were all endocrine factors potentially involved in bone loss, such as cortisol or

thyroid hormones, extensively investigated in all study patients. However, the high number of patients included in the AN with OC and AN without OC groups counterbalances these limitations and shows a positive effect of hormone substitution. Although the two AN groups showed similar disease characteristics, such as the duration of AN, they differed in terms of minor parameters such as age, weight, FFST, and age at AN onset, which may potentially interfere with bone mass (20). It is worth noting that multiple adjustments on these covariables did not modify the differences between the groups. In addition, the evaluation of OC use in a setting of current medical practice is a strong point of this study because it reinforces the potential utility of this therapy to partially protect bone tissue in young AN patients, who are seen routinely in our clinical practice.

CONCLUSION

In this study, a cluster of arguments converges to encourage the prescription of contraceptives to limit bone loss in young patients with AN, particularly in those presenting as extremely underweight. However, as contraceptive use does not provide total protection of bone mass, our results should not be taken as an encouragement to clinicians to rely solely on this treatment to protect bone. Instead, they should encourage continued vigilance in monitoring these patients for variations in bone mass. New therapeutic approaches must be developed in association of contraceptives to obtain a normalization of the bone mass in these patients.

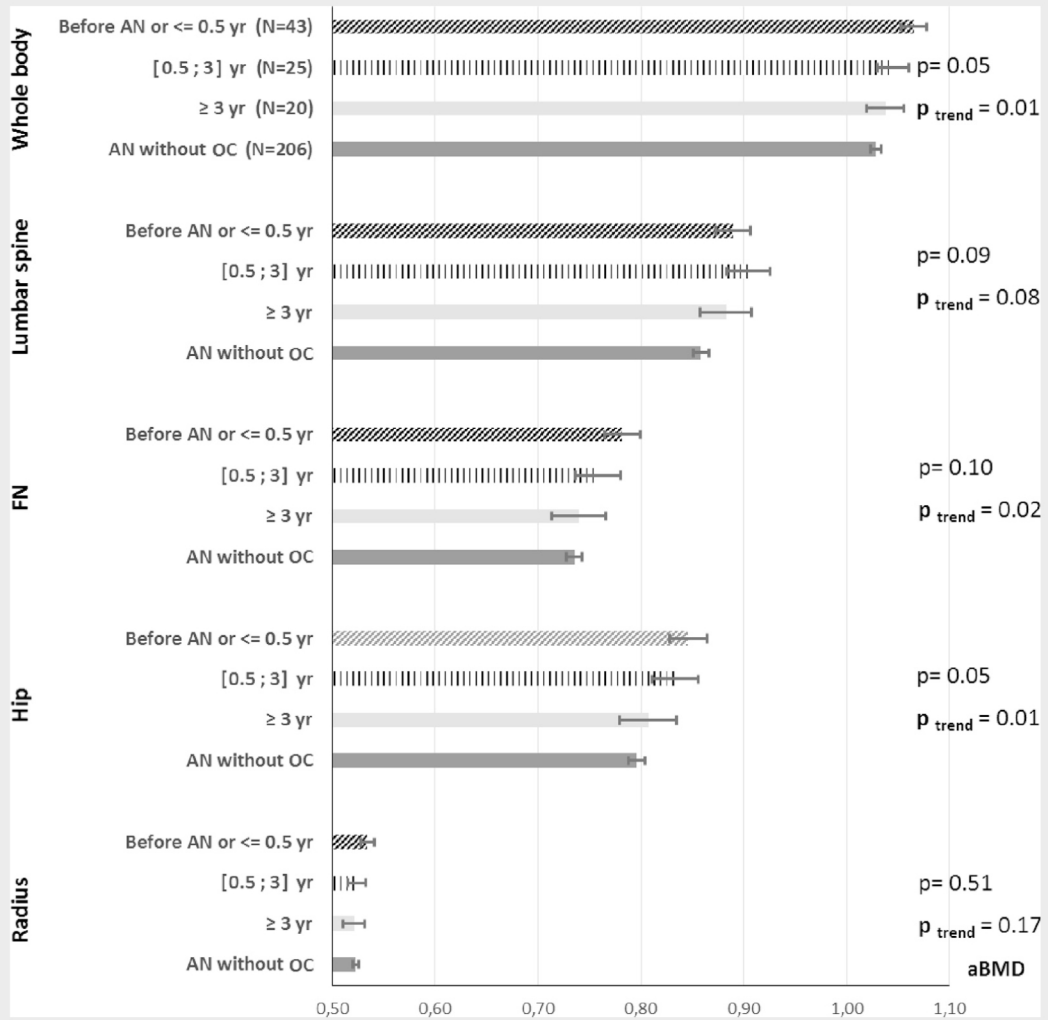
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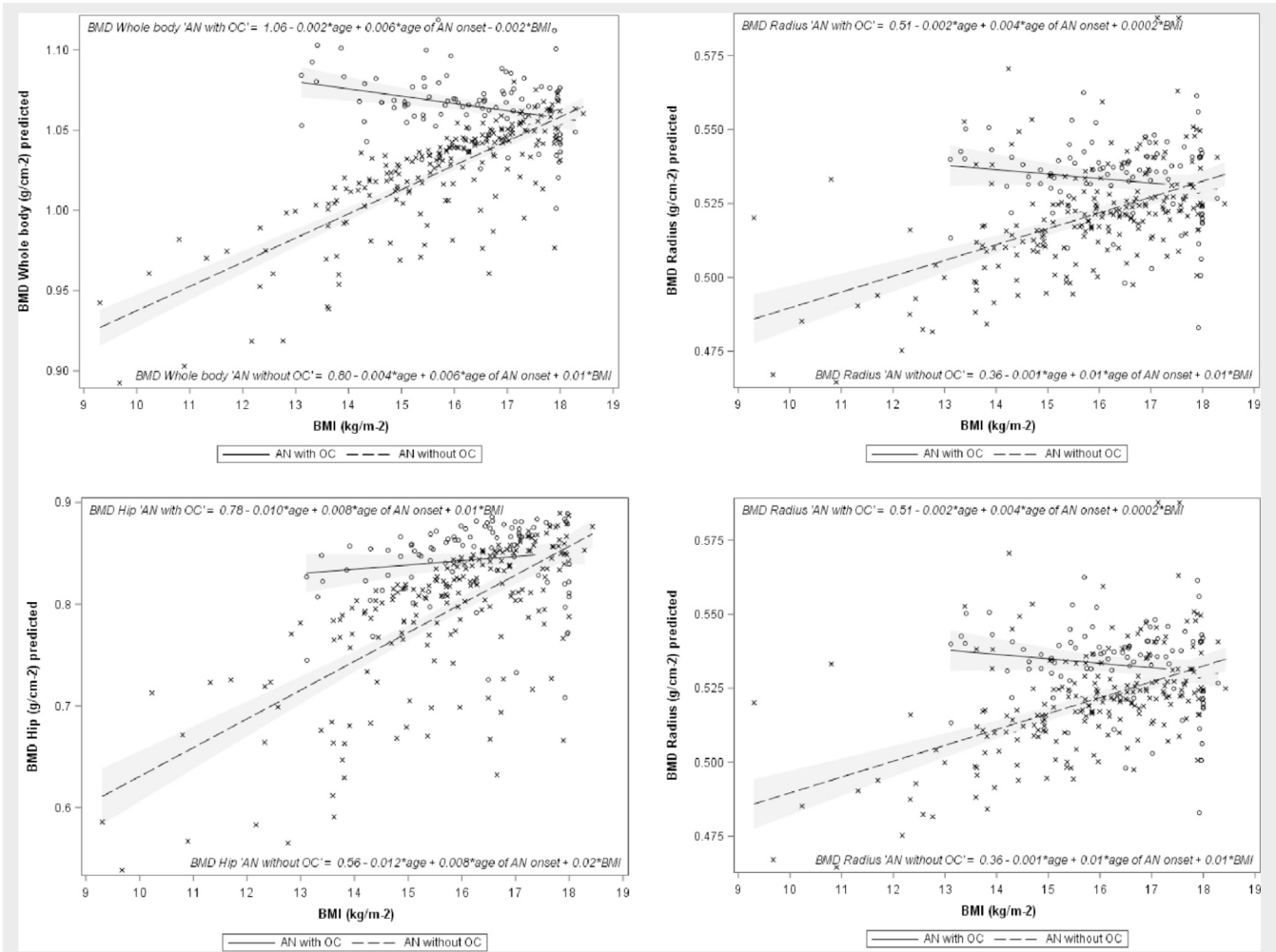
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SUPPLEMENTAL FIGURE 1



In patients, areal bone mineral density (aBMD) adjusted on age, weight, and age of AN onset according to the delay between anorexia nervosa (AN) onset and the start of the contraceptive use. Data are presented as mean aBMD (g/cm²) adjusted ± standard error of the mean. *P* trend = trend test adjusted; FN = femoral neck; OC = oral contraceptives.

SUPPLEMENTAL FIGURE 2



Relationship between each areal bone mineral density (aBMD) site and body mass index according to the oral contraceptive (OC) status. The dashed curve represents the 95% confidence interval of the adjusted mean. AN = anorexia nervosa.