

Bone mineral density in well-trained females with different hormonal profiles

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Summary

Objective: The association between sex hormones and bone mineral density (BMD) has been studied in sedentary women, whereas only few studies have evaluated trained females. Therefore, the aim of this study was to assess the influence of sex hormones on BMD in well-trained females with different hormonal profiles: eumenorrheic females, oral contraceptive (OC) users and postmenopausal women. The secondary purpose was to determine if maximal oxygen consumption ($\dot{V}O_{2max}$) or maximal back squat strength (1RM) could be good predictors of BMD in this population.

Methods: Sixty-eight eumenorrheic, forty-one monophasic-OC users and sixteen postmenopausal well-trained females participated in this study. A Dual-energy X-ray Absorptiometry scan (DXA), a basal blood sample and a maximal back squat and/or a maximal treadmill test were performed. In order to measure all volunteers under similar hormonal conditions (low sex hormone levels), all tests were carried out during the early follicular phase for the eumenorrheic females and in the withdrawal phase for the OC group.

Results: One way ANCOVA reported lower values of BMD in postmenopausal ($1.13 \pm 0.07 \text{ g/cm}^2$) than in eumenorrheic ($1.19 \pm 0.08 \text{ g/cm}^2$) ($p=0.003$) and OC users ($1.17 \pm 0.07 \text{ g/cm}^2$) ($p=0.030$). Pearson's correlation showed a positive relationship between BMD and 1RM ($p<0.001$), but not with $\dot{V}O_{2max}$.

Conclusions: Lower BMD has been reported in postmenopausal women compared to both, eumenorrheic females and OC users. BMD loss after menopause seems to be not fully compensated by exercise, but this could effectively mitigate it. Moreover, 1RM back squat reported a slight association to BMD. Hence, strength training may be the best choice to prevent BMD loss.

Key words:

17 β -estradiol. Progesterone. Oral contraception. Exercise. Postmenopause. Eumenorrheic.

Densidad mineral ósea en mujeres entrenadas con diferente perfil hormonal

Resumen

Objetivo: La asociación entre hormonas sexuales y densidad mineral ósea (DMO) ha sido bastante estudiada en mujeres sedentarias, pero no en mujeres entrenadas. Por tanto, el objetivo de este estudio fue analizar la influencia de las hormonas sexuales en la DMO de deportistas con diferentes perfiles hormonales: mujeres eumenorreicas, usuarias de la píldora anti-conceptiva y mujeres postmenopáusicas. El segundo objetivo fue analizar si el consumo máximo de oxígeno ($\dot{V}O_{2max}$) o la sentadilla trasera (1RM) serían buenos predictores de DMO en dicha población.

Metodología: Sesenta y seis mujeres eumenorreicas, cuarenta y una usuaria de píldora monofásica y dieciséis mujeres postmenopáusicas bien entrenadas participaron en el estudio. Una densitometría ósea (DXA), una analítica basal y una prueba de esfuerzo y/o de 1RM en sentadilla trasera fueron llevados a cabo. Con el objetivo de que todas las voluntarias fueran medidas bajo las mismas condiciones (bajos niveles de hormonas sexuales), todas las pruebas fueron realizadas en la fase folicular temprana para las mujeres eumenorreicas y en la fase no hormonal para las usuarias de píldora.

Resultados: ANCOVA de una vía mostró valores de DMO más bajos en mujeres postmenopáusicas ($1,13 \pm 0,07 \text{ g/cm}^2$) comparado con las eumenorreicas ($1,19 \pm 0,08 \text{ g/cm}^2$) ($p=0,003$) y las usuarias de píldora ($1,17 \pm 0,07 \text{ g/cm}^2$) ($p=0,030$). La correlación de Pearson mostró una relación positiva entre DMO y sentadilla ($p<0,001$), pero no mostró asociación con el $\dot{V}O_{2max}$.

Conclusión: Las mujeres postmenopáusicas presentan valores de DMO más bajo que las mujeres eumenorreicas y las usuarias de píldora. El descenso de DMO tras la menopausia parece no ser completamente compensado por la práctica de actividad física, aunque ésta puede atenuar ese descenso. Además, la sentadilla mostró una ligera asociación positiva con la DMO, por lo que el entrenamiento de fuerza podría ser la mejor opción para prevenir el descenso de DMO.

Palabras clave:

17 β -estradiol. Progesterona. Píldora anticonceptiva. Ejercicio. Postmenopausia. Eumenorrea.

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Introduction

Osteoporosis is a skeletal disease represented by low bone mineral density (BMD) due to an imbalance between rates of bone formation and bone resorption. BMD homeostasis depends on two bone cells: osteoblasts (which stimulate bone formation) and osteoclasts (which stimulate bone resorption)¹. Osteoclasts also produce a glycoprotein called sclerostin, which inhibits bone formation²⁻⁴. The activity of these two cells is affected by many factors such as pregnancies, tobacco, calcium intake, 17 β -estradiol (E2) levels, age, oral contraceptive (OC) use and physical activity⁵.

Sex hormones, specifically E2, play a key role in bone growth. These sex hormones are essential for the maintenance of bone tissue, since E2 decrease osteoclasts formation and generation as well as stimulate their apoptosis⁶⁻⁹. In short, E2 suppress bone resorption and the production of sclerostin by inhibiting the osteoclasts activity. Moving on to the osteoblasts, in the last years some studies have proved the positive effect E2 has over these cells. It seems that these sex hormones stimulate osteoblasts activity, encouraging bone formation¹⁰⁻¹². Although the role of the progesterone on BMD metabolism is still unclear¹³, it seems to have, together with E2, complementary bone action such as preserving peak bone mass and preventing pre- and perimenopausal bone loss¹⁴. Despite osteoporosis can also occur in young individuals, is most common in elderly population⁵, mainly due to the loss of the ovarian function and the decrease in sex hormones¹. The drop in E2 produces an imbalance in bone formation and resorption, accelerating bone loss during the first years of the menopause¹⁵.

Furthermore, the use of OC pills has been widespread among females in the last few years, inducing a reduction of endogenous hormones production in this population. Depending on the dosages of exogenous sex hormones (ethinyl estradiol and progestin) presented in the contraceptive formulations, bone tissue metabolism might be affected¹⁶. Studies related to OC and BMD are still inconclusive possibly because of the differences in studies design, formulations and time of use of OC, different methods for measuring BMD and population characteristics¹⁶.

Exercise is advocated to be one of the best tools to increase bone mass and prevent its loss in elderly^{17,18}. Physical and functional performance has been positive correlated with BMD as well as with the maximal oxygen consumption ($\dot{V}O_{2max}$) and strength¹⁹⁻²³, hence BMD may be associated with these two performance variables. Hence, it seems that increasing bone mass before the postmenopause it's a good way to prevent osteoporosis in elderly females²⁴. In fact, an increase of 10% of bone tissue during the adulthood may reduce fracture risk in half in the future²⁵.

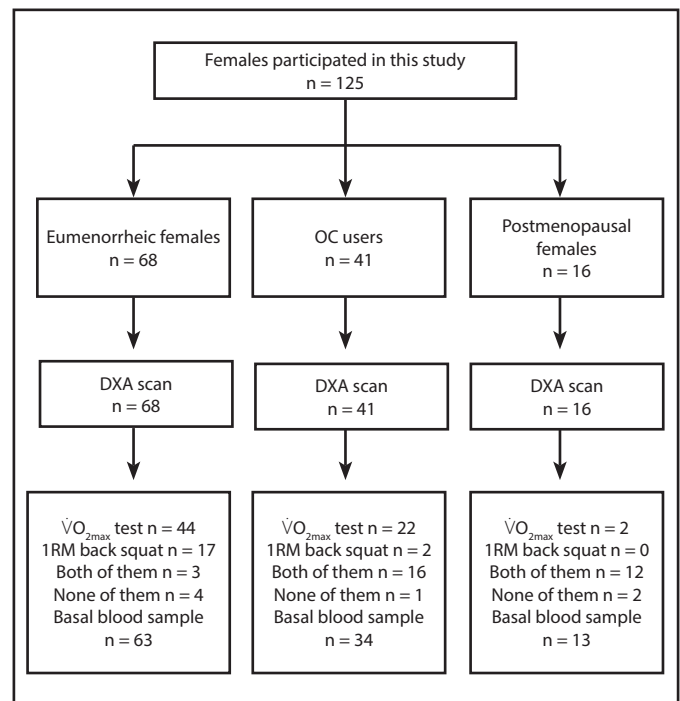
Taking into account all the data aforementioned, we hypothesized that, in active population, OC users may have lower values of BMD regarding eumenorrheic females whereas postmenopausal athletes might have similar values of BMD to eumenorrheic. Thus, the aim of this study was to analyse the influence of sex hormone concentration on BMD in female athletes, comparing three different hormonal profiles: eumenorrheic, monophasic OC users and postmenopausal female athletes. Furthermore, a secondary objective of the present study was to determine if $\dot{V}O_{2max}$ and maximal squat strength are good predictors of BMD in this population.

Material and method

Participants

Sixty-eight eumenorrheic females (26-32 days cycles length), forty-one low dose monophasic OC users (4.13 \pm 3.83 years intaking them) and sixteen postmenopausal females (at least one year without menstruation) participated in this study. Volunteers characteristics are shown in Table 1 and the flow chart for participation is shown in Figure 1. At the start of the data collection, all participants conducted a questionnaire gathering information about training status, health conditions, dietary supplements consumption and type of OC pills when appropriate. Brands and formulation of OC pills used were: Cecilia (n=3): ethinyl estradiol 0.03 mg and dienogest 2 mg; Drosure (n=2): ethinyl estradiol 0.03 mg and drospirenone 3 mg; Yasmin (n=9): ethinyl estradiol 0.03 mg and drospirenone 3 mg; Loette (n=4): ethinyl estradiol 0.02 mg and levonorgestrel 0.1 mg; Levobel (n=2): ethinyl estradiol 0.02 and levonorgestrel 0.1; Diane (n=4): ethinyl estradiol 0.035 mg and cyproterone 2 mg; Edelsin (n=1): ethinyl estradiol 0.035 and Norgestimate 0.25 mg; Drosbelallex (n=2): ethinyl estradiol 0.02 mg and Drospirenone 3 mg; Melodene (n=2): ethinyl estradiol 0.015 mg and gestodene 0.06 mg; Linelle (n=3): ethinyl estradiol 0.02 mg and levonorgestrel 0.1 mg; Stada (n=1): ethinyl estradiol 0.02 mg and drospirenone 3 mg; Sibilla (n=3): ethinyl estradiol 0.03 mg and dienogest 2 mg. Thereby, exogenous sex hormones concentration mean for the OC group was 0.03 \pm 0.01 mg/day of ethinyl estradiol and 1.79 \pm 1.28 mg/day of progestin. All of them were well-trained in endurance and/or in strength training (1.31 \pm 0.41 hours per session, 3.9 \pm 1.1 sessions per week with 7.65 \pm 5.15 years of

Figure 1. Flow chart with the sample we had for each test.



OC: oral contraceptive; DXA: Dual-energy X-ray Absorptiometry scan; $\dot{V}O_{2max}$: maximal oxygen consumption; 1RM back squat: back squat maximal strength.

Table 1. Characteristics of the study population.

	Eumenorrhic		OC users		Postmenopausal		p
	Mean±SD	n	Mean±SD	n	Mean±SD	n	
Age (yr)	32.90±10.22	68	26.48±4.74	48	51.71±3.69	16	0.000 [¶]
Height (cm)	163.76±5.96	68	163.01±5.94	48	160.97±5.31	16	0.233
Weight (kg)	59.25±9.54	68	58.23±5.95	48	56.08±8.32	16	0.386
BMI (kg/m ²)	22.09±3.25	68	21.92±2	48	21.7±3.33	16	0.866
$\dot{V}O_{2max}$ (ml/kg/min)	49.69±4.18	47	48.80±5.73	38	46.01±98	14	0.076
1RM back squat (kg)	74.55±16.73	20	66.83±15.24	18	50.33±4.19	13	0.000 [§]
FSH (mIU/mL)	8.03±3.65	63	5.24±4.53	34	76.73±47.69	13	0.000 [§]
LH (mIU/mL)	6.22±2.58	63	3.44±3.17	34	41.74±21.14	13	0.000 [§]
E2 (pg/mL)	48.59±34.55	63	26.47±27.45	34	19.97±26.13	13	0.001 [¶]
Progesterone (ng/mL)	0.46±0.72	63	0.28±0.17	34	0.21±0.17	13	0.169

BMI: body mass index; $\dot{V}O_{2max}$: maximal oxygen consumption; 1RM: maximal back squat strength; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: 17 β -estradiol.

[¶]Significant differences between all groups ($p < 0.001$).

[§]Significant differences in postmenopausal regarding eumenorrhic females and OC users ($p < 0.001$).

[¶]Significant differences between eumenorrhic females and OC users ($p < 0.05$).

experience for eumenorrhic females; 1.39±2.08 hours per session, 3.68±1.15 sessions per week with 6.57±4.48 years of experience for the OC group; 1.17±0.31 hours per session, 3.9±1.16 sessions per week with 7.9±3.31 years of experience for postmenopausal women). Females with metabolic pathologies, hormonal disorders, smoking habits, intaking supplementation or with injuries/surgeries in the last 6 months were excluded from this study. To be included in the study participants were required to be healthy adult females, without iron deficiency anemia (serum ferritin <20 μ g/l, hemoglobin <115 μ g/l and transferrin saturation <16%), non-pregnant or oophorectomized, not to consume medication that alters vascular function (e.g., tricyclic antidepressants, α -blockers, β -blockers, etc.) and they had to perform endurance and 7or strength training between 3 and 12 hours per week. All participants were informed about the procedures and risks involved and an informed consent was obtained from each participant. The experimental protocol was approved by the ethical Committee of the Universidad Politécnicade Madrid and it is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki)²⁶ (Figure 1).

Procedures

Volunteers first came to the laboratory to perform a Dual-energy X-ray Absorptiometry scan (DXA) to evaluate body composition, and a basal blood sample to discard possible diseases. After 60-90 min of ad-libitum meal, volunteers performed a 1RM back squat test (20 eumenorrhic females, 18 OC users and 12 postmenopausal) or a maximal treadmill test (47 eumenorrhic females, 38 OC users and 14 postmenopausal) to determine their 1RM back squat and their maximal $\dot{V}O_2$. Some volunteers performed both tests, conducting them in different menstrual cycles. In order to measure all groups under similar hormonal environment (low sex hormone levels), all these tests were carried out during the early follicular phase (between the 2nd and 5th day of the menstrual cycle, being the onset of the cycle the first day of menstrual bleeding) for the eumenorrhic females and in the withdrawal phase (between de 3rd and the 7th day of the placebo week) for the OC group.

Dual-energy X-ray Absorptiometry scan

A DXA scan (Version 6.10.029GE Encore 2002, GE Lunar Prodigy; GE Healthcare, Madison, WI, USA) was done between 8-10 am in fasting state to obtain the whole BMD. The scan was calibrated per two days using the phantom supplied by the manufacturer. All volunteers performed the test in underwear, with their body and hands in a supine position and their feet joined by a tape. During the measurements, moving and talking were forbidden. DXA scan was always carried out by the same researcher.

Maximal treadmill test

After a warm-up of 3 min walking on the treadmill at 6 km/h, the maximal running test started at 8km/h increasing its speed 0.2 km/h each 12 seconds until exhaustion. The test was carried out with a computerized treadmill (H/P/COSMOS 3PW 4.0; H/P/COSMOS Sports & Medical, Nussdorf-Traunstein, Germany). $\dot{V}O_2$ was measured with the gas analyser Jaeger Oxycon Pro (Erich Jaeger; Viasys Healthcare, Hoechberg, Germany).

1RM back squat test

The 1RM in the back squat exercise was determined by using the PowerliftApp²⁷. Participants performed a 5-min cycle-ergometer warm-up and some mobility and dynamic stretching exercises. Then, volunteers performed 4 sets of 1 rep with submaximal loads proportionally increased between 70% and 90% of participants' maximum reported. A box was set just under participants tights in order to fix the point where tights were parallel to floor.

Sex hormones analysis

Basal blood samples were taken between 8-10 am in fasting state to ensure that females were healthy and without hormonal disorders. Samples were obtained by venipuncture into a vacutainer containing clot activator. Following the inversion and clotting, the blood was

centrifuged (Biosan LMC-300 version V.5AD) for 10 min at 3000 rpm and transferred into Eppendorf tubes and stored frozen at -80°C until further analysis. Then, the serum samples were delivered to the clinical laboratory to determine sex hormones and verify menstrual cycle phase. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), E2 and progesterone were measured via ADIVA Centaur® solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens city, Germany). Coefficients of variation reported by the laboratory were 7.74 for FSH, 10.77 for LH, 7.84 for E2 and 14.11 for progesterone.

Statistical analysis

All data are reported as means ± SD. One way ANCOVA was performed for comparisons among groups and age was used as a covariable. Scheffé test was applied to examine the pairwise comparison of each significant fixed factor. Pearson's correlation was performed to verify the association between BMD and $\dot{V}O_{2max}$ 1RM back squat and sex hormones concentrations. All tests were conducted with a 5% significance level. All data were initially tested for normality with the Kolmogorov-Smirnov test. Statistical analyses were performed using SPSS software for windows, version 20.1 (IBM corp., Armonk, NY, USA).

Secondly, Cohen effects sizes (ES) were calculated to verify the magnitude of the mean differences between menstrual phases. The ES were interpreted based on the following criteria: <0.2 = trivial, 0.2 to 0.6 = small effect, 0.6 to 1.2 = moderate effect, 1.2 to 2.0 = large effect, and >2.0 = very large²⁸. The 90% confidence interval (CI) was also calculated. Magnitude Based Inferences were carried out to determine the beneficial, trivial or harmful effect, of the menstrual cycle phases. When a clear interpretation was possible, a qualitative inference was given as follows: 0.5% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possibly; 75% to 95%, likely; 95% to 99.5%, very likely; and >99.5%, most likely²⁹.

Results

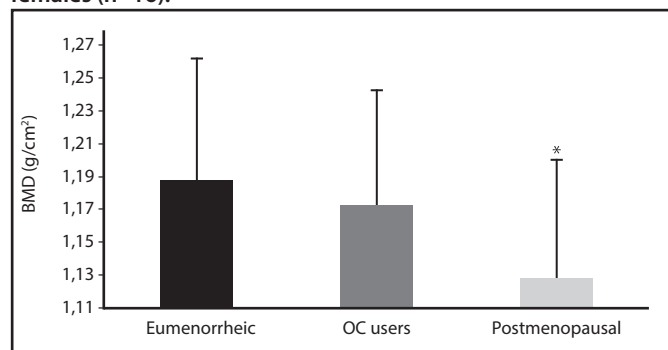
The one-way ANCOVA showed, as expected, significant differences between groups in age ($F_{2,122}=55.202$) and weight ($F_{2,121}=4.339$), having the postmenopausal females lower values in comparison with the eumenorrhic group ($p=0.013$) and OC users ($p=0.023$). Nonetheless, no significant differences were reported for $\dot{V}O_{2max}$ ($F_{2,95}=1.742$) and 1RM back squat ($F_{2,46}=2.706$) among groups. In accordance with sex hormones, significant differences were found for FSH ($F_{2,106}=62.064$) and LH ($F_{2,106}=82.820$), where postmenopausal females presented higher values than eumenorrhic and OC users ($p<0.001$ for all comparisons). Moreover, E2 levels ($F_{2,104}=7.344$) in eumenorrhic were significantly

higher than in OC users ($p=0.006$); whereas, not significant differences were reported for progesterone ($F_{2,106}=1.705$) among groups (Table 1).

Significant differences were observed for BMD ($F_{2,121}=5.708$; $p=0.004$) among different hormonal profiles (Figure 2). Scheffé test reported lower values of BMD in postmenopausal females in relation to the eumenorrhic ($p=0.003$) and to the OC ($p=0.030$) group. Effect sizes and magnitude-based inferences for BMD among groups are shown in Table 2. A small and unclear effect for eumenorrhic and OC users was found; whereas a large and likely effect was reported when comparing postmenopausal females with eumenorrhic and OC users.

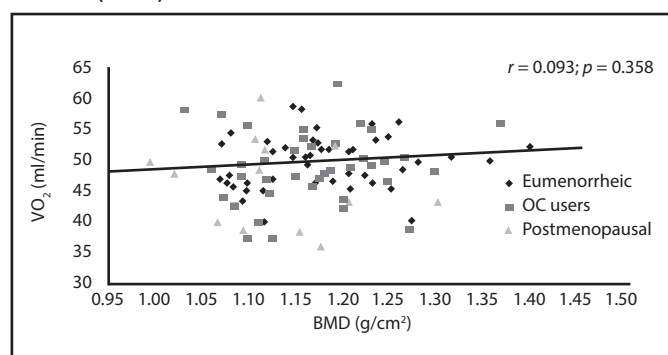
Pearson's correlation did not show significant association between BMD and $\dot{V}O_{2max}$ (Figure 3), whereas a positive relationship between BMD and 1RM was found ($r=0.446$; $p=0.001$) (Figure 4). Regarding sex hormones,

Figure 2. BMD comparisons among different hormonal profiles: eumenorrhic (n=68), OC users (n=41) and postmenopausal females (n=16).



* Significant differences regarding eumenorrhic females ($p<0.05$).

Figure 3. Relationship between BMD and $\dot{V}O_{2max}$ in well-trained females (n=99).



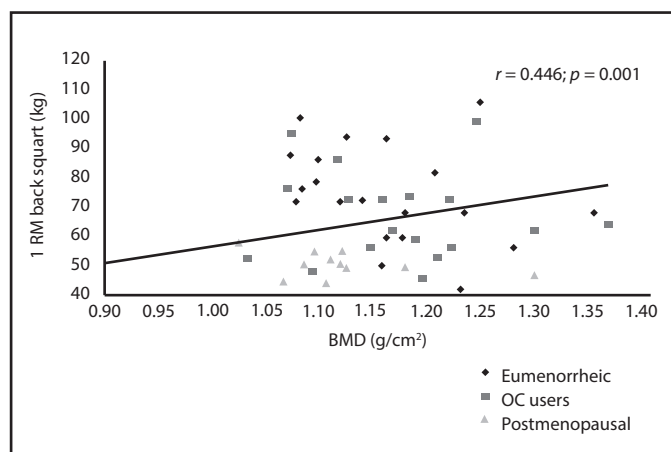
BMD: bone mineral density; $\dot{V}O_{2max}$: maximal oxygen consumption.

Table 2. Pairwise comparison for BMD. Results expressed as effect size and magnitude based inference.

Variable	Pairwise comparisons	ES (90% CI)	Chances of being negative/trivial/ positive	Qualitative inference
BMD (g/cm ²)	OC users vs Eumenorrhic	-0.22 (-2.29, 1.85)	31.4/17.9/50.8	Unclear
	Postmenopausal vs Eumenorrhic	-1.27 (-2.75, 0.21)	2.6/3.2/94.2	Likely
	Postmenopausal vs OC users	-1.19 (-2.90, 0.53)	4.4/4.8/90.8	Likely

BMD: bone mineral density

Figure 4. Relationship between BMD and maximal back squat strength in well-trained females (n=50).



BMD: bone mineral density; 1RM: 1RM back squat.

no significant association was found when correlating BMD with LH ($r=-0.186$; $p=0.052$), E2 ($r=0.063$; $p=0.518$) and progesterone ($r=0.054$; $p=0.574$). The FSH hormone reported a light negative correlation with BMD ($r=-0.240$; $p=0.012$).

Discussion

The aim of this study was to compare three different hormonal profiles (eumenorrheic, OC users and postmenopausal) to observe the influence of sex hormones on BMD in well-trained females. The main finding was that postmenopausal females have lower values of BMD compared with the eumenorrheic women and there is a light association between maximal back squat strength values and BMD.

Regarding BMD differences when comparing eumenorrheic and postmenopausal females, our results are in accordance with previous literature which reported a decrease in BMD because of the fall of plasmatic E2 in the elderly³⁰⁻³². However, it is worth mentioning that previous studies carried out with sedentary healthy females³³⁻³⁵ showed decreases of BMD higher than those seen in our female athletes. In this line, the World Health Organization (WHO) showed that BMD loss in lumbar spine and femur in women, ranged from 10% to 20% in elderly³³. These percentages were similar to the ones reported by Martin *et al.*³⁴. Additionally, another research studying the whole body revealed a 20% reduction in bone mass at the age of 60 years old compared with the group between 20-40 years old³⁵. In contrast, our results showed a difference of 5% on BMD comparing eumenorrheic with postmenopausal women. These differences between our results and previous studies might be explained by the positive effect that exercise exerts on bone mass, since exercise is well known as a good method for preserving bone tissue³⁶⁻³⁸.

According to young females the positive effect of OC use pointed out by some previous studies^{30,39-41} has not been confirmed in the present investigation, since no differences were found between eumenorrheic and OC users. Our findings, however, are in accordance

with others new findings that reported no differences in BMD with the use of OC pills^{16,42-48}. The dosages of sex hormone concentrations could explain this discrepancy. Currently, ethinyl estradiol and progestin levels in OC pills are lower than they used to be in the past (e.g. ethinyl estradiol concentration was 150 mg/day but today is 15 mg/day; progestin concentration was 9.85 mg/day but today is 0.35 mg/day)⁴⁹. This could be determinant when comparing BMD, since sex hormones, specially ethinyl estradiol, play an important role in bone metabolism. On the one hand, ethinyl estradiol decrease osteoclasts formation and generation as well as stimulate their apoptosis⁶⁻⁹. Hence, ethinyl estradiol suppress bone resorption by inhibiting the osteoclasts activity. On the other hand, recent studies showed that ethinyl estradiol may stimulate osteoblasts activity, encouraging bone formation¹⁰⁻¹². Moreover, the time of OC use should be considered, since the period reported in the studies is different: 6 months¹⁶, 12 months⁴⁷, 24 months⁴³, 36 months³⁰ or even 7 years⁴⁶. Finally the only study found with athletes (rowers) didn't study BMD but bone metabolism markers⁵⁰. All metabolic markers studied, osteocalcin (bone formation) and type I carboxyterminal telopeptide (ICTP) (bone resorption), were lower in OC users compared with eumenorrheic females which could suggest that no differences in BMD may exist between groups.

Another objective of the present study was to determine if $\dot{V}O_{2max}$ and maximal back squat strength are good predictors of BMD in this population. Our results didn't show significant association between BMD and $\dot{V}O_{2max}$, whereas a positive correlation between BMD and 1RM was found. Although previous literature reported a strong positive correlation between BMD and maximal $\dot{V}O_{2max}$ in sedentary females^{48,51-53}, our data did not support these findings. These differences might be explained by the training status of the samples. Our volunteers were physically active so they all have high levels of $\dot{V}O_{2max}$ and BMD, whereas previous literature has been carried out with sedentary females^{48,51-53}. This could have led to a spurious correlation, since so the women with higher levels of $\dot{V}O_{2max}$ might be those more active, and therefore with more frequent stimuli for BMD increase as well. So that, $\dot{V}O_{2max}$ could not be a good predictor of BMD when studying active population since training status could be a confounding variable. However, regarding 1RM back squat, a positive correlation with BMD was found. This result confirmed the strong association between BMD and muscle strength previously documented in female athletes⁵⁴⁻⁵⁸ as well as the good predictor that muscle strength is for BMD recorded in advance with healthy females^{20,21,59,60}. Thus, not only might strength levels be determinant for BMD in sedentary population but also in active people. This association could be explained by the fact that the greater the muscle mass, the higher the forces exerted by the tendons over the bones. So that, bones have to resist these mechanical forces, which makes them become stronger⁶¹.

Finally, the association between sex hormones and BMD were not significant for any but for FSH, where a negative correlation was observed. On the basis of the hormones released by the anterior pituitary, negative correlations between LH and BMD as well as FSH and BMD were reported in previous studies carried out with healthy Chinese women^{62,63}, there foreign line with our results. The negative correlation found between FSH and BMD could be explained by the fact that this sex hormone could stimulate osteoclasts and induce immune cells to

excrete TNF- α promoting bone resorption⁶². Indeed, another study confirmed that FSH is related to bone turnover indicators⁶⁴ and the risk of osteoporosis⁶³. Moving on to the ovarian sex hormones, the critical role of E2 in bone metabolism is consistent with several previous reports⁶⁻¹² as well as progesterone, which preserves peak bone mass and prevents bone loss¹⁴. Despite previous studies reported a positive association between E2 and BMD^{65,66} as well as between progesterone and BMD⁶⁷ in sedentary females, our findings didn't show these correlations. These discrepancies may be due to population characteristics, since previous researches were carried out with sedentary women, whereas the current study with female athletes. It is well known that exercise is one of the best ways for increasing bone mass and preventing its loss^{17,18}. Thus, although sex hormones play a key role in bone metabolism, their influence becomes less crucial in trained females, since exercise is an important positive factor. Furthermore, a recent study carried out with healthy Chinese women, reported no association between E2 and BMD, suggesting that decreases in BMD in the elderly is associated with the increased of FSH and LH levels, rather than the decreased of E2⁶².

The current study attempts to address a gap in the research through investigation of an important variable like BMD in well-trained females. The strengths of our study included the inclusion of different female hormonal profiles and the recruitment of a homogenous group of active and healthy women for all of them: eumenorrheic females, OC users and postmenopausal women. However, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes throughout life span. Finally, a limitation of this study could be not have taken into account the direct relation between FSH and E2, hence the increase in FSH after menopause is related to the absence of E2. Thus, it would have been interesting to evaluate how long have postmenopausal women been in menopause.

The present study showed lower BMD in postmenopausal than eumenorrheic in spite of the regular practice of exercise. Nonetheless, it's worth mentioning that previous studies carried out with sedentary healthy females showed decreases of BMD higher than those seen in our well-trained postmenopausal females. Differences between study results might be explained by the positive effect that exercise could exert on bone mass. Therefore, the BMD loss after menopause seems to be not fully compensated by exercise, but this could effectively mitigate it during this stage. Interestingly, maximal oxygen consumption did not correlate to BMD in this population; while 1RM back squat reported a slight association to BMD. Hence, strength training may be the best choice to prevent BMD loss.

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Conflict of interest

The authors do not declare a conflict of interest.

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