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ORIGINAL

BODY COMPOSITION IN PREMENOPAUSAL AND POSTMENOPAUSAL WELL-TRAINED FEMALES

COMPOSICIÓN CORPORAL EN MUJERES DEPORTISTAS PREMENOPÁUSICAS Y POSTMENOPÁUSICAS

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ABSTRACT

Purpose: The aim was to analyse the influence of sex hormones on body composition in well-trained females with different hormonal environments.

Methods: Sixty-six eumenorrheic, forty-one low-dose-monophasic oral contraceptive users and sixteen postmenopausal well-trained females participated in this study. Volunteers underwent a Dual-energy X-ray

Absorptiometry scan (DXA) and a bioimpedance during the early-follicular and the withdrawal phase, verified with blood samples.

Results: ANCOVA test reported no differences neither in DXA measurements (weight, fat free mass, fat mass, android and gynoid fat mass) nor in bioimpedance variables (weight, fat free mass, fat mass and total body water) among study groups.

Conclusion: Sex hormones seems not to influence body composition in active women. Curiously, premenopausal and postmenopausal active women present the same fat mass distribution. It could be explained by the positive effect exercise has on body composition, and this in turn on preventing cardiovascular and metabolic diseases in this population.

KEY WORDS: Eumenorrheic, oral contraceptive, sex hormones, fat mass, fat free mass, exercise.

RESUMEN

Objetivo: El propósito fue analizar la influencia de las hormonas sexuales en la composición corporal de deportistas con diferente estatus hormonal.

Metodología: 46 mujeres eumenorreicas, 41 usuarias de píldora anticonceptiva monofásica y 16 mujeres postmenopáusicas bien entrenadas participaron en el estudio. Las voluntarias realizaron un Dual-energy X-ray Absorptiometry scan (DXA) y una bioimpedancia durante la fase folicular temprana y no hormonal, verificado con una analítica.

Resultados: La prueba ANCOVA no mostró diferencias ni en las variables medidas con DXA (peso, masa grasa androide y ginoide, masa grasa total y masa libre de grasa) ni en las de bioimpedancia (peso, masa grasa, masa libre de grasa y agua corporal total).

Conclusión: Las hormonas sexuales parecen no influir en la composición corporal de mujeres activas. Las mujeres postmenopáusicas activas presentan una distribución de masa grasa similar a las premenopáusicas, lo que podría explicarse por el efecto positivo de la actividad física.

PALABRAS CLAVE: Eumenorreicas, píldora anticonceptiva, hormonas sexuales, masa grasa, masa libre de grasa, ejercicio.

INTRODUCTION

Endogenous sex hormones (17 β -estradiol and progesterone) play a key factor in female reproductive system, but they also have an important role influencing lipid metabolism, body fat distribution and skeletal muscle in women. This is because of the presence of sex hormones receptors in non-reproductive tissues such as hypothalamus, cardiovascular system (1), kidney tubules, liver, skeletal muscle and adipose tissue (2).

Sex hormones, specifically estradiol, increase lipoprotein lipase action, stimulating lipid oxidation process and decreasing carbohydrate oxidation (3). However, more than the individual effect of the estradiol is estradiol and

progesterone interaction what seems to be crucial. Indeed, the increase of estradiol/progesterone ratio might be important in determining the final effect of these sex hormones on fat metabolism (4). These hormones are also important for the maintenance of skeletal muscle, promoting not only muscle growth but also its regeneration (5). In accordance with body fluid regulation, sex hormones play a crucial role influencing water retention in females. Due to the presence of estradiol receptors in the hypothalamus nuclei, where an important hormone involved in the regulation of renal water is produced; arginine vasopressin (AVP), this sex hormone may cause shifts in body fluid regulation (1). Moreover, high levels of progesterone might stimulate water retention as well, because of the increase of aldosterone hormone, hence this sex hormone increases aldosterone production (hormone which acts on kidney tubes stimulating Na^+ resorption and K^+ excretion) (6).

All these physiological effects caused by endogenous sex hormones might be affected in those females who are using oral contraceptive (OC) pills. In the last decades, the use of OC pills has been widespread among females, inducing a reduction of endogenous hormones production in this population due to the intake of exogenous ones (ethinyl estradiol and progestin) (7). In accordance with exogenous sex hormones, there is less knowledge about the effects of its administration on females' physiology. It has been suggested that ethinyl estradiol has mineralocorticoid actions, which activates renin-angiotensin-aldosterone system encouraging Na^+ and fluid retention, whereas progestin has anti-mineralocorticoid actions which antagonizes the effect of Na^+ and fluid retention (8-10). On the contrary, other studies reported that ethinyl estradiol and progestin administration increase plasma volume and even the combination of both exogenous sex hormones causes the greatest increase (11). Furthermore, a recent review concluded that ethinyl estradiol administration could inhibit lipolysis process (12). Thereby, due to the different hormonal environment presented in OC users regarding eumenorrheic females, it is speculated that differences in females' physiology may exist between both groups. Postmenopausal females might also have a different behavior due to the loss of the ovarian function and therefore their decrease in sex hormones concentration (13). Aging is associated with a decrease in fluid volume (14), a drop in lean body mass and an increase in fatty tissue, specially android fat mass (15-17), and this in turn rises metabolic and cardiovascular risks after menopause (18). However, it seems that these postmenopausal effects could be attenuated by physical activity (19).

According to the current literature, it appears that sex hormones concentrations affect substrate metabolism during exercise (increasing lipids oxidation in OC users and reducing it in postmenopausal women) (4). Nevertheless, it still remains unclear how these changes in females' metabolism during exercise could affect body composition in these population when training frequently. Although the influence of sex hormones on body fluid regulation and lipid metabolism has been recently studied in sedentary healthy females (15, 20, 21), it remains unclear how this influence could modify body water (TBW), fat mass (FM) or fat free mass (FFM) in well-trained females.

OBJECTIVES

The aim of this study is to analyse the influence of sex hormones concentration on body composition variables in well-trained females, comparing three different hormonal profiles: eumenorrheic females, low dose monophasic OC users and postmenopausal women.

METHODS

Subjects

The present work is an observational cross-sectional study performed by sixty-six eumenorrheic females (32.9 ± 10.2 years; 163.7 ± 5.9 cm), forty-one low dose monophasic OC users (26.5 ± 4.7 years; 163.1 ± 5.9 cm) and sixteen postmenopausal women (51.7 ± 3.7 years; 160.9 ± 5.3 cm) participated in this study. Brands and formulation of OC pills used were: Cecilia® (n=4): 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=5): ethinyl estradiol 0.02 mg and levonorgestrel 0.1 mg; Levobel® (n=3): ethinyl estradiol 0.02 and levonorgestrel 0.1; Diane® (n=5): ethinyl estradiol 0.035 mg and cyproterone 2 mg; Edelsin® (n=2): 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. All of them were well-trained in endurance and/or in strength training: (i) 1.3 ± 0.4 hours per session, 3.9 ± 1.1 sessions per week with 7.7 ± 5.2 years of experience for eumenorrheic females; (ii) 1.4 ± 2.1 hours per session, 3.7 ± 1.2 sessions per week with 6.6 ± 4.5 years of experience for the OC group; (iii) 1.2 ± 0.3 hours per session, 3.9 ± 1.2 sessions per week with 7.9 ± 3.3 years of experience for postmenopausal women. Running, obstacle races, crossfit and triathlon were the sport activities they trained. Exclusion criteria included smoking, thyroid problems, medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, α -blockers, β -blockers, etc.), pregnancy and ovariectomy. At the start of the data collection, all participants conducted a questionnaire gathering information about training experience, health status, the absence of dietary supplements consumption and type of OC pills when appropriate. An informed consent was obtained from each participant with all the information about the procedures and risks involved. The experimental protocol was approved by the ethical Committee of the Universidad Politécnica de Madrid, with DEP2016 code, and it is in accordance with The Code of Ethics of the World Medical Association (22).

Experimental protocol

Body composition tests were carried out under similar hormonal environments for all groups (low sex hormonal levels): 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 eumenorrheic females, in the withdrawal phase (between the 3rd and the 7th day of the placebo week) for the OC group and at any time for postmenopausal women. Volunteers performed, in different days, a Dual-energy X-ray Absorptiometry scan (DXA) and a bioimpedance between 8-10 am following the standard recommendations (23). During the first day, DXA test was carried out by all participants. Nonetheless, due to the drop out of 21 volunteers, only fifty-six eumenorrheic females, thirty-five OC users and thirteen postmenopausal females did the bioimpedance test. DXA test was performed to analyse body composition variables such as weight, FM and FFM, in the same way that bioelectrical impedance was conducted to analyse weight, FM, FFM and TBW.

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 body composition variables such as weight, FM and FFM. Volunteers did not perform physical activity 24h previous the test. The scan was calibrated per two days using the phantom supplied by the manufacturer. All volunteers performed the test in underwear, with their body 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.

Bioimpedance

A bioimpedance (Biológica Tecnología Médica SL: Tanita BC-418 MA, Tokyo, Japan) was done between 8-10 am to obtain weight, FM, FFM, and TBW. Volunteers either performed physical activity or drank coffee 24h previous the bioimpedance. Firstly, the researcher introduced the age, sex and height of the volunteers, which was previously measured with a stadiometer (SECA-213, Valencia, Spain; range 20-205cm). Then, each subject stood erect with bare feet placed on the contact electrode on the bioimpedance device. Bioimpedance test was always carried out by the same researcher.

Blood samples

All blood samples were obtained by venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for ten minutes at 3000 rpm. After that, serum was transferred into eppendorf tubes and stored frozen at -80°C until further analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones in order to verify hormonal profiles.

Blood sample analyses were carried out at Agencia Española de Protección de la Salud en el Deporte (AEPSAD) laboratory, in Madrid, Spain. Total 17 β -estradiol, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured with a COBAS E411 (Roche Diagnostics, GmbH,

Mannheim, Germany), using electrochemiluminescence immunoassay (ECLIA) technology. Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were, respectively: 11.9% and 8.5% at 93.3 pg/ml and 6.8% and 4.7% at 166 pg/ml for 17 β -Estradiol; 23.1% and 11.8% at 0.7 ng/ml and 5.2% and 2.5% at 9.48 ng/ml for Progesterone; 5.3% and 1.8% at 1.2 mIU/ml for FSH; 5.2% and 1.8% at 0.54 mIU/ml for LH; 5.0% and 4.0% at 300 mIU/l for Prolactin; 4.6% and 1.5% at 3.82 μ IU/ml for TSH; 8.5% and 6.0% at 17.3 pg/ml for IL-6.

Statistical analysis

All data are reported as mean \pm Standard Deviation (SD). Throughout Kolmogorov-Smirnov test, data showed a normal distribution. Thus, comparisons among study groups (eumenorrheic, OC users and postmenopausal) were performed by one-way ANCOVA and age was used as a covariable. Scheffé test was applied to examine the pairwise comparison. All tests were conducted with a 5% significance level. Statistical analyses were performed using SPSS software for windows, version 20.1 (SPSS Inc, Chicago, IL, USA).

RESULTS

One-way ANCOVA test showed a mean difference among all groups in age ($F_{2,96}=138.716$; $p<0.001$), while no differences were reported for height ($F_{2,96}=1.364$; $p=0.261$). In accordance with training status, no significant differences were found for experience ($F_{2,126}=0.868$; $p=0.422$), sessions per week ($F_{2,126}=0.906$; $p=0.407$), time per session ($F_{2,126}=0.178$; $p=0.837$). Finally, hormone results (Figure 1) reported significant differences for FSH and LH ($p<0.001$ for both sex hormones), presenting postmenopausal females the highest values ($p=0.034$). Nevertheless, no significant differences were observed either for estradiol ($p=0.103$), progesterone ($p=0.169$) or estradiol/progesterone ratio ($p=0.287$) among study groups.

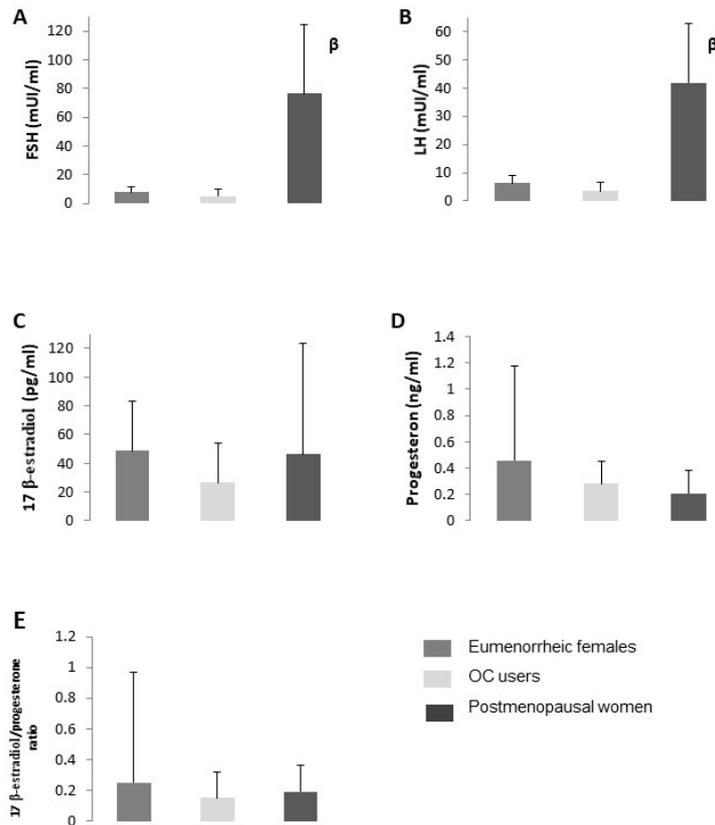


Figure 1: Sex hormones concentrations among females with different hormonal profiles: eumenorrheic females, low-dose monophasic OC users and postmenopausal women. FSH: folliculostimulating hormone; LH: luteinizing hormone; OC: oral contraceptive. ^β Significant differences in postmenopausal women regarding eumenorrheic females and OC users ($p < 0.001$).

With regard to body composition variables (Table 1), DXA measurements did not show significant differences either for weight ($F_{2,119}=1.184$), FFM percentage ($F_{2,119}=0.233$) or FM percentage ($F_{2,119}=0.233$) among well-trained females with different hormonal profiles. Moreover, percentages of android FM and gynoid FM did not vary among study groups ($F_{2,119}=1.037$ and $F_{2,119}=0.515$ respectively). Moving on to bioimpedance results, no significant differences were found for none of the variables measured: weight ($F_{2,100}=0.739$), FFM percentage ($F_{2,101}=1.033$), FM percentage ($F_{2,100}=2.245$) and TBW ($F_{2,100}=0.915$).

Table 1: Body composition variables measured by DXA and bioimpedance in well-trained females with different hormonal profiles.

		Eumenorrheic		OC users		Postmenopausal		<i>p</i>	η^2
		Mean±SD	n	Mean±SD	n	Mean±SD	n		
DXA	Weight (Kg)	59.74±8.73	66	57.80±6.02	41	56.69±8.16	16	0.309	0.020
	FFM (%)	75.35±6.73	66	74.59±5.64	41	74.56±7.13	16	0.793	0.004
	FM (%)	24.65±6.73	66	25.41±5.64	41	25.44±7.13	16	0.793	0.004
	Android FM (%)	5.77±1.41	66	6.07±1.08	41	6.31±1.96	16	0.358	0.017
	Gynoid FM (%)	25.73±3.15	66	25.51±2.26	41	24.81±4.10	16	0.599	0.009
BIA	Weight (Kg)	58.28±6.93	56	57.73±5.86	35	54.95±3.71	13	0.423	0.017
	FFM (%)	79.16±5.24	56	79.66±4.09	35	79.21±7.22	13	0.360	0.020
	FM (%)	20.96±5.22	56	20.49±4.14	35	22.54±4.20	13	0.111	0.043
	TBW (kg)	33.84±3.40	56	33.63±2.27	35	31.15±1.57	13	0.404	0.018

OC: Oral contraceptive; BIA: bioimpedance; DXA: Dual-energy x-ray absorptiometry; FFM: fat free mass; FM: fat mass; TBW: total body water.

DISCUSSION

The aim of this study was to analyse the influence of sex hormones concentration on body composition variables in well-trained females, comparing three different hormonal profiles: eumenorrheic females, low dose monophasic OC users and postmenopausal women. The main finding was the lack of differences between postmenopausal women and premenopausal females. Moreover, monophasic OC pills did not impact on body composition.

Retrospective studies strongly suggested that a decrease in FFM occurs after menopause. Muscle mass loss seems to occur in postmenopausal females because of the decrease of sex hormones, specially estradiol, with the ovarian failure (24). Evidence is accumulating that estradiol deficiency induces apoptosis in skeletal muscle contributing to loss of muscle mass and, therefore, strength (24). Nonetheless, outcomes from this study did not support previous literature. For instance, Distefano, et al. (2018) showed a decrease in muscle mass of 10% in sedentary postmenopausal females (50 years old) (25), Baumgartner et al. (1998) reported a drop of 23.6% in sedentary females at the age of 70 years old compared to the premenopausal group (26), while our well-trained postmenopausal women did not suffer a reduction in their FFM.

Meanwhile, an important reduction in TBW has been observed in sedentary postmenopausal women (15). These lower values of TBW could be related to the lower values of FFM in this population, because of the large amount of water stored in the muscle, as the water content in the muscle represents the major component of body weight (about 76%) (27). In addition, aging is associated with a higher plasma osmolality and a reduction of thirst sensation, leading to a decrease in fluid (dehydration), which could be another explanation for this drop of water in the elderly (14). However, results from this work did not support previous literature, as no differences in TBW has been reported in our postmenopausal women.

The lack of agreement regarding body composition could be explained by differences in physical activity status, since previous studies have been carried out with sedentary women. It is well known that exercise is crucial to prevent the muscle mass decrease in this population, or at least to avoid a pronounced decline (25, 28). Therefore, it could be hypothesized that the drop of muscle mass, and consequently TBW, seems to be less pronounced, or even averted, in females who practice exercise regularly. Finally, it is worth mentioning the lack of differences in FM distribution between study groups. Nonetheless, previous research reported an increase in android FM and a decrease in gynoid FM in sedentary postmenopausal women (16, 17), and this in turn rises metabolic and cardiovascular risks in elderly women (18). Hence, exercise could be a key factor to avoid an increase in android FM in postmenopausal female, preventing them of suffering cardiovascular and metabolic pathologies associated with FM distribution.

In terms of OC users, body composition outcomes obtained in this study are not backed up by previous literature. On the one hand, there is a recent review which concluded a decrease of FFM due to the use of OC pills (29). Nonetheless, there are some aspects we should take into consideration, such as volunteers' characteristics and the type of OC pills. Our participants were well-trained females, using low dose monophasic OC users; whereas the review previously mentioned did not take into consideration physical activity status and mixed all types of OC pills. It is accepted that exercise stimulates the gain of muscle mass (30), so this factor may be crucial when studying FFM. In any case, if OC had a deleterious effect on FFM, it seems to be counteracted by exercise, specially strength training. On the other hand, when it comes to fluid regulation, controversial results have been reported. Although here is a strongly propose that mineralocorticoid effects of ethinyl estradiol are counterbalanced by the anti-mineralocorticoid actions of progestin (8-10), some authors reported that ethinyl estradiol and progestin administration increase plasma volume and even the combination of both exogenous sex hormones causes the greatest increase (11). Nevertheless, our data reported no differences in TBW for the OC group. The different dosages of exogenous hormones used in the present study may explain this lack of differences in TBW. Nowadays, sex hormones dosages in OC pills are lower than they used to be (e.g. ethinyl estradiol concentration was 150 mg/day whereas today is 15 mg/day and progestin concentration was 9.85 mg/day whereas today is 0.35 mg/day) (31). Hence, exogenous sex hormones levels from today's monophasic OC pills might not be high enough to promote shifts in TBW in well-trained females. Similar to our findings, other study did not find differences in TBW when comparing eumenorrheic females and OC users in athletes (20).

The current study attempts to address a gap in the research through investigation of important variables of body composition in well-trained females. The strengths of our study included the inclusion of different endogenous and exogenous female hormone profiles and the recruitment of an homogenous group for all of them; eumenorrheic females, OC users and postmenopausal women (active and healthy women). Nonetheless, it is worth mentioning that the phase in which we have done the measurements could influence the results. OC users were measured during their withdrawal phase, so no exogenous sex

hormones were intaking, while eumenorrhic women were measured during their early follicular phase, which is characterized by low dosages of sex hormones and postmenopausal female always have low levels of sex hormones because of their ovarian failure. Therefore, all groups had a similar sex hormones environment the day of measurement. Additionally, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes throughout life span. It should be noted that different hormonal stages throughout the menstrual cycle and OC cycle in well-trained females might be also interesting to analyse.

CONCLUSIONS

According to our results, sex hormones from different hormonal profiles (eumenorrhic, low dose monophasic OC users and postmenopausal females) do not influence body composition in physically active women. Therefore, the FFM loss, due to the age and the decrease in sex hormones concentrations, seems to be compensated by exercise. Interestingly, android FM do not vary in active postmenopausal female, which could be explain by the positive effect exercise has on body composition, and this in turn on female's health. However, more data are needed in order to elucidate the influence of different hormonal profiles in body composition variables when studying physically active women, since most of the previous studies have been carried out without considering physical activity.

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