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RESEARCH ARTICLE

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Steroid hormone levels in postmenopausal hysterectomised women with and without ovarian conservation: the continuous endocrine function of the ovaries

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ABSTRACT

This study aims to clarify the effect of postmenopausal bilateral oophorectomy on plasma steroid hormone levels. Women who were submitted in the postmenopausal period to hysterectomy for uterine benign conditions were divided into two groups: 18 women had isolated hysterectomy and 11 had hysterectomy with bilateral salpingo-oophorectomy. In both groups serum hormone levels were quantified by solid phase extraction and gas chromatography and tandem mass spectrometry. Differences in dehydroepiandrosterone (DHEA), testosterone, androstenedione and oestradiol were determined in both groups. The analysis revealed lower steroid levels in the bilateral salpingo-oophorectomy group when compared to the isolated hysterectomy group with statistically significant differences found for DHEA (5.8 ± 3.2 vs. 9.4 ± 4.4 ng/mL; $p = 0.019$) and oestradiol (0.69 ± 0.4 vs. 1.48 ± 4.3 ng/mL; $p = 0.007$). The results are consistent with a significant endocrine activity of the postmenopausal ovary. The clinical consequences of these findings need to be clarified and postmenopausal prophylactic bilateral salpingo-oophorectomy re-evaluated.

IMPACT STATEMENT

- **What is already known on this subject?** Although it is consensual that premenopausal prophylactic bilateral oophorectomy should not be performed because it has harmful effects on women's health, the evidence regarding the effects of postmenopausal prophylactic bilateral oophorectomy is scarce and this procedure continues to be a regular practice. Few studies have demonstrated that postmenopausal ovaries still have endocrine activity that may impact older women's health.
- **What do the results of this study add?** This is the first study to compare hormone levels of postmenopausal women based on their hysterectomy and oophorectomy status using GC-MS/MS, a highly sensitive bioanalytical assay for the measurement of steroid hormones. Previous studies relied on immunoassays and did not compare DHEA levels, which according to the intracrinology theory is a precursor for androgens and oestrogens. In this study, statistically significant lower levels of DHEA and oestradiol were found after postmenopausal bilateral salpingo-oophorectomy.
- **What are the implications of these findings for clinical practice and/or further research?** This is a pilot study that may lead to further investigation in this area to clarify the impact of the prophylactic removal of postmenopausal ovaries on older women's health and lead to changes in surgical procedures.

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Introduction

The period of a woman's life that elapses since menopause tends to increase further due to the increase in average life expectancy. Several observational studies have revealed that removal of the ovaries before the menopause has repercussions on the health of postmenopausal women. An increased incidence of cardiovascular disease (Colditz *et al.* 1987, Parker *et al.* 2007, Rivera *et al.* 2009), osteoporosis (Gallagher 2007), neurological disorders (Rocca *et al.* 2007, Rocca *et al.* 2008a), depression (Rocca *et al.* 2008b) and changes in sexual function (Nathorst-Böös *et al.* 1993, Celik *et al.* 2008) has been shown.

While it is now relatively consensual that premenopausal ovaries should be preserved whenever possible, there is less consistent scientific evidence with regard to removal of the ovaries after menopause. The idea that the ovaries after menopause would be dispensable, since they stop producing oestrogens and progesterone has led to the indiscriminate conduct of prophylactic bilateral oophorectomy during hysterectomy in this population, in an attempt to reduce ovarian cancer risk, even in low risk ovarian cancer women.

Although there is no strong scientific evidence, removal of the ovaries after menopause appears to have a negative impact on the health of older women. The ovaries seem to

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continue to have an endocrine activity, contributing to maintain the levels of androgens, which may play an important role at the cardiovascular level (Laughlin *et al.* 2010), in sexual function (Kaplan and Owett 1993, Warnock *et al.* 1997, Palacios 2007), in bone remodelling (Marshall *et al.* 1977, Davidson *et al.* 1982, Greendale *et al.* 1997, van Geel *et al.* 2009) and in cognitive function (Genazzani *et al.* 2007, Ryan *et al.* 2012, Blair *et al.* 2015).

It seems important to clarify the impact of postmenopausal BSO on androgen levels in order to optimise our surgical practice without harming the health of elderly women.

Materials and methods

A cross-sectional study was conducted after approval by the Ethics Committee of Hospital Amato Lusitano, a Portuguese tertiary hospital.

Between 2017 and 2019, postmenopausal women were recruited during routine Gynecological evaluation in a hospital setting for a study involving plasma steroid levels measurements. Postmenopausal status was based on 12 months of amenorrhoea and serum follicle stimulating hormone levels greater than 30 mIU/mL. A total of 29 women were hysterectomised after menopause for benign conditions: 18 had isolated hysterectomy and 11 women had hysterectomy with bilateral salpingo-oophorectomy (BSO). It was our intention to compare women who underwent hysterectomy with or without BSO after the menopause and not simply postmenopausal women with intact ovaries (irrespective of hysterectomy status) *vs.* women who underwent postmenopausal BSO to eliminate the possible interference of the surgery in the ovarian vascularisation when ovaries were retained.

A fasting blood sample was obtained from each woman, between 8 and 10 am, after written informed consent.

Current or past users of systemic hormonal therapy or corticosteroid treatment were excluded. Other exclusion criteria included smoking, narcotic addiction, alcoholism and chronic hepatic or renal diseases. All participants were Caucasian.

Plasma samples were stored at −80°C and protected from light until analysis. The studied compounds, dehydroepiandrosterone (DHEA), androstenedione (A), 17β-oestradiol (E2) and testosterone (T) were quantified by solid phase extraction (SPE) and gas chromatography and tandem mass spectrometry (GC-MS/MS). Briefly, 1 mL of plasma was diluted with 1 mL of phosphate buffer saline (PBS) (pH = 7) and spiked with 100 µL of internal standard (DHEA-d6). SPE cartridges (Oasis® HLB 3 cc, Waters, USA) were conditioned with 2 mL of methanol and 2 mL of 0.1% acetic acid. After passing of the sample through the cartridge, this was washed with 2 mL of deionised water. The columns were afterwards dried under full vacuum for 30 min and the analytes were eluted with 2 mL methanol. The extracts were concentrated to dryness under a gentle nitrogen stream, and were afterwards dissolved in 20 µL of methanol, from which a 3 µL aliquot was injected into the GC-MS/MS system. The remaining residue was further evaporated to dryness under a gentle nitrogen stream at 36 °C, and 20 µL of *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added. Derivatization took place in a domestic digital microwave oven (Candy CMG 2017 M, Portugal) for 2 min at 800 W, and 3 µL was injected. This step was deemed necessary because some of the analytes under study (E2 and T) present active moieties and need to be derivatized before analysis by GC-based procedures.

The statistical analysis software used was SPSS 27.0. Hysterectomy and oophorectomy status was stratified into two categories: hysterectomy with ovarian conservation and hysterectomy with BSO. Descriptive statistics were reported as means ± standard deviation (SD) for continuous variables and as frequencies (%) for categorical variables. Statistical analyses were obtained using the Mann-Whitney test for comparison of central tendency between groups for numerical variables after normality check. The Chi-Square Test was used to analyse the association between categorical variables. When necessary, Fisher's Exact Test was used as an alternative to Chi-square test. A p value of 0.05 or less was considered statistically significant.

Results

The mean age of patients in both groups did not reveal statistically significant differences. Both groups are equally homogeneous with regard to body mass index (BMI) and age of hysterectomy (Table 1).

When comparing the two groups according to the years from menopause to the date of blood collection using Fisher's Exact Test, no statistically significant differences were found ($p = 0.735$).

Although we found differences in both groups concerning time of hysterectomy to blood collection we did not detect any statistically significant association between time of hysterectomy to blood collection and hormone concentrations.

Vaginal oestrogens were used, two to three times a week, by 44% of the women in the group of hysterectomy with ovarian conservation and by 36.4% of the women in the group submitted to hysterectomy with BSO, but again no statistically significant differences were found using Fisher's Exact Test ($p = 0.717$).

Once established that the variables above were not statistically different amongst both groups, the analysis of plasma steroid levels in both groups revealed differences. In the hysterectomy and BSO group lower plasma steroid levels were found: 54% lower levels of oestradiol; 38.3% lower levels of DHEA; 36.1% lower levels of testosterone; 31.4% lower levels of androstenedione. Statistically significant differences were found for E2 and DHEA (Table 2).

Discussion

Our study was the first to compare plasma steroid levels of postmenopausal women based on their hysterectomy and oophorectomy status using GC-MS/MS, a highly sensitive hormone assay method.

This study revealed lower plasma steroid levels when BSO was performed in the postmenopausal period when compared with patients not submitted to BSO, with statistically significant differences in DHEA and E2 levels, suggesting that

Table 1. Characterisation of the groups under study: hysterectomy with ovarian conservation and hysterectomy with BSO (mean ± standard deviation).

	Hysterectomy with ovarian conservation (n = 18)	Hysterectomy with BSO (n = 11)	*p*-Value[a]
Age (years)	70.6 ± 7.1	72.5 ± 9.6	0.521
BMI (Kg/m^2)	29.1 ± 4.1	30.9 ± 5.5	0.296
Age of hysterectomy (years)	67.9 ± 8.1	66.4 ± 9.2	0.580
Time from menopause to blood collection (years)	22.3 ± 8.5	20.1 ± 9.3	0.642
Time from menopause to hysterectomy (years)	19.1 ± 8.8	14.0 ± 8.9	0.173
Time from hysterectomy to blood collection (years)	2.7 ± 3.6	6.1 ± 3.8	0.002

[a]Mann–Whitney test.

Table 2. Hormonal concentrations in the study groups: hysterectomy with ovarian conservation and hysterectomy with BSO (mean ± standard deviation).

	Hysterectomy with ovarian conservation (n = 18)	Hysterectomy with BSO (n = 11)	*p*-Value[a]
E2 (ng/mL)	1.48 ± 4.3	0.69 ± 0.4	0.007
DHEA (ng/mL)	9.4 ± 4.4	5.8 ± 3.2	0.019
T (ng/mL)	1.6 ± 3.4	1.0 ± 0.9	0.173
A (ng/mL)	1.4 ± 1.5	0.97 ± 0.5	0.774

[a]Mann–Whitney test.

the postmenopausal ovary does have a positive impact on steroid plasma levels and a continuous endocrine function.

The idea that the postmenopausal ovary continues to have endocrine activity is not recent but the mechanisms of its contribution to the production of androgens have evolved.

Pioneer studies in the 70 s attempted to determine the relative importance of the ovary and adrenal gland in the production of androgens after the menopause. Some of these studies demonstrated a gradient of androgen concentrations between the ovarian vein and peripheral blood while others used pharmacological suppression of the adrenal gland (Judd *et al.* 1974, Maroulis and Abraham 1976, Vermeulen 1976). These studies confirmed that postmenopausal ovary continued to have an important endocrine function.

The Rancho Bernardo Study, the largest populational study to examine the association between hormone levels, oophorectomy and time since menopause, revealed a reduction in 30% of testosterone levels in postmenopausal women who were previously oophorectomized (Laughlin *et al.* 2000). This study did not specify whether oophorectomy was performed before or after menopause.

Another study published in 2015 using a subset of 2251 participants from the Nurse's Health Study (NHS) revealed 25% lower testosterone levels in postmenopausal women previously submitted to bilateral oophorectomy. This difference was also confirmed when adjusted for postmenopausal oophorectomy, with a smaller sample size (30 participants were previously submitted to postmenopausal oophorectomy) (Kotsopoulos *et al.* 2015).

Although consistent with our results, these studies used immunoassays for steroid measurements. However, concerns about specificity of these methods when steroid levels are low have led to implementation of MS-based techniques as the gold standard methodology for steroid hormone analysis. Mass spectrometry offers a unique identification profile of each of the study analytes, eliminating interferences and thus allowing greater sensitivity and specificity (Andrew and Homer 2020). In fact there are different published works about the determination of these compounds using GC-MS/MS (Hansen *et al.* 2011, McDonald *et al.* 2011, Caron *et al.* 2015, Matysik and Schmitz 2015). In this work excellent limits of detection and quantitation were achieved (0.05 ng/mL for E2; 0.1 ng/mL for A and DHEA, and 0.5 ng/mL for T) using only 1 mL of sample.

A key point to note is that most of the earlier studies did not include DHEA in the analysis or included DHEA-S which is almost exclusively produced by the adrenal gland.

However, Labrie demonstrated that after menopause the only source of sex steroids is circulating DHEA which is converted into androgens and oestrogens by peripheral tissues using the process of intracrinology (Labrie 1991). In a study comparing 442 intact and 71 ovariectomized postmenopausal women aged 42–74 years, Labrie *et al.* confirmed that the postmenopausal ovary contributes to approximately 20% of the total pool of circulating DHEA (Labrie *et al.* 2011). The results are consistent with our findings, a statistically significant decrease in DHEA in women submitted to postmenopausal BSO, which could explain the decrease in other steroid levels. Interestingly, the finding of statistically significant lower levels of oestradiol could also be a reasonable explanation for the worst clinical outcomes after postmenopausal bilateral oophorectomy with respect to sexual function or bone density, as most of the studies addressing these issues focussed mainly on androgen levels. Also, if this significant reduction in oestradiol levels in postmenopausal women submitted to BSO is confirmed by other studies, a role of the postmenopausal BSO in the treatment of ER-positive breast cancer should be investigated. More studies are needed to clarify if reduction in DHEA after postmenopausal BSO has a significant clinical impact in the health of older women.

Limitations of our study include a small sample size and the totality of participants being Caucasian, which makes it difficult to generalise the results. This study focused on women who underwent hysterectomy with or without BSO after the menopause and that is the reason why the sample size was small. It is not so frequent to carry out a hysterectomy after the menopause for benign conditions as it is in the premenopausal period. Another important limitation is related to the study design. This is a cross-sectional study of women already submitted to hysterectomy and we do not know the criteria used for the conservation or removal of the ovaries. The majority of women whose ovaries were preserved underwent vaginal hysterectomy, which suggests it was a technical issue, given that BSO is more difficult to perform in this type of surgery. It would also have been ideal to have had pre-surgery hormone values to be sure that it is in fact the intervention that is responsible for the lower steroid hormone levels.

Despite the limitations, our results are consistent with previous studies and in agreement with intracrinology theory that holds that after menopause sex steroid hormones derive from DHEA. The postmenopausal ovary continues to have a

relevant endocrine function and a positive impact on steroid hormone levels. More studies with larger sample sizes and MS-based techniques are needed to provide a more accurate evidence over the benefits of ovarian conservation after menopause, so that appropriate counselling can be given to all postmenopausal women considering a prophylactic bilateral oophorectomy during hysterectomy for benign conditions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

References

Andrew, R. and Homer, N.Z.M., 2020. Mass spectrometry: future opportunities for profiling and imaging steroids and steroid metabolites. *Current Opinion in Endocrine and Metabolic Research*, 15, 71–78.

Blair, J.A., *et al.*, 2015. Hypothalamic-pituitary-gonadal axis involvement in learning and memory and Alzheimer's disease: more than 'just' estrogen. *Frontiers in Endocrinology*, 6, 45.

Caron, P., Turcotte, V., and Guillemette, C., 2015. A chromatography/tandem mass spectrometry method for the simultaneous profiling of ten endogenous steroids, including progesterone, adrenal precursors, androgens and estrogens, using low serum volume. *Steroids*, 104, 16–24.

Celik, H., *et al.*, 2008. The effect of hysterectomy and bilaterally salpingo-oophorectomy on sexual function in post-menopausal women. *Maturitas*, 61 (4), 358–363.

Colditz, G.A., *et al.*, 1987. Menopause and the risk of coronary heart disease in women. *The New England Journal of Medicine*, 316 (18), 1105–1110.

Davidson, B.J., *et al.*, 1982. Total and free estrogens and androgens in postmenopausal women with hip fractures. *The Journal of Clinical Endocrinology and Metabolism*, 54 (1), 115–120.

Gallagher, J.C., 2007. Effect of early menopause on bone mineral density and fractures. *Menopause*, 14 (3 Pt 2), 567–571.

Genazzani, A.R., *et al.*, 2007. Androgens and the brain. *Maturitas*, 57 (1), 27–30.

Greendale, G.A., Edelstein, S., and Barrett-Connor, E., 1997. Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. *Journal of Bone and Mineral Research*, 12 (11), 1833–1843.

Hansen, M., *et al.*, 2011. Determination of steroid hormones in blood by GC-MS/MS. *Analytical and Bioanalytical Chemistry*, 400 (10), 3409–3417.

Judd, H.L., *et al.*, 1974. Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. *The Journal of Clinical Endocrinology and Metabolism*, 39 (6), 1020–1024.

Kaplan, H.S. and Owett, T., 1993. The female androgen deficiency syndrome. *Journal of Sex & Marital Therapy*, 19 (1), 3–24.

Kotsopoulos, J., *et al.*, 2015. The relationship between bilateral oophorectomy and plasma hormone levels in postmenopausal women. *Hormones & Cancer*, 6 (1), 54–63.

Labrie, F., 1991. Intracrinology. *Molecular and Cellular Endocrinology*, 78 (3), C113–C118.

Labrie, F., Martel, C., and Balser, J., 2011. Wide distribution of the serum dehydroepiandrosterone and sex steroid levels in postmenopausal women: role of the ovary? *Menopause (New York, N.Y.)*, 18 (1), 30–43.

Laughlin, G.A., *et al.*, 2000. Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study. *The Journal of Clinical Endocrinology & Metabolism*, 85 (2), 645–651.

Laughlin, G.A., Goodell, V. and Barrett-Connor, E., 2010. Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. *The Journal of Clinical Endocrinology and Metabolism*, 95 (2), 740–747.

Maroulis, G.B. and Abraham, G.E., 1976. Ovarian and adrenal contributions to peripheral steroid levels in postmenopausal women. *Obstetrics and Gynecology*, 48 (2), 150–154.

Marshall, D.H., Crilly, R.G., and Nordin, B.E., 1977. Plasma androstenedione and oestrone levels in normal and osteoporotic postmenopausal women. *British Medical Journal*, 2 (6096), 1177–1179.

Matysik, S. and Schmitz, G., 2015. Determination of steroid hormones in human plasma by GC-triple quadrupole MS. *Steroids*, 99 (Pt B), 151–154.

McDonald, J.G., Matthew, S., and Auchus, R.J., 2011. Steroid profiling by gas chromatography-mass spectrometry and high performance liquid chromatography-mass spectrometry for adrenal diseases. *Hormones & Cancer*, 2 (6), 324–332.

Nathorst-Böös, J., von Schoultz, B., and Carlström, K., 1993. Elective ovarian removal and estrogen replacement therapy–effects on sexual life, psychological well-being and androgen status. *Journal of Psychosomatic Obstetrics and Gynaecology*, 14 (4), 283–293.

Palacios, S., 2007. Androgens and female sexual function. *Maturitas*, 57 (1), 61–65.

Parker, W.H., *et al.*, 2007. Ovarian conservation at the time of hysterectomy for benign disease. *Clinical Obstetrics and Gynecology*, 50 (2), 354–361.

Rivera, C.M., *et al.*, 2009. Increased cardiovascular mortality after early bilateral oophorectomy. *Menopause*, 16 (1), 15–23.

Rocca, W.A., *et al.*, 2007. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology*, 69 (11), 1074–1083.

Rocca, W.A., *et al.*, 2008a. Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology*, 70 (3), 200–209.

Rocca, W.A., *et al.*, 2008b. Long-term risk of depressive and anxiety symptoms after early bilateral oophorectomy. *Menopause*, 15 (6), 1050–1059.

Ryan, J., *et al.*, 2012. Hormone levels and cognitive function in postmenopausal midlife women. *Neurobiology of Aging*, 33 (7), 1138–1147.

van Geel, T.A.C.M., *et al.*, 2009. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study. *European Journal of Endocrinology*, 160 (4), 681–687.

Vermeulen, A., 1976. The hormonal activity of the postmenopausal ovary. *The Journal of Clinical Endocrinology and Metabolism*, 42 (2), 247–253.

Warnock, J.K., Bundren, J.C. and Morris, D.W., 1997. Female hypoactive sexual desire disorder due to androgen deficiency: clinical and psychometric issues. *Psychopharmacology Bulletin*, 33 (4), 761–766.