UNIVERSIDADE
BEIRA INTERIOR

The Function of the Ovaries after Menopause

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Tese para obtenção do Grau de Doutor em
Medicina
(3º ciclo de estudos)

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Data: 2024.03.25 09:45:22 +0000

Acknowledgments

First and foremost, I would like to thank three people who were fundamental to the execution of this doctoral thesis: Professor José Fonseca Moutinho, Professor Eugenia Gallardo and Professor Sara Morgado Nunes.

To Professor José Fonseca Moutinho, my PhD supervisor, for his patience and motivational strength, but above all for inspiring me to think outside the box but always focusing on the patient. His example of a life dedicated to the pursuit of knowledge, the improvement of health care, always with a humanist perspective, is an inspiration for me and for all those who had the opportunity to learn from him.

To Professor Eugenia Gallardo, head of the Laboratory of Pharmaco-Toxicology of UBIMedical (Covilhã, Portugal), whose contribution to this thesis goes beyond the laboratory analysis for which she was responsible. Her knowledge and experience were a precious help in writing the articles, but more importantly, her motivation helped me not to give up in the most difficult moments.

To Professor Sara Morgado Nunes, professor of Statistics at the Polytechnic Institute of Castelo Branco (Castelo Branco, Portugal), who was responsible for the statistical analysis of this doctoral work. I will be forever grateful for her permanent availability and for the hours we spent discussing the best options. Her resilience and her spirit kept me optimistic about the outcome of this work.

I gratefully acknowledge the help of the nurses of the Outpatient Department of Amato Lusitano Hospital/ Castelo Branco Local Health Unit (ULSCB), in the consultations and their collaboration in the distribution of the sexual function questionnaires.

I am most grateful to Dr. Mariana Martins, director of the Clinical Pathology Service of ULSCB, and to the Diagnostic and Therapeutic Technicians of ULSCB, especially to Cláudia Lobo and Patrícia Fernandes for their invaluable support in collecting and processing blood samples.

I would like to thank Professor Fátima Santos, of the Health Sciences Research Center of the University of Beira Interior (CICS-UBI), for her precious help in storing the samples at CICS-UBI.

To Dr. José Tomás, Neurologist at the ULSCB, for agreeing to evaluate patients who had lower scores on the cognitive function test.

I would like to express my heartfelt thanks to all the participants in this study, whom I like to refer as “my patients”, for their willingness to participate in the study and also for everything they taught me. I dedicate this study to them.

To my friends, for their unconditional support.

Finally, I want to thank my family. Without them I simply would not have made it.

Thank you all.

Preface

As a young specialist in Obstetrics and Gynecology, having just arrived at a hospital in the interior of Portugal with a significant lack of human resources, I felt the weight of responsibility and was faced with making important decisions on my own. Medical internship does not prepare us for everything. I found myself dealing with a mostly aging population and I realized that these women, with the accumulated ills of life, ask for a more thoughtful approach. With increasing longevity, quality of life after menopause is even more important. In Portugal, prophylactic removal of the ovaries during gynecological surgery for benign pathology after menopause is still a common practice. But one day I thought to myself: “Why am I removing an ovary that looks healthy? What if this ovary still has a relevant endocrine function?” If it is consensual that in premenopausal women the ovaries should be conserved, in postmenopausal women the scientific evidence is less consistent.

Thus arose the idea for this doctoral thesis.

Resumo

Introdução

A remoção de ovários e trompas no mesmo tempo cirúrgico da histerectomia (salpingo-ooforectomia bilateral profilática) é uma prática frequente e realizada com o objetivo principal de prevenir o cancro do ovário. Embora seja consensual que a ooforectomia bilateral profilática na pré-menopausa não deva ser realizada na população de baixo risco de cancro do ovário, por ter efeitos nocivos na saúde da mulher e aumentar as taxas de mortalidade a longo prazo, as evidências sobre os efeitos da ooforectomia bilateral profilática na pós-menopausa são controversas e esse procedimento continua a ser uma prática regular. Alguns estudos demonstraram que os ovários na pós-menopausa continuam a produzir androgénios, que podem desempenhar um papel relevante a nível cardiovascular, na remodelação óssea, na função sexual e na função cognitiva.

A associação entre défice de estrogénio e osteoporose é bem conhecida. Novas evidências revelaram também um possível impacto dos androgénios na densidade mineral óssea (DMO). Em mulheres na pós-menopausa, o tratamento combinado com testosterona e estrogénio revelou maior eficácia no aumento da DMO do que o estrogénio isolado. No entanto, embora a maioria dos estudos tenha revelado uma associação entre androgénios endógenos e DMO, particularmente testosterona, outros não reportaram essa associação.

O défice de estrogénio é responsável pela atrofia vulvovaginal e maior incidência de dispareunia na pós-menopausa. Alguns estudos avaliaram a relação entre androgénios e função sexual em mulheres na pós-menopausa. Os androgénios parecem desempenhar um papel na manutenção da saúde sexual e os ensaios clínicos demonstraram consistentemente que a terapêutica com testosterona melhora a função sexual em mulheres com desejo sexual hipoativo.

O estrogénio tem um papel essencial no cérebro e mulheres pós-menopáusicas com níveis de estradiol mais altos apresentam melhor função cognitiva global, mas alguns estudos revelaram resultados contraditórios. De igual modo, a influência dos andrógenos na função cognitiva pós-menopausa não é bem compreendida. Foi reportada uma associação positiva entre aprendizagem verbal e memória e concentrações fisiológicas de testosterona administrada exogenamente. No entanto, outros estudos revelaram que níveis mais baixos de testosterona endógena estão associados a melhoria da função cognitiva ou não revelaram associação.

Tendo em consideração que, com o aumento da esperança média de vida, o período pós-menopausa vai sendo cada vez maior, torna-se fundamental saber o impacto que a remoção dos ovários pode ter na longevidade e qualidade de vida destas mulheres.
O estudo apresentado tem como objetivos esclarecer o efeito da ooforectomia bilateral pós-menopausa nos níveis séricos de hormonas esteroides e o impacto que estas têm na densidade mineral óssea, função sexual e função cognitiva das mulheres com mais idade.

Materiais e métodos

No período de 1 de janeiro de 2017 a 30 de junho de 2019, 203 mulheres pós-menopáusicas consentiram participar no estudo. Todas tinham ovários intactos na altura da menopausa e nenhuma tinha realizado terapêutica hormonal de substituição. Outros critérios gerais de exclusão foram a presença de patologia ovárica, tratamento atual ou prévio com corticóides, alcoolismo, dependência de narcóticos e doenças hepáticas ou renais crónicas. Cada participante foi submetida a uma colheita de sangue e foram realizados doseamentos séricos de 17β- estradiol (E2), dehidroepiandrosterona (DHEA), testosterona e androstenediona, utilizando cromatografia gasosa associada a espectrometria de massa (GC-MS/MS).
Foram realizados 4 estudos transversais. No primeiro estudo, mulheres submetidas na pós-menopausa a histerectomia por condições benignas foram divididas em dois grupos: 18 mulheres submetidas a histerectomia isolada e 11 submetidas a histerectomia com salpingo-ooforectomia bilateral profilática. Foram determinadas as diferenças nos níveis hormonais em ambos os grupos.
No segundo estudo, 68 mulheres com mais de 65 anos realizaram osteodensitometria óssea pela técnica de absorciometria de raios X com dupla energia (DXA) e foram avaliadas associações entre os níveis hormonais e os valores de T score da coluna lombar e do colo do fémur, controlando as variáveis de confusão.
No terceiro estudo, que incluiu 84 mulheres sexualmente ativas, foram avaliadas associações entre os níveis hormonais e as pontuações nos domínios da resposta sexual feminina avaliados pelo questionário *Female Sexual Function Index* (FSFI), com ajuste para variáveis de confusão.
O quarto estudo incluiu 147 mulheres que completaram o teste *Montreal Cognitive Assessment* (MoCA) para avaliação da função cognitiva e foram determinadas associações entre os níveis hormonais e os parâmetros de função cognitiva (função cognitiva global, função executiva, capacidade visuoespacial, memória de curto prazo, atenção, concentração e memória de trabalho, linguagem, orientação no tempo e no espaço), controlando variáveis de confusão.

Resultados

No primeiro estudo, a análise revelou níveis hormonais mais baixos no grupo de mulheres submetidas a salpingo-ooforectomia bilateral, quando comparado com o grupo submetido a histerectomia isolada, com diferenças estatisticamente significativas para DHEA (5,8± 3,2 vs 9,4± 4,4 ng/mL; p=0,019) e E2 (0,69± 0,4 vs 1,48± 4,3 ng/mL; p=0,007).

O segundo estudo revelou uma associação estatisticamente significativa entre a testosterona e o T score do colo do fémur (p=0,035), mas não da coluna lombar. Nenhuma associação estatisticamente significativa foi encontrada entre as restantes hormonas e os T scores da coluna lombar e colo do fémur.

O terceiro estudo revelou uma associação estatisticamente significativa entre androstenediona e função sexual geral (β=1,23, IC 95% [0,37; 1,98], p=0,010), excitação (β=0,19, IC 95% [0,02; 0,37], p=0,034), orgasmo (β=0,33, IC95% [0,15; 0,45], p=0,001) e satisfação (β=0,25, IC95% [0,11; 0,36], p=0,001). Não foram encontradas associações entre as outras hormonas e os domínios avaliados pelo FSFI.

No quarto estudo foram encontradas correlações negativas entre E2 e os seguintes domínios cognitivos: função executiva (p=0.024), capacidade visuoespacial (p=0.000) e orientação no tempo e no espaço (p=0.020). Nenhuma associação estatisticamente significativa foi encontrada entre DHEA, testosterona e androstenediona e os diferentes parâmetros cognitivos.

Conclusões

Este estudo, utilizando uma técnica laboratorial altamente sensível para a quantificação dos níveis séricos de hormonas esteroides na pós-menopausa, demonstrou que a salpingo-ooforectomia profilática realizada após a menopausa diminui a concentração de hormonas esteroides, com diferenças estatisticamente significativas para o DHEA e estradiol. A redução de DHEA observada neste estudo pode ter um impacto ainda maior na redução das concentrações de testosterona e androstenediona ao nível intracelular, de acordo com a teoria da intracrinologia. Os resultados deste trabalho de doutoramento revelaram uma atividade endócrina significativa do ovário na pós-menopausa, bem como uma associação positiva significativa entre testosterona e densidade mineral óssea do colo do fémur em mulheres com mais de 65 anos de idade e uma associação positiva significativa entre androstenediona e vários parâmetros da função sexual em mulheres pós-menopáusicas. Estes resultados devem ser confirmados por estudos com amostras maiores e doseamentos hormonais por técnicas baseadas em espectrometria de massa, para confirmar o impacto das hormonas esteroides na saúde e qualidade de vida das mulheres na pós-menopausa e a relação risco/benefício da

conservação dos ovários após a menopausa, para que possa ser realizado um aconselhamento adequado relativamente à realização de ooforectomia bilateral profilática na pós-menopausa.

Palavras-chave

Menopausa;Ooforectomia;Hormonas;Estrogénio;Estradiol;Androgénios;Testosterona;
DehidroepiandrosteronaAndrostenediona;DensidadeMineralÓssea;Osteoporose;Funç
ãoSexual;FunçãoCognitiva;Demência

Abstract

Introduction

Removal of ovaries and fallopian tubes at the same surgical time as hysterectomy (prophylactic bilateral salpingo-oophorectomy) is a frequent practice performed with the main objective of preventing ovarian cancer. Although there is consensus that prophylactic bilateral oophorectomy in premenopausal women should not be performed in populations at low risk of ovarian cancer, as it has harmful effects on women's health and increases long-term mortality rates, evidence on the effects of postmenopausal prophylactic bilateral oophorectomy are controversial and this procedure remains a regular practice. Some studies have shown that postmenopausal ovaries continue to produce androgens, which may play an important role in cardiovascular health, bone remodeling, sexual function and cognitive function.

The association between estrogen deficiency and osteoporosis is well known. New evidence has also revealed a possible impact of androgens on bone mineral density (BMD). In postmenopausal women, combined treatment with testosterone and estrogen was more effective in increasing BMD than estrogen alone. However, although most studies have revealed an association between endogenous androgens and BMD, particularly testosterone, others have not reported such an association.

Estrogen deficit is responsible for vulvovaginal atrophy and higher incidence of postmenopausal dyspareunia. Few studies have evaluated the relationship between androgens and sexual function in postmenopausal women. Androgens appear to play a role in maintaining sexual health, and clinical trials have consistently shown that testosterone therapy improves sexual function in women with hypoactive sexual desire.

Estrogen plays an essential role in the brain and postmenopausal women with higher levels of estradiol have better global cognitive function, but some studies have revealed contradictory results. Likewise, the influence of androgens on postmenopausal cognitive function is not well understood. A positive association between verbal learning and memory and physiological concentrations of exogenously administered testosterone has been reported. However, other studies have shown that lower levels of endogenous testosterone are associated with improved cognitive function or have shown no association.

Taking into account that, with the increase in average life expectancy, the post-menopausal period is getting longer, it is essential to know the impact that the removal of the ovaries can have on the longevity and quality of life of these women.

The present study aims to clarify the effect of postmenopausal bilateral oophorectomy on serum levels of steroid hormones and the impact they have on bone mineral density, sexual function and cognitive function in older women.

Materials and methods

In the period from January 1, 2017 to June 30, 2019, 203 postmenopausal women consented to participate in the study. All had intact ovaries at the time of menopause and none had been on hormone replacement therapy. Other general exclusion criteria were the presence of ovarian pathology, current or previous treatment with corticosteroids, alcoholism, narcotic dependence and chronic liver or kidney disease. Each participant underwent a blood collection and serum measurements of 17β-estradiol (E2), dehydroepiandrosterone (DHEA), testosterone and androstenedione were performed using gas chromatography associated with mass spectrometry (GC-MS/MS).

Four cross-sectional studies were performed. In the first study, postmenopausal women undergoing hysterectomy for benign conditions were divided into two groups: 18 women undergoing hysterectomy alone and 11 women undergoing hysterectomy with prophylactic bilateral salpingo-oophorectomy. Differences in hormone levels in both groups were determined.

In the second study, 68 women aged over 65 years underwent bone osteodensitometry using the dual-energy X-ray absorptiometry (DXA) technique, and associations between hormone levels and T-score values of the lumbar spine and femoral neck were evaluated, controlling for confounding variables.

In the third study, which included 84 sexually active women, associations between hormone levels and scores in the domains of female sexual response assessed by the Female Sexual Function Index (FSFI) questionnaire were evaluated, with adjustment for confounding variables.

The fourth study included 147 women who completed the Montreal Cognitive Assessment (MoCA) test to assess cognitive function and determined associations between hormone levels and cognitive function parameters (global cognitive function, executive function, visuospatial ability, short-term memory , attention, concentration and working memory, language, orientation in time and space), controlling for confounding variables.

Results

In the first study, the analysis revealed lower hormone levels in the group of women submitted to bilateral salpingo-oophorectomy, when compared with the group

submitted to isolated hysterectomy, with statistically significant differences for DHEA (5.8± 3.2 vs 9.4 ± 4.4 ng/mL; p=0.019) and E2 (0.69 ± 0.4 vs 1.48 ± 4.3 ng/mL; p=0.007).

The second study revealed a statistically significant association between testosterone and the T-score of the femoral neck (p=0.035), but not of the lumbar spine. No statistically significant association was found between the other hormones and lumbar spine and femoral neck T scores.

The third study revealed a statistically significant association between androstenedione and overall sexual function (β=1.23, 95% CI [0.37; 1.98], p=0.010), arousal (β=0.19, 95% CI [0.02; 0.37], p=0.034), orgasm (β=0.33, 95%CI [0.15; 0.45], p=0.001) and satisfaction (β=0.25, 95%CI [0.11; 0.36], p=0.001). No associations were found between the other hormones and the FSFI domains.

In the fourth study, negative correlations were found between E2 and the following cognitive domains: executive function (p=0.024), visuospatial ability (p=0.000) and orientation in time and space (p=0.020). No statistically significant association was found between DHEA, testosterone and androstenedione and the different cognitive parameters.

Conclusions

This study, using a highly sensitive laboratory technique for quantifying postmenopausal serum levels of steroid hormones, demonstrated that prophylactic salpingo-oophorectomy performed after menopause decreases the concentration of steroid hormones, with statistically significant differences for DHEA and estradiol. The reduction of DHEA observed in this study may have an even greater impact on the reduction of testosterone and androstenedione concentrations at the intracellular level, according to the theory of intracrinology. The results of this doctoral work revealed a significant endocrine activity of the postmenopausal ovary, as well as a significant positive association between testosterone and femoral neck bone mineral density in women over 65 years of age and a significant positive association between androstenedione and several parameters of sexual function in postmenopausal women. These results should be confirmed by studies with larger samples and hormone assays based on mass spectrometry, to confirm the impact of steroid hormones on the health and quality of life of postmenopausal women and the risk/benefit ratio of ovarian conservation after menopause, so that appropriate advice can be given regarding postmenopausal prophylactic bilateral oophorectomy.

Keywords

Menopause;Oophorectomy;Hormones;Estrogen;Estradiol;Androgens;Testosterone;Dehydroepiandrosterone;Androstenedione;BoneMineralDensity;Osteoporosis;SexualFunction;CognitiveFunction;Dementia

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List of abbreviations

A	Androstenedione
AD	Alzheimer`s disease
AMH	Anti-mullerian hormone
BCa	Bias-corrected and accelerated
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
BMD	Bone mineral density
BMI	Body mass index
BRCA	BReast CAncer susceptibility gene
BSO	Bilateral salpingo-oophorectomy
CICS-UBI	Centro de Investigação em Ciências da Saúde - Universidade da Beira Interior
DGS	Direção Geral da Saúde
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
DXA	Dual Energy X-Ray Absorptiometry
E2	17β-estradiol
ELITE	Early versus Late Intervention Trial with Estradiol
FMP	Final menstrual period
FSFI	Female Sexual Function Index
FSH	Follicle stimulating hormone
GC-MS/MS	Gas chromatography and tandem mass spectrometry
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
HRT	Hormone replacement therapy
IQR	Interquartile range
ISCED	International Standard Classification of Education
KEEPS	Kronos Early Estrogen Prevention Study
LC-MS/MS	Liquid chromatography and tandem mass spectrometry
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment test
MPS2	Multipurpose sampler 2
MS	Mass spectrometry
NHS	Nurse`s Health Study
RR	Relative risk
SD	Standard deviation
SPE	Solid-phase extraction
SPSS	Statistical Package for the Social Sciences
STRAW	Stages of Reproductive Aging Workshop
T	Testosterone
ULSCB	Unidade Local de Saúde de Castelo Branco
WHI	Women´s Health Initiative study
WHIMS	Women´s Health Initiative Memory Study
WHO	World Health Organization

1. Introduction

The average life expectancy of a Western woman in the early 20th century was 56 years. At the beginning of the 21st century it was 80 years. About 1/3 of a woman's life is spent in the postmenopausal period[1]. According to data from the National Institute of Statistics, the life expectancy of women at birth in Portugal, in 2019-2021, was estimated at 83.37 years. Menopause happens naturally on average at age 51 in white Caucasians, with ethnic and regional variations[2]. It is predicted that at the beginning of the next century the average life expectancy of a woman will be around 100 years, which means that around 50% of a woman's life will be spent after menopause. Menopause is defined as the final menstrual period (FMP) and results from the cessation of ovarian follicular activity. As a consequence, the production of estrogen and progesterone is reduced. However, at the level of ovarian stromal cells there is still some production of androgens, which are thought to have some importance in the quality of life of older women.

In 2011, the gold standard criteria for staging reproductive aging were defined by the Stages of Reproductive Aging Workshop + 10 (STRAW + 10). This staging system summarizes the changes in hypothalamic-pituitary-ovarian function that occur before and after the FMP, and provides a more comprehensive basis for assessing reproductive aging in clinical and research contexts (Figure 1)[3].

	Menarche					FMP (0)				
Stage	-5	-4	-3b	-3a	-2	-1	+1 a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION		POSTMENOPAUSE			
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	*variable*				*variable*	1-3 years	2 years (1+1)		3-6 years	*Remaining lifespan*
PRINCIPAL CRITERIA										
Menstrual Cycle	Variable to regular	Regular	Regular	Subtle changes in Flow/ Length	*Variable Length* Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of >=60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B			Low Low	Variable* Low Low	↑ Variable* Low Low	↑ >25 IU/L** Low Low	↑ Variable* Low Low		Stabilizes Very Low Very Low	
Antral Follicle Count			Low	Low	Low	**Low**	Very Low		Very Low	
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms *Likely*	Vasomotor symptoms *Most Likely*			*Increasing* symptoms of urogenital atrophy

* Blood draw on cycle days 2-5 ↑ = elevated
**Approximate expected level based on assays using current international pituitary standard[67-69]

Figure 1 - The Stages of Reproductive Aging Workshop + 10 staging system for reproductive aging in women. Reprinted with permission from Harlow et al. *STRAW+10 Staging Reproductive Aging* Climacteric15(2):105-14, Fertil Steril 97:843-51, J Clin Endocrinol Metab 97:1159-68, Menopause 19:387-95, 2012

Regarding the postmenopausal period, it was initially divided into early and late postmenopause, but the Straw +10 recommended that early postmenopause be divided into three substages based on the increase of FSH and decrease of estradiol. Stages +1a and +1b each last 1 year and end at the time point at which FSH and estradiol levels stabilize. Stage +1a marks the end of the 12-month period of amenorrhea required to define that the FMP has occurred. It corresponds to the end of "perimenopause," a term still in common usage that means the time around menopause and begins at Stage –2 and ends 12 months after the FMP. Stage +1c represents the period of stabilization of high FSH levels and low estradiol values that is estimated to last 3 to 6 years. The late postmenopause (Stage +2) represents the period in which further changes in reproductive endocrine function are more limited and processes of somatic aging become of paramount concern. Studies are needed to characterize the hormonal changes of postmenopause from Stage +1 to +2 because data across these stages are limited[3].

1.1. Steroid hormones after menopause

At the menopause a dramatic decline in plasma estradiol level occurs, the postmenopausal ovary will cease to contribute do estradiol levels in blood and peripheral conversion of androstenedione into estrone becomes prominent[1].

Androgen production in women takes place in three compartments: the ovary, adrenal, and peripheral tissues. The peripheral compartment involves the interconversion of androgens, as well as the conversion of androgen to estrogen (via aromatase activity)[4].

The major androgens in women, listed in descending order of serum concentration, include dehydroepiandrosterone sulphate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione (A), testosterone (T), and dihydrotestosterone (DHT). However, the first three are more correctly considered as pro-androgens, which require conversion to T to express their androgenic effects (Figure 2)[5].

DHEAS is a unique secretory product of the adrenal gland and its decline with age is not related to menopause. DHEA is produced in the adrenal zona reticularis and the ovarian theca, and around 30% derives from circulating DHEAS[6]. DHEA can also be produced intracellularly from DHEAS in the course of peripheral androgen synthesis and its levels decrease steadily with age. Testosterone and androstenedione are secreted by the adrenal zona fasciculata and the ovarian stroma[5]. Testosterone is aromatized to estradiol intracellularly. Aromatization may increase with age, altering the effects of testosterone in older women[7].

In women of reproductive age, daily production of testosterone is shared equally between the ovaries and adrenal glands and accounts for approximately one-third of the testosterone in circulation. Peripheral conversion of androgen precursor steroids to testosterone in non-steroid producing tissues accounts for the remaining two-thirds of testosterone in circulation. These ratios change after menopause when the ovaries are in senescence[8]. Current evidence suggests that the fall in androgens is not as clearly defined as the sharp fall in estradiol at menopause, as perhaps may be expected with the decline in ovarian function[9]. The postmenopausal ovary continues to produce androgens in a proportion that varies across studies, as will be discussed later in more detail.

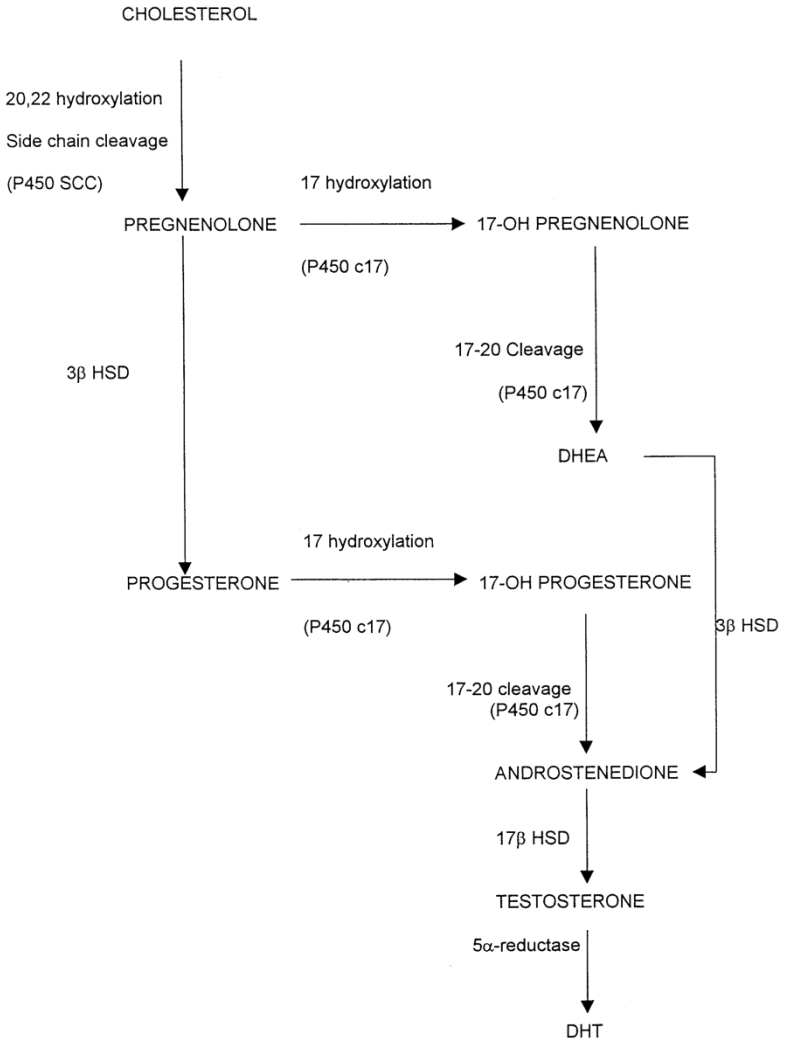

Figure 2 - Schema of androgen biosynthesis
Reprinted from Fertil Steril, 2002 Apr;77 Suppl 4, Burger HG, Androgen production in women, pages S3-5, Copyright 2002, with permission from Elsevier

A group of Canadian researchers suggested an innovative theory based on intracrinology. Labrie *et al* stated that after menopause the only source of sex steroids

is circulating DHEA which is converted into androgens and estrogens by peripheral tissues using the process of intracrinology[10]. The steroids produced inside the cells are also inactivated intracellularly at their site of synthesis, maintaining the circulating levels of both estrogens and androgens at very low and biologically inactive concentrations, thus avoiding stimulation of the endometrium and a possible action in other tissues[11]. According to these researchers, about 80% of DHEA comes from the adrenal gland and 20% from the ovary, and its production decreases over time[12]. Menopause is associated with a 60% decline in adrenal androgens, including DHEA. They therefore consider that the decrease in DHEA production over time is responsible for menopausal symptoms[11].

Research data suggests that some changes associated with menopause and aging may be related to the lack of hormones other than estrogen, in particular of the androgen class. Postmenopausal androgen deficit has been associated with decreased feelings of well-being and depression[13,14], changes in sexual fuction[15–20], cognitive impairment and memory loss[21–23], loss of muscle mass[24,25] and increased risk of osteoporosis and fractures[25–29]. The relationship between androgens and cardiovascular risk is rather complex. A few studies linking androgen levels with cardiovascular risk suggest that both low and high levels can be harmful[30]. Nevertheless, postmenopausal women with lower androgen levels had a higher incidence of carotid and coronary atherosclerosis[31–34].

At the beginning of the 21st century, the theory of the existence of an Androgen Insufficiency Syndrome was taking shape and in 2002, after a consensus meeting, its components were established (Table 1)[35].

Table 1 - Components of female androgen insufficiency syndrome

Symptoms
Low libido with global decrease in sexual desire or fantasy
Persistent unexplained fatigue
Decreased sense of well-being
Signs
Thinning or loss of pubic hair
Decreased lean body mass
Osteopenia or osteoporosis
Other indications
Symptoms persist despite having normal estrogen production if premenopausal or being on adequate estrogen replacement therapy if hypogonadal
Onset following an event associated with decreased androgen production
Other causes of symptoms have been evaluated and ruled out

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Failure of these signs and symptoms to resolve with estrogen replacement therapy is an important indicator of postmenopausal androgen deficiency. However, the determination of androgen levels still lacks scientific validation due to the lack of standardization, especially in postmenopausal women who in themselves have very low values, and that still present variations with some factors (race, body mass index, tobacco). For these reasons, androgen insufficiency syndrome remains to be validated.
Several studies have tried to demonstrate an improvement in these parameters with androgen therapy or with the addition of androgens to estrogen therapy[36–44]. However, androgen therapy in ovariectomized women, or in other conditions associated with low androgen levels, is not recommended, as there is limited evidence regarding its benefits and there are no long-term studies to determine its risks[45].

1.1.1. Steroid hormones and bone mineral density

As average life expectancy increases, more women will suffer the consequences of bone loss and osteoporosis. Osteoporosis was defined as a systemic skeletal disease characterized by low bone mass and deterioration in microarchitecture which results in an increase in bone fragility and increased susceptibility to fracture[46]. In recent decades, rather than being seen as an unavoidable disease in older women, osteoporosis has come to be seen as a preventable disease, and much progress has been made in its treatment. Osteoporosis is estimated to affect one-fifth of women aged 70, two-fifths of women aged 80, and three-fifths of women aged 90[47]. A recent meta-analysis reported that the worldwide prevalence of osteoporosis in the elderly women is 35.3%[48]. In Europe, in 2010, there were 3.5 million fractures related to osteoporosis. Due to demographic changes, it is estimated that the number of fragility fractures will reach 4.5 million in 2025[49].
Bone mineral density (BMD) is the amount of bone mass per unit volume (volumetric density), or per unit area (areal density), and both can be measured in vivo by densitometric techniques. Dual-energy X-ray absorptiometry (DXA) is the most widely used bone densitometric technique. Areal density (g/cm2) rather than a true volumetric density (g/cm3) is measured since the scan is two dimensional. Bone mineral density is most often described as a T-score or Z-score, both of which are units of standard deviation (SD). The T-score describes the number of SDs by which the BMD in an individual differs from the mean value expected in young healthy individuals[49]. A WHO study group in 1994 defined osteoporosis as a BMD of 2.5 or more SDs below the mean value of young healthy white women[50].

Since Albright`s pioneering studies[51], the association between estrogen deficiency and osteoporosis has been well recognized, and the estrogen-centered theory of the pathogenesis of postmenopausal osteoporosis prevailed[52].

The last decade has seen a paradigm shift, and new evidence has also revealed a possible role for androgens in osteoporosis. Androgens affect bone directly via interactions with androgen receptors, and indirectly via binding to estrogen receptors α and β after aromatization in fat or other tissues[53]. Furthermore, in postmenopausal women the combined treatment of androgens plus estrogens revealed more efficacy in increasing BMD than isolated estrogen[37,54]. However, studies that examined the association of androgens and bone mineral density have shown contradictory results. Although most of the studies revealed an association between androgens, particularly testosterone and BMD, others have failed to reach this conclusion.

1.1.2. Steroid hormones and sexual function

Although the frequency of sexual activity declines with age[55,56], older women engage in frequent and satisfying sexual activity[57]. Women want to talk more often about sexuality-related problems with their doctors and there is a growing awareness in the medical community regarding this issue. Often seen as a minor problem in relation to all of the problems that exist at this stage of life, studies carried out in recent decades have shown that this issue is important for the maintenance of quality of life[58]. In a large study from the United States investigating the sexual experiences of 31 000 women aged 18-102 years the prevalence of sexual problems increased with age: 27.2% (age 18–44 years), 44.6% (age 45–64 years), and 80.1% (age 65 years and older)[59]. However, a few studies mention that distress related to sexual problems changes little with age[60] or even declines[59] and the worldwide prevalence of sexual dysfunction among 40- to 80-year-old women is estimated to be 43%[61]. The etiology of female sexual dysfunction includes biological, psychological, interpersonal and sociocultural risk factors[62]. In addition to lower self-esteem, relationship issues, mental health problems with higher prevalence of depression and chronic diseases such as diabetes, postmenopausal hormonal changes play an important role[63].

Estrogen deprivation is responsible for vulvovaginal atrophy and higher incidence of dyspareunia and topical vaginal estrogen therapy improves sexual function in postmenopausal women[64]. In a recent large study of premenopausal women the authors showed that testosterone, dehydroepiandrosterone and androstenedione have small but significant positive associations with sexual desire, pleasure and self-image[65]. However, few studies have been conducted regarding androgens and sexual function in

postmenopausal women. Androgens appear to play a role in maintaining sexual health and clinical trials have consistently demonstrated that testosterone therapy improves sexual function in women with hypoactive sexual desire disorder[66].

In 2018, a consensus document of the International Society for the Study of Women´s Sexual Health was published, which stated that vaginal administration of DHEA has been shown to be effective in the treatment of dyspareunia, whereas vaginal testosterone administration may be effective[67]. In 2019, a consensus of the most important international societies (*Global Consensus Position Statement on the Use of Testosterone Therapy for Women*) established that the only current indication for testosterone therapy is hypoactive sexual desire, with available evidence revealing a moderate therapeutic effect. Its long-term safety has not yet been established[68].

1.1.3. Steroid hormones and cognitive function

Approximately 50 million people worldwide live with dementia, with Alzheimer's disease (AD) being the most frequent cause[69]. Women are more impacted by AD than men, presenting significantly greater risk of developing AD and a greater cognitive deterioration than men at the same disease stage[70]. Women live longer than men and the average life expectancy is increasing, but despite this, there seem to be sex differences in the brain that depend on multiple social and biological factors. An age-related loss of sex steroid hormones has been associated with an increased risk of cognitive decline[71].

The majority of the studies on the impact of sex hormones on the postmenopausal brain have focused on the lack of estrogen. Estrogen is known to have an essential role in the brain: promotes neurotrophin synthesis, modulates cholinergic and dopaminergic neurotransmitter systems and protects the brain against stress and inflammation[72]. A prolonged reproductive period (indicative of a greater lifetime exposure to female sex hormones) may be associated with higher cognitive performance and delayed cognitive decline[73–76]. Nulliparity was also associated to decreased cognitive decline[77]. Bilateral oophorectomy resulting in surgical menopause, with a steep decline in estrogen production, was inversely associated with cognitive performance[78–80].

Postmenopausal women with higher remaining circulating estradiol levels have better global cognitive function[81,82], verbal memory[83,84], and better semantic memory performance[22], but other studies have revealed contradictory results[85–87]. High levels of estrogen have been associated with enhanced verbal fluency[71,72,81], but reports are not consistent[88].

The literature also reports conflicting evidence regarding the effect of systemic hormone replacement therapy (HRT): observational studies have suggested some beneficial effects of HRT on cognition, although some researchers have identified cognitive decline or an increased risk of dementia associated with HRT; interventional studies indicated detrimental effects of HRT on older women, leading to cognitive decline and a greater risk of dementia[89].

Likewise, the influence of androgens on postmenopausal cognitive function is not well understood and the scientific evidence is contradictory. Although there has been an interest in conducting studies on the effects of androgen therapy on cognitive function, there are very few studies demonstrating an association of endogenous androgen levels with cognitive function. Research studies have shown a positive association between verbal learning and memory and physiological concentrations of testosterone administered to postmenopausal women exogenously[42,89]. However, it was also reported that higher endogenous testosterone levels were associated to lower scores of cognitive function[90], and lower endogenous testosterone levels were associated to an improvement in verbal episodic memory[22], or showed no association[87].

1.1.4. Measurement of steroid hormone levels

In the 1960s, 70s and 80s, radioimmunoassay was the main technique used for the dosing of steroid hormones. Despite the high throughput presented by these methods, the use of radioisotopes makes decontamination mandatory. Currently, non-radioactively labeled detection techniques (such as chemiluminescence or electrochemiluminescence) are widely implemented. Instruments for immunoassay-based methods are relatively easy to use, while sample preparation steps are not required and the cost is reasonable. However, these assays lack specificity due to the cross-reactivity of the antibodies with other steroid hormones[91].

Interest has recently arisen in the use of new analytical techniques based on mass spectrometry for the ultrasensitive determination of androgen concentration[92–94]. Mass spectrometry offers a unique identification profile of each of the study analytes, eliminating interferences, thus allowing greater sensitivity and specificity[95]. Mass spectrometry based (MS-based) techniques are now the gold standard for measuring steroid hormones in postmenopausal women, as they have greater accuracy and specificity than immunoassays, due to the very low serum concentrations of these hormones in the postmenopausal period[96].

Although liquid chromatography and tandem mass spectrometry (LC -MS/MS) has become the preferred method for simple bioanalysis of an extended range of compound

classes, gas chromatography and tandem mass spectrometry (GC-MS/MS) has higher accuracy, precision, sensitivity and specificity when it comes to measuring estrogen and androgens in the postmenopausal period[97]. Derivatization is necessary for some of these compounds when gas chromatographic methods are used, since it improves the sensitivity and resolution of the separation. This is necessary to achieve the low concentrations usually found in biological specimens[91,97]. Concerning LC-based procedures, ion suppression can be directly related to inadequate sample preparation, and it is a major problem of LC-MS/MS techniques[98]. For instance, regarding the determination of testosterone in plasma from postmenopausal women, Thakur et al have stated that GC/MS-MS provides excellent sensitivity and specificity when compared to liquid chromatographic methods, and helps elucidating the pharmacokinetic parameters of testosterone-related therapy, allowing as well monitoring endogenous testosterone as a pharmacodynamic biomarker[97]. In fact, there are different published works about the determination of these compounds using GC-MS/MS[99–102].

1.2. The ovaries after menopause

The idea that the ovary after menopause does not become inert, and continues to have an endocrine function, is not recent. For decades, there has been controversy over whether the postmenopausal ovary is an androgen production site. In vitro studies have demonstrated the presence of all enzymes necessary for steroidogenesis in postmenopausal ovarian stroma[103,104]. Pioneering studies that began in the 1970s were carried out with the aim of determining the relative importance of the ovary and the adrenal gland in postmenopausal androgen production[105–113]. Some of these studies, with reduced samples, were based on the measurement of the gradient of androgen concentration in the ovarian vein and peripheral blood, in postmenopausal women undergoing oophorectomy[105,109–111,113,114]. Judd et al were the first investigators to demonstrate a reduction in testosterone and androstenedione concentrations after bilateral oophorectomy[105]. Other studies resorted to pharmacological stimulation or suppression of the adrenal gland, also confirming that the ovary maintains endocrine activity in the postmenopausal period[106–108]. In 2000 the Rancho Bernardo Study was published, the first population-based study that examined the association between plasma hormone levels, previous oophorectomy and time after menopause. With a sample of 684 women between 50 and 89 years old, they found that total and free testosterone levels decreased by 30% in women who underwent hysterectomy and

bilateral oophorectomy compared to women who underwent hysterectomy with preservation of the ovaries. Interestingly, in non-ovariectomized women, testosterone levels increased with age, reaching premenopausal levels in the group of women aged 70-79 years[115].

However, a study published in 2001 by Couzinet et al provided evidence that in a very specific sample of postmenopausal women with adrenal insufficiency, oophorectomy had no impact on circulating plasma androgen levels, thus concluding that the adrenal gland would be the main responsible for the production of androgens after menopause and the ovary would have a negligible role[116]. This study had a great scientific impact at that time.

In 2007, Fogle et al published a study with a methodology similar to that of studies carried out in the 1970s and 1980s, using more adequate techniques for collecting blood from the ovarian vein and more sensitive radioimmunoassay analytical technology, confirming once again the existence of a difference in the gradient between the concentration of androgens in the ovarian vein vs peripheral blood, before and after performing bilateral oophorectomy in 13 postmenopausal women[117].

More recent studies have also demonstrated a significant decline in postmenopausal plasma testosterone levels after bilateral oophorectomy[93,118–120]. Labrie et al reported a 20% contribution of the ovary to the total pool of DHEA which, according to the intracrinology theory, could possibly explain the reported negative effect of oophorectomy on longevity[12].

In fact, several studies have shown that the postmenopausal ovary continues to produce androgens and this potential seems to persist for many years.

1.2.1. The impact of bilateral oophorectomy

The removal of apparently normal ovaries and fallopian tubes at the same surgical time as the hysterectomy (prophylactic bilateral salpingo-oophorectomy or adnexectomy) is a frequent practice and is performed with the main objective of preventing ovarian cancer. It is a cancer with insidious clinical manifestations and ineffective screening, so that in more than half of the cases it is detected at an advanced stage and, as such, with a poor prognosis. If there is a consensus that bilateral salpingo-oophorectomy (BSO) should be performed in women with genetic mutations that confer a high risk of ovarian and breast cancer (BRCA 1 and 2)[121], in women who do not carry high risk genetic mutations, the risk/benefit balance of this intervention is uncertain and remains a controversial topic among gynecologists. On the one hand, prophylactic BSO significantly and consistently decreases the risk of ovarian cancer. A 24-year follow-up of the Nurses' Health Study (NHS) revealed that only 34 of 13,035 women (0.26%) who

had ovarian conservation at the time of hysterectomy died from ovarian cancer[122]. In the cohort of the Women's Health Initiative Observational Study (WHI), which included women who had a history of hysterectomy and BSO (n = 14 254) or hysterectomy with ovarian conservation (n = 11 194) and no family history of ovarian cancer, it was found that ovarian cancer was rare in both groups. In the hysterectomy-only group, 0.33% of the women developed ovarian cancer compared with 0.02% of the women in the BSO group[123]. A recent study, published in 2021, included 195,282 women who underwent hysterectomy for benign pathology: BSO was performed in 46 661 women and ovarian conservation in 148 621 women. BSO was associated with absolute reductions of 0.38% in ovarian cancer incidence and 0.18% in ovarian cancer death for >20 years follow-up. Among women over 50 years, absolute reductions in incidence and death were 0.62% and 0.42%, respectively[124]. On the other hand, the incidence of ovarian cancer after isolated hysterectomy is also decreased[123,125] and the incidence of ovarian cancer is never reduced to zero after BSO, as there is always a residual risk of peritoneal carcinoma.
Regarding the risk of breast cancer in the general population, a risk reduction was detected among women who underwent prophylactic BSO[122,126]. The greatest reduction was observed in women with BSO performed before age 45, but even in women older than 55 there was a significant decrease in breast cancer incidence with ovarian removal. It is noteworthy that simple hysterectomy before age 45 years was also associated with a lower risk of breast cancer[126].

The beneficial effects of premenopausal prophylactic BSO in ovarian and breast cancer should be balanced against potential negative effects on women's health. An increased incidence of cardiovascular disease[122,127–132], osteoporosis[133], neurological diseases[80,134–136], depression[137] and changes in sexual function[138–140] has been demonstrated after premenopausal BSO. Other observational studies have also shown that despite reduction in ovarian and breast cancer, BSO performed before age 45 or 50 years is associated with increased all-cause mortality[131,141–145].
A study published in 2005, based on a Markov model to assess the optimal age for BSO at the time of hysterectomy, found that BSO should be delayed to age 65 years to mitigate mortality[146]. This article significantly affected the gynecology community for decades and still continues to be cited as justification to delay BSO to age 65 years. A recently published study revised the original model to include contemporary research and calculating proportion alive to age 80 years based on age at surgery, and they found that the age at which BSO can safely be performed without undue increased mortality was at age 50 years or older[147].

If it is currently relatively consensual that the ovaries of premenopausal women should be preserved whenever possible, in postmenopausal women undergoing bilateral oophorectomy, the scientific evidence is less consistent.
Although the NHS showed that postmenopausal BSO could be associated to higher mortality rates[141], other studies did not support this[123,142,144,148].

Regarding the cardiovascular impact, it was found that bilateral oophorectomy performed after the age of 50 increases the risk of myocardial infarction[148]. In a meta-analysis of 18 observational studies, cardiovascular risk doubled in postmenopausal women undergoing bilateral oophorectomy (RR:2.62; IC 95%: 2.05-3.35)[149]. However, some flaws were pointed out to this study and a subsequent systematic review did not find an increase in cardiovascular risk in women undergoing prophylactic bilateral oophorectomy compared to hysterectomy alone[150]. Other studies, which included the WHI study, were also in agreement with this finding[123,129]. However, in 2013, updated NHS cohort data were published, which continued to demonstrate an increase in coronary pathology and stroke, even in women undergoing bilateral oophorectomy after age 55[141]. The follow-up period for women was longer in the NHS study than in the WHI (28 years *vs* 8 years). Additionally, a study published in 2023, also showed an increased cardiovascular mortality in women with BSO performed over 53 years[132]. Another recent study, reported that women who underwent hysterectomy alone were also at greater risk of cardiovascular disease and coronary revascularization compared with no surgery, although the risk was higher when BSO was performed, even after age 50 at surgery[151]. It should be noted that most of these studies stratified participants by age rather than by menopausal status.

A study of 340 postmenopausal women undergoing prophylactic bilateral oophorectomy revealed 54% more osteoporotic fractures in these women than in women with ovarian conservation. The authors concluded that the results were in line with the hypothesis that androgens produced by the postmenopausal ovary would be important in the prevention of osteoporosis[152]. Subsequently, some studies revealed contradictory results. A study showed that postmenopausal women undergoing prophylactic bilateral oophorectomy had lower free testosterone levels, but did not have lower bone mineral density values or greater risk of fracture than women with intact ovaries[153]. The NHS and the WHI studies also showed no increased risk of femoral neck fracture in women undergoing prophylactic bilateral oophorectomy before or after menopause[122,123]. In 2014, a secondary analysis of a randomized controlled trial in 222 postmenopausal women found a significant decline in bone mineral density values in

the lumbar spine and femoral neck in previously oophorectomized women, which was greater the longer the time elapsed since menopause, although they did not present data regarding if women underwent bilateral oophorectomy before or after menopause[154].

The impact of BSO on sexual function in postmenopausal women has not been well studied. A study of 92 postmenopausal women, aged 45–55 years, who were scheduled for hysterectomy and BSO due to benign causes, compared preoperative sexual function and six months after surgery, and showed a significant reduction in sexual function scores after surgery[155]. Another study included postmenopausal women aged 57-85 years: 356 women reported prior bilateral oophorectomy and 996 women had retained their ovaries. Among women with prior bilateral oophorectomy, 75.4% had their ovaries removed prior to menopause. No significant differences in the report of sexual ideation or sexual problems were found between women with prior bilateral oophorectomy and women who retained their ovaries. The menopausal status at the time of oophorectomy did not impact the results[156].

As for the impact of postmenopausal BSO on cognitive function, the available scientific evidence is also sparse. Two studies showed that unlike premenopausal BSO, postmenopausal BSO was not associated with cognitive decline[79,80]. A recent study using the Montreal Cognitive Assessment test (MoCA) showed that bilateral oophorectomy was significantly associated with mild cognitive impairment, regardless being performed above or below 45 years, but did not specify menopausal status at the time of surgery[157].

1.3. The scope of the problem

Hysterectomy is the most common surgery performed in non-pregnant women worldwide[148]. Removal of the ovaries during hysterectomy is the most frequent prophylactic intervention and the risk/benefit of this intervention remains unclear in the postmenopausal period.
In Portugal, the overall rate of hysterectomy in 2014 was 171/100 000 women per year. In women submitted to hysterectomy for benign pathology, the rate of bilateral adnexectomy decreased from 71.0% in 2000 to 51.9% in 2014. This rate significantly reduced in all age groups with the exception of women over 70 years old (Table 2)[158].

Some hospital services maintain systematic bilateral oophorectomy protocols above 50, 60 or 65 years of age for ovarian cancer prophylaxis, in apparently normal ovaries.

In the United States of America, more than 4 out of 10 women aged 70-79 years reported having had a hysterectomy[159]. Between 1998 and 2011, more than 60% of women aged 45-65 years underwent bilateral adnexectomy at the time of hysterectomy[160].

Table 2 - Hysterectomy route and concomitant adnexal surgery stratified by age at time of hysterectomy, in Portugal (2000 vs 2014)

			Hysterectomy route			Concomitant adnexal surgery#	
Age at time of hysterectomy (years)	**Year of the surgery**	**Percentage from the total of hysterectomies (n)**	**Laparotomy**	**Vaginal**	**Laparoscopy**	**Bilateral adnexectomy**	**Bilateral salpingectomy**
Younger than 40	2000	9.2% (n = 1041)	95.0% (n = 989)	3.8% (n = 39)	1.2% (n = 12)	22.1% (n = 142)	2.2% (n = 14)
	2014	5.9% (n = 553)***	81.3% (n = 448)***	6.0% (n = 33)*	12.7% (n = 70)***	12.1% (n = 36)***	25.6% (n = 76)***
40–49	2000	43.0% (n = 4872)	94.8% (n = 4614)	4.0% (n = 195)	1.2% (n = 60)	67.1% (n = 2483)	1.1% (n = 41)
	2014	33.7% (n = 3144)***	80.7% (n = 2535)***	6.7% (n = 209)***	12.8% (n = 401)***	32.1% (n = 811)***	22.6% (n = 571)***
50–59	2000	25.4% (n = 2876)	88.0% (n = 2532)	10.7% (n = 307)	1.2% (n = 35)	90.2% (n = 1621)	0.5% (n = 9)
	2014	27.2% (n = 2532)**	72.0% (n = 1822)***	17.5% (n = 444)***	10.4% (n = 264)***	76.7% (n = 1222)***	6.8% (n = 109)***
60–69	2000	12.4% (n = 1403)	64.1% (n = 898)	34.5% (n = 483)	1.1% (n = 15)	92.7% (n = 341)	0.0% (n = 0)
	2014	17.6% (n = 1642)***	50.8% (n = 831)***	43.2% (n = 708)***	5.7% (n = 94)***	85.1% (n = 343)**	2.5% (n = 10)**
70 or older	2000	10.0% (n = 1127)	57.1% (n = 643)	42.2% (n = 475)	0.7% (n = 8)	88.9% (n = 168)	0.0% (n = 0)
	2014	15.6% (n = 1455)***	55.1% (n = 801)	40.0% (n = 582)	4.1% (n = 60)***	85.6% (n = 226)	0.8% (n = 2)
Total	2000	100% (n = 11 319)	85.5% (n = 9676)	13.3% (n = 1499)	1.2% (n = 130)	71.0% (n = 4755)	1.0% (n = 64)
(any age)	2014	100% (n = 9326)	69.1% (n = 6437)***	21.2% (n = 1976)***	9.5% (n = 889)***	51.9% (n = 2638)***	15.1% (n = 768)***

Proportion of hysterectomy in each age group was analysed by the $\chi 2$ test (2000 vs. 2014). In each age group, each route of hysterectomy and concomitant adnexal surgery were analysed by the $\chi 2$ test (2000 vs. 2014). # In the group of women with hysterectomy for benign pathology; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Reprinted from Gante I, et al. Hysterectomies in Portugal (2000-2014): What has changed? Eur J Obstet Gynecol Reprod Biol. 2017 Jan;208:97–102. (CC BY-NC-ND 4.0)

Prophylactic removal of ovaries, like prophylactic removal of other organs (breast, colon, or appendix) should only be performed when the benefits outweigh the risks.

In the last decade, a theory has emerged based on several studies that demonstrated the fallopian tubes were the origin of most serous carcinomas of the ovary and peritoneum. This paradigm shift will have implications for surgical management, whereby bilateral salpingectomy with preservation of the ovaries in situations of hysterectomy for benign pathology will reduce the rate of ovarian cancer, avoiding the morbidity caused by bilateral oophorectomy[161]. Based on the current understanding of ovarian carcinogenesis and the safety of salpingectomy, the American College of Obstetricians and Gynecologists developed a Committee Opinion and in 2019 recommended that the surgeon and patient should discuss the potential benefits of the removal of the fallopian tubes during a hysterectomy in women at population risk of ovarian cancer who are not

having an oophorectomy. Counseling women who are undergoing routine pelvic surgery about the risks and benefits of salpingectomy should include an informed consent discussion about the role of oophorectomy and bilateral salpingo-oophorectomy[162].

In Portugal, the rate of bilateral salpingectomy during hysterectomy for benign pathology increased from 1.0% in 2000 to 15.1% in 2014, but strongly depended on age: in women under 40 the rate increased from 2.2% to 25.6% but in those aged 60 or more the rate remained low[158], most likely because above this age the BSO rates were high.

The decision regarding concurrent BSO with hysterectomy is also influenced by reoperation rates due to subsequent ovarian pathology or adnexal pain from postsurgical adhesions, but the incidence of oophorectomy after hysterectomy is only 9.2% at 30-year follow-up and is only 1.9 percentage points higher than the incidence of oophorectomy in referent women with intact reproductive organs[163].

The physician's recommendation to perform an elective BSO at the time of a hysterectomy for a benign condition is strongly influenced by the patients' age[164] but there is marked between-surgeon variation in BSO rates, which suggests ongoing uncertainty in practice[148]. Although it is consensual that premenopausal prophylactic bilateral oophorectomy should not be performed because it has harmful effects on women`s health and increases long-term mortality rates, 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.

It is thought that the ovaries continue to produce androgens after menopause, but their real influence on the consequences of menopause is still unknown. Do the ovaries have a secondary role in relation to the adrenal gland and as such are subject to prophylactic removal after menopause? Or, on the other hand, could the continuous production of androgens throughout menopause, even in small amounts, be beneficial at multiple levels in aging?

Taking into account that the average life expectancy tends to increase and prophylactic bilateral oophorectomy continues to be performed frequently, it is essential to determine the role of the ovary in the health of older women.

2. Aims

This study aims to clarify the role of the ovaries in the postmenopausal period, by determining the effect of postmenopausal bilateral oophorectomy on plasma steroid hormone levels and its impact on older women's health.

The specific objectives of this study are:

- To compare serum concentrations of steroid hormones in women who underwent hysterectomy after menopause, with and without concomitant bilateral salpingo-oophorectomy;
- To determine if lower levels of steroid hormone levels are associated with lower levels of bone mineral density in older women;
- To assess the association between steroid hormone levels and sexual function in postmenopausal women;
- To assess the association between steroid hormone levels and cognitive function in postmenopausal women.

3. Materials and methods

The study was performed according to established ethical guidelines and approval of the Ethics Committee of Amato Lusitano Hospital/ Castelo Branco Local Health Unit (ULSCB), a tertiary hospital in Castelo Branco, Portugal. A written informed consent was obtained from each participant in the study.

To carry out this study, the creation of a Gynecology/Menopause consultation was approved by the ULSCB Board of Directors. The referral for this consultation was done through the usual form of referral or by registering the patient directly at the Outpatient Department counter of the hospital. The only requirement was to be in the postmenopausal period.

From January 1, 2017 to June 30, 2019, 261 postmenopausal women were evaluated in this consultation, 203 of which met the inclusion criteria and consented to participate in this study.

The general inclusion criteria were postmenopausal women who had intact ovaries at the time of menopause. Postmenopausal status was based on 12 months of amenorrhea and serum follicle stimulating hormone levels greater than 30 mUI/mL. The general exclusion criteria were: current or past users of systemic HRT or corticosteroid treatment, current adnexal pathology, alcoholism, narcotic addiction and chronic hepatic or renal diseases. Adnexal pathology was excluded by performing transvaginal ultrasound to all the participants.

3.1. Participants and study design

3.1.1. Steroid hormone levels in postmenopausal hysterectomized women with and without ovarian conservation

Of the 203 participants, a total of 29 women were hysterectomized after menopause for benign conditions: 18 had isolated hysterectomy and 11 women had hysterectomy with BSO. It was our intention to compare steroid hormone levels in 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, in order to eliminate the possible interference of the surgery in the ovarian vascularization when ovaries were retained.

3.1.2. Steroid hormone levels and bone mineral density in women over 65 years of age

Bone densitometry was performed on women over 65 years of age, according to the general health department`s guidelines. In Portugal, there is a guideline issued by the General Health Department (DGS) that recommends that all women over 65 should undergo bone densitometry of the lumbar spine and femoral neck using DXA technology[165]. For all patients included in this study, measurement of the BMD of the lumbar spine and femoral neck was performed in the same center using a GE Lunar Prodigy DXA system (GE Healthcare, Madison, WI, USA).

A total of 68 participants were enrolled in a cross-sectional study on the association between steroid hormone levels and bone mineral density. Other specific exclusion criteria for this study included history of taking any medication for osteoporosis, and history of endocrine or rheumatologic diseases.

3.1.3. Postmenopausal sexual function and steroid hormone levels

In the consultation, participants were asked to complete the Portuguese version of the Female Sexual Function Index (FSFI) questionnaire[166], validated in the Portuguese population[167]. The FSFI questionnaire is the most widely used screening tool and outcome measure of female sexual function and consists of a 19-item written questionnaire concerning sexual activity in the last 4 weeks. Each answer is assigned a value and the sum of the values of specific questions corrected by a factor allows to assess six domains of sexual function: desire, arousal, lubrication, orgasm, satisfaction and pain (dyspareunia). The domain scores are added, resulting in a final score that can range from 2 to 36. Higher scores indicate a better degree of sexual function and an FSFI total score of 26.55 or less indicates sexual dysfunction[168].

The FSFI questionnaire was completed by 168 women, but only 50% had been sexually active in the previous four weeks and were included in this study. Among the women who refused to fill in the questionnaire, some did not explain the reason, others did not know how to read or did not bring their reading glasses.

A total of 84 postmenopausal women were enrolled in a cross-sectional study on the association between steroid hormone levels and sexual function.

3.1.4. Postmenopausal cognitive function and steroid hormone levels

Cognitive function was assessed in 147 women, using the 7.1 original version of the Montreal Cognitive Assessment (MoCA) test created in Canada[169] and validated for the Portuguese population[170]. The MoCA test assesses six domains (memory, visuospatial capacity, executive function, attention, language and orientation to time and place),

having a maximum score of 30 points. The short-term memory recall task (5 points) involves two learning trials of five nouns and delayed recall after approximately 5minutes. Visuospatial abilities are assessed using a clock-drawing task (3 points) and a three-dimensional cube copy (1 point). Multiple aspects of executive functions are assessed using an alternation task adapted from the Trail Making B task (1 point), a phonemic fluency task (1 point), and a two-item verbal abstraction task (2 points). Attention, concentration, and working memory are evaluated using a sustained attention task (target detection using tapping; 1 point), a serial subtraction task (3 points), and digits forward and backward (1 point each). Language is assessed using a three-item confrontation naming task with low-familiarity animals (lion, camel, rhinoceros; 3 points), repetition of two syntactically complex sentences (2 points), and the aforementioned fluency task. Finally, orientation to time and place is evaluated (6 points). In the original version, the cut-off point to define mild cognitive impairment (MCI) is 26 points[169].

It should be noted that during the period in which this study was carried out, certification for administering the MoCA test was not mandatory, and only became so since September 1st 2019. The MoCA test was administered by only one clinician within the scope of the consultation, who also recorded her personal and family anamnestic data, and referral to Neurology was prompted when the final score was less than 18 points (moderate or severe cognitive impairment).

3.2. Laboratory analysis

The laboratory methodology was common to all studies that are part of this thesis.

One blood sample was obtained from each woman between 8 and 10 am at the hospital laboratory, either on the day of consultation and completion of the questionnaires, or on the following day. All blood samples were centrifuged within 1 h of collection to separate serum. Serum samples were transported in refrigerated bags to the Health Sciences Research Center of University of Beira Interior (CICS-UBI), where they were stored at −80 °C and protected from light until analysis.

In the laboratory of Pharmaco-Toxicology of UBIMedical, a method was developed and validated for the identification and quantification of 17-beta-estradiol (E2), testosterone (T), androstenedione (A) and dehydroepiandrosterone (DHEA) in blood samples by solid-phase extraction (SPE) and GC-MS/MS.

Testosterone measurement performed refers to total testosterone. The Global Consensus Position Statement on the Use of Testosterone Therapy for Women

published in 2019 recommends that current research into testosterone physiology and clinical effects should mainly focus on measuring total testosterone as the main biomarker rather than the free testosterone because evidence that the free testosterone is the biologically active fraction is lacking[171].

3.2.1. Reagents and standards

The analytical standards DHEA, A, E2 and T as well as the internal standards, DHEA-d6, were purchased as 1 mg/mL solutions from Sigma-Aldrich (Sintra, Portugal). Methanol LiChrosolv® (HPLC grade) and acetic acid (analytical reagent grade) were purchased from VWR International (Carnaxide, Portugal). Lastly, deionized water was obtained from a Milli-Q System (Millipore Billerica, MA, USA). Oasis® HLB Solid Phase extraction cartridges (3cc) were obtained from Waters (Milford, MA, USA). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Sigma-Aldrich (Sintra, Portugal). Working solutions of the compounds of interest were prepared at the final concentrations of 1µg/mL and 10 and 100 ng/mL with methanol. In the case of internal standards, a working solution at 10 µg/mL was prepared also in methanol. All standard solutions were stored at -20 °C and protected from light.

3.2.2. Gas chromatographic and mass spectrometric conditions

An HP 7890A gas chromatography system, equipped with a model 7000 triple quadrupole mass selective detector, both from Agilent Technologies (Soquimica, Portugal), was used. This system also had an automatic MPS2 autosampler injector from Gerstel (Mülheim an der Ruhr, Germany). The separation of the analytes was achieved using a capillary column of fused silica (30 m ×0.25 mm, 0.25 µm i.d.) with 5% phenylmethylsiloxane (HP-5 MS), supplied by J & W Scientific (Folsom, CA, USA). The oven temperature started at 1500 °C, followed by an increase of 12 °C per minute until 310 °C, which was maintained for 4 min. The total time of the chromatographic run was 17.33 min. The volume of injection was 3 µL in splitless mode, and the injector and detector temperatures were 280 °C and 310 °C, respectively; the source temperature was 230 °C. The carrier gas, helium, was set at a constant flow rate of 1 mL/min.

The mass spectrometer was operated with a filament current of 35 µA and electron energy 70 eV in the positive electron ionization mode. The transitions were chosen based on selectivity and abundance in order to maximize the signal-to-noise ratio in matrix extracts (Table 3).

Table 3 - Retention times and selected ions for the identification of the steroids

Analyte	Transitions (m/z) Precursor ion – Product ion	Retention time (min)	Collision Energy (eV)	Dwell time (µs)
DHEA	202.4-174.1*	11.39	50	5
	202.4-187.2		50	5
Androstenedione	285.4-124.1*	12.24	100	5
	285.4-285.4		100	2
17β-estradiol	285.5-73.1*	12.26	100	20
	285.5-285.5		100	5
Testosterone	207.8-73.1*	12.26	250	20
	207.8-193.1		250	5
DHEA-d6	293.3-293.3	11.39	150	5
DHEA-d6 (derivatized)	284.9-179.1	11.39	15	150

*Represents the transition of the quantifying ion; Dehydroepiandrosterone (DHEA) and internal standard DHEA-d6

3.2.3. Solid-phase extraction procedure

For this study, the extraction was performed with SPE using Oasis® HLB3cc. Before the extraction procedure, 1mL of plasma was diluted with 1 mL of phosphate buffer saline (pH=7) and spiked with 100 µL of internal standard (DHEA-d6). SPE cartridges (Oasis® HLB 3cc, Waters, USA) were conditioned with 2 mL of methanol and 2 mL of 0.1 % acetic acid. After the sample passed through the cartridge, a washing step was performed with 2mL of deionized water. Following this step, the columns were dried under full vacuum for 30 min. Subsequently, the analytes of interest were eluted with 2 mL methanol. The resulting extracts were evaporated to dryness under a steam of nitrogen. The remaining residues were dissolved in 20 µL of methanol and vortex mixed and 3 µL was injected into the GC-MS/MS system. After this step, the remaining residue was evaporated to dryness under a gentle nitrogen stream at 36 °C. The analytes under study present active moieties, and therefore derivatization is deemed necessary to the analysis of E2 and T prior to their analysis by GC-based procedures. To accomplish this, 20 µL of BSTFA was added to the dry extracts, and 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 into the GC-MS/MS system.

3.2.4. Results of validation process

Linearity was achieved between 0.05 to 100 ng/mL for E2; 0.1 to 100 ng/mL for DHEA and A and 0.5 to 100 ng/mL for T. 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; 0.5 ng/mL for T) using only 1mL of sample.

Figure 3 - Chromatogram relative to the lower limit of E2 quantification (C=0.05 ng/mL)

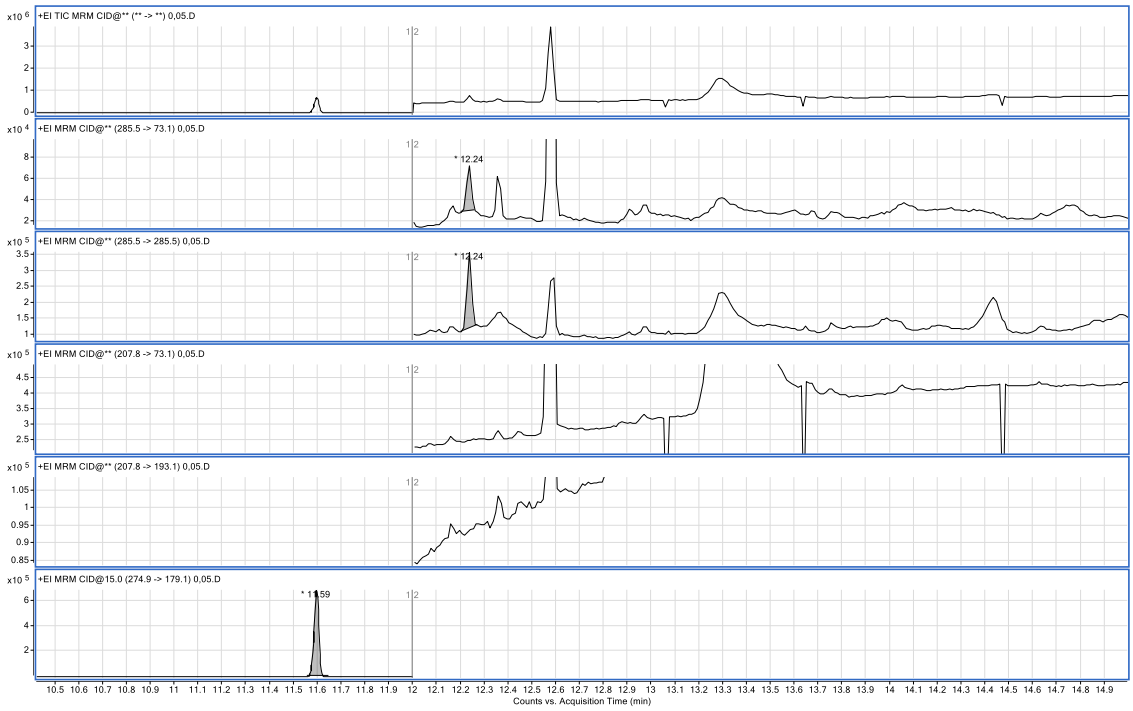


Figure 4 - Chromatogram relative to the lower limit of DHEA quantification (C=0.1 ng/mL)

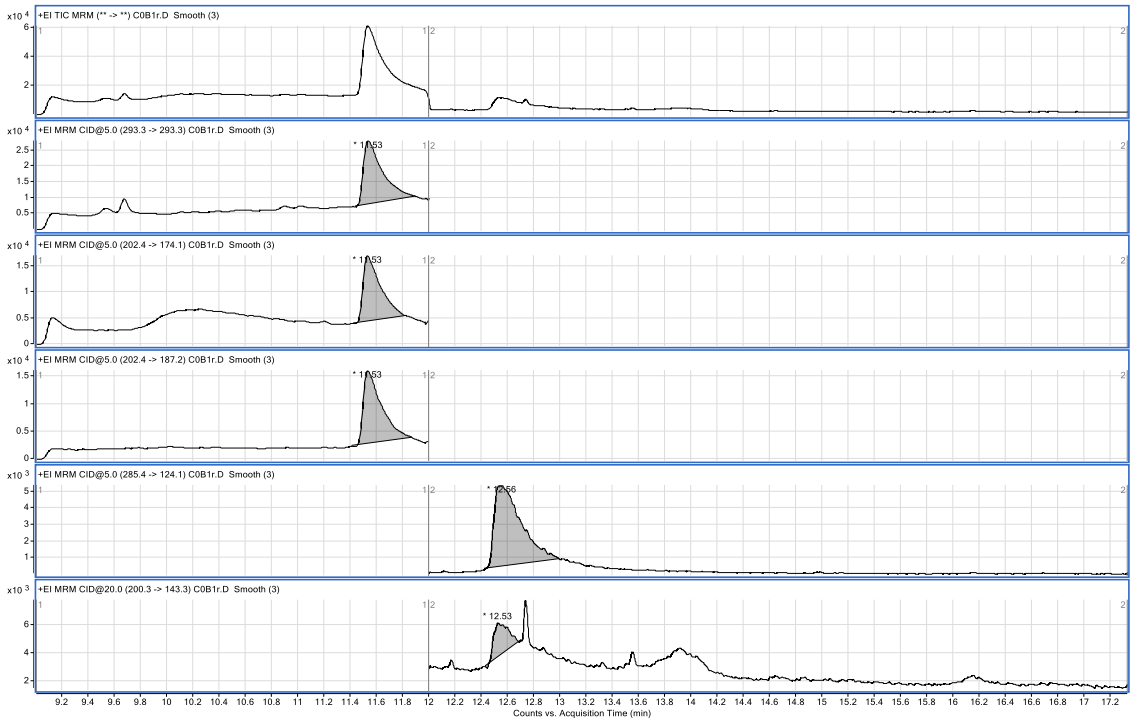


Figure 5 - Chromatogram relative to the lower limit of androstenedione quantification (C=0.1 ng/mL)

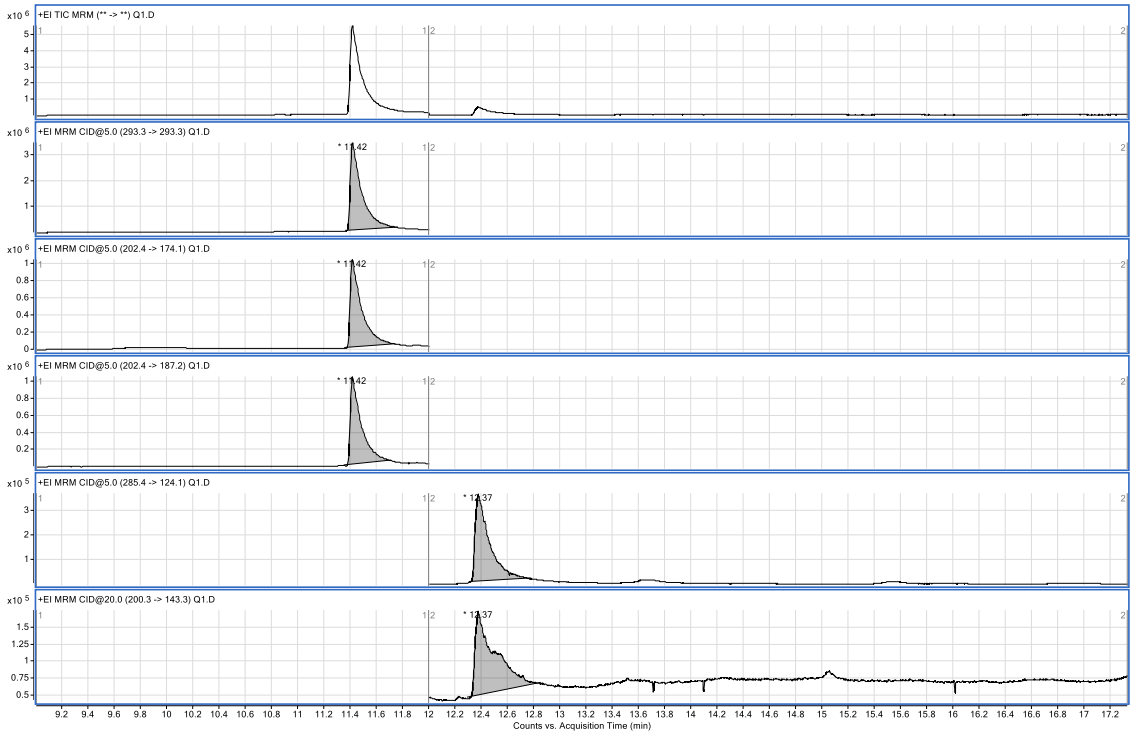
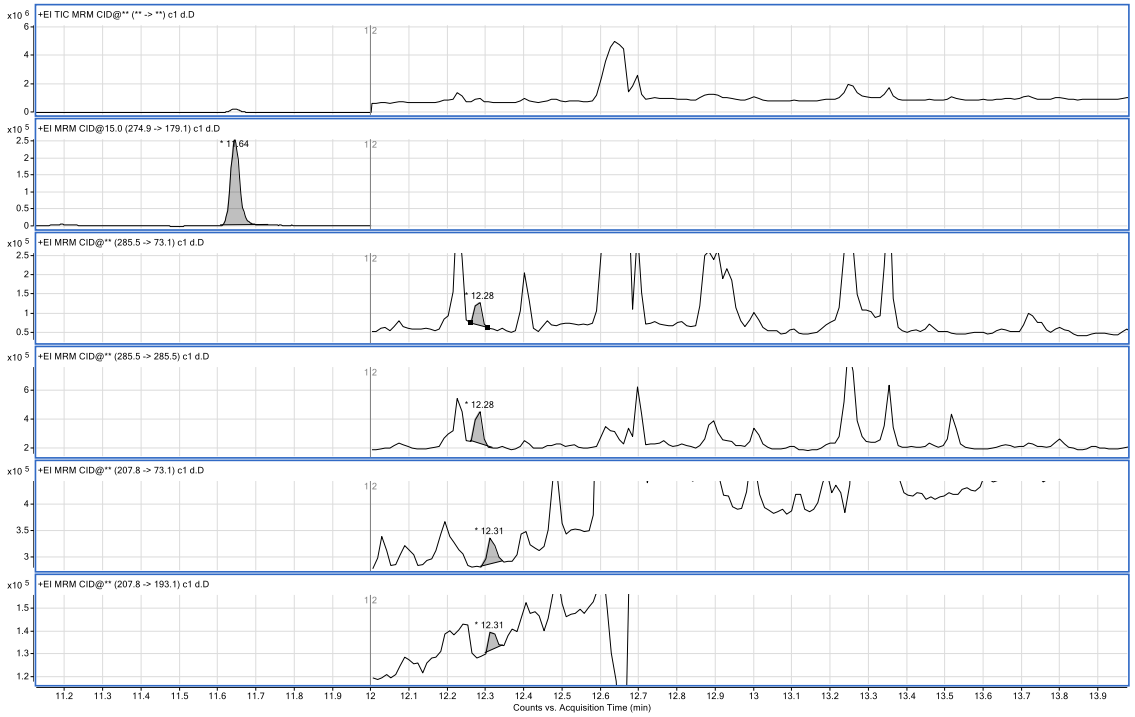


Figure 6 - Chromatogram relative to the lower limit of testosterone quantification (C=0.5 ng/mL)



3.3. Statistical analysis

3.3.1. Steroid hormone levels in postmenopausal hysterectomized women with and without ovarian conservation

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 analyze 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.

3.3.2. Steroid hormone levels and bone mineral density in women over 65 years of age

The statistical analysis software used was SPSS 27.0. Descriptive statistics were reported as means ± SD for continuous variables and as frequencies (%) for categorical variables. Statistical analyses were obtained using Pearson`s correlations to examine associations between variables. In order to analyze the joint effect of the variables under study in the T score of the hip and in the T score of the lumbar spine, two multiple linear regression analyses were performed: one with T score of the hip as the dependent variable and the other with T score of the lumbar spine as the dependent variable. The independent variables were “Age”, “Race”, “Body mass index” (Kg/m2), “Regular alcohol habits”, “Smoking habits”, “Age of menarche”, “Type of menstrual cycles”(regular vs irregular), “Parity”, “Age of menopause”, “Years since menopause“, “Vaginal estrogen use”, “Estradiol levels (ng/mL)”, “Testosterone levels (ng/mL)”, “DHEA levels (ng/mL)”, and “Androstenedione levels (ng/mL)”. Qualitative variables were treated as dummy variables, and the regression method used was the stepwise, in which only regression variables significantly related to the dependent variable entered the regression model. These variables were entered successively according to their degree of association with the dependent variable. A p value of 0.05 or less was considered statistically significant.

3.3.3. Postmenopausal sexual function and steroid hormone levels

The statistical analysis software used was SPSS 27.0. Descriptive statistics were reported as means ± SD and median ± interquartile range (IQR) for continuous variables, and as frequencies (%) for categorical variables. Serum levels of estradiol, testosterone, DHEA and androstenedione were our primary predictive variables and the confounding variables that could interfere with the results were age, reproductive lifespan, parity, years since menopause, body mass index (BMI), smoking habits, regular alcohol habits, diagnosis of depression, diabetes, vaginal estrogen use (2 to 3 times weekly), and education level. Education levels were classified according to International Standard Classification of Education (ISCED). In Portugal, ISCED level 1 corresponds to basic education (the first 6 years), ISCED level 2 to basic education (the next three-year cycle), ISCED level 3 is upper secondary education and ISCED level superior to 4 corresponds to higher education[172]. Reproductive lifespan was defined as the difference between ages at menopause and menarche and categorized into quartiles (<33, 33-37, 38-40, or >40 years). BMI was calculated as weight in kilograms divided by height in meters squared, and categorized according to standard cut points.

Multiple linear regression models were performed considering each of the FSFI domain scores as dependent variables and hormone concentrations as regressors. In a first phase, univariate regression models were performed in order to analyze the effect of each of the hormone concentrations separately on each of the FSFI domain scores. In a second phase, the analysis was adjusted, including the variables age, reproductive lifespan, body mass index, depression, smoking habits and vaginal estrogen use, in order to detect possible changes in the estimated coefficients. The variables parity, regular alcohol habits, diabetes and education levels were not considered in the analysis due to their reduced variability and the variable years since menopause was not considered in the models due to collinearity with the variable age. Qualitative variables were treated as dummy variables and the regression method used was Enter. A p value of 0.05 or less was considered statistically significant. To calculate the confidence intervals for the parameters, bootstrap was used based on a resampling of 1000 bootstrap samples. In particular, we used the bootstrap wild as resampling method and we calculated bootstrap bias-corrected and accelerated (BCa) confidence intervals.

3.3.4. Postmenopausal cognitive function and steroid hormone levels

The statistical analysis software used was SPSS 27.0. Descriptive statistics were reported as means ± SD and range for continuous variables, and as frequencies (%) for categorical variables.

In order to assess the association between cognitive domains measured by the MoCA test (global cognitive function, executive function, visuospatial abilities, short-term memory, attention, concentration and working memory, language, orientation to time and place) and hormone levels (estradiol, testosterone, DHEA and androstenedione), nonparametric partial correlation coefficients were calculated, controlling for confounding variables: age, education level, years since menopause, depression, smoking habits and BMI. Education levels were classified according to International Standard Classification of Education (ISCED). In Portugal, ISCED level 1 corresponds to basic education (the first 6 years), ISCED level 2 to basic education (the next three-year cycle), ISCED level 3 is upper secondary education and ISCED level superior to 4 corresponds to higher education[172]. BMI was calculated as weight in kilograms divided by height in meters squared. The diagnosis of depression was assessed by the current use of antidepressants.

A p value of 0.05 or less was considered statistically significant.

4. Results

4.1. Steroid hormone levels in postmenopausal hysterectomized women with and without ovarian conservation

The mean age of patients in both groups did not reveal statistically significant differences. Both groups are equally homogeneous with regard to BMI and age of hysterectomy (Table 4).

Table 4 - Characterization 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/m²)	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

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 estrogens 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 estradiol; 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 5).

Table 5 - 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**
T (ng/mL)	1.6 ± 3.4	1.0 ± 0.9	0.173
DHEA (ng/mL)	9.4 ± 4.4	5.8 ± 3.2	**0.019**
A (ng/mL)	1.4 ± 1.5	0.97 ± 0.5	0.774

[a] Mann-Whitney test

4.2. Steroid hormone levels and bone mineral density in women over 65 years of age

Table 6 presents the demographic and laboratorial parameters of the participants. The majority of patients were Caucasian (98.5%). Obesity was confirmed in 44.1% of patients: 26.5% obesity class I, 13.2% obesity class II, 4.4% obesity class III; 41.2% of the patients were overweight, and only 14.7% had a normal weight. Most patients (98.5%) did not have alcoholic or smoking habits. Regular menstrual cycles throughout their reproductive life were reported by 88.2% of women. Only 7.4% of women were nulliparous and 75% had given birth to 2 or more children. The mean age of menopause was 50.2 years. The number of years since menopause (years from menopause to date of blood collection) was on average 22 years and in 40 women (58.8%) it was over 20 years. Vaginal estrogen cream was used two to three times weekly by 37 women (54.4%).

Table 6 - Demographic characteristics and mean hormone levels of the participants (n=68)

	Frequency n (%)	**Mean**	**SD**	**Minimum**	**Maximum**
Age (years)		72.2	6.6	65	89
Race (Caucasian)	67 (98.5%)				
BMI (Kg/m2)		29.7	5.2	20	45
Underweight (<18.5Kg/m2)	0 (0%)				
Normal weight (18.5-24.9Kg/m2)	10 (14.7%)				
Overweight (25-29.9Kg/m2)	28 (41.2%)				
Obesity class I (30-34.9Kg/m2)	18 (26.5%)				
Obesity class II (35-39,9Kg/m2)	9 (13.2%)				
Obesity class III (>=40 Kg/m2)	3 (4.4%)				
Age of menarche (years)		12.9	1.6	10	16
Menstrual cycles					
Regular	60 (88.2%)				
Irregular	8 (11.8%)				
Parity		1.9	0.9	0	4
Nulliparous	5 (7.4%)				
Multiparous					
1	12 (17.6%)				
2	34 (50%)				
>2	17 (25%)				
Age of menopause (years)		50.2	4.4	37	58

Years since menopause		22.0	7.3	10	38
10-19	28 (41.2%)				
20-29	29 (42.6%)				
>=30	11 (16.2%)				
Regular alcohol habits	1 (1.5%)				
Smoking habits	1 (1.5%)				
Vaginal estrogen use	37 (54.4%)				
E2 (ng/mL)		0.94	2.49	0.05	18.00
Testosterone (ng/mL)		1.66	3.11	0.50	17.19
DHEA (ng/mL)		9.89	5.01	1.82	32.24
Androstenedione (ng/mL)		1.31	1.02	0.10	5.55

Controlling for all possible confounding variables (age, race, BMI, alcohol and smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, and vaginal estrogen use), positive correlations were found between the T score of the lumbar spine and the femoral neck and all four tested steroid hormones (Table 7). However, only the positive correlation between the testosterone concentration and the T score of the hip was statistically significant (p=0.035).

Table 7 - Correlation coefficients for hormone levels and T scores of the hip and lumbar spine

Hormone levels	**T score hip**		**T score lumbar spine**	
	R	P	R	P
E2	0.162	0.234	0.144	0.289
T	0.283	**0.035**	0.155	0.254
DHEA	0.151	0.266	0.04	0.769
A	0.245	0.069	0.131	0.337

Variables controlled: age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use

By multiple linear regression analysis, it was found that testosterone and BMI positively affected the T score of the hip. Age negatively affected the T score of the hip, being the most predictive variable (Table 8). The type of regression used was stepwise and therefore only the statistically significant variables were expressed in the regression models. The first variable to enter the model was age (Model 1), followed by testosterone (Model 2) and BMI (Model 3). As the variables entered the models, the coefficients remained approximately constant, which means that there are no strong dependency relationships between these variables and therefore multicollinearity problems were not detected. The ANOVA table shows that, globally, the adjusted

models are statistically significant and the proportion of variance explained by the regression models increased as successive variables entered the models (Table 9). We have also observed that only BMI positively affected the T score of the lumbar spine (Table 10) and the model is statistically significant (Table 11).

Table 8 - Beta coefficients for testosterone, age and BMI in adjusted models of prediction of T score of the hip, using stepwise regression

Model		β	Std. Error	P	95% CI
1	Age	-0.056	0.020	0.007	-0.097; -0.016
2	Age	-0.051	0.020	0.012	-0.091; -0.011
	Testosterone (ng/mL)	0.086	0.041	0.037	0.005; 0.167
3	Age	-0.045	0.019	0.024	-0.084; -0.006
	Testosterone (ng/mL)	0.089	0.039	0.028	0.010; 0.167
	Body mass index (Kg/m2)	0.053	0.024	0.029	0.006; 0.101

a. Dependent variable: T score hip
b. Predictors: E2, testosterone, DHEA, androstenedione, age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use

Table 9 - ANOVA table for stepwise regression models adjusted for the dependent variable T score of the hip

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	8.524	1	8.524	7.714	0.007b
	Residual	71.831	65	1.105		
	Total	80.355	66			
2	Regression	13.283	2	6.641	6.337	0.003c
	Residual	67.072	64	1.048		
	Total	80.355	66			
3	Regression	18.187	3	6.062	6.144	0.001d
	Residual	62.167	63	0.987		
	Total	80.355	66			

a. Dependent Variable: T score hip
b. Predictors: Age
c. Predictors: Age, Testosterone levels (ng/mL)
d. Predictors: Age, Testosterone levels (ng/mL), Body mass index (Kg/m2)

Table 10 - Beta coefficient for BMI in adjusted model of prediction of T score of the lumbar spine, using stepwise regression

Model		β	Std. Error	p	95% CI
1	Body mass index (Kg/m2)	0.124	0.038	0.002	0.049; 0.198

a. Dependent variable: T score lumbar spine
b. Predictors: E2, testosterone, DHEA, androstenedione, age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use

Table 11 - ANOVA table for stepwise regression model adjusted for the dependent variable T score of the lumbar spine

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	26.954	1	26.954	10.847	0.002b
	Residual	161.518	65	2.485		
	Total	188.472	66			

a. Dependent Variable: T score lumbar spine
b. Predictors: Body mass index (Kg/m2)

4.3. Postmenopausal sexual function and steroid hormone levels

All participants were Caucasian and mean age was 59.4 years. Table 12 presents descriptive statistics of demographic, clinical and laboratorial parameters of the participants.

Table 12 - Basic descriptive parameters of the participants (n=84)

	Frequency n (%)	Mean (SD)	Min.	Max.	Median (IQR)
Age		59.4 (6.06)	49	77	58 (9)
40-49 y	1 (1.2%)				
50-59 y	50 (59.5%)				
60-69 y	28 (33.3%)				
70-79 y	5 (6%)				
Age of menarche		12.5 (1.51)	10	16	12 (2)
Age of menopause		50.2 (3.57)	37	56	51 (3)
Reproductive lifespan		37.7 (3.75)	21	44	38 (4)
<37 y	25 (29.8%)				
37-38 y	19 (22.6%)				
39-40 y	22 (26.2%)				
>40 y	18 (21.4%)				
Parity		1.9 (0.68)	0	4	2 (1)
Nulliparous	1 (1.2%)				
Multiparous	83 (98.8%)				
Years since menopause		9.2 (6.86)	1	28	8 (9)
<10 y	48 (57.1%)				
10-19 y	28 (33.3%)				
20-29 y	8 (9.5%)				
Body mass index (Kg/m2)		27.9 (4.86)	20	46	27 (7)
Underweight (<18.5Kg/m2)	0 (0%)				
Normal weight (18.5-24.9Kg/m2)	21 (25%)				
Overweight (25-29.9Kg/m2)	33 (39.3%)				
Obesity class I (30-34.9Kg/m2)	24 (28.6%)				
Obesity class II (35-39,9Kg/m2)	4 (4.8%)				
Obesity class III (>=40 Kg/m2)	2 (2.4%)				

Smoking habits	11 (13.1%)				
Regular alcohol habits	0 (0%)				
Depression	19 (22.6%)				
Diabetes	6 (7.1%)				
Vaginal estrogen use	27 (32.1%)				
Education levels					
None	3 (3.6%)				
ISCED 1	37 (44%)				
ISCED 2	10 (11.9%)				
ISCED 3	12 (14.3%)				
ISCED 4	22 (26.2%)				
Hormone levels (ng/mL)					
Estradiol		1.15 (2.12)	0.05	10.98	0.27 (1.14)
Testosterone		2.89 (5.59)	0.50	33.80	0.50 (1.45)
DHEA		10.99 (5.97)	2.33	32.24	10.17 (5.99)
Androstenedione		1.54 (1.20)	0.16	6.87	1.23 (0.85)
FSFI domain score					
Desire		2.8 (1.03)	1.2	5.4	3 (1.8)
Arousal		3.4 (1.05)	1.2	5.4	3.6 (1.8)
Lubrication		3.9 (1.17)	1.2	6.0	3.9 (1.7)
Orgasm		3.8 (1.30)	1.2	6.0	3.9 (2.0)
Satisfaction		4.6 (1.05)	1.2	6.0	4.8 (1.2)
Pain		4.5 (1.27)	1.2	6.0	4.8 (2.3)
Total		22.9 (5.26)	10.7	34.8	23.4 (7.8)

Table 13 shows the estimated coefficients for the multiple linear regression associations, the respective 95% bootstrap BCa confidence intervals and p-values.

The univariate analysis revealed a statistically significant association between testosterone and the orgasm (β=0.04, BCa95%CI [0.00; 0.07], p=0.047) and satisfaction domains (β=0.03, BCa95%CI [0.00; 0.05], p=0.031) and also between DHEA and satisfaction (β=0.03, BCa95%CI [0.00; 0.06], p=0.023). However, no significant associations were found between estradiol, testosterone or DHEA and any of the domain scores, after adjustment for the variables age, reproductive lifespan, body mass index, depression, smoking habits and vaginal estrogen use (Model 2).

It was found that androstenedione positively affects sexual function, with this association being statistically significant for the FSFI total score (β=1.24, BCa95%CI [0.47; 1.78, p=0.003), and for arousal (β=0.20, BCa95%CI [0.02; 0.37], p=0.044),

lubrication (β=0.21, BCa95%CI [0.03; 0.33], p=0.038), orgasm (β=0.32, BCa95%CI [0.13; 0.46], p=0.004), and satisfaction domains (β=0.26, BCa95%CI [0.12; 0.35], p=0.001). After adjustment for the predictor variables (model 2) the results remain significant for FSFI total score (β=1.23, BCa95%CI [0.37; 1.98], p=0.010), arousal (β=0.19, BCa95%CI [0.02; 0.37], p=0.034), orgasm (β=0.33, BCa95%CI [0.15; 0.45], p=0.001) and satisfaction (β=0.25, BCa95%CI [0.11; 0.36], p=0.001), but no longer having statistical significance for the lubrication domain.

Table 13 - Univariate and multivariate regression models for FSFI domain scores as a function of hormone concentrations

		Total B-coefficient BCa95%CI P	**Desire** B-coefficient BCa95%CI P	**Arousal** B-coefficient BCa95%CI P	**Lubrication** B-coefficient BCa95%CI P	**Orgasm** B-coefficient BCa95%CI P	**Satisfaction** B-coefficient BCa95%CI P	**Pain** B-coefficient BCa95%CI P
Estradiol	Model 1	0.22 -0.26; 0.85 0.338	0.05 -0.03; 0.14 0.212	0.02 -0.08; 0.13 0.706	0.03 -0.08; 0.16 0.573	0.05 -0.07; 0.17 0.391	0.06 -0.03; 0.16 0.092	0.03 -0.09; 0.17 0.577
	Model 2	0.10 -0.50; 0.70 0.731	0.03 -0.09; 0.15 0.594	-0.01 -0.13; 0.11 0.897	0.03 -0.11; 0.20 0.621	0.01 -0.13; 0.14 0.963	0.05 -0.11; 0.19 0.357	0.02 -0.14; 0.23 0.749
Testosterone	Model 1	0.11 -0.05; 0.24 0.181	0.01 -0.02; 0.04 0.503	0.01 -0.04; 0.05 0.751	0.01 -0.04; 0.06 0.689	0.04 0.00; 0.07 **0.047**	0.03 0.00; 0.05 **0.031**	0.01 -0.02; 0.05 0.419
	Model 2	0.06 -0.17; 0.38 0.578	0.00 -0.05; 0.06 0.875	0.00 -0.06; 0.07 0.926	0.00 -0.05; 0.07 0.884	0.02 -0.04; 0.07 0.578	0.03 0.00; 0.05 0.105	0.02 -0.02; 0.04 0.390
DHEA	Model 1	0.13 -0.05; 0.30 0.128	0.02 -0.02; 0.05 0.386	0.02 -0.02; 0.06 0.368	0.03 -0.01; 0.07 0.128	0.04 -0.01; 0.09 0.084	0.03 0.00; 0.06 **0.023**	0.01 -0.03; 0.05 0.667
	Model 2	0.10 -0.13; 0.41 0.291	0.02 -0.02; 0.05 0.343	0.01 -0.04; 0.08 0.659	0.02 -0.04; 0.09 0.306	0.03 -0.03; 0.11 0.225	0.03 -0.01; 0.08 0.138	0.00 -0.05; 0.07 0.935
Androstenedione	Model 1	1.24 0.47; 1.78 **0.003**	0.18 0.00; 0.34 0.094	0.20 0.02; 0.37 **0.044**	0.21 0.03; 0.33 **0.038**	0.32 0.13; 0.46 **0.004**	0.26 0.12; 0.35 **0.001**	0.12 -0.07; 0.26 0.267
	Model 2	1.23 0.37; 1.98 **0.010**	0.20 0.01; 0.34 0.079	0.19 0.02; 0.37 **0.034**	0.18 -0.04; 0.42 0.092	0.33 0.15; 0.45 **0.001**	0.25 0.11; 0.36 **0.001**	0.11 -0.09; 0.28 0.322

Model 1. Univariate analysis
Model 2. Multivariate analysis adjusted for age, reproductive lifespan,BMI, depression, smoking habits and vaginal estrogen use

4.4. Postmenopausal cognitive function and steroid hormone levels

Table 14 shows the demographic, clinical and laboratorial parameters of the participants. The majority of the participants were Caucasian and mean age was 61.7 years, ranging from 50 to 86 years. The number of years since menopause (years from menopause to date of blood collection) was on average 10.8 years. Most patients (99.3%) did not have alcoholic habits. Only 9.5% of the patients had smoking habits. Depression was present in 44 patients (29.9%). It should be noted that 48.3% of patients had only basic education ISCED level 1.

Table 14 - Basic descriptive parameters of the participants (n=147)

	Frequency n (%)	Mean (SD)	Range
Age (years)		61.7 (8.0)	50-86
Race (Caucasian)	146 (99,3%)		
Education level			
ISCED 1	71 (48.3%)		
ISCED 2	25 (17%)		
ISCED 3	19 (12.9%)		
ISCED 4	32 (21.8%)		
Years since menopause		10.8 (8.5)	1-38
Depression	44 (29.9%)		
Alcohol habits	1 (0.7%)		
Smoking habits	14 (9.5%)		
BMI (Kg/m2)		28.6 (5.1)	18-46
Hormone levels			
E2 (ng/mL)		1.11(2.40)	0.05-18.00
Testosterone (ng/mL)		2.88 (6.02)	0.50-34.89
DHEA (ng/mL)		10.82 (5.77)	2.33-33.81
Androstenedione (ng/mL)		1.41 (0.96)	0.10-6.87
Cognitive domains			
Global cognitive function		24.3 (3.3)	12-30
Executive function		3.0 (0.8)	1-4
Visuospatial abilities		3.0 (0.9)	0-4
Short-term memory		2.5 (1.5)	0-5
Attention, concentration and working memory		4.8 (1.2)	1-6
Language		4.2 (0.9)	1-5
Orientation to time and place		5.9 (0.3)	3-6

Table 15 presents the correlation coefficients for hormone levels and cognitive domains. Controlling for possible confounding variables (age, education level, years since menopause, depression, smoking habits and BMI), negative correlations were found between estradiol and the following cognitive domains: executive function (p=0.024), visuospatial abilities (p=0.000) and orientation to time and place (p=0.020). Although a negative correlation was also found between estradiol and global cognitive function, it did not reach a statistical significance at a level of 5%. No statistically significant associations were found between DHEA, testosterone and androstenedione and cognitive domains.

The variables race and alcohol habits were not considered in the analysis due to their reduced variability.

Table 15 - Correlation coefficients for hormone levels and cognitive domains

		E2	T	DHEA	A
Global cognitive function	Correlation	-0.149	0.074	0.000	0.005
	Sig. (2-tailed)	0.078	0.387	0.999	0.949
Executive function	Correlation	-0.190	-0.049	-0.004	0.019
	Sig. (2-tailed)	**0.024**	0.566	0.965	0.821
Visuospatial abilities	Correlation	-0.333	-0.119	-0.041	0.006
	Sig. (2-tailed)	**0.000**	0.161	0.634	0.941
Short-term memory	Correlation	0.028	0.128	0.099	-0.006
	Sig. (2-tailed)	0.746	0.132	0.246	0.944
Attention, concentration and working memory	Correlation	-0.074	0.026	0.003	0.047
	Sig. (2-tailed)	0.387	0.764	0.967	0.583
Language	Correlation	-0.086	0.041	-0.060	0.071
	Sig. (2-tailed)	0.312	0.632	0.483	0.405
Orientation to time and place	Correlation	-0.196	-0.165	0.050	0.022
	Sig. (2-tailed)	**0.020**	0.052	0.560	0.794

Variables controlled: age, education level, years since menopause, depression, smoking habits, BMI

5. Discussion

5.1. Steroid hormone levels in postmenopausal hysterectomized women with and without ovarian conservation

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 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 and studies in the 70`s demonstrated a gradient of androgen concentrations between the ovarian vein and peripheral blood while others used pharmacological suppression of the adrenal gland[105–107]. These studies suggested that the postmenopausal ovary continues 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 with bilateral oophorectomy compared to women with ovarian conservation[115]. However, this study did not specify whether oophorectomy was performed before or after menopause. Davison et al, showed lower levels of testosterone among older postmenopausal submitted to BSO, suggesting ongoing ovarian production of androgens many years beyond the time of natural menopause[118].
Another study, published in 2015, using a subset of 2251 participants from the NHS study 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)[119].
Stanczyk et al, found a significant decrease in testosterone and estradiol 2 weeks after surgery, in 9 postmenopausal women submitted to hysterectomy and BSO for benign pathology[173].

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. When our study was published we had the idea that it was the first study to compare hormone levels of postmenopausal women based on their hysterectomy and oophorectomy status using GC-MS/MS. However, a study published very shortly before ours, used GC-MS/MS to compare steroid hormone levels in postmenopausal women submitted to BSO (180 cases) versus women with intact ovaries (38 controls), but in a study population of women at increased risk of breast and/or ovarian cancer. They also found a significant reduction in estradiol levels in the BSO group compared to the control group, as we did, but no significant differences were seen for the other hormones, which included testosterone, androstenedione and DHEA[174]. If this significant reduction in estradiol levels in postmenopausal women submitted to BSO is confirmed by other studies, a role for postmenopausal BSO in the treatment of breast cancer should be considered. Regarding the androgen levels, our study had a significantly higher mean age at surgery than the study of Mai et al, even though there were no differences in our study groups regarding age and time since menopause. Could the ovary have a more important role than the adrenals in the production of androgens as age increases? The Rancho Bernardo study reported an increase in testosterone levels with age among women with intact ovaries, reaching premenopausal levels for the 70–79 decade with relatively stable levels thereafter[115]. This outcome was also suggested by Davison et al[118]. A recent prospective observational study, using LC-MS/MS, included 37 postmenopausal women submitted to risk-reducing BSO and found a decrease of testosterone after surgery[175].

Regarding androstenedione, the scientific evidence is weaker. Some studies showed no decline in androstenedione after postmenopausal BSO[115,173–175], whilst others reported a trend towards a decrease in androstenedione levels but not statistically significant[117,118], in agreement with our study.

The finding of a significant decrease in DHEA in our study is a novelty. 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 produced by the adrenal gland. In a study comparing 442 intact and 71 ovariectomized postmenopausal women aged 42 to 74 years , Labrie et al confirmed that the postmenopausal ovary contributes to approximately 20% of the total pool of circulating DHEA[12]. The results are consistent with our findings, a statistically significant decrease in DHEA in women submitted to postmenopausal BSO, which according to the intracrinology theory could explain the decrease in other steroid

levels[10]. 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 generalize the results. However, the reasons why the sample is reduced are based on current clinical practice and time constraints for conducting this study. It is not so frequent to carry out a hysterectomy after the menopause for benign conditions as it is in the premenopausal period and it is even more rare to preserve the ovaries after the menopause. The majority of women whose ovaries were preserved underwent vaginal hysterectomy, which suggests it was more of a technical issue rather than clinical, given that BSO is more difficult to perform in this type of surgery. In addition, the previous use of systemic HRT was an exclusion criterion, and the prescription of HRT was an almost generalized practice a few years ago, which aggravated the difficulty in obtaining the study sample.

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. 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. Prospective randomized clinical trials, through a double-blind process using treatment and control groups, are the optimal method for determining best evidence on interventions used to address a specific medical problem. However, because of economic and ethical issues, it is often not feasible to conduct those types of studies[176].

Despite the limitations, our study was the first to use an MS-based technique for the measurement of steroid levels in women submitted to hysterectomy for benign reasons, comparing BSO with ovarian conservation in the postmenopausal period.

5.2. Steroid hormone levels and bone mineral density in women over 65 years of age

The results showed a statistically significant association between testosterone and bone mineral density of the hip in women over 65 years. Although positive correlations were found between E2, DHEA and androstenedione and bone mineral density of the hip and lumbar spine, we did not find statistical significance after adjustments for possible confounding factors.

Older studies using immunoassays for steroid measurements are conflicting. Our results are in line with the studies that showed an association between testosterone and BMD of the hip [25,177–179]. Tok et al, in a sample of 147 postmenopausal women (mean age 52y) found that serum free testosterone levels were correlated positively with the BMD at the lumbar spine and femoral neck [178]. Likewise, Van Geel et al found the same associations in 329 postmenopausal women, after adjustment for age [25].

Lambrinoudaki et al, in the study with the largest sample to date (884 postmenopausal women not on hormone therapy), found that testosterone and androstenedione were significantly associated with BMD at the hip. This study also confirmed an association between estradiol and BMD of the lumbar spine and hip [179]. It should be noted that the mean age of the participants in this study was 52.4 years.

However, other previous studies have failed to demonstrate that association [28,180–182]. Murphy et al found significant positive correlations between the free estradiol and testosterone indices and bone mineral density at all sites but these relationships remained significant only for the free estradiol index after adjustment for age and body mass index [180]. Greendale et al, in a large population-based study (The Rancho Bernardo Study) of elderly women (mean age of 72.2y), found the association between bioavailable testosterone and BMD was statistically significant only at the ultradistal radius, after accounting for covariates (age, BMI, alcohol, thyroid hormone, thyazides, exercise, cigarette use, and estrogen use) [28]. The other study that included women over 65 as participants (223 women) found that free testosterone was positively related to hip BMD, but after excluding estrogen users the sample was reduced, and there was a decrease in the magnitude and statistical significance of that relationship, which was even more attenuated after adjusting for estradiol [181].

We did not find a statistical significance between estradiol and BMD, and this can be explained by the small sample size of women included in our study but also by the pathophysiology of osteoporosis. An earlier classification of osteoporosis, although no longer used, divided osteoporosis in 2 types. In type I osteoporosis, there was a more

pronounced effect of estrogen deprivation. In older women with type II osteoporosis, other factors could be additionally responsible for bone loss [183]. Using immunoassays for steroid measurements, Slemenda et al also found that in older postmenopausal women, depending on skeletal site, both higher testosterone and estrogen concentrations were associated with slower bone loss [177] and Stone et al demonstrated in 9704 community-dwelling white women over 65 years of age that estradiol levels > 10 pg/ml were associated with 0.1% annual hip bone loss and levels below 5 pg/ml with an average of 0.8% [184]. Today it is known that estrogen decline in menopause is predominantly associated with trabecular bone loss. In women over the age of 65, most bone loss is cortical, not trabecular[185]. This could explain the absence of association of E2 and BMD in our sample of older women.

We found a weak positive correlation, with no statistical significance, between DHEA and BMD of the hip and lumbar spine. In theory, being a prohormone for the synthesis of estradiol and androgens, DHEA should correlate positively to BMD. Recently, Jankowsi et al concluded, in a pooled analysis of four clinical trials, that women on treatment with oral DHEA had increased lumbar spine and trochanter BMD and maintained total hip BMD [186]. However the relationship between endogenous DHEA and BMD is not well recognized. Most of the studies analyzed DHEA sulfate (DHEAS), and the results are discrepant, either showing a positive association with BMD [178,187,188] or no association [179,180,189].

The finding of a significant positive influence of testosterone in bone mineral density of the hip in older women should encourage further research into testosterone deficiency in elderly women, with a potential impact in the prevention and treatment of postmenopausal osteoporosis. The effects of testosterone on the bone of older postmenopausal women are not very well documented but it is known that testosterone may have direct effects on bone via the androgen receptor, or indirect effects via aromatization [53]. The major limitations of this study are the small sample size and the cross-sectional design of the study, which does not allow us to make causal inferences. Despite the limitations, our study is pioneering, because it is the first study analyzing associations between sex steroid hormone levels and bone mineral density using GC-MS/MS. We have shown that GC-MS/MS is a conveyable technique for future larger prospective studies conducted to provide accurate evidence that in older postmenopausal women androgen deficit plays an important role in bone loss and senile osteoporosis.

5.3. Postmenopausal sexual function and steroid hormone levels

The main finding in this study is the important and independent contribution of androstenedione to sexual function in postmenopausal women. This is evident after excluding a significant association between multiple variables known to influence sex hormone concentrations and sexual function and the inclusion in the models of demographic and clinical variables that seemed to influence any of the sexual function domain scores.
In this study no significant associations were found for testosterone, DHEA or estradiol and any of the sexual function domain scores.

An Australian study which included a subgroup of 678 women aged 45-75 years, although without reference to menopausal status, found no evidence of association between low scores of sexual domains and low testosterone levels. DHEA and estradiol were not measured in this study but they found DHEAS to be associated with low self-reported sexual function. Also, in this group of older women, androstenedione was highly associated with pleasure[190]. Another study of 149 healthy postmenopausal Iranian women also found no association between testosterone and FSFI domains[191].
However these previous studies used immunoassays for steroid measurements, with the limitations mentioned previously for this methodology, especially when hormone concentrations are low, as in the postmenopausal period.
A recent prospective cohort study of 99 women aged 26-90 years treated for rectal cancer (median age of 61 years and predominantly postmenopausal) analyzed the association between serum levels of endogenous androgens measured by liquid chromatography-mass spectrometry and FSFI scores. This study also found no association with free testosterone and sexual function domain scores (except for lubrication) and androstenedione was found to be significantly associated with all sexual domain scores except for desire and satisfaction[192]. Although this study was not performed in an exclusively postmenopausal population it is in agreement with the results of our study.
The fact that we did not reach statistical significance in regard to the sexual desire domain may be related to sample size. In a study of 560 women using LC-MS/MS for determination of hormone levels, the group of women aged 45-65 years (n=160) showed a strongly statistically significant correlation between androstenedione and sexual desire[193].

Overall our results are consistent with previous investigations showing a trend towards a significant association between androstenedione and postmenopausal sexual function and a lower influence of other hormones, which turns out to be a little different from what was found in premenopausal women[65,194].
Research on androstenedione supplementation in women is scarce. A study showed that the administration of oral androstenedione increased serum testosterone and estrone levels in postmenopausal women and could potentially be used to provide androgenic supplementation[195].

A Cochrane review stated that DHEA may slightly improve sexual function in postmenopausal women when compared with placebo[196]. Although our study did not find an association between serum levels of DHEA and sexual function in postmenopausal women, the results are not in conflict with the use of DHEA supplementation for improving sexual function because DHEA is converted at various levels into active androgens and/or estrogens in specific peripheral tissues by the process of intracrinology[197].

In this study, we chose not to measure estrone because, despite being the predominant estrogen in menopause, it has a very weak action and we had funding constraints for hormone assays. However, as we surprisingly found a statistically significant association between androstenedione and various parameters of sexual function, given that estrone is derived mainly from androstenedione and in lesser extent from estradiol, estrone should be considered in future studies.
The main strengths of our study when compared to previous studies are the inclusion of exclusively postmenopausal women and the use of GC-MS/MS, a highly sensitive bioanalytical assay to measure low hormonal concentrations. Also the adjustment for multiple variables known to influence hormonal levels and sexual function has an advantage over previous studies and makes our results more valid.
The main weaknesses of this study are the small sample size and the cross-sectional design of the study, which does not allow us to make causal inferences between endogenous hormone levels and self-reported sexual function scores. It should also be noted that this study did not assess sexual distress. As previously mentioned, sexual dysfunction has a complex etiology and many other potential contributors were not controlled in this study.

5.4. Postmenopausal cognitive function and steroid hormone levels

After adjustment for age and other confounding factors, an association between estradiol and executive function, visuospatial abilities and orientation to time and place was found, evidencing a tendency to obtain lower scores when evaluating these cognitive domains in patients with higher concentrations of estradiol. Although a negative correlation was also found between estradiol and global cognitive function, it did not reach a statistical significance at a level of 5%.

Contrary to a few studies that suggested a positive association between estradiol and cognitive function[81,82], our study is in line with studies that showed a null or negative influence of estrogen[85–87,198].

Drake et al reported that E2 is associated with enhanced verbal memory but also showed negative associations with visuospatial skills[83].

The Rotterdam Study, a population-based follow-up study on chronic diseases, including dementia, in women aged 55 years or older, showed that higher levels of total estradiol were associated with an increased 6 year risk of dementia (age-adjusted hazard ratio per standard deviation increase 1.38; 95% CI 1.04-1.84)[86].

In the Rancho Bernardo Study cohort, which included 343 postmenopausal women with a median age 70 years, higher estradiol predicted a greater four year decline in performance on a category fluency test of cognitive flexibility and executive function[88].

There are many observational studies and several meta-analyses regarding the effects of systemic HRT on cognitive function, and more than half suggest benefit but nearly all long term clinical trials fail to show benefit and the longer trials tend to show harm[199]. Recent observational studies continue to provide conflicting results[200,201]. However, the Women's Health Initiative Memory Study (WHIMS), the largest postmenopausal estrogen trial of dementia, including a subset of 2947 women aged 65 or older, did not confirm a positive effect of HRT in aged women, and even showed a higher risk of MCI and dementia in the HRT groups[202]. In this cohort subjects were older than in other studies. The accumulated evidence has led to the theory that there is a critical period for the beneficial effect of HRT when initiated around the age of menopause, or in the first few years after menopause. Treatment initiated many years after the menopause does not have a benefit and may even be harmful. However, the newer Early versus Late Intervention Trial with Estradiol (ELITE)-cog and Kronos Early Estrogen Prevention Study (KEEPS) trials have reported no beneficial or adverse effects of HRT on cognition among recently postmenopausal women within 6 years of the menopause diagnosis[203,204].

Our study showed no significant associations between androgens and cognitive function, after adjustment for possible confounding factors.
Levels of testosterone were related positively to verbal fluency[83], verbal memory[84] and predicted better categorical performance on the Mini-Mental State Examination (MMSE) and the World component of the MMSE[85]. Davis et al reported in 92 postmenopausal women aged 55–65 years (on no systemic sex hormone therapy), a small but statistically significant effect of testosterone treatment on verbal learning and memory[42].
However, in line with our study, other studies showed no association between testosterone and cognitive function[87,198,205].
A prospective cohort study of 3044 women of the NHS in which women aged 70 years and older were administered the Telephone Interview of Cognitive Status, a telephone version of the MMSE, could not demonstrate that testosterone levels were associated with either objective or subjective measures of cognitive function[205]. It should be noted that in this study the blood draw was performed during the early postmenopausal stage.
In another study of 402 postmenopausal women, higher testosterone concentration was associated with lower scores for neurocognition index, memory and psychomotor speed[90].

Our study is one of the few to attempt to show an association between endogenous DHEA levels and cognitive function, although there are multiple studies showing no effect of DHEA supplementation on cognitive function[206,207].
Most of the epidemiological studies focused on the role of DHEAS decline in the onset of cognitive impairment occurring with age with a large number of observations showing no association[208].
Bojar et al reported that postmenopausal women with a high normal level of DHEA scored significantly better in verbal and visual memory[90]. Nonetheless, a recent Australian study, published in 2023, showed that in 5511 women 70 years or older, DHEA was not associated with cognitive performance[198].

There is a paucity of data regarding androstenedione and cognitive function, but a few studies reported no association between androstenedione and cognitive function[83,205].

It is difficult to compare our results to others due to the use of different diagnostic instruments, applied to ethnically different populations, as well as differences in mean

age. Most of the studies used the MMSE. The main advantage of the MoCA test when compared to MMSE is the superior sensitivity in the diagnosis of mild cognitive impairment, a transitional state between normal aging and dementia, especially Alzheimer's disease[169]. A limitation that can be pointed to our study has to do with the high percentage of women with a low level of education (48.3% of patients had only ISCED level 1). The MoCA test is more suitable for cognitive screening of the population with higher education[169].

Most of the previous studies used immunoassays for the measurement of steroid hormone levels, but mass spectrometry based techniques are now the gold standard for measuring steroid hormones in postmenopausal women, due to its higher accuracy. Despite the limitations of sample size and cross-sectional design, this is the first study to analyze associations between steroid hormone levels and cognitive function in postmenopausal women, using GC-MS/MS for hormone measurements and the MoCA test as a cognitive diagnostic tool.

6. Conclusions and future perspectives

This study, using a highly sensitive technique for serum steroid levels quantification, demonstrated that prophylactic oophorectomy performed after menopause decreases the concentration of steroid hormones, having found statistical significance for DHEA and estradiol, although there was also a decrease in testosterone and androstenedione concentrations without statistical significance. Other studies have shown a statistically significant reduction in testosterone after postmenopausal BSO, so it is undeniable that the ovary contributes in some way to the steroid hormone pool after menopause. Either through the direct production of androgens, as some studies suggest, or, according to the theory of intracrinology, through the production of DHEA which is transformed into testosterone and androstenedione at the intracellular level. Therefore, the reduction of DHEA after postmenopausal BSO observed in our study may have an even greater impact on the reduction of testosterone and androstenedione concentrations at the intracellular level, not accessible to laboratory quantification. The reduction of testosterone and androstenedione can have an impact at different levels and contribute to the decrease in quality of life and morbidity associated with aging.

We have shown that testosterone has a significant positive influence in bone mineral density of the hip in older women, consistent with the results of previous studies. The prevalence and burden of hip fractures has increased with increasing average life expectancy, and is one of the most serious health care problems affecting older women. In Portugal, as in Europe, the lifetime probability of hip fracture in women aged 70 is around 15% [209], which is actually higher than the lifetime probability of ovarian cancer (<1%)[210]. In women above 65 years of age, hip fracture is responsible for a 2-fold increased mortality in the first year after its occurrence [211].

We found a significant positive influence of androstenedione in overall sexual function of postmenopausal women, with higher impact on specific domains such as arousal, lubrication, orgasm and satisfaction. Could androstenedione be the most important hormone for sexual function after menopause? This is an interesting finding, which revives the discussion about the role of androgens in sexual dysfunction and future therapeutic options. Even though sexual dysfunction is seen as a minor problem as aging increases, this paradigm is changing as postmenopausal women's quality of life improves and average life expectancy increases, and nowadays women are already giving more importance to these issues.

Regarding cognitive function, age is the most important factor for cognitive decline. However, accumulating evidence shows that steroid hormones influence cognition, but different hormones can influence different aspects of cognition during aging, and this may explain the different results obtained from the studies. Estrogen, known to have an association with cognitive function in pre-menopause and in the early stages of menopause, may in fact have a negative impact on some aspects of cognitive function in older women, which explains the results of studies showing that estrogen therapy has no effect or may be deleterious in older women.

It should be noted that the differences observed in the various studies carried out on the association between circulating levels of steroid hormones and BMD, sexual function and cognitive function are also probably related to the heterogeneity of the studies, with regard to the design of the study and methodology used. There are also concerns regarding the bioavailability of these compounds. In addition, variations in the androgen receptor need to be considered, which may result in variability in end organ response to absolute circulating levels of androgens[212].
More studies with larger sample sizes and MS-based techniques are needed to provide a more accurate evidence over the impact of steroid hormones in the health of postmenopausal women and the benefits of ovarian conservation after menopause, so that appropriate counseling can be given to all postmenopausal women considering a prophylactic bilateral oophorectomy during hysterectomy for benign conditions.

Given the fact that ovarian cancer incidence remains low after hysterectomy with ovarian preservation in a low-risk population and that scientific evidence suggests bilateral salpingectomy as a safe and effective alternative to reduce the incidence of ovarian cancer in women undergoing hysterectomy for benign pathology, it is important for the clinicians to know that the postmenopausal ovary continues to have a relevant endocrine function in the late postmenopause stage (STRAW +2).

Lastly, future investigations should also focus on frailty. Frailty is an emerging concept that is theoretically defined as a state of increased vulnerability to poor resolution of homeostasis following a stress and is a consequence of cumulative decline in multiple physiological systems over a lifespan[213]. Changes in postmenopausal hormones and inflammatory factors appear to be mediating factors for the risk of frailty in older women[214], but the consequences of prophylactic BSO on the risk of frailty are not well understood[215,216].

7. References

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Appendices

Appendix I. Deliberations of study approval

ULS
Unidade Local de Saúde
de Castelo Branco, EPE

Ofício: Elsa Filipa Henriques Roque Nunes

Assunto: Pedido autorização para realização de trabalho de investigação para tese de doutoramento em Medicina

Requerente: Dra. Elsa Filipa Henriques Roque Nunes – Assistente de Ginecologia/Obstetricia da ULSCB

Titulo: Pedido de autorização para realização de um estudo intitulado "A função dos ovários na menopausa", tem como objectivo avaliar o impacto da realização de ooforectomia bilateral após a menopausa na saúde e longevidade das mulheres especificamente a nível cardiovascular, ósseo, sexual e cognitivo", sendo feito através de uma consulta de menopausa da ULSCB, para a qual solicita abertura de um tempo de consulta fora do horário normal de trabalho.

Orientador: Prof. Dr. José Alberto Fonseca Moutinho

População do estudo: Utentes da consulta de menopausa da ULSCB

Data do pedido: Ofício datado no HAL em 23 de Agosto de 2016

A Comissão de Ética da ULSCB, concorda com o referido estudo desde que seja mantida a confidencialidade dos sujeitos do mesmo, todos os princípios éticos inerentes ao processo de investigação sejam respeitados e com a devida autorização do Director do Serviço.

ULS de Castelo Branco, E.P.E., 30 de Setembro de 2016

A Comissão de Ética

ULS
Unidade Local de Saúde
de Castelo Branco, EPE

Presidente do Conselho de Administração

De: António Vieira Pires – Presidente do Conselho de Administração

Para: Exma. Sra. Dra. Elsa Filipa Henriques Roque Nunes – Assistente de Ginecologia/Obstetrícia

C/C: Exmo. Sr. Dr. Paulo Lima – Director do Serviço de Obstetrícia/Ginecologia

Serviço de Informática

SCAD/Consulta Externa

Gabinete de Planeamento e Apoio à Gestão

Administracao HAL
17 19012 2016-11-18 15:35:54

Assunto: Estudo – *"A Função dos ovários na menopausa"* – Abertura de Consulta de Menopausa

Data: 18 de Novembro de 2016

No seguimento do pedido formulado por V. Exa. para realização do estudo supracitado, e consequente abertura de um tempo de consulta fora do horário normal de trabalho – Consulta de Menopausa, inserido na Tese de Doutoramento em Medicina que V. Exa. se encontra a realizar, informo que o Conselho de Administração deliberou, em 27.10.2016, homologar o parecer favorável da Comissão de Ética, pelo que se autoriza o solicitado, desde que seja mantida a confidencialidade dos sujeitos do estudo, devendo ser respeitados todos os princípios éticos inerentes ao processo de investigação e com devida autorização do Director de Serviço.

Com os melhores cumprimentos,

O Presidente do Conselho de Administração da ULSCB, EPE

Dr. António Vieira Pires

/MP

Appendix II. Informed consent

CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM INVESTIGAÇÃO

de acordo com a Declaração de Helsínquia[1] e a Convenção de Oviedo[2]

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Título do estudo: **A FUNÇÃO DOS OVÁRIOS NA MENOPAUSA**

Enquadramento:

O presente estudo tem como população-alvo todas as mulheres pós-menopáusicas vigiadas em consulta hospitalar de Ginecologia na Unidade Local de Saúde de Castelo Branco durante o período em que decorre o estudo.

O período da vida da mulher que decorre desde a menopausa tem tendência a alargar-se cada vez mais, com o aumento da esperança média de vida. Alguns estudos mostraram que a remoção dos ovários após a menopausa parece ter um impacto negativo na saúde das mulheres com mais idade. Os ovários continuam a produzir hormonas (androgénios) após a menopausa, em quantidades inferiores, mas que têm um papel importante a diversos níveis no corpo humano. Este estudo pretende avaliar o impacto da remoção dos ovários após a menopausa na saúde e qualidade de vida das mulheres, especificamente a nível ósseo (risco de osteoporose), função sexual e função cognitiva (atenção, memória, raciocínio, etc.).

Explicação do estudo:

Na primeira consulta, é realizada uma história clínica e de seguida procede-se ao exame ginecológico. Posteriormente, será solicitada a sua participação, com o apoio do médico, em alguns exercícios e questões no âmbito de um questionário de função cognitiva. Por fim será solicitado o preenchimento individual de um questionário de função sexual. No final da consulta serão prescritos exames complementares de diagnóstico, nomeadamente colheita de sangue para análises hormonais, ecografia endovaginal e osteodensitometria óssea (exames a realizar no hospital). Será agendada uma consulta para comunicação dos resultados dos exames solicitados e eventual orientação terapêutica e/ou vigilância.

Condições e financiamento:

Este estudo não comporta qualquer tipo de financiamento. A participação no estudo é de carácter voluntário e não serão atribuídos quaisquer prejuízos, assistenciais ou outros, caso não queira participar.

Confidencialidade e anonimato:

Será garantida a confidencialidade dos dados recolhidos, que serão utilizados exclusivamente para o presente estudo. Estará também assegurado o anonimato dos participantes através da ausência de registo de dados de identificação.

[1] http://portal.arsnorte.min-saude.pt/portal/page/portal/ARSNorte/Comiss%C3%A3o%20de%20%C3%89tica/Ficheiros/Declaracao_Helsinquia_2008.pdf

[2] http://dre.pt/pdf1sdip/2001/01/002A00/00140036.pdf

Agradeço a sua participação,

Elsa Filipa Henriques Roque Nunes
Assistente Hospitalar de Ginecologia/Obstetrícia da Unidade Local de Saúde de Castelo Branco
Aluna de doutoramento em Medicina da Universidade da Beira Interior

Assinatura do Médico proponente: ____

-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-o-

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela/s pessoa/s que acima assina/m ou um representante das mesmas. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo/a investigador/a.

Nome: ____

Assinatura: ____ Data: ___ /___ /______

SE NÃO FOR O PRÓPRIO A ASSINAR POR IDADE OU INCAPACIDADE

NOME: …
BI/CD Nº: .... DATA OU VALIDADE ….. /….. /……..
GRAU DE PARENTESCO OU TIPO DE REPRESENTAÇÃO: ....
ASSINATURA …

ESTE DOCUMENTO É COMPOSTO DE 2 PÁGINAS E FEITO EM DUPLICADO:
UMA VIA PARA O/A INVESTIGADOR/A, OUTRA PARA A PESSOA QUE CONSENTE

Appendix III. Questionnaire layouts

III.1. FSFI questionnaire

ÍNDICE DE FUNCIONAMENTO SEXUAL FEMININO
(FSFI; Rosen et al., 2000; traduzido e adaptado por Pedro Nobre, 2001)

Coloque uma cruz na resposta que mais se adequa à sua situação tendo em conta as **últimas quatro semanas**

1. Com que frequência sentiu desejo ou interesse sexual?
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

2. Como classifica o seu nível de desejo ou interesse sexual?
- ☐ Muito elevado
- ☐ Elevado
- ☐ Moderado
- ☐ Baixo
- ☐ Muito baixo/nenhum

3. Com que frequência se sentiu sexualmente excitada durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

4. Como classifica o seu nível (grau) de excitação sexual durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Muito elevado
- ☐ Elevado
- ☐ Moderado
- ☐ Baixo
- ☐ Muito baixo/nenhum

5. Qual a sua confiança em conseguir excitar-se durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Confiança muito elevada
- ☐ Confiança elevada
- ☐ Confiança moderada
- ☐ Confiança baixa
- ☐ Confiança muito baixa/nenhuma

6. Com que frequência se sentiu satisfeita com a sua excitação sexual durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

7. Com que frequência ficou lubrificada (molhada) durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

8. Qual a dificuldade que teve em ficar lubrificada (molhada) durante qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Extremamente difícil ou impossível
- ☐ Muito difícil
- ☐ Difícil
- ☐ Ligeiramente difícil
- ☐ Nenhuma dificuldade

9. Com que frequência manteve a sua lubrificação até ao fim da actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

10. Qual a dificuldade que teve em manter a sua lubrificação até ao fim de qualquer actividade ou relação sexual?
- ☐ Não tive actividade sexual
- ☐ Extremamente difícil ou impossível
- ☐ Muito difícil
- ☐ Difícil
- ☐ Ligeiramente difícil
- ☐ Nenhuma dificuldade

11. Quando teve estimulação sexual ou relações sexuais, com que frequência atingiu o orgasmo (clímax)?
- ☐ Não tive actividade sexual
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

12. Quando teve estimulação sexual ou relações sexuais qual a dificuldade que teve para atingir o orgasmo (clímax)?
- ☐ Não tive actividade sexual
- ☐ Extremamente difícil ou impossível
- ☐ Muito difícil
- ☐ Difícil
- ☐ Ligeiramente difícil
- ☐ Nenhuma dificuldade

13. Qual foi o seu nível de satisfação com a sua capacidade para atingir o orgasmo (clímax) durante qualquer actividade sexual?
- ☐ Não tive actividade sexual
- ☐ Muito satisfeita
- ☐ Moderadamente satisfeita
- ☐ Igualmente satisfeita e insatisfeita
- ☐ Moderadamente insatisfeita
- ☐ Muito insatisfeita

14. Qual foi o seu nível de satisfação com o grau de proximidade emocional entre si e o seu parceiro durante a actividade sexual?
- ☐ Não tive actividade sexual
- ☐ Muito satisfeita
- ☐ Moderadamente satisfeita
- ☐ Igualmente satisfeita e insatisfeita
- ☐ Moderadamente insatisfeita
- ☐ Muito insatisfeita

15. Qual o seu nível de satisfação com o relacionamento sexual que mantém com o seu parceiro?
- ☐ Muito satisfeita
- ☐ Moderadamente satisfeita
- ☐ Igualmente satisfeita e insatisfeita
- ☐ Moderadamente insatisfeita
- ☐ Muito insatisfeita

16. Qual o seu nível de satisfação com a sua vida sexual em geral?
- ☐ Muito satisfeita
- ☐ Moderadamente satisfeita
- ☐ Igualmente satisfeita e insatisfeita
- ☐ Moderadamente insatisfeita
- ☐ Muito insatisfeita

17. Com que frequência sentiu dor ou desconforto durante a penetração vaginal?
- ☐ Não tentei ter relações sexuais
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

18. Com que frequência sentiu dor ou desconforto após a penetração vaginal?
- ☐ Não tentei ter relações sexuais
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

19. Como classifica o seu nível de dor ou desconforto durante ou após a penetração vaginal?
- ☐ Não tentei ter relações sexuais
- ☐ Muito elevado
- ☐ Elevado
- ☐ Moderado
- ☐ Baixo
- ☐ Muito baixo/nenhum

20. Com que frequência a contracção dos músculos da sua vagina dificultou ou impediu a penetração do pénis durante qualquer relação sexual ?
- ☐ Não tentei ter relações sexuais
- ☐ Quase sempre/sempre
- ☐ A maior parte das vezes (mais de metade das vezes)
- ☐ Algumas vezes (cerca de metade das vezes)
- ☐ Poucas vezes (menos de metade das vezes)
- ☐ Quase nunca/nunca

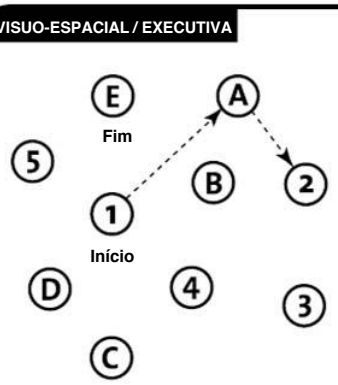
III.2. MoCA questionnaire

MONTREAL COGNITIVE ASSESSMENT (MOCA)

VERSÃO PORTUGUESA – 7.1 VERSÃO ORIGINAL

Nome: _____ **Idade:** _____
Género: _____ **Data de Nascimento:** _____
Escolaridade: _____ **Data de Avaliação:** _____

VISUO-ESPACIAL / EXECUTIVA — **Pontos**



Copiar o cubo

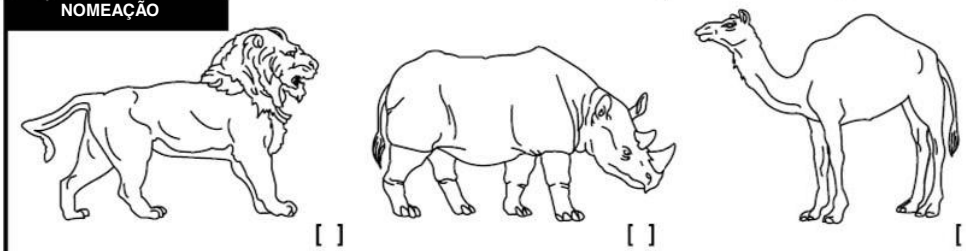
[ ] [ ]

Desenhar um Relógio (onze e dez) (3 pontos)

[ ] Contorno [ ] Números [ ] Ponteiros

__/5

NOMEAÇÃO



[ ] [ ] [ ]

__/3

MEMÓRIA

Leia a lista de palavras. O sujeito deve repeti-la. Realize dois ensaios. Solicite a evocação da lista **5 minutos mais tarde.**

	Boca	Linho	Igreja	Cravo	Azul
1º ensaio					
2º ensaio					

Sem Pontuação

ATENÇÃO

Leia a sequência de números. (1 número/segundo)

O sujeito deve repetir a sequência. [ ] 2 1 8 5 4

O sujeito deve repetir a sequência na ordem inversa. [ ] 7 4 2

__/2

Leia a série de letras (1 letra/segundo). O sujeito deve bater com a mão cada vez que for dita a letra A. Não se atribuem pontos se ≥ 2 erros.

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Subtrair de 7 em 7 começando em 100. [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 ou 5 subtracções correctas: **3 pontos;** 2 ou 3 correctas: **2 pontos;** 1 correcta: **1 ponto;** 0 correctas: **0 pontos**

__/3

LINGUAGEM

Repetir: Eu só sei que hoje devemos ajudar o João. [ ] **O gato esconde-se sempre que os cães entram na sala.** [ ]

__/2

Fluência verbal: Dizer o maior número possível de palavras que comecem pela letra "P" (1 minuto). [ ]____ (N ≥ 11 Palavras)

__/1

ABSTRACÇÃO

Semelhança p.ex. entre banana e laranja = fruta [ ] **comboio - bicicleta** [ ] **relógio - régua**

__/2

EVOCAÇÃO DIFERIDA

	Deve recordar as palavras SEM PISTAS	Boca	Linho	Igreja	Cravo	Azul	
		[ ]	[ ]	[ ]	[ ]	[ ]	Pontuação apenas para evocação **SEM PISTAS**
Opcional	Pista de categoria						
	Pista de escolha múltipla						

__/5

ORIENTAÇÃO

[ ] **Dia do mês** [ ] **Mês** [ ] **Ano** [ ] **Dia da semana** [ ] **Lugar** [ ] **Localidade**

__/6

© Z.Nasreddine MD

Examinador: _____

TOTAL __/30

Versão Portuguesa: Freitas, S., Simões, M. R., Santana, I., Martins, C. & Nasreddine, Z. (2013). *Montreal Cognitive Assessment (MoCA): Versão 1.* Coimbra: Faculdade de Psicologia e de Ciências da Educação da Universidade de Coimbra.

Appendix IV. Scientific production

IV.1. Published articles

IV.1.1. Steroid hormone levels in postmenopausal hysterectomised women with or without ovarian conservation: the continuous endocrine function of the ovaries

The Journal of Obstetrics and Gynaecology

Taylor & Francis Group

Journal of Obstetrics and Gynaecology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijog20

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

Elsa Nunes, Eugenia Gallardo, Sara Morgado-Nunes & José Fonseca-Moutinho

To cite this article: Elsa Nunes, Eugenia Gallardo, Sara Morgado-Nunes & José Fonseca-Moutinho (2023) Steroid hormone levels in postmenopausal hysterectomised women with and without ovarian conservation: the continuous endocrine function of the ovaries, Journal of Obstetrics and Gynaecology, 43:1, 2141618, DOI: 10.1080/01443615.2022.2141618

To link to this article: https://doi.org/10.1080/01443615.2022.2141618

Published online: 04 Nov 2022.

JOURNAL OF OBSTETRICS AND GYNAECOLOGY
2023, VOL. 43, NO. 1, 2141618
https://doi.org/10.1080/01443615.2022.2141618

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

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.

ARTICLE HISTORY
Received 25 July 2022
Accepted 25 October 2022

KEYWORDS
Menopause; oophorectomy; androgens; oestrogens; dehydroepiandrosterone; intracrinology

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).

Funding

This work was funded by the Portuguese Society of Gynaecology and partially supported by CICS-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT) and by Fundo Europeu de Desenvolvimento Regional (FEDER) under the scope of PORTUGAL 2020 and Programa Operacional do Centro (CENTRO 2020), with the project reference UIDB/00709/2020.

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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.

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IV.1.2. Steroid hormone levels and bone mineral density in women over 65 years of age

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Steroid hormone levels and bone mineral density in women over 65 years of age

Elsa Nunes[1✉], Eugenia Gallardo[1,2], Sara Morgado-Nunes[3] & José Fonseca-Moutinho[1]

Previous studies using immunoassays for steroid measurements have focused on the association between steroid hormone levels and bone mineral density (BMD) in postmenopausal women, obtaining contradictory results. This study aimed to assess this association using a highly sensitive bioanalytical method. A total of 68 postmenopausal women, aged 65–89 years, were enrolled in a cross-sectional study. Measurements of the BMD of the hip and lumbar spine were performed using dual energy X-ray absorptiometry, and serum hormone levels were quantified by gas chromatography and tandem mass spectrometry. Associations between estradiol (E2), testosterone, dehydroepiandrosterone (DHEA), androstenedione and T score levels of the hip and lumbar spine were evaluated, after adjustment for confounding variables. The analysis revealed a statistically significant association between testosterone and the T score of the hip ($p = 0.035$), but not that of the lumbar spine. No statistically significant associations were found between E2, DHEA, androstenedione and the T scores of the hip and the lumbar spine. Using a highly sensitive hormone assay method, our study identified a significant association between testosterone and BMD of the hip in women over 65 years of age, suggesting that lower testosterone increases the risk of osteoporosis.

As average life expectancy increases, more women will suffer the consequences of bone loss and osteoporosis. In recent decades, rather than being seen as an unavoidable disease in older women, osteoporosis has come to be seen as a preventable disease, and much progress has been made in its treatment. Osteoporosis is estimated to affect one-fifth of women aged 70, two-fifths of women aged 80, and three-fifths of women aged 90[1]. A recent meta-analysis reported that the worldwide prevalence of osteoporosis in the elderly women is 35.3%[2].

Since Albright's pioneering studies[3], the association between estrogen deficiency and osteoporosis has well been recognized, and for some time the theory of estrogen-centered pathogenesis of postmenopausal osteoporosis prevailed[4]. In the past decade a shift in paradigm was observed, and new evidence revealed a possible role for androgens in the prevention of osteoporosis.

Androgens affect bone directly via interactions with androgen receptors, and indirectly via binding to estrogen receptors α and β after aromatization in fat or other tissues[5]. Furthermore, in postmenopausal women the combined treatment of androgens plus estrogens revealed more efficacy in increasing bone mineral density (BMD) than isolated estrogen[6,7].

Menopause is associated with a 70% decline in adrenal androgens, including dehydroepiandrosterone (DHEA), which is converted at various levels into active androgens and/or estrogens in specific peripheral tissues by the process of intracrinology[8]. For decades, there has been controversy over whether the postmenopausal ovary is an androgen production site[9–12]. Postmenopausal bilateral oophorectomy was related to lower levels of BMD[13] and to an increased risk of osteoporotic fractures[14]. More recently, it was stated that around 20% of serum DHEA originates from the postmenopausal ovary[15], which in accordance with the intracrinology theory could explain the impact of postmenopausal oophorectomy on androgen levels. However, studies that have examined the association of androgens and bone mineral density have shown contradictory results, and the low specificity of the immunoassays used may have contributed. Although most of the studies revealed an association between androgens, particularly testosterone and BMD, others have failed to reach this conclusion.

In the 1960s, 70s and 80s, radioimmunoassay (RIA) was the main technique used for the dosing of steroid hormones. Despite the high throughput presented by these methods, the use of radioisotopes makes decontamination mandatory. Currently, non-radioactively labeled detection techniques (such as chemiluminescence or

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electrochemiluminescence) are widely implemented. Instruments for immunoassay-based methods are relatively easy to use, while sample preparation steps are not required and the cost is reasonable. However, these assays lack specificity due to the cross-reactivity of the antibodies with other steroid hormones[16].

Mass spectrometry based (MS-based) techniques are now the gold standard for measuring steroid hormones in postmenopausal women, as they have greater accuracy and specificity than immunoassays, due to the very low serum concentrations of these hormones in the postmenopausal period[17]. Although high performance liquid chromatography and tandem mass spectrometry (LC–MS/MS) has become the preferred method for simple bioanalysis of an extended range of compound classes, gas chromatography and tandem mass spectrometry (GC–MS/MS) has higher accuracy, precision, sensitivity and specificity when it comes to measuring estrogen and androgens in the postmenopausal period[18].

The goal of this study is to determine if lower levels of testosterone, androstenedione and DHEA are in fact associated with lower levels of bone mineral density in older women, using GC–MS/MS, a highly sensitive bio-analytical assay for steroid measurements in the postmenopausal period.

Materials and methods

A cross-sectional study was conducted after approval by the Ethics Committee of Hospital Amato Lusitano, a Portuguese tertiary hospital. This study was carried out in accordance with relevant local regulations and the Declaration of Helsinki. Written informed consent was obtained for each participant. Between 2017 and 2019, 68 women over 65 years old evaluated in a gynecology consultation met the criteria for the performance of bone densitometry, according to the general health department`s guidelines. In Portugal, there is a government norm that recommends that all women over 65 should undergo bone densitometry of the lumbar spine and femoral neck using DXA technology (dual energy X-ray absorptiometry). For all patients included in this study, measurement of the bone mineral density (BMD) of the lumbar spine and femoral neck was performed in the same center using a GE Lunar Prodigy DXA system (GE Healthcare, Madison, WI, USA).

The inclusion criteria were women over 65 years of age who had intact ovaries at the time of menopause. Adnexal pathology was excluded by performing transvaginal ultrasound to all the participants. Current or past users of systemic hormonal therapy or corticosteroid treatment were excluded. Other exclusion criteria included history of taking any medication for osteoporosis, chronic hepatic or renal diseases, and history of endocrine or rheumatologic diseases.

One blood sample was obtained from each woman between 8 and 10 am. All blood samples were centrifuged within 1 h of collection to separate serum, which was stored at − 80 °C and protected from light until analysis. The studied compounds, dehydroepiandrosterone (DHEA), androstenedione, 17β-estradiol (E2), and testosterone 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 deionized water. The columns were then 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; then, they were 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. Descriptive statistics were reported as means ± standard deviation (SD) for continuous variables and as frequencies (%) for categorical variables. Statistical analyses were obtained using Pearson`s correlations to examine associations between variables. In order to analyze the joint effect of the variables under study in the T score of the hip and in the T score of the lumbar spine, two multiple linear regression analyses were performed: one with T score of the hip as the dependent variable and the other with T score of the lumbar spine as the dependent variable. The independent variables were "Age", "Race", "Body mass index" (Kg/m2), "Regular alcohol habits", "Smoking habits", "Age of menarche", "Type of menstrual cycles"(regular vs irregular), "Parity", "Age of menopause", "Years since menopause", "Vaginal estrogen use", "Estradiol levels (ng/mL)", "Testosterone levels (ng/mL)", "DHEA levels (ng/mL)", and "Androstenedione levels (ng/mL)". Qualitative variables were treated as dummy variables, and the regression method used was the stepwise, in which only regression variables significantly related to the dependent variable entered the regression model. These variables were entered successively according to their degree of association with the dependent variable. A p value of 0.05 or less was considered statistically significant.

Results

Table 1 presents the demographic and laboratorial parameters of the participants. The majority of patients were Caucasian (98.5%). Obesity was confirmed in 44.1% of patients: 26.5% obesity class I, 13.2% obesity class II, 4.4% obesity class III; 41.2% of the patients were overweight, and only 14.7% had a normal weight. Most patients (98.5%) did not have alcoholic or smoking habits. Regular menstrual cycles throughout their reproductive life were reported by 88.2% of women. Only 7.4% of women were nulliparous and 75% had given birth to 2 or more children. The mean age of menopause was 50.2 years. The number of years since menopause (years from menopause to date of blood collection) was on average 22 years and in 40 women (58.8%) it was over 20 years. Vaginal estrogen cream was used two to three times weekly by 37 women (54.4%).

	Frequency n (%)	Mean	SD	Minimum	Maximum
Age (years)		72.2	6.6	65	89
Race (Caucasian)	67 (98.5%)				
BMI (kg/m^2)		29.7	5.2	20	45
Underweight (< 18.5 kg/m^2)	0 (0%)				
Normal weight (18.5–24.9 kg/m^2)	10 (14.7%)				
Overweight (25–29.9 kg/m^2)	28 (41.2%)				
Obesity class I (30–34.9 kg/m^2)	18 (26.5%)				
Obesity class II (35–39,9 kg/m^2)	9 (13.2%)				
Obesity class III (≥ 40 kg/m^2)	3 (4.4%)				
Age of menarche (years)		12.9	1.6	10	16
Menstrual cycles					
Regular	60 (88.2%)				
Irregular	8 (11.8%)				
Parity		1.9	0.9	0	4
Nulliparous	5 (7.4%)				
Multiparous					
1	12 (17.6%)				
2	34 (50%)				
> 2	17 (25%)				
Age of menopause (years)		50.2	4.4	37	58
Years since menopause		22.0	7.3	10	38
10–19	28 (41.2%)				
20–29	29 (42.6%)				
≥ 30	11 (16.2%)				
Regular alcohol habits	1 (1.5%)				
Smoking habits	1 (1.5%)				
Vaginal estrogen use	37 (54.4%)				
E2 (ng/mL)		0.94	2.49	0.05	18.00
Testosterone (ng/mL)		1.66	3.11	0.50	17.19
DHEA (ng/mL)		9.89	5.01	1.82	32.24
Androstenedione (ng/mL)		1.31	1.02	0.10	5.55

Table 1. Demographic characteristics and mean hormone levels of the participants (n = 68).

Controlling for all possible confounding variables (age, race, BMI, alcohol and smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, and vaginal estrogen use), positive correlations were found between the T score of the lumbar spine and the femoral neck and all four tested steroid hormones (Table 2). However, only the positive correlation between the testosterone concentration and the T score of the hip was statistically significant ($p = 0.035$).

By multiple linear regression analysis, it was found that testosterone and BMI positively affected the T score of the hip. Age negatively affected the T score of the hip, being the most predictive variable (Table 3). The type of regression used was stepwise and therefore only the statistically significant variables were expressed in the regression models. The first variable to enter the model was Age (Model 1), followed by Testosterone (Model 2) and BMI (Model 3). As the variables entered the models, the coefficients remained approximately constant, which means that there are no strong dependency relationships between these variables and therefore multicollinearity

	T score hip		T score lumbar spine	
Hormone levels	R	P	R	P
E2	0.162	0.234	0.144	0.289
Testosterone	0.283	0.035	0.155	0.254
DHEA	0.151	0.266	0.04	0.769
Androstenedione	0.245	0.069	0.131	0.337

Table 2. Pearson`s correlation coefficients for hormone levels and T score hip and lumbar spine. Variables controlled: age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use.

Model		β	Std. error	*P*	95% CI
1	Age	−0.056	0.020	0.007	−0.097; −0.016
2	Age	−0.051	0.020	0.012	−0.091; −0.011
	Testosterone (ng/mL)	0.086	0.041	0.037	0.005; 0.167
3	Age	−0.045	0.019	0.024	−0.084; −0.006
	Testosterone (ng/mL)	0.089	0.039	0.028	0.010; 0.167
	Body mass index (kg/m^2)	0.053	0.024	0.029	0.006; 0.101

Table 3. Beta coefficients for testosterone, age and BMI in adjusted models of prediction of T score of the hip, using stepwise regression. a. Dependent variable: T score hip. b. Predictors: E2, testosterone, DHEA, androstenedione, age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use.

problems were not detected. The ANOVA table shows that, globally, the adjusted models are statistically significant and the proportion of variance explained by the regression models increased as successive variables entered the models (Table 4). We have also observed that only BMI positively affected the T score of the lumbar spine (Table 5) and the model is statistically significant (Table 6).

Discussion

The main objective of this study was to assess the positive associations between serum E2, DHEA and androstenedione and the bone mineral density of the hip and lumbar spine, by using a more sensitive laboratorial technique than those used in previous studies. Our results showed a statistically significant association between testosterone and bone mineral density of the hip in women over 65 years. Although positive correlations were

Model		Sum of squares	Df	Mean square	F	Sig
1	Regression	8.524	1	8.524	7.714	0.007b
	Residual	71.831	65	1.105		
	Total	80.355	66			
2	Regression	13.283	2	6.641	6.337	0.003c
	Residual	67.072	64	1.048		
	Total	80.355	66			
3	Regression	18.187	3	6.062	6.144	0.001d
	Residual	62.167	63	0.987		
	Total	80.355	66			

Table 4. ANOVA table for stepwise regression models adjusted for the dependent variable T score of the hip. a. Dependent variable: T score hip. b. Predictors: Age. c. Predictors: Age, Testosterone levels (ng/mL). d. Predictors: Age, Testosterone levels (ng/mL), Body mass index (Kg/m2).

Model		β	Std. error	*p*	95% CI
1	Body mass index (kg/m^2)	0.124	0.038	0.002	0.049; 0.198

Table 5. Beta coefficients for testosterone, age and BMI in adjusted models of prediction of T score of the lumbar spine, using stepwise regression. a. Dependent variable: T score lumbar spine. b. Predictors: E2, testosterone, DHEA, androstenedione, age, race, BMI, alcohol, smoking habits, age of menarche, type of menstrual cycles, parity, age of menopause, years since menopause, vaginal estrogen use.

Model		Sum of squares	Df	Mean square	F	Sig
1	Regression	26.954	1	26.954	10.847	0.002b
	Residual	161.518	65	2.485		
	Total	188.472	66			

Table 6. ANOVA table for stepwise regression model adjusted for the dependent variable T score of the lumbar spine. a. Dependent Variable: T score lumbar spine. b. Predictors: Body mass index (kg/m^2).

found between E2, DHEA and androstenedione and bone mineral density of the hip and lumbar spine, we did not find statistical significance after adjustments for possible confounding factors.

Older studies using immunoassays for steroid measurements are conflicting. Our results are in line with the studies that showed an association between testosterone and BMD of the hip[19–22]. Tok et al. in a sample of 147 postmenopausal women (mean age 52 years) found that serum free testosterone levels were correlated positively with the BMD at the lumbar spine and femoral neck[20]. Likewise, Van Geel et al. found the same associations in 329 postmenopausal women, after adjustment for age[22].

Lambrinoudaki et al. in the study with the largest sample to date (884 postmenopausal women not on hormone therapy), found testosterone and androstenedione were significantly associated with BMD at the hip. This study also confirmed an association between estradiol and BMD of the lumbar spine and hip[21]. It should be noted that the mean age of the participants in this study was 52.4 years.

However, other previous studies have failed to demonstrate that association[23–26]. Murphy et al. found significant positive correlations between the free estradiol and testosterone indices and bone mineral density at all sites but these relationships remained significant only for the free estradiol index after adjustment for age and body mass index[23]. Greendale et al. in a large population-based study (The Rancho Bernardo Study) of elderly women (mean age of 72.2 years), found the association between bioavailable testosterone and BMD was statistically significant only at the ultradistal radius, after accounting for covariates (age, BMI, alcohol, thyroid hormone, thyazides, exercise, cigarette use, and estrogen use)[24]. The other study that included women over 65 as participants (223 women) found that free testosterone was positively related to hip BMD, but after excluding estrogen users the sample was reduced, and there was a decrease in the magnitude and statistical significance of that relationship, which was even more attenuated after adjusting for estradiol[25].

Concerns about the specificity of immunoassays when serum 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, thus allowing greater sensitivity and specificity [27]. GC–MS/MS has been reported to be the more precise and accurate than LC–MS/MS in this type of analysis[18]. Derivatization is necessary for some of these compounds when gas chromatographic methods are used, since it improves the sensitivity and resolution of the separation. This is necessary to achieve the low concentrations usually found in biological specimens[16,18]. Concerning LC-based procedures, ion suppression can be directly related to inadequate sample preparation, and it is a major problem of LC–MS/MS techniques[28]. For instance, concerning the determination of testosterone in plasma from postmenopausal women, Thakur et al. have stated that GC/MS–MS provides excellent sensitivity and specificity when compared to liquid chromatographic methods, and helps elucidating the pharmacokinetic parameters of testosterone-related therapy, allowing as well monitoring endogenous testosterone as a pharmacodynamic biomarker[18]. In fact there are different published works about the determination of these compounds using GC–MS/MS[29–32]. 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; 0.5 ng/mL for T) using only 1 mL of sample. Our study is the first to analyze associations between sex steroid levels and bone mineral density using GC–MS/MS, and for that reason, a small number of patients were included, aiming to be a pilot study.

We did not find a statistical significance between estradiol and BMD, and this can be explained by the small sample size of women included in our study but also by the pathophysiology of osteoporosis. An earlier classification of osteoporosis, although no longer used, divided osteoporosis in 2 types. In type I osteoporosis, there was a more pronounced effect of estrogen deprivation. In older women with type II osteoporosis, other factors could be additionally responsible for bone loss[33]. Using immunoassays for steroid measurements, Slemenda et al. also found that in older postmenopausal women, depending on skeletal site, both higher testosterone and estrogen concentrations were associated with slower bone loss[19] and Stone et al. demonstrated in 9704 community-dwelling white women over 65 years of age that estradiol levels > 10 pg/mL were associated with 0.1% annual hip bone loss and levels below 5 pg/mL with an average of 0.8%[34]. Today it is known that estrogen decline in menopause is predominantly associated with trabecular bone loss. In women over the age of 65, most bone loss is cortical, not trabecular[35]. This could explain the absence of association of E2 and BMD in our sample of older women.

We found a weak positive correlation, with no statistical significance, between DHEA and BMD of the hip and lumbar spine. In theory, being a prohormone for the synthesis of estradiol and androgens, DHEA should correlate positively to BMD. Recently, Jankowsi et al. concluded, in a pooled analysis of four clinical trials, that women on treatment with oral DHEA had increased lumbar spine and trochanter BMD and maintained total hip BMD[36]. However the relationship between endogenous DHEA and BMD is not well recognized. Most of the studies analyzed DHEA sulfate (DHEAS), and the results are discrepant, either showing a positive association with BMD[20,37,38] or no association[21,23,39].

The finding of a significant positive influence of testosterone in bone mineral density of the hip in older women should encourage further research into testosterone deficiency in elderly women, with a potential impact in the prevention and treatment of postmenopausal osteoporosis. The effects of testosterone on the bone of older postmenopausal women are not very well documented but it is known that testosterone may have direct effects on bone via the androgen receptor, or indirect effects via aromatization[5]. The prevalence and burden of hip fractures has increased with increasing average life expectancy, and is one of the most serious health care problems affecting older women. In Portugal, as in Europe, the lifetime probability of hip fracture in women aged 70 is around 15%[40]. In women above 65 years of age, hip fracture is responsible for a twofold increased mortality in the first year after its occurrence[41].

The major limitations of this study are the small sample size and the cross-sectional design of the study, which does not allow us to make causal inferences. Despite the limitations, our study is pioneering, because it is the first study analyzing associations between sex steroid hormone levels and bone mineral density using GC–MS/MS. We have shown that GC–MS/MS is a conveyable technique for future larger prospective studies conducted

to provide accurate evidence that in older postmenopausal women androgen deficit plays an important role in bone loss and senile osteoporosis.

Data availability

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

Received: 7 January 2023; Accepted: 22 March 2023
Published online: 25 March 2023

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Acknowledgements
This work was partially supported by CICS-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT) and by Fundo Europeu de Desenvolvimento Regional (FEDER) under the scope of PORTUGAL 2020 and Programa Operacional do Centro (CENTRO 2020), with the project reference UIDB/00709/2020.

Author contributions
E.N.: Project development, data collection, manuscript writing; EG: Data management; SMN: Data analysis; JFM: Manuscript editing.

Competing interests
The authors declare no competing interests.

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IV.1.3. Postmenopausal sexual function and steroid hormone levels: a hospital-based cross-sectional study

CLIMACTERIC
2023, VOL. 26, NO. 2, 143–148
https://doi.org/10.1080/13697137.2023.2171286

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ORIGINAL ARTICLE

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Postmenopausal sexual function and steroid hormone levels: a hospital-based cross-sectional study

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ABSTRACT

Objective: Steroid hormone levels, particularly androgens, play an important role in sexual function in premenopausal women, but this relationship is not so well determined after menopause. This study aimed to assess the association between steroid hormone levels and sexual function in postmenopausal women.

Methods: A total of 84 postmenopausal women with intact ovaries, who had never used systemic hormone therapy, were enrolled in a cross-sectional study. Sexual function was assessed using the Female Sexual Function Index (FSFI) questionnaire and serum levels of steroid hormones were quantified by gas chromatography and tandem mass spectrometry. Associations between estradiol, testosterone, dehydroepiandrosterone, androstenedione and FSFI domain scores were evaluated.

Results: After adjustment for confounding variables, the analysis revealed a statistically significant association between androstenedione and overall sexual function ($\beta = 1.23$, 95% confidence interval [CI] [0.37; 1.98], $p = 0.010$), arousal ($\beta = 0.19$, 95% CI [0.02; 0.37], $p = 0.034$), orgasm ($\beta = 0.33$, 95% CI [0.15; 0.45], $p = 0.001$) and satisfaction ($\beta = 0.25$, 95% CI [0.11; 0.36], $p = 0.001$). No associations were found between the other hormones and FSFI domains.

Conclusion: The main finding of this study is the association of androstenedione with sexual function in postmenopausal women, not verified for other steroid hormones. Further studies are necessary to determine the importance of androstenedione for postmenopausal sexual function.

ARTICLE HISTORY
Received 5 June 2022
Revised 28 December 2022
Accepted 17 January 2023
Published online 2 February 2023

KEYWORDS
Menopause; sexual function; androgens; estradiol

Introduction

With the increase in average life expectancy, nearly half of a woman's life will be spent in the postmenopausal period. Although the frequency of sexual activity declines with age [1,2], older women engage in frequent and satisfying sexual activity [3]. Women want to talk more often about sexuality-related problems with their doctors and there is growing awareness in the medical community regarding this issue. Often seen as a minor problem in relation to all of the problems that exist at this stage of life, studies carried out in recent decades have shown that this issue is important for the maintenance of quality of life [4]. In a large study from the USA investigating the sexual experiences of 31,000 women aged 18–102 years, the prevalence of sexual problems increased with age: 27.2% for age 18–44 years, 44.6% for age 45–64 years and 80.1% for age 65 years and older [5]. However, a few studies mention that distress related to sexual problems changes little with age [6] or even declines [5] and the worldwide prevalence of sexual dysfunction among women 40–80 years old is estimated to be 43% [7]. The etiology of female sexual dysfunction includes biological, psychological, interpersonal and sociocultural risk factors [8]. In addition to lower self-esteem, relationship issues, mental health problems with higher prevalence of depression and chronic diseases such as diabetes, postmenopausal hormonal changes play an important role [9].

Estrogen deprivation is responsible for vulvovaginal atrophy and higher incidence of dyspareunia and topical vaginal estrogen therapy improves sexual function in postmenopausal women [10]. Androgens appear to play a role in maintaining sexual health and clinical trials have consistently demonstrated that testosterone therapy improves sexual function in women with hypoactive sexual desire disorder [11]. In a recent large study of premenopausal women, the authors showed that testosterone, dehydroepiandrosterone (DHEA) and androstenedione have small but significant positive associations with sexual desire, pleasure and self-image [12]. However, few studies have been conducted regarding androgens and sexual function in postmenopausal women. Adrenal androgens decline with age and by the time of menopause have already decreased 60% [13]. For decades there has been controversy over whether the postmenopausal ovary is an androgen production site [14–17]. More recently it was stated that around 20% of serum DHEA originates from the postmenopausal ovary [18]. Bilateral oophorectomy performed during the postmenopausal period reduces sexual function scores significantly [19].

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The purpose of this study is to determine the association between steroid hormone levels and sexual function in postmenopausal women with intact ovaries.

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 evaluated in a hospital gynecology consultation and formally consented to participate in the study. Postmenopausal status was based on 12 months of amenorrhea. At the time of menopause all women had intact ovaries and adnexal pathology was excluded by performing transvaginal ultrasound to all participants. Current or past users of systemic hormonal therapy or corticosteroid treatment were excluded. Other exclusion criteria were alcoholism, narcotic addiction and chronic hepatic or renal diseases.

Participants were asked to complete the Portuguese version of the Female Sexual Function Index (FSFI) questionnaire [20], validated in the Portuguese population [21]. The FSFI is a 19-item written questionnaire concerning sexual activity in the last 4 weeks and each answer is assigned a value. The sum of the values of specific questions corrected by a factor allows to assess six domains of sexual function: desire, arousal, lubrication, orgasm, satisfaction and pain (dyspareunia). The domain scores are added, resulting in a final score that can range from 2 to 36. Higher scores indicate a better degree of sexual function, and an FSFI total score of 26.55 or less indicates sexual dysfunction [22].

One blood sample was obtained from each woman between 8 and 10 am. Plasma samples were stored at −80 °C and protected from light until analysis. The studied compounds (DHEA, androstenedione, 17β-estradiol and testosterone) were quantified by solid phase extraction and gas chromatography and tandem mass spectrometry (GC-MS/MS). Briefly, 1 ml of plasma was diluted with 1 ml of phosphate buffer saline (pH 7) and spiked with 100 µl of internal standard (DHEA-d6). Solid phase extraction cartridges (Oasis® HLB 3 cc; Waters, USA) were conditioned with 2 ml of methanol and 2 ml of 0.1% acetic acid. After the sample passed through the cartridge, a washing step was performed with 2 ml of deionized water. Following this step, the columns were dried under full vacuum for 30 min. Subsequently, the analytes of interest were eluted with 2 ml methanol. The resulting extracts were evaporated to dryness under a steam of nitrogen. The remaining residues were dissolved in 20 µl of methanol and vortex mixed, and 3 µl was injected into the GC-MS/MS system. After this step, the remaining residue was evaporated to dryness under a gentle nitrogen stream at 36 °C. The analytes under study present active moieties, and therefore derivatization is deemed necessary to the analysis of 17β-estradiol and testosterone prior to their analysis by GC-based procedures. To accomplish this, 20 µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide was added to the dry extracts, and 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 into the GC-MS/MS system.

Estrone was not considered in the analysis because, despite being the predominant estrogen in menopause, it has a very weak action and also due to funding constraints.

The statistical analysis software used was SPSS 27.0. Descriptive statistics were reported as the mean ± standard deviation and median ± interquartile range for continuous variables, and as the frequency (%) for categorical variables. Serum levels of estradiol, testosterone, DHEA and androstenedione were our primary predictive variables and the confounding variables that could interfere with the results were age, reproductive lifespan, parity, years since menopause, body mass index (BMI), smoking habits, regular alcohol habits, diagnosis of depression, diabetes, vaginal estrogen use (two to three times weekly) and education level. Education levels were classified according to the International Standard Classification of Education (ISCED). In Portugal, ISCED level 1 corresponds to basic education (the first 6 years), ISCED level 2 to basic education (the next 3-year cycle), ISCED level 3 is upper secondary education and ISCED level superior to 4 corresponds to higher education. Reproductive lifespan was defined as the difference between ages at menopause and menarche, and categorized into quartiles (<33, 33–37, 38–40 or >40 years). BMI was calculated as weight in kilograms divided by height in meters squared, and categorized according to standard cut-off points.

Multiple linear regression models were performed considering each of the FSFI domain scores as dependent variables and hormone concentrations as regressors. In a first phase, univariate regression models were performed in order to analyze the effect of each of the hormone concentrations separately on each of the FSFI domain scores. In a second phase, the analysis was adjusted, including the variables age, reproductive lifespan, BMI, depression, smoking habits and vaginal estrogen use, in order to detect possible changes in the estimated coefficients. The variables parity, regular alcohol habits, diabetes and education levels were not considered in the analysis due to their reduced variability and the variable years since menopause was not considered in the models due to collinearity with the variable age. Qualitative variables were treated as dummy variables and the regression method used was Enter. A *p*-value of 0.05 or less was considered statistically significant. To calculate the confidence intervals (CIs) for the parameters, the bootstrap method was used based on a resampling of 1000 bootstrap samples. In particular, we used bootstrap wild as a resampling method and we calculated bootstrap bias-corrected and accelerated (BCa) CIs.

Results

A total of 168 postmenopausal women completed the FSFI questionnaire but only 84 women (50%) had been sexually active in the previous 4 weeks and were included in this study. All participants were Caucasian and their mean age was 59.4 years. Table 1 presents descriptive statistics of demographic, clinical and laboratorial parameters of the participants.

Table 1. Basic descriptive characteristics of the study sample ($n = 84$).

Characteristic	*Frequency, n (%)*	*Mean (standard deviation)*	*Minimum*	*Maximum*	*Median (interquartile range)*
Age (years)		59.4 (6.06)	49	77	58 (9)
40–49	1 (1.2%)	–	–	–	–
50–59	50 (59.5%)	–	–	–	–
60–69	28 (33.3%)	–	–	–	–
70–79	5 (6%)	–	–	–	–
Age of menarche (years)	–	12.5 (1.51)	10	16	12 (2)
Age of menopause (years)	–	50.2 (3.57)	37	56	51 (3)
Reproductive lifespan (years)	–	37.7 (3.75)	21	44	38 (4)
<37	25 (29.8%)	–	–	–	–
37–38	19 (22.6%)	–	–	–	–
39–40	22 (26.2%)	–	–	–	–
>40	18 (21.4%)	–	–	–	–
Parity		1.9 (0.68)	0	4	2 (1)
Nulliparous	1 (1.2%)	–	–	–	–
Multiparous	83 (98.8%)	–	–	–	–
Years since menopause		9.2 (6.86)	1	28	8 (9)
<10	48 (57.1%)	–	–	–	–
10–19	28 (33.3%)	–	–	–	–
20–29	8 (9.5%)	–	–	–	–
Body mass index (kg/m^2)		27.9 (4.86)	20	46	27 (7)
Underweight (<18.5 kg/m^2)	0 (0%)	–	–	–	–
Normal weight (18.5–24.9 kg/m^2)	21 (25%)	–	–	–	–
Overweight (25–29.9 kg/m^2)	33 (39.3%)	–	–	–	–
Obesity class I (30–34.9 kg/m^2)	24 (28.6%)	–	–	–	–
Obesity class II (35–39.9 kg/m^2)	4 (4.8%)	–	–	–	–
Obesity class III (≥40 kg/m^2)	2 (2.4%)	–	–	–	–
Smoking habit	11 (13.1%)	–	–	–	–
Regular alcohol habit	0 (0%)	–	–	–	–
Depression	19 (22.6%)	–	–	–	–
Diabetes	6 (7.1%)	–	–	–	–
Vaginal estrogen use	27 (32.1%)	–	–	–	–
Education level					
None	3 (3.6%)	–	–	–	–
ISCED 1	37 (44%)	–	–	–	–
ISCED 2	10 (11.9%)	–	–	–	–
ISCED 3	12 (14.3%)	–	–	–	–
ISCED 4	22 (26.2%)	–	–	–	–
Hormone levels (ng/ml)					
Estradiol	–	1.15 (2.12)	0.05	10.98	0.27 (1.14)
Testosterone	–	2.89 (5.59)	0.50	33.80	0.50 (1.45)
DHEA	–	10.99 (5.97)	2.33	32.24	10.17 (5.99)
Androstenedione	–	1.54 (1.20)	0.16	6.87	1.23 (0.85)
FSFI domain score					
Desire	–	2.8 (1.03)	1.2	5.4	3 (1.8)
Arousal	–	3.4 (1.05)	1.2	5.4	3.6 (1.8)
Lubrication	–	3.9 (1.17)	1.2	6.0	3.9 (1.7)
Orgasm	–	3.8 (1.30)	1.2	6.0	3.9 (2.0)
Satisfaction	–	4.6 (1.05)	1.2	6.0	4.8 (1.2)
Pain	–	4.5 (1.27)	1.2	6.0	4.8 (2.3)
Total	–	22.9 (5.26)	10.7	34.8	23.4 (7.8)

DHEA, dehydroepiandrosterone; FSFI, Female Sexual Function Index; ISCED, International Standard Classification of Education.

Table 2 presents the estimated coefficients for the multiple linear regression associations, the respective 95% bootstrap BCa CIs and *p*-values.

The univariate analysis revealed a statistically significant association between testosterone and the orgasm ($\beta = 0.04$, BCa 95% CI [0.00; 0.07], $p = 0.047$) and satisfaction domains ($\beta = 0.03$, BCa 95% CI [0.00; 0.05], $p = 0.031$), and also between DHEA and satisfaction ($\beta = 0.03$, BCa 95% CI [0.00; 0.06], $p = 0.023$). However, no significant associations were found between estradiol, testosterone or DHEA and any of the domain scores, after adjustment for the variables age, reproductive lifespan, BMI, depression, smoking habits and vaginal estrogen use (model 2).

It was found that androstenedione positively affects sexual function, with this association being statistically significant for the FSFI total score ($\beta = 1.24$, BCa 95% CI [0.47; 1.78, $p = 0.003$) and for arousal ($\beta = 0.20$, BCa 95% CI [0.02; 0.37], $p = 0.044$), lubrication ($\beta = 0.21$, BCa 95% CI [0.03; 0.33], $p = 0.038$), orgasm ($\beta = 0.32$, BCa 95% CI [0.13; 0.46], $p = 0.004$) and satisfaction domains ($\beta = 0.26$, BCa 95% CI [0.12; 0.35], $p = 0.001$). After adjustment for the predictor variables (model 2) the results remain significant for FSFI total score ($\beta = 1.23$, BCa 95% CI [0.37; 1.98], $p = 0.010$), arousal ($\beta = 0.19$, BCa 95% CI [0.02; 0.37], $p = 0.034$), orgasm ($\beta = 0.33$, BCa 95% CI [0.15; 0.45], $p = 0.001$) and satisfaction ($\beta = 0.25$, BCa 95% CI [0.11; 0.36], $p = 0.001$), but no longer having statistical significance for the lubrication domain.

Discussion

The main finding in this study is the important and independent contribution of androstenedione to sexual function in postmenopausal women. This is evident after excluding a

Table 2. Univariate and multivariate regression models for FSFI domain scores as a function of hormone concentrations.

Hormone	*Model*	*Total*	*Desire*	*Arousal*	*Lubrication*	*Orgasm*	*Satisfaction*	*Pain*
Estradiol	1	0.22, [−0.26; 0.85], 0.338	0.05, [−0.03; 0.14], 0.212	0.02, [−0.08; 0.13], 0.706	0.03, [−0.08; 0.16], 0.573	0.05, [−0.07; 0.17], 0.391	0.06, [−0.03; 0.16], 0.092	0.03, [−0.09; 0.17], 0.577
	2	0.10, [−0.50; 0.70], 0.731	0.03, [−0.09; 0.15], 0.594	−0.01, [−0.13; 0.11], 0.897	0.03, [−0.11; 0.20], 0.621	0.01, [−0.13; 0.14], 0.963	0.05, [−0.11; 0.19], 0.357	0.02, [−0.14; 0.23], 0.749
Testosterone	1	0.11, [−0.05; 0.24], 0.181	0.01, [−0.02; 0.04], 0.503	0.01, [−0.04; 0.05], 0.751	0.01, [−0.04; 0.06], 0.689	0.04, [0.00; 0.07], **0.047**	0.03, [0.00; 0.05], **0.031**	0.01, [−0.02; 0.05], 0.419
	2	0.06, [−0.17; 0.38], 0.578	0.00, [−0.05; 0.06], 0.875	0.00, [−0.06; 0.07], 0.926	0.00, [−0.05; 0.07], 0.884	0.02, [−0.04; 0.07], 0.578	0.03, [0.00; 0.05], 0.105	0.02, [−0.02; 0.04], 0.390
DHEA	1	0.13, [−0.05; 0.30], 0.128	0.02, [−0.02; 0.05], 0.386	0.02, [−0.02; 0.06], 0.368	0.03, [−0.01; 0.07], 0.128	0.04, [−0.01; 0.09], 0.084	0.03, [0.00; 0.06], **0.023**	0.01, [−0.03; 0.05], 0.667
	2	0.10, [−0.13; 0.41], 0.291	0.02, [−0.02; 0.05], 0.343	0.01, [−0.04; 0.08], 0.659	0.02, [−0.04; 0.09], 0.306	0.03, [−0.03; 0.11], 0.225	0.03, [−0.01; 0.08], 0.138	0.00, [−0.05; 0.07], 0.935
Androstenedione	1	1.24, [0.47; 1.78], **0.003**	0.18, [0.00; 0.34], 0.094	0.20, [0.02; 0.37], **0.044**	0.21, [0.03; 0.33], **0.038**	0.32, [0.13; 0.46], **0.004**	0.26, [0.12; 0.35], **0.001**	0.12, [−0.07; 0.26], 0.267
	2	1.23, [0.37; 1.98], **0.010**	0.20, [0.01; 0.34], 0.079	0.19, [0.02; 0.37], **0.034**	0.18, [−0.04; 0.42], 0.092	0.33, [0.15; 0.45], **0.001**	0.25, [0.11; 0.36], **0.001**	0.11, [−0.09; 0.28], 0.322

Data presented as β-coefficient, [BCa 95% CI], p-value. Model 1: univariate analysis. Model 2: multivariate analysis adjusted for age, reproductive lifespan, body mass index, depression, smoking habits and vaginal estrogen use. BCa, bootstrap bias-corrected and accelerated; CI, confidence interval; DHEA, dehydroepiandrosterone; FSFI, Female Sexual Function Index.

significant association between multiple variables known to influence sex hormone concentrations and sexual function and the inclusion in the models of demographic and clinical variables that seemed to influence any of the sexual function domain scores. In this study, no significant associations were found for testosterone, DHEA or estradiol and any of the sexual function domain scores, after adjustment for predictor variables.

An Australian study which included a subgroup of 678 women aged 45–75 years, although without reference to menopausal status, found no evidence of association between low scores of sexual domains and low testosterone levels. DHEA and estradiol were not measured in this study but they found DHEAS to be associated with low self-reported sexual function. Also, in this group of older women, androstenedione was highly associated with pleasure [23].

Another study of 149 healthy postmenopausal Iranian women also found no association between testosterone and FSFI domains [24].

These previous studies used immunoassays for steroid measurements. Concerns about specificity of immunoassays when serum steroid levels are low have led to implementation of MS-based techniques as the gold standard methodology for steroid hormone analysis. MS offers a unique identification profile of each of the study analytes, eliminating interferences and thus allowing greater sensitivity and specificity [25]. There are different published works about the determination of these compounds using GC-MS/MS [26–29]. In our work, excellent limits of detection and quantitation were achieved (0.05 ng/ml for 17β-estradiol; 0.1 ng/ml for androstenedione and DHEA, and 0.5 ng/ml for testosterone) using only 1 ml of sample.

A recent prospective cohort study of 99 women aged 26–90 years treated for rectal cancer (median age of 61 years and predominantly postmenopausal) analyzed the association between serum levels of endogenous androgens measured by liquid chromatography–MS and FSFI scores. This study also found no association with free testosterone and sexual function domain scores (except for lubrication), and androstenedione was found to be significantly associated with all sexual domain scores except for desire and satisfaction [30]. Although that study was not performed in an exclusively postmenopausal population it is in line with the results of our study.

The fact that we did not reach statistical significance in regard to the sexual desire domain may be related to sample size. In a study of 560 women using LC-MS/MS for determination of hormone levels, the group of women aged 45–65 years ($n = 160$) showed a strongly statistically significant correlation between androstenedione and sexual desire [31].

Overall, our results are consistent with previous investigations showing a trend toward a significant association between androstenedione and postmenopausal sexual function and a lower influence of other hormones, which turns out to be a little different from what was found in premenopausal women [12,32].

Research on androstenedione supplementation in women is scarce. A study showed that the administration of oral androstenedione increased serum testosterone and estrone levels in postmenopausal women and could potentially be used to provide androgenic supplementation [33].

A Cochrane Review stated that DHEA may slightly improve sexual function in postmenopausal women when compared with placebo [34]. Although our study did not find an association between serum levels of DHEA and sexual function in postmenopausal women, the results are not in conflict with the use of DHEA supplementation for improving sexual function because DHEA is converted at various levels into active androgens and/or estrogens in specific peripheral tissues by the process of intracrinology [35].

The main strengths of our study when compared to previous studies are the inclusion of exclusively postmenopausal women and the use of a highly sensitive bioanalytical assay to measure low hormonal concentrations. Also, the adjustment for multiple variables known to influence hormonal levels and sexual function has an advantage over previous studies and makes our results more valid.

The main weaknesses of this study are the small sample size and the cross-sectional design of the study, which does not allow us to make causal inferences between endogenous hormone levels and self-reported sexual function scores. It should also be noted that this study did not assess sexual distress. As previously mentioned, sexual dysfunction has a complex etiology and many other potential contributors were not controlled in this study.

The finding of a significant positive influence of androstenedione in sexual function in postmenopausal women should deserve more attention in future research projects. Larger studies using MS-based techniques are necessary to determine whether androstenedione is the most important steroid hormone for sexual function during the postmenopausal period.

Potential conflict of interest No potential conflict of interest was reported by the authors.

Source of funding This work was partially supported by CICS-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT); Fundo Europeu de Desenvolvimento Regional (FEDER) [under the scope of PORTUGAL 2020]; Programa Operacional do Centro (CENTRO 2020) [project reference UIDB/00709/2020].

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IV.2. Posters

Presented at the international scientific meeting "18th World Congress on Menopause".

International IMS Menopause Society
Promoting education and research on midlife women's health

18 WORLD CONGRESS ON MENOPAUSE

26–29 OCTOBER 2022
LISBON, PORTUGAL

Certificate of Attendance

Elsa Nunes

attended the 18th World Congress on the Menopause

held in Lisbon, Portugal from 26 to 29 October 2022

Abstracts presented by Elsa Nunes:

- P142. Postmenopausal cognitive function and steroid hormone levels
  by Elsa Nunes, Eugenia Gallardo, Sara Morgado-Nunes, José Fonseca-Moutinho - Poster Session Posters

- P118. Postmenopausal sexual function and steroid hormone levels
  by Elsa Nunes, Eugenia Gallardo, Sara Morgado-Nunes, José Fonseca-Moutinho - Poster Session Posters

PROF. PAULINE MAKI
CHAIRMAN OF THE SCIENTIFIC PROGRAM COMMITTEE

PROF. STEVEN GOLDSTEIN
CHAIRMAN OF THE ORGANISING COMMITTEE

IMS 18 WORLD CONGRESS ON MENOPAUSE
LISBON, PORTUGAL
26–29 OCTOBER 2022

POSTMENOPAUSAL SEXUAL FUNCTION AND STEROID HORMONE LEVELS

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INTRODUCTION

Although the frequency of sexual activity declines with age[1,2], older women engage in frequent and satisfying sexual activity[3]. In a large study from the United States investigating the sexual experiences of 31 000 women aged 18-102 years the prevalence of sexual problems increased with age: 27.2% (age 18–44 years), 44.6% (age 45–64 years), and 80.1% (age 65 years and older)[4]. However, a few studies mention that distress related to sexual problems changes little with age[5] or even declines[4] and the worldwide prevalence of sexual dysfunction among 40 to 80-year-old women is estimated to be 43%[6]. In addition to lower self-esteem, relationship issues, mental health problems with higher prevalence of depression and chronic diseases such as diabetes, postmenopausal hormonal changes play an important role in sexual function[7]. Estrogen deprivation is responsible for vulvovaginal atrophy and higher incidence of dyspareunia and topical vaginal estrogen therapy improves sexual function in postmenopausal women[8]. Androgens appear to play a role in maintaining sexual health and clinical trials have consistently demonstrated that testosterone therapy improves sexual function in women with hypoactive sexual desire disorder[9]. In a recent large study of premenopausal women the authors showed that testosterone, dehydroepiandrosterone and androstenedione have small but significant positive associations with sexual desire, pleasure and self-image[10]. However, few studies have been conducted regarding androgens and sexual function in postmenopausal women.

AIMS

The purpose of this study was to assess the association between steroid hormone levels and sexual function in postmenopausal women.

METHODS

A total of 168 postmenopausal women were enrolled in a cross-sectional study but only 84 women (50%) had been sexually active in the previous four weeks and were included in this study. Postmenopausal status was based on 12 months of amenorrhea and serum follicle stimulating hormone (FSH) levels greater than 30 mIU/mL. At the time of menopause all women had intact ovaries and adnexal pathology was excluded by performing ultrasound to all participants. Current or past users of systemic hormonal therapy or corticosteroid treatment were excluded. Other exclusion criteria were alcoholism, narcotic addiction and chronic hepatic or renal diseases. Sexual function was assessed after self-completion of the *Female Sexual Function Index* (FSFI) questionnaire and serum levels of estradiol, testosterone, DHEA and androstenedione were quantified by solid phase extraction and gas chromatography and tandem mass spectrometry (GC-MS/MS). Multiple linear regression models were performed considering each of the FSFI domain scores as dependent variables and hormone concentrations as regressors, as well as possible confounding variables: age, reproductive lifespan, body mass index, depression, smoking habits and vaginal estrogen use. The variables parity, regular alcohol habits, diabetes and education levels were not considered in the analysis due to their reduced variability and the variable years since menopause was not considered in the models due to collinearity with the variable age.

RESULTS

Table 1. Demographic and clinical characteristics of the participants

	Frequency n (%)	Mean (SD)	Range
Age (years)		59.4 (6.1)	49-77
Race (Caucasian)	84 (100%)		
Education level			
None	3 (3.6%)		
ISCED 1 (basic education 4th grade)	37 (44%)		
ISCED 2 (basic education 9th grade)	10 (11.9%)		
ISCED 3 (upper secondary education)	12 (14.3%)		
ISCED 4 (higher education)	22 (26.2%)		
Reproductive lifespan		37.7 (3.75)	21-44
Parity (multiparous)	83 (98.8%)		
Years since menopause			
<10 y	48 (57.1%)		
10-19 y	28 (33.3%)		
20-29 y	8 (9.5%)		
Vaginal estrogen use	27 (32.1%)		
Diabetes	6 (7.1%)		
Depression	19 (22.6%)		
Alcohol habits	0 (0%)		
Smoking habits	11 (13.1%)		
BMI (Kg/m2)		27.9 (4.9)	20-46

Table 2.Descriptive statistics for hormones and sexual function domains

Hormone levels	Mean (SD)	Range
E2 (ng/mL)	1.15 (2.12)	0.05-10.98
Testosterone (ng/mL)	2.89 (5.59)	0.05-33.80
DHEA (ng/mL)	10.99 (5.97)	2.33-32.24
Androstenedione (ng/mL)	1.54 (1.20)	0.16-6.87
FSFI domains		
Total	22.9 (5.26)	10.7-34.8
Desire	2.8 (1.03)	1.2-5.4
Arousal	3.4 (1.05)	1.2-5.4
Lubrication	3.9 (1.17)	1.2-6.0
Orgasm	3.8 (1.30)	1.2-6.0
Satisfaction	4.6 (1.05)	1.2-6.0
Pain	4.5 (1.27)	1.2-6.0

Table 3. Beta Coefficients for the regression linear models. Estradiol, testosterone, DHEA, androstenedione, age, reproductive lifespan, body mass index, depression, smoking habits and vaginal estrogen use are the regression variables and FSFI domain scores are the dependent variables

		Unstandardized Coefficients		Standardized Coefficients	t	p
		B	Std. Error	Beta		
Total score	Estradiol	-.030	.488	-.012	-.072	.959
	Testosterone	.022	.162	.024	.140	.881
	DHEA	-.069	.140	-.079	-.504	.616
	Androstenedione	1.412	.618	.321	2.407	**.026**
Desire	Estradiol	.029	.067	.060	.346	.721
	Testosterone	-.013	.030	-.070	-.396	.699
	DHEA	-.006	.024	-.034	-.209	.833
	Androstenedione	.220	.131	.257	1.857	.120
Arousal	Estradiol	-.014	.073	-.029	-.176	.853
	Testosterone	-.005	.034	-.028	-.166	.882
	DHEA	-.013	.026	-.073	-.468	.654
	Androstenedione	.238	.102	.271	2.051	**.021**
Lubrication	Estradiol	.023	.102	.042	.260	.837
	Testosterone	-.014	.042	-.069	-.416	.732
	DHEA	.004	.038	.021	.139	.917
	Androstenedione	.172	.158	.176	1.350	.261
Orgasm	Estradiol	-.057	.151	-.092	-.606	.892
	Testosterone	.018	.046	.077	.497	.744
	DHEA	-.013	.039	-.058	-.405	.735
	Androstenedione	.367	.140	.338	2.772	**.017**
Satisfaction	Estradiol	-.013	.079	-.026	-.150	.893
	Testosterone	.022	.031	.117	.670	.491
	DHEA	-.011	.025	-.062	-.387	.713
	Androstenedione	.266	.107	.304	2.218	**.021**
Pain	Estradiol	-.002	.109	-.003	-.018	.993
	Testosterone	.023	.038	.103	.575	.552
	DHEA	-.027	.035	-.126	-.762	.474
	Androstenedione	.165	.154	.155	1.100	.305

*Significance level 0.05

DISCUSSION/CONCLUSION

The main finding of this study is the significant association between androstenedione and overall sexual function, arousal, orgasm and satisfaction. No significant associations were found for estradiol, testosterone or DHEA and any of the sexual function domain scores. Despite the limitations, the small sample size and the cross-sectional design of the study, our results are consistent with previous investigations showing a trend towards a significant association between androstenedione and postmenopausal sexual function and a lower influence of other hormones[11–13]. Further studies, with larger samples and using MS-based techniques, are necessary to determine whether androstenedione is the most important steroid hormone for postmenopausal sexual function.

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POSTMENOPAUSAL COGNITIVE FUNCTION AND STEROID HORMONE LEVELS

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INTRODUCTION

Women are more impacted by Alzheimer's disease than men, presenting significantly greater risk of developing Alzheimer's disease and a greater cognitive deterioration than men at the same disease stage[1]. An age-related loss of sex steroid hormones has been associated with an increased risk of cognitive decline[2]. Estrogen is known to have an essential role in the brain: promotes neurotrophin synthesis, modulates cholinergic and dopaminergic neurotransmitter systems and protects the brain against stress and inflammation[3]. Postmenopausal women with higher remaining circulating estradiol levels have better global cognitive function [4,5], episodic memory [6,7], and better semantic memory performance[8], but other studies have revealed contradictory results[9,10]. The literature also reports conflicting evidence regarding the effect of hormone therapy[11]. Likewise, the influence of androgens on postmenopausal cognitive function is not well understood. Studies have shown a positive association between verbal learning and memory and physiological concentrations of testosterone administered to postmenopausal women exogenously[11,12]. However, higher endogenous testosterone levels have been associated to lower scores of cognitive function[13]. In addition, lower endogenous testosterone levels have been associated to an improvement in verbal episodic memory[8], or showed no association[10].

AIMS

The objective of this study was to assess the association between steroid hormone levels and cognitive function in postmenopausal women, using a highly sensitive bioanalytical assay for steroid measurement.

METHODS

A total of 147 postmenopausal women, recruited at a hospital Gynecology consultation, were enrolled in a cross-sectional study. All women had undergone natural menopause and current or past users of systemic hormonal therapy or corticosteroid treatment were excluded. Cognitive function was assessed using the *Montreal Cognitive Assessment* (MoCA) test and serum levels of estradiol, dehydroepiandrosterone (DHEA), testosterone and androstenedione were quantified by solid phase extraction and gas chromatography and tandem mass spectrometry (GC-MS/MS). In order to assess the association between cognitive domains measured by the MoCA test (global cognitive function, executive function, visuospatial abilities, short term memory, attention, concentration and working memory, language, orientation to time and place) and hormone levels, nonparametric partial correlation coefficients were calculated, controlling for possible confounding variables: age, education level, years since menopause, depression, smoking habits and body mass index (BMI).

RESULTS

Table 1. Demographic and clinical characteristics of the participants

	Frequency n (%)	Mean (SD)	Range
Age (years)		61.7 (8.0)	50-86
Race (Caucasian)	146 (99,3%)		
Education level			
ISCED 1 (basic education 4th grade)	71 (48.3%)		
ISCED 2 (basic education 9th grade)	25 (17%)		
ISCED 3 (upper secondary education)	19 (12.9%)		
ISCED 4 (higher education)	32 (21.8%)		
Years since menopause		10.8 (8.5)	1-38
Depression	44 (29.9%)		
Alcohol habits	1 (0.7%)		
Smoking habits	14 (9.5%)		
BMI (Kg/m2)		28.6 (5.1)	18-46

Table 2. Descriptive statistics for hormones and cognitive domains

Hormone levels	Mean (SD)	Range
E2 (ng/mL)	1.11(2.40)	0.05-18.00
Testosterone (ng/mL)	2.88 (6.02)	0.50-34.89
DHEA (ng/mL)	10.82 (5.77)	2.33-33.81
Androstenedione (ng/mL)	1.41 (0.96)	0.10-6.87
Cognitive domains		
Global cognitive function	24.3 (3.3)	12-30
Executive function	3.0 (0.8)	1-4
Visuospatial abilities	3.0 (0.9)	0-4
Short-term memory	2.5 (1.5)	0-5
Attention, concentration and working memory	4.8 (1.2)	1-6
Language	4.2 (0.9)	1-5
Orientation to time and place	5.9 (0.3)	3-6

Table 3. Correlation coefficients for hormone levels and cognitive domains

		Estradiol	Testosterone	DHEA	Androstenedione
Global cognitive function	Correlation	-0.149	0.074	0.000	0.005
	Sig. (2-tailed)	0.078	0.387	0.999	0.949
Executive function	Correlation	-0.190	-0.049	-0.004	0.019
	Sig. (2-tailed)	0.024*	0.566	0.965	0.821
Visuospatial abilities	Correlation	-0.333	-0.119	-0.041	0.006
	Sig. (2-tailed)	0.000*	0.161	0.634	0.941
Short-term memory	Correlation	0.028	0.128	0.099	-0.006
	Sig. (2-tailed)	0.746	0.132	0.246	0.944
Attention, concentration and working memory	Correlation	-0.074	0.026	0.003	0.047
	Sig. (2-tailed)	0.387	0.764	0.967	0.583
Language	Correlation	-0.086	0.041	-0.060	0.071
	Sig. (2-tailed)	0.312	0.632	0.483	0.405
Orientation to time and place	Correlation	-0.196	-0.165	0.050	0.022
	Sig. (2-tailed)	0.020*	0.052	0.560	0.794

*. Correlation is significant at the 0.05 level
Variables controlled: age; education level; years since menopause; depression; smoking habits; BMI

DISCUSSION/CONCLUSION

An association between estradiol and executive function, visuospatial abilities and orientation to time and place was found, evidencing a tendency to obtain lower scores when evaluating these cognitive domains in patients with higher concentrations of estradiol. Although a negative correlation was also found between estradiol and global cognitive function, it did not reach a statistical significance at a level of 5%. No significant associations between testosterone, DHEA, androstenedione and cognitive function were found. Previous studies used immunnoassays for the measurement of steroid hormone levels, but mass spectrometry based techniques are now the gold standard for measuring steroid hormones in postmenopausal women, due to its higher accuracy. Despite the limitations of sample size and cross-sectional design, this is the first study to analyse associations between steroid hormone levels and cognitive function using GC-MS/MS. Further studies, with larger samples and using MS-based techniques, are necessary to better clarify the influence of steroid hormone levels in cognitive function.

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