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RESEARCH ARTICLE

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Estrogen receptor genes polymorphisms determine serum lipid profile in healthy postmenopausal women treated with calcium, vitamin D, and genistein

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Abstract

Cardiovascular risk increases in women after menopause. Unfavorable lipid-lipoprotein changes due to a lack of estrogens may have an important role in this context. Estrogen actions are mainly mediated by their binding to two estrogen receptors (ERs) whose signaling may be conditioned by different factors. Calcium, vitamin D, and genistein, among others, cause a beneficial effect on serum lipid profile by its modulation. Some genetic factors can also determine this signal. We determined the possible additive effect of genistein on calcium and vitamin D supplementation regarding serum lipid profile changes and whether ER polymorphisms may mediate in this effect.

We performed a prospective, double blind study in which women were randomized in two groups: one group received calcium and vitamin D and the other group received calcium, vitamin D and genistein. Subsequently, we studied rs9340799, rs928554, and rs4986938 ER polymorphisms in both groups. Our results showed that being a carrier of the variant allele G of rs928554 polymorphism was associated with a greater decrease in triglyceride levels and that the homozygous AA genotype of rs9340799 polymorphism was associated with a greater decrease in total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels after calcium, vitamin D, and genistein supplementation. This is the first report showing an association between polymorphisms in ER genes and an improvement of the serum lipid profile after taking calcium, vitamin D, and genistein supplementation in postmenopausal women. It reinforces the hypothesis that genetic factors are crucial in ER signalling.

KEYWORDS

calcium, cardiovascular, estrogen receptor, genistein, polymorphism, vitamin D

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1 | INTRODUCTION

Cardiovascular disease (CVD) is the principal cause of mortality around the world, being responsible of 17 million deaths a year, almost one-third of the total deaths in the world. In women, risk of CVD increases after menopause, when unfavorable lipid-lipoprotein changes due to a lack of estrogens occurs.[1] This hypothesis is supported by a large number of observational studies showing a lower CVD risk in women taking estrogens.[2]

Estrogens, such as 17 β-estradiol, are steroid hormones derived from cholesterol by the successive action of steroidogenic enzymes. They are involved in multiple physiological processes by acting on various tissues. They regulate the reproductive organs but also participate in many other physiological processes such as bone remodeling or immune regulation, among others.[3]

Regarding CVD, estrogens have a potential cardioprotective action as an immunomodulator of the inflammatory response in atherosclerosis.[4] Estrogens also regulate lipid metabolism in the liver and can decrease CVD risk mainly by increasing the hepatic synthesis of low-density lipoprotein (LDL) receptor, resulting in decreased LDL circulating levels. Estrogens also increase the activity of the enzyme lipoprotein lipase, raising high-density lipoprotein (HDL) levels.[5]

Estrogen actions are mainly mediated by their binding to two estrogen receptors (ERs), ERα and ERβ. ERs are generated from two different genes that are localized on chromosome 6 and chromosome 14, respectively.[6] In the absence of hormone, ERs remain inactive in the cytosol. Upon hormone binding, ERs experience a dimerization and translocation into the nucleus. In the nucleus, ERs bind to genomic sequences denominated estrogen responsive elements (ERE) at target genes promoters, which regulate the transcription of those genes.[7] This mechanism of transcriptional regulation represents more than 90% of estrogens target genes.[8] ERE are found in the promoter region of genes implicated, among others, in lipid metabolism.[9-11]

Furthermore, there is previous evidence about the association of ERs polymorphisms and changes in plasma lipid and apolipoprotein levels.[12]

ER signalling can be modulated by different agents other than estrogens. Calcium has been proposed to bind to amino acids in sites A-D of ERα and directly activate it. It is also possible that the binding of calcium indirectly activates the receptor, for example, by the recruitment of coactivators.[13] 1, 25-Dihydroxyvitamin D can bind to a vitamin D response element within the ER promoter and inhibit ER gene transcription.[14] Phytoestrogens are plant-derived compounds that have structural similarity to estrogen and that are able to bind to the ERs. Genistein, a phytoestrogen, is one of the main isoflavones in soy and it modulates ER signalling by a genomic effect.[15-17]

Taking into account the above and focusing on the effect of the estrogens on the lipid profile, the primary outcome of our study was to determine whether calcium, vitamin D and genistein supplementation had a beneficial additive effect on serum lipid profile. The secondary outcome was to determine whether this effect was mediated by polymorphisms in ER genes. The novelty of this study is that, to our knowledge, this is the first time that a possible effect of polymorphisms in ER genes on serum lipid profile in patients taking calcium, vitamin D, and genistein is studied.

2 | SUBJECTS AND METHODS

2.1 | Subjects

We included 102 healthy postmenopausal women (no menstruation within the last year) referred from West Valladolid primary healthcare centres. Exclusion criteria were a menopause history of more than 10 years, a recorded medical history of osteoporosis, nephrolithiasis, hyperparathyroidism, thyroid disease, HIV, neoplasia, smoking, alcohol intake or the use of drugs that alter lipid metabolism (statins, ezetimibe, and fish oil).

First, we performed a prospective, double blind study in which women were randomized in two groups by a computer system (*Research Randomizer*; https://www.randomizer.org). A total of 51 postmenopausal women were included in each group. One group received calcium (1000 mg/day) and vitamin D (cholecalciferol, 800 U/d) and the other group received additionally genistein (90 mg/day). Both group received the supplementation for 12 weeks. Every patient received two different pills: one with calcium and vitamin D and another with genistein or placebo (same envelope, form and colour). We selected a dosage of 1000 mg/day of calcium and 800 U/d of vitamin D (cholecalciferol) due to the fact that these dosages are those recommended for adults between 19 to 60 years old by the US Institute of Medicine.[18] We selected a dosage of 90 mg/day of genistein because it has been reported that this is the minimum dose needed to observe beneficial effects.[19-21] We ensure adherence to treatment by asking the patient each visit every month. In addition, we quantified the serum calcium and serum vitamin D levels at baseline and at week twelve.

Blood samples were obtained after 8 hours fasting. Clinical and analytical variables such as sex, age, age of menopause, body mass index (BMI), calcium, vitamin D, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were collected from each subject before and after of 12 weeks of taking supplementation.

The experimental protocol was in accordance with the Declaration of Helsinki (2008) of the World Medical Association, approved by the University Hospital of Valladolid Ethics Committee and in compliance with the Spanish data protection law (LO 15/1999) and specifications (RD 1720/2007). All who accepted to participate in the study signed a written consent.

2.2 | DNA isolation and polymorphism genotyping

Genomic DNA was extracted from peripheral blood with commercial methods (Qiagen or GE Healthcare). We analysed the rs9340799, rs4986938, and rs928554 polymorphism. The rs9340799 is a intronic polymorphism (A>G) in the ER gene 1 (ESR1). rs4986938 (G>A) and rs928554 (A>G) are intronic polymorphisms in the ER gene 2 (ESR2). Genotyping was performed using TaqMan 5′-exonuclease allelic discrimination assays that contain sequence-specific forward and reverse primers to amplify the polymorphic sequences and two probes labelled with VIC and FAM dyes to detect both alleles of each polymorphism.[22] Polymerase chain reaction (PCR) reactions were carried out using TaqMan universal PCR Master Mix following instructions in a Step-One Plus Real-time PCR system (Applied Biosystems, Foster City, CA). To assess reproducibility, a random selection of 5% of the samples were regenotyped, all of these genotypes matched with the genotypes initially designated.

2.3 | Data analysis

Hardy-Weinberg equilibrium was tested with an exact test implemented in HWSIM software. rs9340799 ESR1, rs4986938 ESR2, and rs928554 ESR2 polymorphisms were in Hardy-Weinberg equilibrium. The statistical analyses were performed using SPSS software. For the analysis, differences with a $P < 0.05$ were considered as statistically significant.

3 | RESULTS

Baseline characteristics were similar between the two groups included in the study (Table 1). All women were caucasians. All women referred to perform daily physical activity (≥1 hour per day). Our results showed that a supplementation with calcium and vitamin D for 12 weeks caused a decrease in the total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride serum levels (Table 2). Our results also showed the additive effect of genistein supplementation as those postmenopausal women on supplementation with genistein for 12 weeks had a statistically significant decrease in the total cholesterol and LDL-cholesterol serum levels, although it did not cause a variation in the HDL-cholesterol and triglyceride levels (Table 2). All the biochemical variables were in normal distribution (Table S1). Serum calcium and serum vitamin D levels at baseline and at week 12 after calcium, vitamin D, and genistein supplementation are summarized in Table 3.

According to the study of ESR1 rs9340799, ESR2 rs4986938, and ESR2 rs928554 polymorphisms, there were no differences in baseline total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides in the subjects included in the study (Table 4). We found statistically significant differences in serum lipid profile changes after any of the two regimens of supplementation depending of ESR1 rs9340799 and ESR2 rs928554 polymorphisms. Being a carrier of the variant allele G of ESR2 rs928554 polymorphism was associated with a greater decrease in triglyceride levels after supplementation therapy. Regarding ESR1 rs9340799 polymorphism, homozygous AA genotype was associated with a greater decrease in total cholesterol, LDL-cholesterol and triglyceride levels after supplementation therapy. *P*-value was adjusted by BMI, age, sex, and genistein supplementation (Table 4).

4 | DISCUSSION

Risk of CVD increases after menopause in women. Unfavorable lipid-lipoprotein changes due to a lack of

TABLE 1 Comparison of clinical and analytical variables between the two study groups

	Calcium and vitamin D	Calcium, vitamin D and genistein	*P*-value
Age, mean ± SD, y	55.04 ± 3.44	55.42 ± 3.81	0.597
Age of menopause, mean ± SD, y	49.54 ± 3.43	49.13 ± 4.12	0.059
BMI, mean ± SD	25.02 ± 3.87	25.35 ± 3.75	0.671
Cholesterol, mean ± SD, mg/dL	231.98 ± 42.52	244.57 ± 33.45	0.107
LDL-cholesterol, mean ± SD, mg/dL	134.22 ± 36.62	139 ± 30.39	0.061
HDL-cholesterol, mean ± SD, mg/dL	71.83 ± 15.98	65.79 ± 15.22	0.058
Triglycerides, mean ± SD, mg/dL	120.77 ± 52.15	142 ± 75.19	0.671

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL-cholesterol, low-density lipoprotein; SD, standard deviation.

TABLE 2 Serum lipid profile changes caused by calcium, vitamin D and genistein supplementation for 12 wk in postmenopausal women. *P*-value was adjusted by age and BMI

	Calcium and vitamin D	Calcium, vitamin D and genistein	*P*-value
Total cholesterol, mean ± SD, mg/dL	−3.38 ± 1,34	−7.54 ± 2.65	0.042
HDL-cholesterol, mean ± SD, mg/dL	−0.92 ± 0.45	−0.65 ± 0.39	0.781
LDL-cholesterol, mean ± SD, mg/dL	−1.49 ± 0.93	−8.12 ± 2.78	0.036
Triglycerides, mean ± SD, mg/dL	−5.63 ± 2.45	−7.95 ± 3.12	0.841

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

TABLE 3 Serum calcium and serum vitamin D levels at baseline and at week twelve of calcium, vitamin D, and genistein supplementation

	Calcium and vitamin D			Calcium, vitamin D, and genistein		
	Baseline	Week 12	*P*-value	Baseline	Week twelve	*P*-value
Calcium, mg/dL	9.49 ± 0.28	9.46 ± 0.40	>0.05	9.5 ± 0.38	9.42 ± 0.49	>0.05
vitamin D, ng/mL	25 ± 10	33 ± 8	0.0001	24 ± 9	31 ± 8	0.0001

estrogens may have an important role in this context.[1] As we mentioned before, estrogen actions are mainly mediated by their binding to two ERs, ERα and ERβ, whose signaling may be conditioned by different factors as calcium, vitamin D and genistein, among others. As one of the functions of estrogens is the regulation of lipid metabolism in the liver, we first studied whether the addition of genistein to calcium and vitamin D supplementation had an additive beneficial effect on serum lipid profile.

We ensure adherence to treatment by asking the patient at each visit every month. In addition, an increase in vitamin D levels indicates that the patient has taken the pills. Calcium levels did not change substantially as

TABLE 4 Baseline serum lipid profile and serum lipid profile changes after treatment in postmenopausal women according to rs4986938, rs928554, and rs9340799 polymorphisms. *P*-value was adjusted by age, BMI, and genistein supplementation

		Baseline total cholesterol		Baseline LDL-cholesterol		Baseline HLD-cholesterol		Baseline triglycerides	
SNP	Genotype	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value
ESR2 rs4986938	GG	236.4 ± 42.1	0.741	142.2 ± 34.1	0.891	68.14 ± 17.2	0.852	128.1 ± 52.4	0.390
	GA + AA	239.2 ± 37.7		143.5 ± 35,4		68.75 ± 14.6		142.2 ± 67.1	
ESR2 rs928554	AA	240.5 ± 38.8	0.641	143.4 ± 54.5	.866	70.61 ± 15.9	0.293	124.2 ± 52.7	0.187
	AG + GG	236.6 ± 39.6		142.1 ± 35.3		67.19 ± 15.2		145.3 ± 89.1	
ESR1 rs9340799	AA	244.7 ± 36.8	0.209	149.7 ± 31.1	.125	70.61 ± 17.7	0.313	139.3 ± 99.8	0.819
	AG + GG	234.3 ± 40.3		138.4 ± 36.4		67.30 ± 14.1		135.6 ± 61.1	
		Total cholesterol change		LDL-cholesterol change		HDL-cholesterol change		Triglycerides change	
SNP	Genotype	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value	Mean ± SD	*P*-value
ESR2 rs4986938	GG	−6.75 ± 4.21	0.716	−5.31 ± 2.87	0.914	−1.73 ± 0.98	0.261	−4.43 ± 2.98	0.748
	GA + AA	−4.81 ± 1.34		−4.73 ± 1.45		0.52 ± 0.36		−6.34 ± 4.21	
ESR2 rs928554	AA	−7,02 ± 2,46	0.650	−6,31 ± 3,45	0.667	−0,39 ± 0,28	0.812	−3.73 ± 1.85	0.048
	AG + GG	−4.61 ± 2.13		−4.05 ± 2.85		−0.77 ± 0.65		−7.39 ± 4.57	
ESR1 rs9340799	AA	−8.71 ± 3.45	0.039	−7.54 ± 3,01	0.015	−0.75 ± 0.59	0.907	−11.31 ± 7.8	0.022
	AG + GG	−2.85 ± 1.78		−2.17 ± 1.56		−0.56 ± 0.35		−4.29 ± 2.67	

Abbreviations: ESR1, estrogen receptor 1; ESR2, estrogen receptor 2; HDL, high-density lipoprotein; LDL-cholesterol, low-density lipoprotein; SD, standard deviation.

its regulation is done also by parathyroid hormone and vitamin D. Serum calcium and serum vitamin D levels were quantified at baseline and at week twelve. We determined that the duration of treatment was 12 weeks (3 months) for three reasons. The first one is that it has been described that 12 weeks is enough time to observe the beneficial effects of treatment with calcium, vitamin D and genistein.[18,20] Second, as UV light determines the synthesis of vitamin D, we wanted to avoid seasonal variations in our study. The latter is because patients with dyslipidemia starting treatment with statins are usually evaluated after 12 weeks to check if therapeutic goal was reached and we wanted to be as close as possible of practice in real life.

Genistein is the principal isoflavone that is present in soy. It was postulated to have a role in the prevention of cardiovascular risk by regulating lipid and carbohydrate metabolism.[19] Genistein can regulate the lipid profile due to its ability to modulate the ER signalling as it binds to ER forming a complex that interacts with nuclear DNA.[15-17] The estrogenic activity of genistein depends on its concentration, endogenous estrogen levels, and gender.[23] Results of studies on adipose tissue in women showed that genistein inhibits adipose deposition and decreases adipose mass.[24] The results of our study showed that genistein, calcium, and vitamin D supplementation improved the serum lipid profile after 12 weeks. Our results were in agreement with those referred in the bibliography.[19]

ERs signalling can also be conditioned by genetic factors.[2] Taking into account this evidence, we have analysed whether ER polymorphisms could modify the response in lipid profile to supplementation with calcium, vitamin D, and genistein in postmenopausal women. The mechanism by which ESR gene polymorphisms lead to changes in the serum lipid profile is not clear.[2] It has been suggested that these polymorphisms could affect the binding of transcription factors and cause an alteration in ER expression by changing messenger RNA splicing.[25,26]

The main strong point of our study is its design: a prospective, double blind study. Also, this is the first report about the modulation of serum lipid profile (and therefore, cardiovascular risk) after genistein, calcium, and vitamin D supplementation in postmenopausal women by variations in estrogen receptor genes. The polymorphisms that have been associated in this study have a broad physiopathological base and they could have a role in the prevention of cardiovascular risk, as mentioned before.

The main limitation point in our study is the sample size. Our results should be considered preliminary data until they are replicated with larger groups of postmenopausal women.

In conclusion, this is the first report showing an association between polymorphisms in ER genes and an improvement of the serum lipid profile after taking calcium, vitamin D, and genistein supplementation in healthy postmenopausal women, reinforcing the hypothesis that genetic factors are crucial in ER signalling. In the near future, personalized medicine will be a reality. We will possibly be able to select patients with specific SNPs that will benefit from some treatments more than others.

CONFLICT OF INTERESTS

The author declares that there are no conflict of interests.

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