Variants Translating Reduced Expression of the Beta Estrogen Receptor Gene Were Associated With Increased Carotid Intima Media Thickness. A Cross-Sectional Study in Late Postmenopausal Women.

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Abstract

Background There is debate on the role of estrogens in modulating the risk for atherosclerosis in women. Our purpose was to investigate whether the size of the estrogenic impact was independently associated with variation of carotid intima-media thickness (IMT) in healthy late postmenopausal women. The levels of circulating estrogens have been used in previous studies but the influence of SNPs of the estrogen receptors (ER) α and β has not been investigated.

Methods We performed a crossed-sectional study of 91 women in a university hospital. We used a double approach in which, in addition to the measurement of estradiol levels by ultrasensitive methods, genetic variants (SNPs) associated with differing expression of the ER α and β genes were assessed. Multivariable analysis was used to examine the association of candidate factors with the value of IMT and plaque detection at both the carotid wall and the sinus.

Results The levels of glucose were directly associated with IMT at both the carotid wall ($p < 0.001$) and the sinus ($p = 0.001$), while age was positively associated with IMT at the sinus ($p = 0.003$) and vitamin D with IMT at the carotid wall ($P = 0.035$). A genotype combination translating reduced gene expression of the *ER β* was directly associated with IMT at both the carotid wall ($p = 0.001$) and the sinus ($p = 0.002$).

Conclusions Poorer estrogenic impact, as concordant with a SNP variant imposing reduced expression of the *ER β* , was directly associated with IMT at both the carotid wall and the sinus. Glucose level, vitamin D only for the carotid wall, and age only for the sinus, also emerged as independent factors in the IMT variance.

Background

Cardiovascular disease (CVD) is the principal cause of mortality and morbidity in women [1,2]. The disease exhibits specific features in the female, and interest has arisen about whether the influence of risk factors is modified, or even whether the disease may have some distinct risk factors in women [3,4].

There is a debate on whether the extent of the estrogenic impact may be a key factor modulating cardiovascular risk. For example, premature natural or surgical menopause is associated with increased cardiovascular risk [5,6]. The responsibility of hormonal changes associated with menopause has been investigated by measuring the association between endogenous estrogens, usually estradiol (E2), and cardiovascular events in observational studies. But results have been conflicting, with both protective [7,8] and neutral roles [9,10]. The weakness of studies assessing estrogenicity on the basis of only the circulating estradiol levels resides in the physiological oscillations of the hormone. Moreover, accurately measuring very low levels of estradiol, as in postmenopause, represents a technical challenge. Genetic variants (single nucleotide polymorphisms, SNPs) of the estrogen receptors (ER), α and β , which may condition changes in the hormonal message at the tissue level, define an innovative and improved approach. Genetic changes reflect variants that are operative along the whole life, and therefore may translate into considerable difference in the accumulated estrogenic impact. Taking advantage of this

approach, we have focused our study in postmenopause, which represents the wide life period in which atherosclerotic lesions develop and in which risk for cardiovascular events starts being significant.

The objective of the present study was to disclose whether estrogenicity, which in our hands translated an in-depth analysis including the circulating levels of E2 and the SNP variants of both the *ER α* and *β* genes, was associated with variation in the degree of subclinical atherosclerosis in a group of late postmenopausal women. Subclinical atherosclerosis was assessed at the carotid artery by measuring the intima media thickness (IMT) of the far wall in the carotid artery and in the carotid sinus.

Methods

Study design and patients

We designed a crossed study in which one hundred postmenopausal women were invited to participate when coming for their regular health control at our center. The postmenopausal status was confirmed by at least one-year amenorrhea or a surgically induced menopause, together with follicle-stimulating hormone (FSH) levels ≥ 30 mIU/mL and E2 levels within the postmenopausal range. Women were considered eligible if of Caucasian ethnicity, if they were free of any previous or current clinical chronic disease, including CVD, osteoporotic fracture, cancer, or cognitive disease, and had never used menopausal hormonal therapy. Each woman was assessed only once for each of the planned explorations, which were scheduled within the interval of one month.

Study measures

Women were explored according to a protocol designed to analyze a group of variables related with atherosclerotic risk.

Clinical and laboratory assessments

Women were measured their height, weight, and waist circumference, and the body mass index (BMI) was calculated as the ratio between weight (kg) and square height (m^2). Blood pressure was measured in the left arm using an automatic blood pressure monitor (Omron M6, HEM-7001-E, Omron Healthcare Co., Ltd. Kyoto, Japan) and expressed in mmHg. Mean arterial pressure was calculated as diastolic pressure plus $\frac{1}{3}$ pulse pressure, where pulse pressure was systolic pressure minus diastolic pressure [11]. Blood was drawn between 08.00-10.00 a.m. after an overnight fast, and the serum separated. A routine analysis of basic biochemical parameters and a complete lipid profile were performed using enzymatic methods with an auto-analyzer (Olympus AV 5200; Tokyo, Japan).

The levels of the circulating hormones were measured by immunoassay. FSH (mIU/mL) was quantified by chemiluminescence (BioMérieux Inc., Hazelwood, MO, USA, intra- and inter-assay coefficients of variation $\leq 10.0\%$); E2 (pg/mL) was measured using an ultra-sensitive (<1.4 pg/mL) commercial solid phase enzyme-linked immunosorbent assay (ELISA) based on competitive binding (DRG International,

Springfield, NJ, USA, intra- and inter-assay variation coefficients £10.0%). Insulin (mIU/mL) was measured by the C-peptide ELISA kit (IBL International GMBH, Hamburg, Germany, intra- and inter-assay coefficients of variation £6.7% and £10.0%, respectively). Vitamin D was quantified with the Elecsys vitamin D total assay (Roche Diagnostics International, Totkreuz, CH), which measures 25-hydroxyvitamin D by an electro-chemiluminescence binding procedure. The Elecsys coefficients of variation were £6,5% (intra) and £11.5% (inter-assay). The insulin resistance index (homeostasis model assessment, HOMA) was calculated as fasting serum insulin in µIU/mL x (fasting serum glucose in mg/dL x 0.05551)/22.5) [12].

SNPs analyses

Blood samples were collected in tubes with anticoagulant (disodium-EDTA) and kept refrigerated at 4° C. Nucleated cells were used for DNA isolation with a genomic DNA extraction kit (REAL; Durviz, Valencia, Spain) after lysis of red blood cells with ammonium chloride (10 mM KHCO₃, 150 mM NH₄Cl, 0.1 mM EDTA-Na₂, pH 7.4). The 260/280 absorbance ratio of the product ranged from 1.6–2.0, indicating high-quality DNA [13].

The TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA) on a 7900 HT Fast Real-Time PCR System (Applied Biosystems) was used to get the allelic discrimination for genotyping of SNPs: *rs2234369*, *rs9340799*, *rs3798577* and *rs28385619* in the *ESR1* (*ERα*) gene; and *rs1256030* and *rs4986938* in the *ESR2* (*ERβ*) gene. We followed the protocol provided by the manufacturer in which, briefly, 20 ng of genomic DNA was amplified in the presence of 1 x TaqMan probe assay and 1 x TaqMan Universal PCR Master Mix (Applied Biosystems). The 7900 HT thermocycler software was employed for allelic discrimination. Reproducibility was estimated by re-genotyping 5–7% of samples in each plate and was >99%. About 0.5% of the genotypes were ambiguous and samples had to be re-genotyped.

Imaging assessments

IMT was understood as the area of tissue starting at the luminal edge of the artery and ending at the boundary between the media and the adventitia. Both the right and the left carotid artery were explored by B-mode ultrasound with the help of the QLAB-IMT program integrated into a Philips HD-11 XE Scanner. This machine was fitted with a lineal probe with an emission frequency capable of being modulated within a range of 3 to 12 MHz. QLAB-IMT allows an automated measurement of the IMT, with the help of a program that has been specifically designed for escaping from the potential errors entailed in the manual position of cursors. An experimented ultrasonographer, who followed the standards for image acquisition established at the Mannheim consensus [14], performed the examination procedure. The IMT values were obtained in segments free of plaque from 2 locations, the far wall of the common carotid artery at approximately 1 cm proximal to the carotid sinus, and the far wall of the carotid sinus. The mean of the values at both the right and the left carotid arteries was used for analyses.

Carotid plaques were assessed in consistence with the criteria of the Mannheim consensus, i.e., a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value

or demonstrates a thickness 11.5 mm as measured from the media-adventitia interface to the intima-lumen interface [14].

Statistical analysis

Three multivariable models have been carried out using stepwise forward and backward direction based on Akaike's Information Criterion (AIC) [15]. Multiple linear regression was applied to detect linear relationships between dependent variables carotid or sinus IMT and multiple logistic regression for the presence and absence of plaque. Age, BMI, waist perimeter, mean arterial pressure, E2, FSH, triglycerides, high-density lipoprotein (HDL)- cholesterol, low-density lipoprotein (LDL)- cholesterol, total cholesterol, vitamin D, and HOMA index were included as quantitative independent variables and current smoking and SNPs, treated as dummy variables, which were codified as 0 or 1. Smoking status was codified as 0 (absence of smoking) and 1 (current smoker). The SNPs were grouped into two categories according to the inheritance model using the SNPStats software [16].

Collinearity was assessed according to variance-inflation factor (VIF) [17] using Car package [18]. Normality in residuals for both multiple linear regressions was explored using Shapiro-Wilk's test.

R software (3.6.2) [19] was used for all analysis and p value cutoff for significance was set at 0.05.

Results

Baseline data

The group was composed of 91 out of the 100-screened women because 8 participants refused ultrasonographic examination and technical difficulties impaired a satisfactory assessment in one participant (Figure 1). Table 1 shows the clinical and analytical characteristics of the participants who completed the protocol.

The mean age of the participants was 61.8 years and the mean menopausal age was 10.8 years; 21 women (23%) had suffered surgical menopause. There were 10 women who smoked, 24 women who had a body mass index (BMI) $>30 \text{ kg/m}^2$, and 42 women with some hypertensive feature (systolic blood pressure $\geq 140 \text{ mm Hg}$, diastolic blood pressure $\geq 90 \text{ mm Hg}$, or use of anti-hypertensive medication). Also in Table 1, women in the cohort had slight overweight and hypercholesterolemia and reasonable mean vitamin D levels.

Carotid IMT

The mean (SD) IMT values at the carotid wall and in the carotid sinus were 0.672 (0.096) mm and 0.716 (0.122) mm, respectively. Atheromatous plaques were found in 10 women.

Multiple linear regression analysis was used with carotid IMT and sinus IMT as dependent variables (Table 2). The levels of glucose were positively associated with IMT in the carotid wall ($p < 0.001$). The

SNP analysis showed that the genotype TT of the SNP *rs4986938* of the *ERβ* gene, which is associated with lower expression of the gene and therefore interpreted as a reduction of function [20], was directly associated with carotid IMT ($p=0.001$).

The IMT at the sinus also exhibited a positive association with the level of glucose ($p= 0.001$). Age also emerged as a positively associated factor ($p= 0.003$) at this territory. As for the carotid wall, the SNP genotype TT of the SNP *rs4986938* of the *ERβ* gene was directly associated with sinus IMT ($p=0.002$).

Model for presence/absence of plaque did not converge due to low number of presence.

Discussion

Our study focused on a group of women with a mean age of 61.8 years, a period poorly investigated in studies trying to enlighten the role of estrogens in the development of atherosclerosis. That stage, however, is crucial to elucidate the impact of estrogens on atherogenesis, because the lesions of the disease start being detectable during that life period.

The findings of our study are particularly engaging in that regard. We addressed the issue following a double strategy. Firstly, we measured the circulating level of E2 assuming that differences at the precise time-point of our study were representative of a distinct status of estrogenicity in the long haul. Indeed, and although this may be arguable, this has been until now the only approach in studies investigating the impact of endogenous estrogens on CVD in postmenopausal women [7-10] or on other outcomes, like for example breast cancer [21]. We found that E2 did not emerge as an independent variable with an impact on IMT at either the carotid wall or the sinus in our analysis. In contrast, we found that the genotype TT of the SNP *rs4986938* of the *ERβ* gene, which is associated with lower expression of the gene [20], was directly linked with carotid IMT at both the carotid wall ($p = 0.001$) and the sinus ($p=0.002$). This finding is important because it is compatible with a persistently reduced estrogenic function at the target level along the whole life. So, it may be taken as an indication in favor of a protective effect of estrogens in the long-term.

Similar to our study, Finnish investigators could not find an association of IMT with circulating E2 in a cohort of similar age to our group [22]. Other studies have detected some associations between endogenous estrogens and the atherosclerosis burden, but when focusing on the menopausal transition. In a subset of perimenopausal women participating in the Study of Women Across the Nation (SWAN) study, Wildman et al [23] found an inverse association between declining levels of E2 and the adventitia diameter of the common carotid artery. Similar findings were reproduced when a cohort of pre- and perimenopausal women was followed longitudinally for a median of 3.7 years [24] or in women suffering a more rapid menopausal transition [25]. Our study now adds another piece of evidence in that the small, but persistent, changes represented by differences in the functional effect of estrogens at the tissue level may translate into IMT variation.

We also explored the impact of a list of other candidate cardiovascular variables on IMT. Glucose emerged as a predictor at both the carotid wall and the carotid sinus. The impact of glucose on cardiovascular risk has been previously reported in several studies [26,27]. Also, age appeared as an independent predictor of IMT at the sinus. In contrast, other recognized risk factors, such as the lipid profile, BMI, mean arterial pressure, waist perimeter or insulin resistance did not come out as independent predictors in our analysis. While the case of insulin resistance may be interpreted as partly embedded within the impact of glucose, the limited size of our cohort may be argued to explain the reduced sensitivity for identifying other independent candidates. This reinforces the value of our findings concerning the estrogenic action, which showed a significant association with IMT even in conditions in which the independent effect of other recognized predictors of atherosclerosis was undetectable. The case of vitamin D merits a specific comment because the direct association with carotid IMT is against findings in observational studies [28]. However, randomized controlled trials have been unable to demonstrate a beneficial action of vitamin D supplementation on cardiovascular outcomes [29].

To conclude, we analyzed the independent effect of some recognized cardiovascular risk factors on carotid and sinus IMT in a cohort of late postmenopausal women. Poorer estrogenicity, as measured by the SNP variant imposing reduced expression of the *ERβ*, was directly associated with IMT at the two explored territories, carotid wall and sinus. Glucose level, vitamin D only for the carotid wall, and age only for the sinus, also emerged as independent factors in the IMT variance.

Declarations

Ethics approval:

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Institutional review board at our center approved the study (Appr code 090227).

Consent to participate:

Written informed consent was obtained from each woman.

Consent for publication:

Consent for publication was obtained from each woman.

Availability of data and material:

Data are available upon request and permission of the ethical committee at our centre.

Competing interest: None declared.

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Authors' contribution:

Design of the study: AJCM, MAGP, JJT, AMM, JCS, AC.

Clinical assessment of the participants: AJCM, AC.

Laboratory tasks: MAGP, JJT.

Imaging assessment of participants: AJCM, AMM, JCS.

Analysis and interpretation of the data: AJCM, MAGP, JJT, AMM, JCS, AC.

Drafting of manuscript: AC.

Approval of the final version to be submitted: AJCM, MAGP, JJT, AMM, JCS, AC.

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List Of Abbreviations

AIC Akaike's Information Criterion

BMI Body mass index

CVD Cardiovascular disease

E2 Estradiol

ELISA Enzyme-linked immunosorbent assay

ER Estrogen receptor

ESR1 ER α gene

ESR2ER β gene

FSH Follicle-stimulating hormone

HDL High-density lipoprotein

HOMA Homeostasis model assessment

IMT Intima media thickness

LDL Low-density lipoprotein

SNPs Single nucleotide polymorphisms

SWAN Study of Women Across the Nation

VIF Variance-inflation factor

References

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation*. 2017; 135: e146-e603. doi: 10.1161/CIR.0000000000000485.
2. Eurostat. Cardiovascular diseases statistics. http://ec.europa.eu/eurostat/statistics-explained/index.php/Cardiovascular_diseases_statistics (accessed 26th August 2020).
3. Manson JE, Bassuk SS. Biomarkers of cardiovascular disease risk in women. *Metabolism*. 2015; 64, 3 Suppl 1: S33-9. doi: 10.1016/j.metabol.2014.10.028.
4. Young L, Cho L (2019) Unique cardiovascular risk factors in women. *Heart* 2019; 105: 1656–60. doi:10.1136/heartjnl-2018-314268
5. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. *JAMA*. 2019; 322: 2411-21. doi: 10.1001/jama.2019.19191.
6. Schreinlechner M, Noflatscher M, Reinstadler SJ, Sommer P, Lener D, Reiser E, et al. Early onset of menopause is associated with increased peripheral atherosclerotic plaque volume and progression. *Atherosclerosis*. 2020; 297: 25–31. doi: 10.1016/j.atherosclerosis.2020.01.023.
7. Benn M, Voss SS, Holmegard HN, Jensen GB, Hansen AT, Norderstgaard BG. Extreme concentrations of endogenous sex hormones, ischemic heart disease, and death in women. *Arterioscler Thromb Vasc Biol*. 2015; 35: 471-7. doi: 10.1161/ATVBAHA.114.304821.
8. Zhao D, Guallar E, Ouyang P, Subramanya V, Vaidya D, Ndumele CE, et al. Endogenous Sex Hormones and Incident Cardiovascular Disease in Post-Menopausal Women. *J Am Coll Cardiol*. 2018; 71: 2555-66. doi: 10.1016/j.jacc.2018.01.083.
9. Barrett-Connor E, Goodman-Gruen D. Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. *BMJ*. 1995; 311: 1193-6.
10. Rexrode KM, Manson JE, Lee IM, Ridker PM, Sluss PM, Cook NR, et al. Sex hormone levels and risk of cardiovascular events in postmenopausal women. *Circulation*. 2003; 108: 1688-93. doi: 10.1161/01.CIR.0000091114.36254.F3.

11. Abdelfatah AB, Motte G, Ducloux D, Chalopin JM. Determinants of mean arterial pressure and pulse pressure in chronic haemodialysis patients. *J Hum Hypertens*. 2001; 15: 775-9.
12. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1998; 83: 2694-8.
13. Panach L, Serna E, Tarín JJ, Cano A, García-Pérez MA. A translational approach from an animal model identifies CD80 as a candidate gene for the study of bone phenotypes in postmenopausal women. *Osteoporos Int*. 2017; 28: 2445-55. doi: 10.1007/s00198-017-4061-9.
14. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis*. 2012; 34: 290-6. doi: 10.1159/000343145.
15. Sakamoto Y, Ishiguro M, Kitagawa G. Akaike information criterion statistics. Dordrecht, The Netherlands: D Reidel Publishing Co, 1986.
16. <https://www.snpstats.net/start.htm> (accessed 30th December 2019).
17. Fox J, Monette G. Generalized collinearity diagnostics. *J Am Stat Assoc*. 1992; 87: 178–83.
18. Fox J, Weisberg S. An {R} Companion to Applied Regression, 3rd ed. Thousand Oaks CA. US: Sage, 2019. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/> (accessed 17th July 2020).
19. R Core Team. R: A language and environment for statistical computing. In: R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/> (accessed 17th January 2020).
20. GTEx Portal. Available at <https://www.gtexportal.org> (accessed 30th December 2019).
21. James RE, Lukanova A, Dossus L, Becker S, Rinaldi S, Tjønneland A, et al Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case-control study. *Cancer Prev Res (Phila)*. 2011; 4: 1626-35.
22. Bertone-Johnson ER, Virtanen JK, Nurmi T, Niskanen N, Mursu J, Voutilainen S, et al. Follicle-Stimulating Hormone Levels and Subclinical Atherosclerosis in Older Postmenopausal Women. *Am J Epidemiol*. 2018; 187: 16-26. doi: 10.1093/aje/kwx174.
23. Wildman RP, Colvin AB, Powell LH, Matthews KA, Everson-Rose SA, Hollenberg S, et al. Associations of endogenous sex hormones with the vasculature in menopausal women: the Study of Women's Health Across the Nation (SWAN). *Menopause*. 2008; 15: 414-21.
24. El Khoudary SR, Wildman RP, Matthews K, Thurston RC, Bromberger JT, Sutton-Tyrrell K. Progression rates of carotid intima-media thickness and adventitial diameter during the menopausal transition. *Menopause*. 2013; 20: 8-14. doi: 10.1097/gme.0b013e3182611787.
25. Johnson BD, Dwyer KM, Stanczyk FZ, Bittner V, Berga SL, Braunstein GD, et al. The relationship of menopausal status and rapid menopausal transition with carotid intima-media thickness

progression in women: a report from the Los Angeles Atherosclerosis Study. *J Clin Endocrinol Metab.* 2010; 95: 4432-40. doi: 10.1210/jc.2010-0126.

26. Nathan DM, Bayless M, Cleary P, Genuth S, Gubitosi-Klug R, Lachin JM, et al. Diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: advances and contributions. *Diabetes.* 2013; 62: 3976-86. doi: 10.2337/db13-1093.
27. Åkerblom A, Wojdyla D, Steg PG, Wallentin L, James SK, Budaj A, et al. PLATO Investigators. Prevalence and relevance of abnormal glucose metabolism in acute coronary syndromes: insights from the PLATelet inhibition and patient Outcomes (PLATO) trial. *J Thromb Thrombolysis.* 2019; 48: 563-9. doi: 10.1007/s11239-019-01938-2.
28. Norman PE, Powell JT. Vitamin D and cardiovascular disease. *Circ Res.* 2014; 114: 379-93. doi: 10.1161/CIRCRESAHA.113.301241.
29. Pilz S, Verheyen N, Gröbler MR, Tomaschitz A, März W. Vitamin D and cardiovascular disease prevention. *Nat Rev Cardiol.* 2016; 13: 404-17. doi: 10.1038/nrcardio.2016.73.

Tables

Table 1 Clinical characteristics and analytical values of the 91 participating women.

Parameter	Mean	SD
Age (yr)	61.8	7.2
Years since menopause	10.8	7.8
BMI (Kg/m ²)	27.9	4.3
Waist perimeter (cm)	89.0	11.5
Mean arterial pressure (mm Hg)	103.5	13.9
Total cholesterol (mg/dL)	208.5	24.6
HDL-cholesterol (mg/dL)	64.9	15.2
LDL-cholesterol (mg/dL)	122.8	24.0
Triglycerides (mg/dL)	103.7	51.0
FSH (IU/L)	74.6	34.1
Estradiol (pg/mL)	5.0	4.9
Vitamin D (ng/mL)	25.0	9.5
HOMA index	2.7	2.1

BMI: body mass index. HDL: high-density lipoprotein. LDL: low-density lipoprotein. FSH: follicle stimulating hormone. HOMA: homeostasis model assessment.

Table 2 Best model multiple linear regression analysis for carotid artery and sinus according to AIC.

Dependent variable	Independent variables	Estimate	Standard error	t-value	p-value	Adjusted R ²	AIC
Carotid wall	(Intercept)	0.074	0.133	0.557	0.582	0.599	-186.53
	<i>rs3798577 (ESR1)</i>	-0,045	0,027	-1.683	0.103		
	<i>rs28385619 (ESR1)</i>	-0.049	0,029	-1.700	0.100		
	<i>rs4986938 (ESR2)</i>	0.138	0.037	3.771	0.001		
	Age	0.003	0.002	1.715	0.097		
	Glucose	0.003	0.001	4.690	<0.001		
	Triglycerides	<0.001	<0.001	1.431	0.164		
	Vitamin D	0.002	0.001	2.211	0.035		
Carotid bulb	(Intercept)	-0.493	0.233	-2.120	0.043	0.539	-157.99
	<i>rs3798577 (ESR1)</i>	-0.052	0.038	-1.376	0.179		
	<i>rs4986938 (ESR2)</i>	0.185	0.054	3.413	0.002		
	Age	0.009	0.003	3.306	0.003		
	BMI	0.008	0.005	1.605	0.119		
	FSH	0.001	0.001	1.461	0.155		
	Glucose	0.004	0.001	3.662	0.001		
	-	-	-	-	-		

AIC: Akaike's Information Criterion; *ESR1*: estrogen receptor 1. *ESR2*: estrogen receptor 2. BMI: body mass index. FSH: follicle stimulating hormone.

Figures

Figure 1

Flowchart of the study showing details of participants' numbers at each stage of the study.