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# Hot flashes: emerging cardiovascular risk factors in recent and late postmenopause and their association with higher blood pressure

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**Abstract**

***Objective:*** The aim of the study was to compare the endothelial function of symptomatic (self-reported hot flashes >3 on a scale of 0-10) versus asymptomatic (≤3) women in different postmenopause stages, and to examine if the association between hot flashes and endothelial function was independent of classical cardiovascular risk factors observed during the analysis.

***Methods:*** Noninvasive venous occlusion plethysmography within two groups: recent (recent postmenopause [RPM], <10 y, n = 63) and late (late postmenopause [LPM], ≥10 y, n = 67) postmenopause.

***Results:*** Symptomatic women showed lower forearm blood flow and lower percentage increment of it during the reactive hyperemia response; higher systolic ($P < 0.0001$ in RPM and $P = 0.0008$ in LPM) and diastolic ($P = 0.0005$ in RPM and $P = 0.0219$ in LPM) blood pressure; highest score for perimenopausal hot flashes ($P = 0.0007$ in RPM and $P < 0.0001$ in LPM), longer duration of prior oral contraceptive use ($P = 0.009$ in RPM and $P = 0.0253$ in LPM), and higher current sleep disorders ($P < 0.0001$ in RPM and $P = 0.0281$ in LPM) compared with asymptomatic ones. In the LPM group, symptomatic women also had higher prevalence of previous hypertension diagnosis ($P = 0.0092$). During multivariate analysis, blood flow during the reactive hyperemia response was associated with hot flashes after adjusting for age, body mass index, and systolic blood pressure (odds ratio 0.55 [0.36-0.84] in RPM and odds ratio 0.7 [0.5-0.97] in LPM).

***Conclusions:*** In both phases, recent and late post menopause, hot flashes were associated with endothelial dysfunction and higher systolic and diastolic blood pressure, but the relationship between hot flashes and endothelial dysfunction was independent of blood pressure.

***Key Words:*** Blood pressure – Cardiovascular risk – Endothelial dysfunction – Hot flashes – Hypertension – Menopause.

The prevalence of hot flashes during menopause has been described as up to 80% in most societies, being influenced by different factors such as age, ethnicity, education, smoking, and anxiety.[1] Hot flashes were reported to last on an average 7.4 years, but for reasons not entirely clear some women remained symptomatic for more than 11.8 years.[2] Apart from worsening of quality of life,[3] new studies have reported that women with hot flashes have an increased risk of subclinical cardiovascular (CV) disease,[4] as well as of CV events.[5] Different associations, however, have been described according to age and time since menopause. In a reanalysis of the Women's Health Initiative (WHI),[6] in the subgroup of 70 to 79-year-old women with moderate/severe hot flashes, CV events after hormone therapy (HT) were five times more likely to occur compared with the ones of the same age who used placebo. In 50 to 69-year-old women, no increased risk of CV events after HT, however, could be observed related to presence of hot flashes.

The Swan Heart Study, analyzing women during the menopausal transition—42 to 58 years old—described that those with hot flashes presented lower flow-mediated dilatation (FMD) of the brachial artery, evaluated by Doppler, and larger aortic and coronary calcifications, visualized by electron beam tomography, compared with the ones without hot flashes, even adjusting for age, body mass index (BMI), menopausal status, systolic and diastolic blood pressures (SBP and DBP), smoking, levels of cholesterol, triglycerides, glucose, and estradiol.[7] Another article from the same cohort reported increased carotid artery intima thickness ($0.02 \pm 0.01$ mm) in symptomatic versus asymptomatic women.[4] Such discrepancies made us to question at what time during postmenopause and how intense hot flashes must be to be valued as emerging CV risk markers, or if they just come together with classical CV risk factors such as obesity, hypertension, and diabetes.

To encompass CV risk assessment in younger women in recent (<10 y) postmenopause, endothelial function seems to

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be the most appropriate parameter because its dysfunction is considered the earliest sign of atherosclerotic disease,[8] already demonstrated in different CV risk factors, such as hypercholesterolemia, hypertension, smoking, and diabetes,[9] and also in menopause,[10] before any structural vessel change.[11] Endothelial dysfunction results from an imbalance in the production of vasodilator, particularly nitric oxide (NO), and vasoconstrictor substances, causing increased vascular tone, cellular adhesion, and platelet aggregation.[9] Techniques validated for endothelial function assessment include brachial artery ultrasound, video capillaroscopy, and noninvasive venous occlusion plethysmography (VOP).[12] The latter measures forearm blood flow through the variation of its circumference at baseline and after the reactive hyperemia response, induced by ischemia and subsequent release of the segment. The reperfusion maneuver mimics what physiologically happens in daily life by increased physical activity, when shear stress up-regulates endothelial NO production. A lower reactive hyperemia response reflects reduced NO bioavailability to the swirling of blood endogenous stimuli.[13]

The objectives of our study were to compare, in both late and recent postmenopause, symptomatic versus asymptomatic women's endothelial function; and to examine if the association between endothelial function and hot flashes was independent, considering other observed factors that could affect CV risk.

## METHODS

This is a cross-sectional study, conducted according to the project approved by the Ethics Committee of the State University of Rio de Janeiro (CAAE number 11272312.3.0000.5282). The total sample consisted of 130 postmenopausal women between 45 and 70 years, without HT in the past 6 months, recruited during routine appointments at the Female Endocrinology and Gynecology Clinics of the Hospital Federal da Lagoa (HFL) and Instituto Estadual de Diabetes e Endocrinologia do Estado do Rio de Janeiro (IEDE). Exclusion criteria were current smoking, psychiatric illness, prior CV event, uncontrolled hypertension (SBP $>160$ mm Hg or DBP $>100$ mm Hg), thyroid-stimulating hormone (TSH) outside reference values, previous diagnosis of polycystic ovary syndrome or any kind of amenorrhea during reproductive years, and use of drugs that could influence the presence of hot flashes and/or endothelial function such as glucocorticoids, phytoestrogens, calcium channel blockers, sulphonylureas, and central nervous system-acting drugs. The participants were divided into two groups: recent (recent postmenopause [RPM], $<10$ y, $n=63$) and late (late postmenopause [LPM], $\geq 10$ y, $n=67$) postmenopause.

Sampling was performed for convenience, seeking for similar numbers of symptomatic (self-reported score $>3$ on a scale from 0 to 10 for hot flashes intensity) and asymptomatic (grade $\leq 3$)[14] women within groups. Participants were informed about the study objectives and procedures; those who agreed to participate signed the informed consent form and were invited to attend the Laboratory of Clinical and Experimental Research in Vascular Biology (BioVasc), at a scheduled date and after 12 hours fasting. During the same week, participants collected blood at their original clinic, which are linked to the same analytical laboratory, for the following dosages: glucose (ultraviolet enzymatic method; reference values: 60-99 mg/dL), total cholesterol (colorimetric enzymatic method; reference values: desirable $<200$ mg/dL, borderline 200-239 mg/dL), high-density lipoprotein cholesterol (method: enzymatic colorimetric; reference values: $>40$ mg/dL), triglycerides (colorimetric enzymatic method; reference values: normal $<150$ mg/dL, borderline 150-200 mg/dL), albumin (purple brocromocresol method; reference values: 3.6-5.6 g/dL), androstenedione (radioimmunoassay method; reference values: 0.3-3.7 mg/mL), estradiol (electrochemiluminescence method; reference values $<55$ pg/mL), luteinizing hormone (electrochemiluminescence method; reference values: 14.2-52.3 mIU/mL), follicle-stimulating hormone (electrochemiluminescence method; reference values: $>25.8$ mIU/mL), sex hormone-binding globulin (electrochemoluminescence method; reference values: 60-99 mg/dL), estrone (radioimmunoassay method; reference values: 10-60 pg/mL), testosterone (electrochemoluminescence method; reference values: 3-63 ng/mL), insulin (electrochemoluminescence method; reference values for individuals with BMI $<25$: 2-13 μUI/mL; BMI 25-30: 2-19 μUI/mL; BMI $>30$ kg/m$^2$: 2-23 μUI/mL), and dehydroepiandrosterone sulfate (DHEA-S) (electrochemiluminescence method; reference values: 10-331 mg/dL). Low-density lipoprotein cholesterol was calculated using the total cholesterol-HDL cholesterol-(triglycerides/5), with reference values below 160 mg/dL, and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated by insulin (μUI/mL) × glucose (mmol/L)/22.5.[15]

At BioVasc, a single previously trained interviewer applied a questionnaire comprising clinical (Table 1) and gynecological data (Table 2). Physical activity was considered as at least 150 minutes a week; time of hypoestrogenism was defined as years since the last menstrual period less HT duration, and sexual activity was ascertained during the past 2 months. Sleep disorders included difficulties to fall asleep and nocturnal awakening.

Physical examination included weight and height held in a digital scale (Filizola, Personal, São Paulo, SP, Brazil), in addition to abdominal circumference measured horizontally with extendable tape in half the distance between the last rib and the line of the anterior superior iliac crest. BMI was calculated as weight divided by height in meters squared. After the calculation of BMI, participants collected 9 mL of blood, divided into ethylene diamine tetra-acetic acid (EDTA) and serum plasma tubes, to undergo centrifugation (Eppendorf 5804R, Germany) at 22°C at 3,000 rpm for 10 minutes, whereas the tube EDTA plasma was centrifuged at 4°C at a speed of 1,000 rpm for 10 minutes. The centrifuged material was aliquoted and stored at −80°C (Revco Elite Series, Thermo Scientific, Waltham, MA) for later determination of inflammatory markers.

Systolic and diastolic blood pressures (SBP and DBP) and heart rate (HR) were recorded from the average of the two

**TABLE 1.** *Clinical data (RPM and LPM groups)*

| | RPM | | | LPM | | |
|---|---|---|---|---|---|---|
| | Symptomatic (n = 33) | Asymptomatic (n = 30) | *P* | Symptomatic (n = 30) | Asymptomatic (n = 37) | *P* |
| Age, y | 52 (49-55) | 53 (50-56) | 0.5117 | 59.5 (57-64) | 61 (59-64) | 0.1372 |
| Weight, kg | 76.7 (60.5-89.8) | 69.4 (63.3-80.9) | 0.5540 | 65.5 (61.1-76.6) | 64.2 (57.0-73.6) | 0.221 |
| Height, m | 1.6 (1.5-1.6) | 1.56 (1.52-1.60) | 0.3740 | 1.55 (1.5-1.6) | 1.6 (1.5-1.6) | 0.5611 |
| BMI, kg/m$^2$ | 29.9 (25.4-34.8) | 29.2 (26.3-33.6) | 0.7937 | 27.4 (24.9-30.2) | 27.4 (24.6-30.2) | 0.5747 |
| Waist, cm | 97 (85.5-104.5) | 92.5 (84.5-102.0) | 0.3708 | 90.5 (82.0-100.5) | 88 (83.5-97.0) | 0.5918 |
| HR, bpm/min | 66 (58-70) | 64.5 (59.5-70.3) | 0.6740 | 63 (60.0-70.3) | 62 (59.0-66.5) | 0.4713 |
| SPB, mm Hg | 135 (128.5-142.0) | 126 (117.5-129.0) | <0.0001[a] | 137 (128-146) | 127 (120.5-136.0) | 0.0013[a] |
| DPB, mm Hg | 81 (74-84) | 71 (68.8-81.3) | 0.0005[a] | 81.5 (74.8-87.0) | 75 (70.5-83.5) | 0.0272[a] |
| Framingham score | 9 (6.5-11.5) | 8 (4.5-11.0) | 0.1535 | 12.5 (10.3-14.0) | 11 (8-15) | 0.5601 |
| | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| Skin color | | | | | | |
| White | 12 (36.4) | 18 (60) | 0.1142 | 9 (30.0) | 19 (51.4) | 0.1482 |
| Brown | 14 (42.4) | 6 (20) | | 10 (33.3) | 11 (29.7) | |
| Black | 6 (18.2) | 6 (20) | | 11 (36.7) | 7 (18.9) | |
| Marital status | | | | | | |
| Married | 13 (39.4) | 14 (46.7) | 0.5946 | 13 (43.3) | 19 (51,4) | 0.8209 |
| Single | 11 (33.3) | 12 (40.0) | | 10 (33.3) | 12 (32.4) | |
| Divorced | 5 (15.2) | 2 (6.7) | | 2 (6.7) | 1 (2.7) | |
| Widowed | 4 (12.1) | 2 (6.7) | | 5 (16.7) | 5 (13.5) | |
| Education | | | | | | |
| <9 y | 18 (54.5) | 16 (53.3) | 0.5720 | 21 (20.0) | 27 (73.0) | 0.7256 |
| 9-11 y | 12 (36.4) | 9 (30.0) | | 8 (26.7) | 7 (18.9) | |
| >11 y | 3 (9.1) | 5 (16.7) | | 1 (3.3) | 3 (8.1) | |
| Employment | | | | | | |
| Yes | 24 (72.7) | 18 (60) | 0.5176 | 13 (43.3) | 14 (37.8) | 0.8743 |
| No | 6 (18.2) | 9 (30) | | 6 (20.0) | 9 (24.3) | |
| Retired | 3 (9.1) | 3 (10) | | 11 (36.7) | 14 (37.8) | |
| Previous smoking | | | | | | |
| Yes | 17 (51.5) | 11 (36.7) | 0.3118 | 12 (40) | 15 (40.5) | 1.0000 |
| No | 16 (48.5) | 19 (63.3) | | 18 (60) | 22 (59.5) | |
| Alcohol consumption | | | | | | |
| <3 drinks/wk | 10 (30.3) | 13 (43.3) | 0.5325 | 11 (36.7) | 12 (32.4) | 0.6384 |
| >3 drinks/wk | 2 (6.1) | 1 (3.3) | | 6 (20.0) | 5 (13.5) | |
| Not consume | 21 (63.6) | 16 (53.3) | | 13 (43.3) | 20 (54.1) | |
| Physical activity | | | | | | |
| Yes | 3 (9.1) | 5 (16.7) | 0.4616 | 3 (6.7) | 3 (8.1) | 1.0000 |
| No | 30 (90.9) | 25 (83.3) | | 27 (93.3) | 34 (91.9) | |
| Thyroid disease | | | | | | |
| Yes | 8 (24.2) | 6 (20) | 0.7674 | 6 (20) | 7 (18.9) | 1.0000 |
| No | 25 (75.8) | 24 (80) | | 24 (80) | 30 (81.1) | |
| Hypertension | | | | | | |
| Yes | 17 (51.5) | 13 (43.3) | 0.6160 | 25 (83.3) | 19 (51.4) | 0.0092[a] |
| No | 16 (48.5) | 17 (56.7) | | 5 (16.7) | 18 (48.6) | |
| DM | | | | | | |
| Yes | 1 (3) | 0 (0) | 1.0000 | 3 (10) | 5 (13.5) | 0.7225 |
| No | 32 (97) | 30 (100) | | 27 (90) | 32 (86.5) | |
| MS | | | | | | |
| Yes | 15 (45.5) | 9 (30) | 0.2071 | 15 (50) | 11 (29.7) | 0.0904 |
| No | 18 (54.5) | 21 (70) | | 15 (50) | 26 (70.3) | |

BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; HR, heart rate; LPM, late postmenopause; MS, metabolic syndrome; RPM, recent postmenopause; SBP, systolic blood pressure.
[a]Mann-Whitney *U* test and chi-square test, $P < 0.05$.

measurements at least 10 minutes apart, taken after 10 minutes of accommodation with the participant lying down. Clinical and laboratory data were used to calculate the Framingham score[16] and to determine the presence of metabolic syndrome (MS) according to the National Cholesterol Education Program (NCEP) criteria.[17]

Endothelial function assessment was performed by noninvasive forearm VOP,[18] after at least 30 minutes acclimation in a controlled temperature (21°C-23°C) environment. Before and during the exam, HR and blood pressure were recorded, respectively, by electrocardiogram (RT200, Hokanson, Bellevue, WA), and a cuff and three electrodes connected to a digital monitor (Dixtal Biomedical, DX2021, Brazil). Forearm vascular flow (FBF) was quantified using a mercury sensor (AI6 Arterial Inflow System, Hokanson, Bellevue, WA) during four recorded cycles: (1) baseline 1 (FBF-B 1); (2) reactive hyperemia response (FBF-RH), when 3 minutes after the first cycle the upper cuff was inflated to 200 mm Hg for 5 minutes promoting ischemia, and then suddenly deflated; (3) baseline 2 (FBF-B 2), after 15 minutes waiting period to normalize flow; and (4) after 0.4 mg sublingual nitroglycerin (FBF-N) administered 3 minutes after

**TABLE 2.** *Gynecological data (RPM and LPM groups)*

| | RPM | | | LPM | | |
|---|---|---|---|---|---|---|
| | Symptomatic (n = 33) | Asymptomatic (n = 30) | *P* | Symptomatic (n = 30) | Asymptomatic (n = 37) | *P* |
| Age at menarche, y | 13 (12-15) | 12.5 (12-14) | 0.6798 | 12 (12.0-13.3) | 13 (12-15) | 0.0651 |
| Previous OC duration, y | 7 (2.5-10.5) | 2.5 (0-7.3) | 0.0090[a] | 8 (4.5-13.3) | 5 (0.5-8.5) | 0.0140[a] |
| Children, n | 2 (2-3) | 2 (1-3) | 0.3882 | 2 (1-3) | 2 (0-3) | 0.7185 |
| Breast-feeding, mo | 14 (3.0-37.5) | 20.5 (2.0-64.5) | 0.5121 | 12 (0-24) | 5 (0-24) | 0.6762 |
| Age of menopause, y | 48 (45.5-51.0) | 49 (44.8-52.0) | 0.7770 | 46.5 (40.8-49.0) | 47 (41-50) | 0.6899 |
| Years since menopause | 4 (2-6) | 5 (2-7) | 0.4250 | 14 (10.8-17.0) | 15 (12-20) | 0.2286 |
| Hypoestrogenism, y | 4 (2-6) | 4 (2.0-6.5) | 0.7443 | 12.5 (9.8-16.3) | 13 (9.5-18.0) | 0.6355 |
| Menopausal symptoms (0-10) | | | | | | |
| Perimenopausal HFs | 9 (6.5-10.0) | 5.5 (0-8.3) | 0.0007[a] | 9.5 (8-10) | 6 (0-9) | 0.0001[a] |
| Irritability | 7 (3.5-8.0) | 5 (0-8) | 0.1199 | 7 (5-8) | 5 (0-8) | 0.0973 |
| Vaginal dryness | 5 (0-8) | 4 (0-8) | 0.4405 | 6 (4.5-9.3) | 7 (0-8) | 0.4383 |
| Libido | 5 (3-7) | 5 (2.0-6.3) | 0.5599 | 5 (2.5-8.0) | 5 (0-7) | 0.7686 |
| | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| Tubal ligation | | | | | | |
| Yes | 20 (60.6) | 12 (40) | 0.1328 | 15 (50) | 16 (43.2) | 0.6285 |
| No | 13 (39.4) | 18 (60) | | 15 (50) | 21 (56.8) | |
| Type of menopause | | | | | | |
| Surgical | 13 (39.4) | 9 (30) | 0.5973 | 11 (36.7) | 14 (37.8) | 1.0000 |
| Natural | 20 (60.6) | 21 (70) | | 19 (63.3) | 23 (62.2) | |
| Hysterectomy | | | | | | |
| Yes | 11 (33.3) | 9 (30) | 0.7938 | 14 (46.7) | 12 (32.4) | 0.3145 |
| No | 22 (66.7) | 21 (70) | | 16 (53.3) | 25 (67.6) | |
| Oophorectomy | | | | | | |
| Unilateral | 4 (12.1) | 0 (0) | 0.1326 | 3 (10) | 4 (10.8) | 0.4002 |
| Bilateral | 6 (18.2) | 5 (16.7) | | 9 (30) | 6 (16.2) | |
| No | 23 (69.7) | 28 (93.3) | | 18 (60) | 27 (73.0) | |
| Previous HT | | | | | | |
| Yes | 7 (21.2) | 3 (10) | 0.3078 | 11 (36.7) | 11 (29.7) | 0.6069 |
| No | 26 (78.8) | 27 (90) | | 19 (53.3) | 26 (70.3) | |
| Sleep disorder | | | | | | |
| Yes | 26 (78.8) | 8 (26.7) | <0.0001[a] | 19 (63.3) | 13 (35.1) | 0.0281[a] |
| No | 7 (21.2) | 22 (73.3) | | 11 (36.7) | 24 (64.9) | |
| Sexual activity | | | | | | |
| Yes | 14 (42.4) | 14 (46.7) | 0.8026 | 10 (33.3) | 19 (51.4) | 0.2148 |
| No | 19 (57.6) | 16 (53.3) | | 20 (66.7) | 18 (48.6) | |

HF, hot flashes; HT, hormonal therapy; LPM, late postmenopause; OC, oral contraceptive; RPM, recent postmenopause.
[a]Mann-Whitney *U* test and chi-square test, $P < 0.05$.

the third cycle and recorded 5 minutes subsequently (Burns Adler Pharmaceuticals Inc, Charlotte, NC). Each cycle flow was calculated from the average of four measurements, after excluding the minimum and maximum. The percentage of increment during the reactive hyperemia response was calculated in relation to FBF-B 1 (FBF1-% RH), whereas the percentage increase after nitroglycerin was calculated in relation to FBF-B 2 (FBF2-% RN).

Upon termination of all procedures, EDTA plasma samples were diluted 1:8 for inflammatory markers analysis: PECAM-1, sICAM-1, sVCAM-1, sP-selectin, sE-selectin, and PAI-1, through Human Magnetic Adhesion Kit 6-Plex Panel (Life Technologies, Frederick, MD) by multiplexing (simultaneous analysis of multiple analytes) according to the supplier's instructions.

### Statistical analysis

Data analysis was always performed within RPM and LPM groups using GraphPad Prism 5.0 software, by comparing symptomatic versus asymptomatic women. For normality analysis, D'Agostino and Pearson omnibus normality test was used. Bivariate analyses were performed using Mann-Whitney *U* test for mean comparison and chi-square test for categorical variables. Hot flashes intensity (0-10) was correlated with different variables using Spearman test. Logistic regression models were used to measure association, with hot flashes status as independent and FBF-RH as dependent variables. The final model was adjusted by age, BMI, and SBP. The accepted level of significance was $P < 0.05$. IBM SPSS Statistics 19 (IBM, SPSS products, Chertsey, UK) was used for the analysis.

## RESULTS

In the RPM group (n = 63), 33 women were symptomatic and 30 asymptomatic, whereas in the LPM group (n = 67), 30 were symptomatic and 37 asymptomatic for hot flashes.

Within each group, RPM and LPM, there was no difference between symptomatic and asymptomatic women in relation to classical CV risk factors such as age, previous smoking, alcohol consumption, physical activity, BMI, waist circumference, Framingham total score, history of diabetes mellitus, or presence of metabolic syndrome. Only in the LPM group,

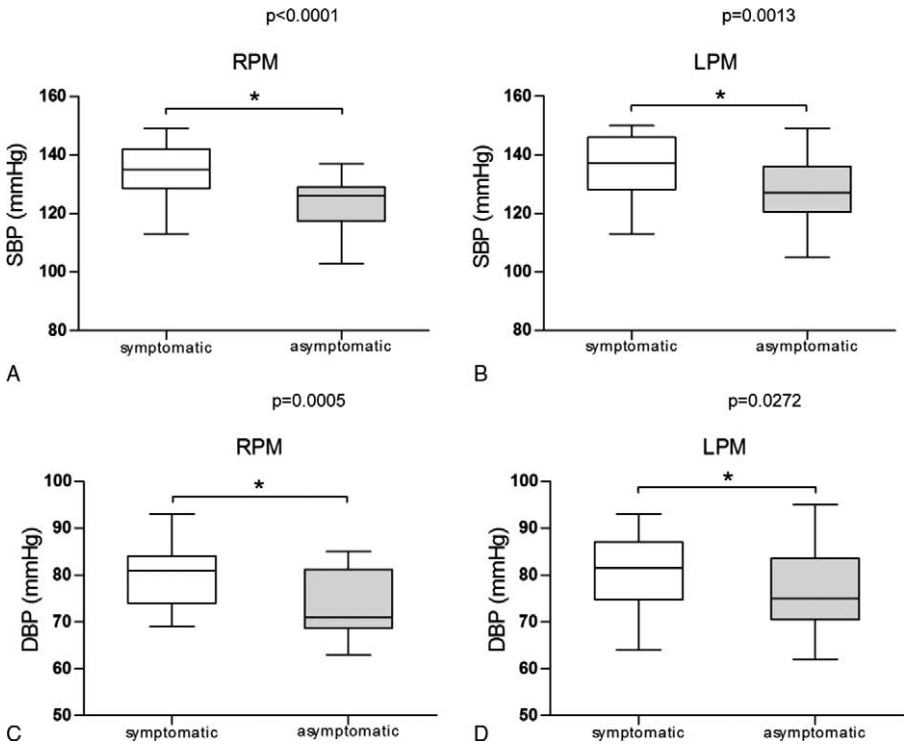


**FIG. 1.** Comparison of systolic (**A** and **B**) and diastolic (**C** and **D**) blood pressure between symptomatic and asymptomatic women within RPM and LPM groups. The box represents median, 25th and 75th percentile, and whiskers minimum and maximum. $^{*}P < 0.05$. DBP, diastolic blood pressure; LPM, late postmenopause; RPM, recent postmenopause; SBP, systolic blood pressure.

previous hypertension diagnosis was more prevalent (83.3%) in symptomatic versus asymptomatic women (51.4%; $P = 0.0092$) (Table 1).

The SBP and DBP were invariably higher in symptomatic compared with asymptomatic women: in the RPM group, SBP was 135.0 (128.5-142.0) versus 126.0 (117.5-129.0) mm Hg ($P = <0.0001$; Fig. 1A); in the LPM group, SBP was 137.0 (128.0-146.0) versus 127.0 (120.5-136.0) mm Hg ($P = 0.0013$; Fig. 1B). Similarly, DBP was 81.0 (74.0-84.0) versus 71.0 (68.8-81.3) mm Hg in the RPM group ($P = 0.0005$; Fig. 1C); and in the LPM group, DBP was 81.5 (74.8-87.0) versus 75.0 (70.5-83.5) mm Hg ($P = 0.0272$; Fig. 1D).

The subjective score given by participants for hot flashes intensity was positively correlated to SBP in both the RPM ($r = 0.4748$, $P < 0.0001$) and the LPM ($r = 0.3220$, $P = 0.0079$) groups. Regarding DBP, this correlation was significant only for the RPM group ($r = 0.3939$, $P = 0.0014$).

Gynecological history was similar, apart from symptomatic women reporting more current sleep disturbances, more intense perimenopausal hot flashes, and longer duration before oral contraceptive (OC) use compared with asymptomatic ones. Ongoing sleep disorders were reported in 78.78% of symptomatic versus 26.66% of asymptomatic women ($P < 0.0001$) in the RPM group; and in 63.33% of symptomatic versus 35.14% of asymptomatic ones ($P = 0.0281$) in the LPM group. Evoked hot flashes score around perimenopause was 9.0 (6.5-10.0) in present symptomatic women versus 5.5 (0-8.3) in asymptomatic ones ($P = 0.0007$) in the RPM group; and 9.5 (8.0-10.0) in symptomatic women versus 6.0 (0-9.0) in asymptomatic ones ($P = 0.0001$) in the LPM group. Previous OC use was 7 (2.5-10.5) in symptomatic versus 2.5 years (0-7.3) in asymptomatic women ($P = 0.009$) in the RPM group; and 8 (4.5-13.3) in symptomatic versus 5 years (0.5-8.5) in asymptomatic women ($P = 0.0140$) in the LPM group (Table 2, Fig. 2). In both groups, the present score for hot flashes was positively correlated with evoked perimenopausal hot flashes score ($r = 0.4746$, $P < 0.0001$ in the RPM group; $r = 0.4704$, $P < 0.0001$ in the LPM group). Only in the RPM group, the correlation between the current score for hot flashes and previous OC duration was significant ($r = 0.3132$, $P = 0.0124$).

Vascular function assessment showed no difference in baseline (FBF-B 1 and 2) or after nitroglycerin (FBF-N and its % increment) flows among symptomatic and asymptomatic women within groups; however, during the reactive hyperemia response, FBF-RH was 3.6 (3.0-5.2) in symptomatic versus 5.9 (4.6-7.8) mL/cm/100 mL in asymptomatic ones in the RPM group ($P < 0.0001$); in the LPM group, FBF-RH was 4.0 (3.0-5.1) versus 5.4 (4.5-7.5) mL/cm/100 mL ($P < 0.0001$), respectively. The percentage increase of baseline blood flow after the reactive hyperemia response (% increment) was 285.1 (203.7-353.3) among symptomatic versus 327.7 (250.9-493.5)% in asymptomatic women ($P = 0.0197$) for the RPM group; and 118.6 (78.9-217.7) in

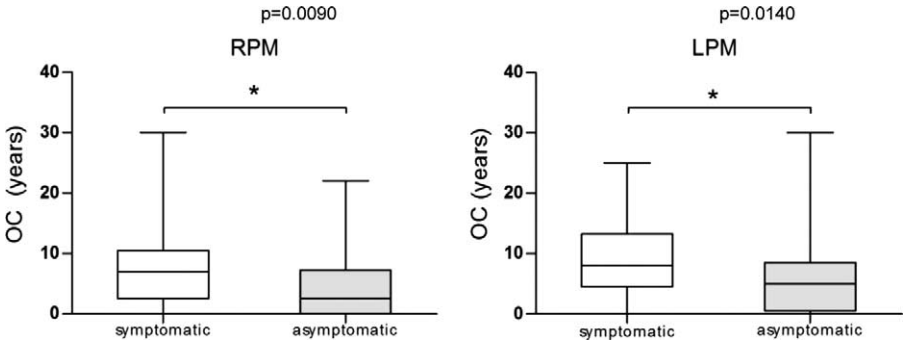


**FIG. 2.** Comparison of years of previous oral contraceptive use between symptomatic and asymptomatic women within RPM and LPM groups. The box represents median, 25th and 75th percentile, and whiskers minimum and maximum. $^{*}P < 0.05$. LPM, late postmenopause; OC, oral contraceptive; RPM, recent postmenopause.

symptomatic versus 218.8 (138.6-272.6)% in asymptomatic women ($P = 0.0015$) in the LPM group (Table 3, Fig. 3).

The score for hot flashes current intensity was negatively correlated with FBF-RH in both groups ($r = -0.4228$, $P = 0.0006$ in the RPM group; $r = -0.3823$, $P = 0.0014$ in the LPM group), and at the same time positively correlated with SBP and DBP (Fig. 4).

In the multivariable model, FBF-RH was associated with hot flashes adjusting for age and BMI in both RPM (odds ratio [OR] 0.54; 95% CI, 0.37-0.78) and LPM groups (OR 0.64; 95% CI, 0.47-0.88). The findings persisted after adjustment for SBP (OR 0.55; 95% CI, 0.36-0.84) in RPM; and OR 0.7; 95% CI, 0.5-0.97 in LPM).

There was no difference between symptomatic and asymptomatic women within RPM and LPM groups in relation to biochemical, hormonal, or inflammatory biomarkers (Table 4).

## DISCUSSION

Age and time of hypoestrogenism[19] affect both endothelial function and hot flashes intensity. To homogenize these variables and place the endothelial impact of hot flashes, for clinical purposes, we have chosen to compare symptomatic and asymptomatic women within RPM and LPM groups, separately.

Amidst each group, symptomatic women showed smaller reactive hyperemia response (FBF-RH) compared with asymptomatic ones, indicating lower endothelium-dependent vasodilatation owing to decreased NO bioavailability. Regarding the RPM group, our observation is in conformity with Thurston et al[7] findings of impaired FMD of the brachial artery in women during the menopausal transition presenting with hot flashes, compared with women with no vasomotor symptoms. Bechlioulis et al[20] also found lower FMD in 42 to 55-year-old women in recent postmenopause with moderate/severe hot flashes compared with the ones with no/mild hot flashes: severity of hot flashes was the most important independent predictor of FMD in these women. Accordingly, we found an inverse correlation between intensity of hot flashes and postreactive hyperemia flow (FBF-RH), both in RPM and LPM groups. Symptomatic women in our study reported higher hot flashes score since perimenopause, suggesting that early in the menopausal transition, intense hot flashes may signal subclinical vascular changes.

We have also observed worse endothelial function in LPM women with hot flashes, compared with their asymptomatic counterparts. If left untreated, endothelial dysfunction may progress to advanced atherosclerosis decades later, with possible unstable plaques, which, subjected to prothrombogenic HT effects, could lead to an increase in the risk of clinical events such as myocardial infarction or stroke, which are currently the major causes of death in LPM women.[21] In concordance, older symptomatic women from the WHI study[6] showed increased CV events after HT. Furthermore, the

**TABLE 3.** *Venous occlusion plethysmography results*

| | RPM | | | LPM | | |
|---|---|---|---|---|---|---|
| | Symptomatic (n = 33) | Asymptomatic (n = 30) | *P* | Symptomatic (n = 30) | Asymptomatic (n = 37) | *P* |
| FBF-B | 1.4 (1.1-2.0) | 1.7 (1.3-2.1) | 0.2285 | 1.7 (1.4-2.2) | 1.8 (1.5-2.3) | 0.5619 |
| FBF-RH | 3.7 (3.0-5.2) | 5.9 (4.6-7.8) | <0.0001[a] | 4 (3.0-5.1) | 5.4 (4.5-7.5) | <0.0001[a] |
| % Increment | 285.1 (203.7-353.3) | 327.7 (250.9-493.5) | 0.0197[a] | 118.6 (78.9-217.7) | 218.8 (138.6-272.6) | 0.0015[a] |
| FBF-B | 1.31 (1.0-1.7) | 1.5 (1.2-1.8) | 0.1283 | 1.5 (1.2-2.0) | 1.5 (1.2-2.1) | 0.8698 |
| FBF-N | 1.31 (1.0-1.6) | 1.6 (1.2-2.0) | 0.0703 | 1.5 (1.3-1.9) | 1.5 (1.2-1.9) | 0.6959 |
| % Increment | −4.1 (−10.8-21.0) | 4.3 (−7.4-26.9) | 0.3710 | 3.3 (−8.1-15.2) | 2.1 (−6.4-12.4) | 0.7918 |

FBF-B,forearm blood flow baseline; FBF-N, forearm blood flow post nitroglycerine; FBF-RH, forearm blood flow during reactive hyperemia; LPM, late postmenopause; RPM, recent postmenopause.
[a]Mann-Whitney *U* test, $P < 0.05$.

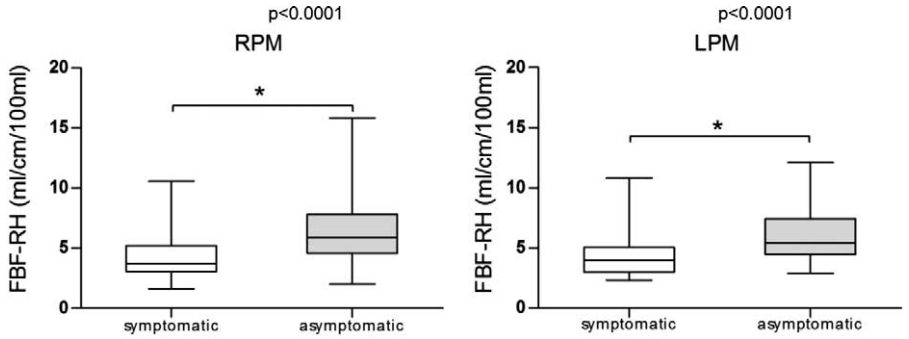


**FIG. 3.** Comparison of forearm blood flow post during the reactive hyperemia response between symptomatic and asymptomatic women within RPM and LPM groups. The box represents median, 25th and 75th percentile, and whiskers minimum and maximum. $^{*}P < 0.05$. FBF-RH, forearm blood flow during reactive hyperemia; LPM, late postmenopause; RPM, recent postmenopause.

randomized double-blind Heart and Estrogen/Progestin Replacement Study (HERS) that initiated HT in 66.7-year-old women previously diagnosed with coronary artery disease, showed that those with significant hot flashes had a relative risk of 9.01 (1.15-70.4) to a new CV event in the first year of HT, compared with women without significant hot flashes, in which case no increased risk (relative risk 1.32; 95% CI, 0.86-2.03; $P = 0.04$) was found.[22]

Interestingly, the total Framingham score, a well-established CV risk assessment tool, did not show significant differences between symptomatic and asymptomatic women within our RPM and LPM groups. Endothelial dysfunction has been associated with the final count, as well as with the individual classical CV risk factors that compose the Framingham score.[23] Age was relatively controlled within our groups, and we can hypothesize that endothelial dysfunction related to hot flashes precedes other CV risk factors evaluated by this instrument, or that hot flashes represent a new unconventional CV risk factor, different from other well described components, that may be useful when evaluating postmenopausal women.

Nonetheless, one of the Framingham risk score predictors, that is treated hypertension or higher SBP, showed associations with hot flashes in our study. Symptomatic women in the LPM group had higher prevalence of previous hypertension diagnosis, similar to the study by Erkal et al,[24] where women up to 65 years with hot flashes showed higher prevalence of essential hypertension (confirmed by 24 h ambulatory monitoring) compared with asymptomatic ones. Conversely, they reported that hypertensive women had more frequent hot flashes than normotensive ones. In our study, symptomatic women of RPM and LPM groups showed higher, although mostly in the normal range, SBP and DBP compared with asymptomatic ones. Gast et al,[25] studying 5,523 women between 46 and 57 years, participating in the Eindhoven Perimenopausal Osteoporosis study, also found 1.92 (0.87-2.97) and 1.25 (0.66-1.84) mm Hg higher SBP and DBP, respectively, in women with hot flashes compared with those without hot flashes. Another study, analyzing women who were $45.6 \pm 10.4$ years old by ambulatory blood pressure, also found higher SBP in the women with hot flashes compared with those without hot flashes, when awake and during sleep, and independent of time since menopause.[26]

Our data are consistent with a recent meta-analysis that includes 11 studies with a total of 19,667 participants, indicating that postmenopausal women with hot flashes had higher SBP compared with those without hot flashes (mean difference: 1.95 mm Hg; 95% CI, 0.27-3.63).[27] The author proposes that increased secretion of epinephrine and norepinephrine owing to sympathetic nerve activity up-regulation is a possible link between symptoms and increased risk of hypertension.

In fact, the connection between hot flashes and higher SBP may occur through the autonomic nervous system, as sympathetic stimulation seems to be associated to both. Although the relation between sympathetic nervous system (SNS) and hypertension is well documented, SNS and hot flashes aspects are unfolding in recent years: increased norepinephrine reduces the thermo neutral zone in the brain[28]; a clinical study described elevated norepinephrine in the brain of women with hot flashes compared with asymptomatic ones[29]; clonidine, an alpha-blocker that decreases norepinephrine release in the brain, increases the thermo neutral zone in symptomatic women and decreases hot flashes.[30] Apart from hot flashes, aging is associated with increased sympathetic tonus.[31] So, the sympathetic tonus increased by hot flashes and aging could potentiate the risk for developing hypertension. Our separation by age groups and especially time since menopause enabled the increased risk of arterial hypertension diagnosis to be evidenced in the LPM group.

Because high blood pressure, which has been consistently associated with hot flashes in our study, is known to reduce endothelium-dependent vasodilatation,[32] when analyzing the association between endothelial function and hot flashes in the multivariate model, SBP has been selected as a covariate. Hot flashes influence was, however, found to persist after adjustment, indicating that other mechanisms, in addition to higher SBP, were responsible for the worse endothelial response in symptomatic women.

A transversal study is not capable of determining whether endothelial dysfunction in women with hot flashes is a consequence of higher blood pressure, or, on the contrary, may be

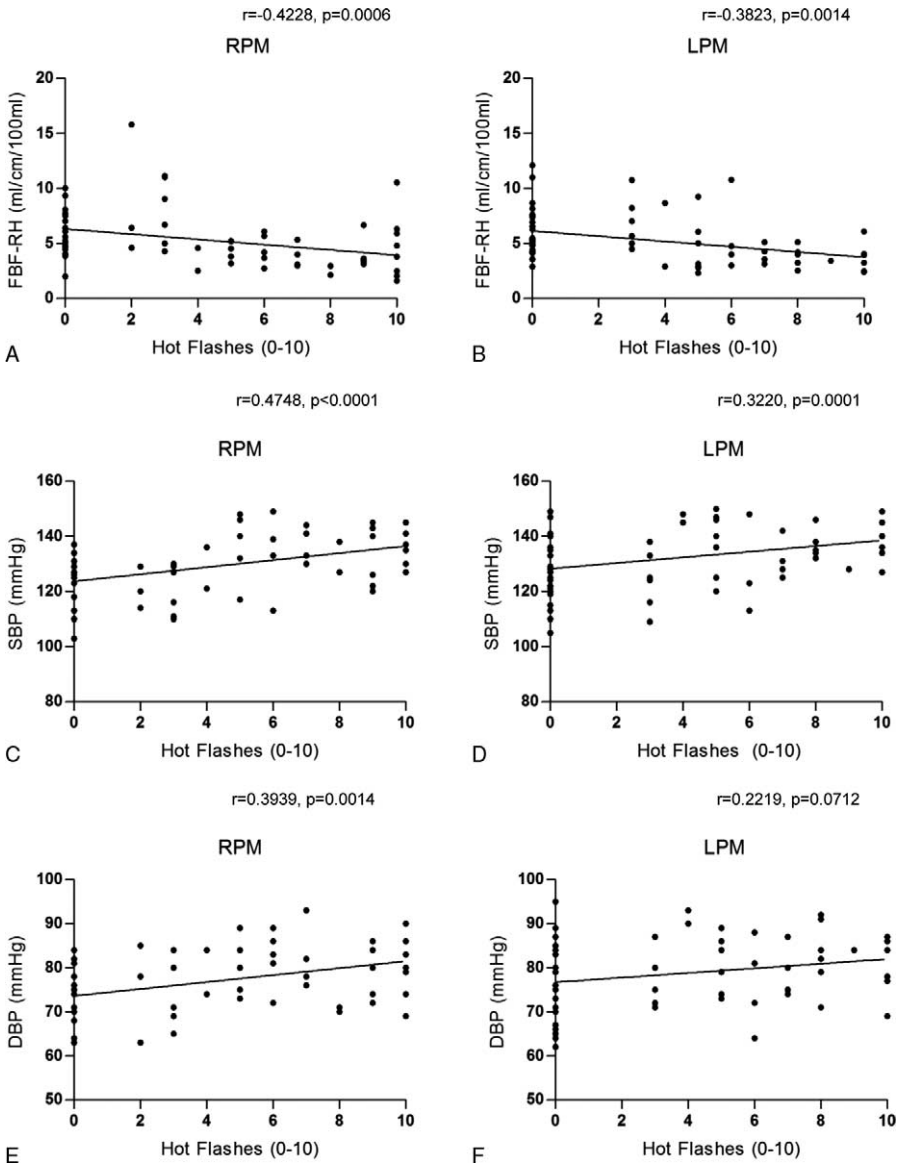


**FIG. 4.** Correlation between hot flashes score and forearm blood flow (**A** and **B**) during the reactive hyperemia response, systolic (**C** and **D**) and diastolic (**E** and **F**) blood pressure in postmenopausal women in RPM and LPM groups. The *x* axis indicates the current score on a scale from 0 to 10 for hot flashes intensity. Each point represents a participant in the study. R of Spearman was used. $^{*}P < 0.05$. DBP, diastolic blood pressure; FBF-RH, forearm blood flow during reactive hyperemia; LPM, late postmenopause; RPM, recent postmenopause; SBP, systolic blood pressure.

the cause. In this sense, a study including women aged 40 to 70 years reported, after adjusting for confounding factors, a twofold risk (2.05; 95% CI, 1.08-3.89) for perimenopausal and postmenopausal hot flashes in those with eclampsia or pre-eclampsia decades before menopause, compared with women without precedent pregnancy hypertension.[33] Endothelial dysfunction could have remained in women who presented hypertension during pregnancy, even after postpartum blood pressure normalization,[34] and predisposed them for hot flashes during the menopausal transition. Rossi et al,[35] following normotensive postmenopausal women aged 44 to 60 years on admission during $3.6 + 0.7$ years, found an OR of 5.77 (95% CI, 4.34-8.10; $P < 0.001$) for hypertension development in those with worse FMD compared with the ones with better FMD at baseline. Thus, endothelial dysfunction found in RPM symptomatic women may be a predictor of hypertension in late postmenopause. An increased sympathetic tonus in younger women, predisposing them to endothelial dysfunction, hypertension, and hot flashes, cannot be discarded.

The prevalence of sleep disorders was higher in symptomatic versus asymptomatic women in both RPM and LPM groups, in agreement with other studies that found relative risks of 2.1 (95% CI, 1.4-3.2)[36] and 5.28 (95% CI, 4.44-6.28; $P < 0.0001$)[37] for difficulty to sleep in women who reported hot flashes compared with those who reported not having had the symptom. Many prospective studies have associated sleep disturbances with worse endothelial function,[38] increased risk

 

**TABLE 4.** *Laboratory data*

| | RPM | | | LPM | | |
|---|---|---|---|---|---|---|
| | Symptomatic (n = 33) | Asymptomatic (n = 30) | *P* | Symptomatic (n = 30) | Asymptomatic (n = 37) | *P* |
| Glucose, mg/dL | 94 (88-101) | 92 (86-99) | 0.1674 | 99 (86.2-105.5) | 95 (89.8-103.0) | 0.5759 |
| Cholesterol, mg/dL | 206 (184-233) | 202 (179.3-231.8) | 0.8317 | 207 (168.3-233.0) | 206 (182.3-236.0) | 0.3998 |
| HDL cholesterol, mg/dL | 48 (41.0-69.5) | 52.5 (45-68) | 0.6732 | 53 (42.5-61.0) | 48 (38.9-71.0) | 0.8321 |
| LDL cholesterol, mg/dL | 128.2 (104.9-157.3) | 122.6 (104.5-138.8) | 0.4024 | 125.2 (95.6-151.8) | 137.6 (108.8-159.9) | 0.3542 |
| Triglycerides, mg/dL | 136 (85-171) | 113 (64.5-160.0) | 0.3090 | 112 (87.3-14.3) | 99 (77.3-128.8) | 0.2855 |
| Albumin, g/dL | 4.4 (4.2-4.6) | 4.4 (4.2-4.6) | 0.6405 | 4.3 (4.2-4.6) | 4.4 (3.9-4.7) | 0.8507 |
| Androstenedione, pg/mL | 0.7 (0.4-1.0) | 0.9 (0.5-1.6) | 0.2079 | 0.9 (0.5-1.4) | 0.7 (0.4-1.4) | 0.9033 |
| Estradiol, pg/mL | 12 (6-18) | 11.9 (7.0-17.2) | 0.9756 | 11.8 (7.0-19.3) | 11.8 (5.0-15.8) | 0.4738 |
| LH, mUI/mL | 27.1 (19.9-36.6) | 31.1 (16.0-41.3) | 0.8081 | 25.2 (18.6-31.6) | 27 (19.9-33.8) | 0.6868 |
| SHBG, mg/dL | 38.8 (29.0-48.3) | 41.5 (29.5-70.5) | 0.4201 | 55.3 (38.2-81.0) | 57.6 (46.3-93.1) | 0.4283 |
| FSH, mUI/mL | 52.4 (35.3-73.2) | 60.8 (33.5-89.2) | 0.6875 | 62.3 (46.9-72.8) | 67.2 (58.2-77.8) | 0.3153 |
| Estrone, pg/mL | 75.5 (55.3-106.6) | 60.9 (49.5-106.0) | 0.3751 | 59 (49.4-75.3) | 56.5 (42.9-72.1) | 0.8472 |
| Testosterone, ng/mL | 14 (6-28) | 12 (7.0-36.1) | 0.7322 | 13 (5.5-24.3) | 16 (6.0-28.9) | 0.4716 |
| Insulin, μUI/mL | 8.7 (5.3-13.5) | 8.1 (6.2-13.0) | 0.8823 | 5.6 (3.3-15.7) | 6.8 (5.1-10.0) | 0.8024 |
| DHEA-S, μg/dL | 66 (40-123) | 82 (50.0-102.5) | 0.8707 | 59.5 (42.0-99.8) | 56 (39.0-89.5) | 0.5988 |
| HOMA-IR | 2.1 (1.3-3.6) | 1.9 (1.4-3.0) | 0.6788 | 1.7 (0.7-3.9) | 1.67 (1.0-2.3) | 0.5214 |
| sPECAM-1, ng/mL | 24.1 (20-26) | 24.6 (19.1-28.5) | 0.8183 | 22.8 (17.2-27.6) | 22.3 (19.3-28.1) | 0.7243 |
| sICAM-1, ng/mL | 137 (117.2-243.3) | 159.5 (109.8-217.4) | 0.8475 | 151 (114.4-265.0) | 129.6 (109.0-163.6) | 0.1775 |
| sVCAM-1, ng/mL | 283.6 (260.0-358.6) | 325.9 (256.9-364.8) | 0.4633 | 291 (262.0-386.6) | 326.4 (294-371) | 0.4935 |
| sE-SELECTIN, ng/mL | 75.6 (56.1-108.6) | 68.9 (50.0-93.2) | 0.3392 | 71.7 (46.8-91.0) | 72.5 (52.0-82.2) | 0.9724 |
| sP-SELECTIN, ng/mL | 96.7 (59.5-123.3) | 93 (63.7-117.3) | 0.6837 | 92 (74.6-128.2) | 84 (69.3-112.0) | 0.1819 |
| PAI-1, ng/mL | 30.7 (21.5-41.5) | 29.2 (23.5-46.4) | 0.8939 | 26.3 (19.9-30.9) | 30.1 (23.3-36.0) | 0.0889 |

DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; LH, luteinizing hormone; LPM, late postmenopause; RPM, recent postmenopause; SHBG, sex hormone-binding globulin.

for high blood pressure, coronary heart disease,[39] and even mortality.[40] Again, SNS can be a common pathway between these results.

Finally, symptomatic women in our study reported longer duration of prior OC use, both in RPM and LPM groups, compared with the asymptomatic ones. Our findings are in agreement with a recent publication by Gallicchio et al,[41] analyzing 732 women, 45 to 54 years old, from the Midlife Women's Health Study, showing an OR of 1.89 (95% CI, 1.16-3.08) for hot flashes in relation to prior history of OC use, after multiple adjustments. One hypothesis to be tested in future studies is that longer estrogen exposure, by means of higher duration and dosage of OCs, activates the estrogen receptor during reproductive life; thus estrogens fall after menopause may be more significant for these women, contributing to presence of hot flashes. Hot flashes intensity was, however, positively correlated with previous OC duration only in the RPM group. Women in the LPM group were at least 10 years off OCs, and this fact may have favored estrogen receptor methylation upon disuse,[42] or may have influenced the information about the exact number of years of previous use because the data were retrospective.

Inflammatory biomarkers were similar in symptomatic and asymptomatic women in both groups, suggesting that endothelial dysfunction precedes metabolic and inflammatory processes that may follow estrogen deficiency.

As a limitation, the study was a cross-sectional one, describing only associations between variables, and could not establish temporal and causal relationships between them. For this purpose, future prospective interventional studies are needed. We analyzed many different characteristics, and, because of the sample size, the logistic regression could include no more than four variables. Therefore, significant results obtained apart from the multivariable model need caution in interpretation. Hot flashes intensity and sleep disturbances were evaluated by self-report, and this kind of subjective quantification may be vulnerable to errors. The quantification of previous OC duration was retrospective, depending on the memory of participants, and did not discriminate different types of hormones.

## CONCLUSIONS

In both RPM and LPM groups, women with hot flashes compared with asymptomatic ones showed worse endothelial function, characterized by lower blood flow during the reactive hyperemia response, higher SBP and DBP and higher prevalence of sleep disorders. In the LPM group symptomatic women also had higher prevalence of previous diagnosis of hypertension. The observed associations, between hot flashes, endothelial dysfunction, higher blood pressure and sleep disorders need more studies to understand the mechanisms. The relationship between hot flashes and longer duration of previous OC use also deserves further examination.

Overall, our data indicate that severe hot flashes seem to be a warning for cardiovascular health at all menopausal stages. Therefore, it is important to observe hot flashes not only as a symptom related to decreased quality of life, but possibly as an early unconventional indicator of cardiovascular risk for this population.

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