UNIVERSITÉ DE GENÈVE

Archive ouverte UNIGE
https://archive-ouverte.unige.ch

Article scientifique | Article | 2017

Published version | Public access

This is the published version of the publication, made available in accordance with the publisher's policy.

The cross-sectional association between vasomotor symptoms and hemostatic parameter levels in postmenopausal women

Harrington, Laura B; Blondon, Marc; Cushman, Mary; Kaunitz, Andrew M; Rossouw, Jacques E; Allison, Matthew A; Martin, Lisa W; Johnson, Karen C; Rosing, Jan; Woods, Nancy F; LaCroix, Andrea Z; Heckbert, Susan R; McKnight, Barbara; Smith, Nicholas L

How to cite

HARRINGTON, Laura B et al. The cross-sectional association between vasomotor symptoms and hemostatic parameter levels in postmenopausal women. In: Menopause, 2017, vol. 24, n° 4, p. 360–370. doi: 10.1097/GME.0000000000000777

This publication URL: https://archive-ouverte.unige.ch/unige:112328
Publication DOI: 10.1097/GME.0000000000000777

© This document is protected by copyright. Please refer to copyright holder(s) for terms of use.

Last update in Archive ouverte UNIGE on 27.01.2025 16:21

Menopause: The Journal of The North American Menopause Society
Vol. 24, No. 4, pp. 360-370
DOI: 10.1097/GME.0000000000000777
© 2016 by The North American Menopause Society

The cross-sectional association between vasomotor symptoms and hemostatic parameter levels in postmenopausal women

Laura B. Harrington, PhD, MPH,[1,2] Marc Blondon, MD, MS,[3] Mary Cushman, MD, MSc,[4] Andrew M. Kaunitz, MD,[5] Jacques E. Rossouw, MD,[6] Matthew A. Allison, MD, MPH,[7] Lisa W. Martin, MD,[8] Karen C. Johnson, MD, MPH,[9] Jan Rosing, PhD,[10] Nancy F. Woods, PhD, RN, FAAN,[11] Andrea Z. LaCroix, PhD,[12] Susan R. Heckbert, MD, PhD,[1] Barbara McKnight, PhD,[13] and Nicholas L. Smith, PhD[1]

Abstract

Objective: Vasomotor symptoms (VMS) may be a marker of cardiovascular risk. We aimed to evaluate the cross-sectional association of VMS presence and severity with hemostatic parameter levels measured at baseline among Women's Health Initiative (WHI) Hormone Therapy trial postmenopausal participants.

Methods: This cross-sectional analysis included 2,148 postmenopausal women with measures of VMS presence and severity reported in the 4 weeks before WHI baseline, who were not using warfarin or hormone therapy and for whom the following baseline hemostatic parameters were measured within the WHI Cardiovascular Disease Biomarker Case-Control Study: antithrombin, plasminogen activator inhibitor-1, protein C antigen, total and free protein S antigen, total and free tissue factor pathway inhibitor, D-dimer, normalized activated protein C sensitivity ratio, and thrombin generation. Using multiple linear regression, we estimated the adjusted average difference in each hemostatic parameter associated with VMS presence and severity. A multiple comparisons-corrected *P* value was computed using the P-min procedure to determine statistical significance of our smallest observed *P* value.

Results: Women were 67 years of age on average and 33% reported VMS presence at baseline. There was some suggestion that VMS presence may be associated with a −0.34 adjusted difference in normalized activated protein C sensitivity ratio compared with no VMS (95% CI, −0.60 to −0.087; $P = 0.009$), but this association was not significant after correction for multiple comparisons ($P = 0.073$). VMS presence or severity was not significantly associated with the other hemostatic parameters.

Conclusions: We found no convincing evidence that VMS presence or severity was associated with levels of hemostatic parameters among postmenopausal women.

Key Words: Epidemiology – Hemostasis – Hot flashes – Menopause.

Vasomotor symptoms (VMS), defined as hot flashes and/or night sweats, are experienced by most women at some point, with estimates of VMS prevalence in postmenopause ranging widely from 30% to 80%.[1] Evidence suggests that VMS may be a marker for vascular change,[2-5] and potentially a marker for

Received May 24, 2016; revised and accepted September 6, 2016.

From the [1]Department of Epidemiology, University of Washington, Seattle, WA; [2]Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA; [3]Division of Angiology and Haemostasis, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland; [4]Department of Medicine, University of Vermont, Burlington, VT; [5]Department of Obstetrics and Gynecology, University of Florida College of Medicine-Jacksonville, Jacksonville, FL; [6]National Heart Lung and Blood Institute, Bethesda, MD; [7]Department of Preventive Medicine, University of California San Diego, San Diego, CA; [8]Department of Medicine, George Washington University, Washington, DC; [9]Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN; [10]Department of Biochemistry, Maastricht University, Maastricht, The Netherlands; [11]School of Nursing, University of Washington, Seattle, WA; [12]Department of Family Medicine and Public Health, University of California San Diego, San Diego, CA; and [13]Department of Biostatistics, University of Washington, Seattle, WA.

The opinions expressed in this manuscript are those of the authors and do not necessarily reflect the views of the National Heart, Lung, and Blood Institute/US Department of Health and Human Services.

Funding/support: The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. This work is supported by grant HL121414 (Smith) from the NHBLI and NHLBI Cardiovascular Disease Training Grants (HL007902: David S. Siscovick; HL098048: Eric B. Rimm).

Financial disclosure/conflicts of interest: A.M.K. reports that his institution receives support from Bayer and TherapeuticsMD and that he serves on advisory boards for Allergan, Bayer, and Pfizer and received payments from Teva. A.Z.L. reports a financial relationship with Sermonix, Pfizer, and Amgen. There are no disclosures for all other coauthors.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website (www.menopause.org).

Address correspondence to: Laura B. Harrington, PhD, MPH, Department of Nutrition, Harvard T.H. Chan School of Public Health, 655 Huntington Avenue, Building 2, Boston, MA 02115.
E-mail: lharring@hsph.harvard.edu

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

cardiovascular event risk.[6] Given the high prevalence of VMS, it has also been suggested that VMS characteristics with greater interindividual variability, such as VMS severity, timing, and duration, may provide information helpful in cardiovascular risk stratification.[6]

The etiology of VMS is incompletely understood, but low endogenous estradiol and high follicle-stimulating hormone levels are associated with VMS presence and greater VMS frequency and severity.[7,8] These hormonal patterns are also associated with mostly prothrombotic changes in hemostatic parameters among midlife women.[9,10] This suggests that VMS may plausibly be a marker of clinically unmeasured endogenous hormonal changes associated with thrombotic risk. In addition, frequent hot flashes have been associated with some hemostatic parameter levels among pre- and perimenopausal women, including higher factor VIIc (FVIIc) and tissue plasminogen activator antigen (tPA-ag) levels.[4] Notably, the association of VMS with most hemostatic parameters among postmenopausal women has not been evaluated.

In a cross-sectional analysis among postmenopausal women not currently using hormone therapy (HT), we tested the hypothesis that VMS presence and severity at baseline in the Women's Health Initiative Hormone Therapy (WHI-HT) trials would be associated with higher levels of hemostatic activation markers and procoagulant and antifibrinolytic parameters, and lower levels of anticoagulant hemostatic parameters. In secondary analyses, we hypothesized that longer VMS duration and postmenopause status at VMS onset, after the hormonal fluctuations that occur during the menopausal transition, would be positively associated with higher levels of markers of ongoing coagulation and procoagulant and antifibrinolytic proteins, and lower levels of anticoagulant proteins.

METHODS

Setting and design

The data for this cross-sectional study are from a nested case-control study of cardiovascular biomarkers in relation to cardiovascular disease (CVD), set within the WHI-HT trials. The WHI-HT trials enrolled 27,347 postmenopausal women aged 50 to 79 years from areas surrounding 40 clinical centers from 1993 to 1998, as previously described.[11-14] The WHI-HT trials were approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

Nested case-control study participants

Women eligible for this study were participants in the CVD Biomarker Case-Control Studies, which included centrally adjudicated cases of coronary heart disease (CHD), defined as myocardial infarction (MI) or coronary death (n = 402), venous thrombosis (VT) (n = 223) occurring between randomization and February 28, 2001, and all centrally adjudicated cases of stroke occurring between randomization and September 12, 2005 (n = 573). Some participants experienced multiple case event types. One control with no MI, VT, or stroke was selected for each case event, with controls selected at the time of the case's event and matched on age, randomization date, hysterectomy status, and prevalent CVD at baseline (n = 1,170).

Excluded were women for whom hemostatic parameter assays of interest were not completed (n = 14), with unknown VMS presence and severity at baseline (n = 20), and who had not completed a full 3-month HT washout before the date of blood sample collection or the date of VMS report (n = 140) (eligible n = 2,148). No eligible participants used warfarin at baseline. Data analyzed in this cross-sectional study were collected at baseline, which collectively encompassed an initial telephone screening eligibility interview and three in-person study screening visits; this baseline screening period was before the WHI-HT trial randomization of women to treatment or placebo.

VMS

Using standardized study forms at the second clinical trial screening visit, women were asked whether hot flashes and night sweats had occurred in the past 4 weeks and whether they were mild, moderate, or severe. Eligible women who were using HT before this screening visit had completed a 3-month period off HT by the time of reporting on current symptoms. Mild symptoms were those that did not interfere with usual activities, moderate symptoms interfered somewhat with usual activities, and severe symptoms were so bothersome that usual activities could not be performed. Women with any mild, moderate, or severe hot flashes were considered to have VMS presence at baseline.

Secondary exploratory analyses relied on historical VMS and menopause data reported by participants at the second baseline screening visit. Separately from the report of VMS presence and severity, women were asked whether they had ever had menopausal symptoms such as hot flashes and/or night sweats. If they had, they reported their age when they first started having symptoms, as well as their age at last symptom, or current age if still having symptoms. Age at menopause was determined using methods previously published,[15] using the age at which a women last had any menstrual bleeding, had a bilateral oophorectomy, or began using HT. For women with a prior hysterectomy without bilateral oophorectomy, age at menopause was defined as the age at HT initiation or the onset of menopausal symptoms. Therefore, women with a prior hysterectomy but no bilateral oophorectomy were excluded from analyses in which menopause status at VMS onset was the exposure of interest (n = 501).

In secondary analyses, menopause status at VMS onset was determined by comparing age at menopause with age at VMS onset and women were thus classified into three categories: as having experienced VMS onset (1) before age at menopause (in pre/perimenopause), (2) within the same year as the age at menopause (year of menopause), or (3) after the age at menopause (postmenopause). VMS duration at the time of

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 1. *Hemostatic parameters for evaluation in relation to vasomotor symptom presence, by primary and secondary hypothesis group*

Group 1 (primary hypotheses)

Name	Abbreviation	Units	Measurement of	Hypothesized direction of association[a]
Antithrombin	—	%	anticoagulation	−
Fibrin D-dimer	D-dimer	μg/mL	global	+
Normalized activated protein C sensitivity ratio	nAPCsr	ratio	APC resistance	+
Plasminogen activator inhibitor-1 antigen	PAI-1	ng/mL	fibrinolysis	+
Protein C	—	%	anticoagulation	−
Protein S (free and total)	—	%	anticoagulation	−
Tissue factor pathway inhibitor (free and total)	TFPI	ng/mL	anticoagulation	−
Thrombin generation	TG			
Lag-time	—	minutes	thrombin initiation	−
Endogenous thrombin potential	ETP	nM.min	thrombin potential	+
Peak	—	nM	peak thrombin concentration	+

Group 2 (secondary hypotheses)

Name	Abbreviation	Units	Measurement of	Hypothesized direction of association[a]
Factor VIII activity	FVIIIc	%	procoagulation	+
Fibrinogen	—	mg/dL	procoagulation	+
Plasmin-alpha 2-antiplamin complex	PAP	nmol/L	fibrinolysis	+
Prothrombin antigen	prothrombin	μg/mL	procoagulation	+
Prothrombin fragment F 1.2	F1.2	nmol/L	procoagulation	+
Thrombin activatable fibrinolysis inhibitor	TAFI	μg/mL	fibrinolysis	+
von Willebrand factor	vWF	%	procoagulation	+

[a]Hypothesized association between vasomotor symptoms (VMS) presence, stronger VMS severity, longer VMS duration, and later menopausal stage at VMS onset and hemostatic parameter levels. This is also the hypothesized direction of the association between the hemostatic parameter and venous thrombosis risk.

WHI baseline was determined by subtracting age at first VMS from age at last VMS, or, age at WHI baseline if VMS were present at baseline or if no VMS end date was reported. Of note, the full VMS duration for women still having VMS at WHI baseline is truncated.

Hemostatic parameter measures

Blood specimens were collected from all participants at baseline, before the collection of baseline VMS information for 1,608 women (mean = 38.5 d prior; range = 1-238 d), after the collection of VMS information for 62 women (mean = 45.1 d after; range = 1-256 d), and on the same day as the collection of VMS information for 478 women. Eligible women who had used HT previously had not used HT for at least 3 months before the date of blood specimen collection. Blood samples were collected into tubes containing 1.8% sodium citrate, centrifuged at 1,300 *g* for 10 minutes at 4°C within 2 hours, and stored at −70°C.[16] Batches used for hemostatic parameter assays included both case and control subjects.[16]

Based on the strength of the published evidence for the association of exogenous HT with factor levels, available hemostatic parameters were divided into two groups a priori (Table 1): a group of primary interest (group 1) and a group of secondary interest (group 2).[17-28] The hemostatic parameters of primary interest were antithrombin, fibrin D-dimer (D-dimer), normalized activated protein C sensitivity ratio (nAPCsr), plasminogen activator inhibitor-1 (PAI-1) antigen, protein C antigen, protein S antigen (free and total), tissue factor pathway inhibitor (TFPI) (free and total), and three parameters of thrombin generation (TG): TG lag-time, the peak thrombin concentration, and the individual's endogenous thrombin potential (ETP). The remaining hemostatic parameters (factor FVIII activity [FVIIIc], fibrinogen, plasmin-alpha 2-antiplasmin complex [PAP], prothrombin antigen, prothrombin fragment F 1.2 [F1.2], thrombin activatable fibrinolysis inhibitor [TAFI], and von Willebrand factor [vWF]) were of secondary interest based on weaker evidence of an association between exogenous HT and these factors.

The hemostatic parameter assays have been described in prior WHI publications and will be summarized in brief.[16,29] After a storage time ranging from 8 to 13 years, the measurement of the three parameters of TG without added APC and of the ETP-based nAPCsr was completed at the Department of Biochemistry at the University of Maastricht, the Netherlands. The nAPCsr is defined as the ratio of the ETPs determined without and with added APC, normalized against normal plasma.[16,30] Total and free TFPI antigen were assayed after a sample storage time of 8 to 13 years using the Asserochrom enzyme-linked immunosorbent assay (Diagnostica Stago, http://www.stago-us.com) at the Department of Hematology, Oslo University Hospital, Oslo. All other hemostatic parameters were measured after a storage time ranging from 3 to 8.5 years. Fibrinogen, FVIIIc, vWF, antithrombin, D-dimer, PAI-1, PAP, prothrombin, F1.2, Protein C, free and total S, and TAFI were measured by the Laboratory for Clinical Biochemistry Research, University of Vermont. Fibrinogen was measured via a clot-rate assay using a STA-R instrument (Diagnostica Stago), FVIIIc by measuring the clotting time after mixing with FVIII-depleted plasma using STA-Deficient VIII (Diagnostica Stago), vWF,

© 2016 The North American Menopause Society

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

antithrombin, and D-dimer by immunoturbidometric or colorimetric assays using a STA-R instrument (Liatest von Willebrand factor, Liatest D-di, Stachrom antithrombin, Diagnostica Stago), PAI-1, PAP, and prothrombin by in-house immunoassays,[31] prothrombin fragment 1.2 by ELISA (Dade-Behring), protein C, and free and total protein S by Asserachrom ELISA (Diagnostica Stago), and TAFI by immunoassay with antibodies from Affinity Biologicals.

Covariates

Baseline demographic, medical history, and lifestyle covariates were collected at baseline screening visits.[12] Self-reported current age, race/ethnicity, age at last menstrual bleeding, prior hysterectomy, education level, smoking status, and physical activity were collected using standardized questionnaires. Participants also self-reported medical history characteristics including prevalent MI, prevalent stroke, prevalent VT, history of DVT or PE among family members, history of treated diabetes, high cholesterol requiring treatment, and physician-diagnosed hypertension. Weight and height were measured at the initial screening visit and were used to calculate body mass index (BMI) in kg/m^2. At the initial telephone eligibility screening interview at baseline, participants reported current use of estrogen and progestogen in the form of pills, skin patches, implants, creams, suppositories, shots, and birth control pills (excluding birth control pills used before age 50). Current use of opposed and unopposed estrogen in the form of pills and patches was also reported at the first in-person screening visit at baseline. We considered women to be current users of HT at baseline if any HT use was reported on either of these two baseline questionnaires. Because we excluded any women without 3 months of HT washout before blood draw or VMS report, any HT use that eligible women reported referred to use before the 3-month HT washout period.

Statistical analysis

We tabulated demographic and medical history characteristics by VMS presence and severity at baseline. We also reported hemostatic parameter level medians and interquartile ranges by VMS presence and severity.

Using multiple linear regression, we estimated the association of VMS presence with each hemostatic parameter separately, adjusting for matching variables and potential confounders: age (linear), hysterectomy (yes/no), prevalent MI, stroke, and VT at baseline, nested case-control study group (control, MI case, stroke case, venous thromboembolism case, multiple case types), race/ethnicity (categorical), BMI (linear, kg/m^2), smoking status (categorical), and current HT use at baseline, which was at least 3 months before blood specimen collection and VMS report (yes/no). In multiple linear regression analyses, missing covariate data were imputed separately for each hemostatic parameter of interest using chained equations, which allows the specification of different analytic models for each variable requiring imputation[32,33]; linear regression analytic models were specified to impute missing BMI values (0.3% missing) and multinomial logistic regression models were specified to impute race/ethnicity (0.2% missing), and smoking status (1.6% missing) using Stata version 14.1 statistical software.[34] Predictors included in the chained equations were VMS presence, hysterectomy, prevalent MI, stroke, and VT, nested case-control study group, current HT use at baseline, and the hemostatic parameter of interest; 10 datasets were imputed.

Linear regression models estimated the adjusted average difference in hemostatic parameters associated with VMS presence (any) compared with absence. We also reported the percent difference in SD units for each hemostatic parameter level associated with VMS presence compared with absence, calculated by dividing the estimated beta by the hemostatic parameter's SD and multiplying by 100. Similar analyses evaluated the association between VMS severity (moderate or severe, compared with mild) and hemostatic parameter levels among women with baseline VMS, and tested for linear trend. Because we were evaluating 12 hemostatic parameters within group 1 in relation to each VMS exposure of interest, and because some hemostatic parameter levels were correlated, we used a P-min procedure to account for multiple comparisons.[35] In three separate sensitivity analyses, we restricted the population to (1) controls from the WHI-HT CVD Biomarker Studies, (2) women not currently using HT at baseline, before VMS report and blood draw, and (3) women who never used HT. An additional sensitivity analysis evaluated differences in hemostatic parameter levels associated with severe versus no VMS and tested for linear trend associated with greater VMS severity than no VMS.

We conducted several secondary analyses. First, we evaluated the presence of interaction between both continuous age and age in 10-year groups at WHI baseline with VMS presence for each of the 12 hemostatic parameters in hypothesis group 1 and evaluated significant interaction using a Bonferroni-adjusted alpha level of 0.0042 (0.05/12 hemostatic parameters in group 1). In addition, among women who reported any history of VMS before or at baseline, secondary analyses evaluated the relation between VMS timing (VMS onset in premenopause, in the same year as menopause, or in postmenopause) and VMS duration ($<$5, 5-9, 10-14, $\geq$15 y) and each hemostatic parameter, separately. We tested for a linear trend in the menopause status at VMS onset and hemostatic parameter relation, and separately, in the VMS duration and hemostatic parameter relation by modeling these exposures using grouped-linear terms. Secondary analyses of VMS duration and menopausal stage at VMS onset used a P-min procedure[35] to account for multiple comparisons.

RESULTS

Table 2 shows demographic and health characteristics of eligible WHI-HT CVD Biomarkers Study participants, by VMS presence and severity. At baseline, 67% of eligible participants reported no VMS in the prior 4 weeks (n = 1,430), 22% reported mild (n = 465), 9% reported moderate (n = 199), and 3% reported severe VMS (n = 54). At baseline,

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 2. *Participant characteristics at WHI baseline, by baseline VMS presence and severity*

			Any VMS (n = 718)		
	All participants (n = 2,148)	No VMS (n = 1,430)	Mild (n = 465)	Moderate (n = 199)	Severe (n = 54)
Age at enrollment, mean (SD), y	67.2 (6.6)	68.3 (6.2)	65.4 (6.7)	63.8 (7.1)	63.1 (6.7)
CVD nested case/control event status, n (%)					
CHD case	383 (17.8)	243 (17.0)	85 (18.3)	40 (20.1)	15 (27.8)
Stroke case	527 (24.5)	343 (24.0)	107 (23.0)	61 (30.7)	16 (29.6)
VT case	213 (9.9)	145 (10.1)	50 (10.8)	14 (7.0)	4 (7.4)
No event (control)	1,068 (49.7)	726 (50.8)	234 (50.3)	88 (44.2)	20 (37.0)
HT use at baseline, pre 3-month washout, n (%)					
Never	1,148 (53.5)	809 (56.6)	226 (48.6)	85 (42.7)	28 (51.9)
Past	959 (44.6)	599 (41.9)	223 (48.0)	111 (55.8)	26 (48.0)
Current	41 (1.9)	22 (1.5)	16 (3.4)	3 (1.5)	0 (0.0)
Years since menopause, n (%)					
<10 y	246 (11.5)	120 (8.4)	76 (16.3)	37 (18.6)	13 (24.1)
10-19 y	663 (30.9)	435 (30.4)	150 (32.3)	64 (32.2)	14 (25.9)
≥20 y	926 (43.1)	647 (45.2)	176 (37.9)	78 (39.2)	25 (46.3)
Unknown	313 (14.6)	228 (15.9)	63 (13.6)	20 (10.1)	2 (3.7)
Ethnicity, n (%)					
American Indian/Alaskan Native	13 (0.61)	6 (0.42)	6 (1.3)	0 (0.0)	1 (1.9)
Asian/Pacific Islander	23 (1.1)	21 (1.5)	2 (0.43)	0 (0.0)	0 (0.0)
Black	244 (11.4)	98 (6.9)	78 (16.8)	47 (23.6)	21 (38.9)
Hispanic	65 (3.0)	41 (2.9)	11 (2.4)	11 (5.5)	2 (3.7)
White not of Hispanic Origin	1,777 (82.7)	1,246 (87.1)	362 (77.9)	139 (69.9)	30 (55.6)
Unknown	26 (1.2)	18 (1.3)	6 (1.3)	2 (1.0)	0 (0.0)
Education, n (%)					
High school diploma or less	678 (31.6)	421 (29.4)	154 (33.1)	74 (37.2)	29 (53.7)
School after high school	847 (39.4)	556 (38.9)	189 (40.7)	83 (41.7)	19 (35.2)
College degree or higher	607 (28.3)	446 (31.2)	118 (25.4)	38 (19.1)	5 (9.3)
Unknown	16 (0.74)	7 (0.49)	4 (0.86)	4 (2.0)	1 (1.9)
Family history of VT, n (%)	194 (9.0)	137 (9.6)	32 (6.9)	15 (7.5)	10 (18.5)
Ever hypertension, n (%)	966 (45.0)	606 (42.4)	221 (47.5)	103 (51.8)	36 (66.7)
High cholesterol, n (%)	364 (17.0)	247 (17.3)	74 (15.9)	29 (14.6)	14 (25.9)
Ever diabetes, n (%)	267 (12.4)	157 (11.0)	69 (14.8)	28 (14.1)	13 (24.1)
BMI, mean (SD), kg/m^2	29.2 (5.8)	28.8 (5.7)	29.6 (5.6)	30.5 (6.5)	31.7 (4.6)
Smoking, n (%)					
Never	1,073 (50.0)	735 (51.4)	228 (49.0)	91 (45.7)	19 (35.2)
Past	808 (37.6)	538 (37.6)	173 (37.2)	76 (38.2)	21 (38.9)
Current	233 (10.9)	135 (9.4)	57 (12.3)	29 (14.6)	12 (22.2)
Unknown	34 (1.6)	22 (1.5)	7 (1.5)	3 (1.5)	2 (3.7)
Physical activity, n (%)					
<5 MET-h/wk	877 (40.8)	567 (39.7)	181 (38.9)	97 (48.7)	32 (59.3)
5 to <12 MET-h/wk	426 (19.8)	284 (19.9)	102 (21.9)	33 (16.6)	7 (13.0)
≥12 MET-h/wk	598 (27.8)	414 (29.0)	121 (26.0)	54 (27.1)	9 (16.7)
Unknown	247 (11.5)	165 (11.5)	61 (13.1)	15 (7.5)	6 (11.1)

BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; HT, hormone therapy; MET, metabolic equivalent of task; VMS, vasomotor symptoms; VT, venous thrombosis; WHI, Women's Health Initiative.

women were 67.2 years of age on average and 43% of women had experienced menopause ≥20 years earlier. Women with severe VMS were more likely to have experienced VMS <10 years earlier (24%), to be of black race (39%) and to be current smokers (22%) than women with no VMS or less severe VMS. On average, women with severe VMS also had a higher BMI (31.7 kg/m^2) than women with no VMS or less severe VMS. Table 3 shows unadjusted medians and interquartile ranges of hemostatic parameter levels by VMS presence and severity.

In adjusted analyses evaluating the association between VMS presence and severity and group 1 hemostatic parameters (Table 4), there was some suggestion that the presence of any VMS at baseline was associated with lower nAPCsr values than VMS absence ($\beta = -0.34$; 95% CI, −0.60 to −0.087; $P = 0.009$); however, this did not survive correction for multiple comparisons ($P = 0.073$). There was no evidence of an association between VMS presence or severity and any other group 1 hemostatic parameter levels. In sensitivity analyses (1) restricted to controls, (2) restricted to women who did not currently use HT at baseline, and (3) restricted to women who never used HT, results were similar. In sensitivity analyses that estimated differences in hemostatic parameter levels associated with severe versus no VMS, we did not detect any significant difference; linear tests for trend also suggested no statistically significant trend, after correcting for multiple comparisons. We found no evidence of an association of VMS presence or severity (Table 5) with any of the group 2 hemostatic parameter levels.

In secondary analyses that evaluated the interaction between age at WHI baseline and VMS presence for each of the 12 hemostatic parameters in group 1, there was some suggestion that the association between VMS presence and TG ETP levels may differ by continuous age (P value for

© 2016 The North American Menopause Society

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 3. *Unadjusted median measures and interquartile ranges of hemostatic parameter levels, by VMS presence and severity*

	All participants		No VMS		Any VMS					
					Mild VMS		Moderate VMS		Severe VMS	
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)
Group 1 hemostatic parameters										
Antithrombin, %	1,001	91 (20)	674	91 (19)	227	89 (20)	79	92 (22)	21	91 (16)
D-dimer, μg/mL	1,608	0.37 (0.41)	1,060	0.39 (0.41)	356	0.32 (0.37)	149	0.32 (0.39)	43	0.32 (0.35)
nAPCsr, ratio	1,601	3.4 (3.3)	1,073	3.4 (3.3)	349	3.1 (3.0)	141	3.6 (3.6)	38	3.3 (3.2)
PAI-1, ng/mL	1,500	41.5 (50.8)	989	39.6 (48.5)	331	45.2 (53.1)	140	49.5 (59.1)	40	51.3 (64.3)
Protein C, %	732	109 (27)	487	108 (26)	171	114 (32)	57	109 (22)	17	104 (22)
Protein S, total, %	730	108 (22)	487	107 (23)	170	108 (20)	56	111 (19)	17	115 (25)
Protein S, free, %	725	101 (26)	483	100 (26)	171	104 (26)	55	103 (32)	16	99 (33)
TFPI, total, ng/mL	1,810	86.5 (25.5)	1,222	86.6 (26.3)	388	85.7 (24.6)	159	87.8 (27.3)	41	81.9 (26.0)
TFPI, free, ng/mL	1,826	15.6 (8.7)	1,232	15.6 (8.7)	391	15.5 (8.3)	162	15.8 (11.0)	41	14.9 (9.5)
Thrombin generation										
Lag-time, min	1,602	2.3 (0.58)	1,075	2.3 (0.59)	348	2.3 (0.58)	141	2.3 (0.59)	38	2.4 (0.66)
ETP, nM.min	1,602	681 (176)	1,075	680 (182)	348	690 (170)	141	677 (148)	38	698 (175)
Peak, nM	1,602	219 (87)	1,076	219 (85)	348	220 (94)	141	212 (88)	38	235 (83)
Group 2 hemostatic parameters										
FVIIIc, %	1,612	110 (71)	1,061	110 (64)	357	107 (75)	151	110 (84)	43	115 (72)
Fibrinogen, mg/dL	1,612	317 (118)	1,061	315 (113)	357	307 (121)	151	327 (127)	43	345 (160)
PAP, nmol/L	1,499	4.4 (2.3)	988	4.5 (2.4)	331	4.3 (2.2)	140	4.1 (2.5)	40	3.7 (2.0)
Prothrombin, μg/mL	998	108 (26)	671	108 (26)	226	109 (27)	80	112 (27)	21	108 (29)
F1.2, nmol/L	1,489	1.3 (0.47)	984	1.3 (0.46)	328	1.3 (0.50)	139	1.3 (0.50)	38	1.3 (0.42)
TAFI, μg/mL	1,571	5.0 (2.4)	1,032	5.1 (2.4)	349	4.9 (2.4)	147	5.4 (2.6)	43	4.5 (1.7)
vWF, %	1,605	95 (58)	1,059	97 (59)	354	95 (60)	149	93 (51)	43	89 (59)

ETP, endogenous thrombin potential; F1.2, prothrombin fragment F 1.2; FVIIIc, factor VIII activity; IQR, interquartile range; nAPCsr, normalized activated protein C sensitivity ratio; PAI-1, plasminogen activator inhibitor-1 antigen; PAP, plasmin-alpha 2-antiplasmin complex; TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; VMS, vasomotor symptoms; vWF, von Willebrand factor.

interaction = 0.034); however, this association did not remain statistically significant after correcting for multiple comparisons, using the Bonferroni-adjusted alpha level of 0.0042. For other hemostatic parameter levels, we found no significant evidence of an interaction between VMS presence and continuous age or age in 10-year groups at WHI baseline.

In secondary analyses among women who ever had VMS, there were no associations between any of the hemostatic parameters with menopause status at VMS onset (Table 1, Supplemental Digital Content 1, http://links.lww.com/MENO/A197). There was also no evidence of an association between linear VMS duration and any hemostatic parameter, other than some suggestion of a linear trend between longer VMS duration and higher PAI-1 antigen levels ($P = 0.024$), which was nonsignificant after adjusting for multiple comparisons ($P = 0.21$) (Table 2, Supplemental Digital Content 1, http://links.lww.com/MENO/A197).

DISCUSSION

In this cross-sectional analysis among postmenopausal women not currently using HT, there was minimal evidence of an association between VMS presence, severity, duration, or timing and measures of hemostasis. These data are not supportive of the hypothesis that VMS is a marker of a more thrombotic profile among postmenopausal women.

VMS and hemostasis

The lack of an association between VMS presence and hemostatic parameter levels observed here contrasts with results of a longitudinal study of pre- and perimenopausal women. In Study of Women's Health Across the Nation (SWAN), participants aged 42 to 52 years at baseline (n = 3,199), more frequent hot flashes were associated with higher FVIIc and tPA-ag levels, which is suggestive of an association between greater VMS frequency and a more thrombotic hemostatic profile.[4] In this same study, PAI-1 and fibrinogen were also positively associated with more frequent VMS, but not after adjustment for confounders. Measures of tPA-ag and FVIIc were unavailable in the WHI-HT CVD Biomarkers Study for evaluation in relation to VMS.

Several differences between the WHI-HT and SWAN study populations may explain why the SWAN study identified a VMS-hemostatic parameter association and our WHI-HT analysis identified minimal evidence of such an association. The SWAN analysis evaluated associations between VMS and hemostatic parameters longitudinally across eight study visits over 8 years, among women with an average age of 46 years at cohort entry.[4] At each study visit, women reported VMS frequency in the 2 weeks prior. The average age of SWAN participants at cohort entry is over 20 years younger than that of women included in this WHI-HT analysis (mean = 67 y). Also, 54% of SWAN participants were premenopausal and 46% were early perimenopausal, in contrast to the predominantly late postmenopausal population included in this WHI-HT analysis. Therefore, VMS reported in SWAN were occurring in the context of the menopausal transition but in WHI-HT were occurring in postmenopause, among women for whom the transition into postmenopause occurred ≥20 years prior in 43% of participants.

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 4. *Adjusted associations between VMS presence and severity and primary hypothesis group hemostatic parameter levels*

		VMS presence (no VMS as reference)			VMS severity (mild VMS as reference)					
						Moderate		Severe		
Group 1 hemostatic parameters	Mean (SD)	n (no VMS/ VMS)	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	n (mild/ moderate/ severe)	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	*P* trend
Antithrombin, %	91 (16)	674/327	0 (−2 to 3)	1.7% (−12.2 to 15.6)	227/79/21	0 (−4 to 4)	2.5% (−22.3 to 27.3)	−6 (−13 to 2)	−35% (−80.0 to 9.7)	0.34
D-dimer, μg/mL	0.52 (0.57)	1,060/548	−0.040 (−0.10 to 0.021)	−7.0% (−17.7 to 3.8)	356/149/43	0.029 (−0.067 to 0.13)	5.1% (−11.7 to 21.9)	−0.090 (−0.25 to 0.071)	−15.8% (−44.0 to 12.5)	0.64
nAPCsr, ratio	3.9 (2.3)	1,074/527	−0.34 (−0.60 to −0.087)[b]	−14.8% (−26.0 to −3.8)	348/141/38	0.23 (−0.21 to 0.67)	10.0% (−9.0 to 29.3)	0.29 (−0.47 to 1.1)	12.6% (−20.5 to 45.9)	0.26
PAI-1, ng/mL	59.8 (56.2)	989/511	−1.1 (−7.3 to 5.3)	−1.79% (−13.0 to 9.4)	331/140/40	0.68 (−10.2 to 11.5)	1.2% (−18.1 to 20.5)	1.9 (−16.2 to 20.1)	3.4% (−28.9 to 35.8)	0.83
Protein C, %	111 (21)	487/245	1 (−2 to 5)	5.8% (−10.6 to 22.3)	171/57/17	1 (−6 to 7)	3.6% (−28.7 to 35.7)	−3 (−14 to 9)	−9.3% (−67.5 to 42.1)	0.84
Protein S, total, %	108 (17)	487/243	0 (−3 to 3)	0.14% (−16.3 to 16.6)	170/56/17	2 (−4 to 7)	10.4% (−21.0 to 41.2)	3 (−6 to 12)	15.6% (−36.9 to 68.1)	0.43
Protein S, free, %	102 (20)	483/242	2 (−2 to 5)	7.9% (−8.7 to 24.4)	171/55/16	1 (−5 to 7)	6.2% (−25.8 to 38.3)	−4 (−15 to 7)	−19.4% (−75.1 to 36.2)	0.79
TFPI, total, ng/mL	88.6 (20.7)	1,222/588	0.55 (−1.6 to 2.7)	2.6% (−7.6 to 12.9)	388/159/41	1.2 (−2.7 to 5.1)	5.7% (−13.1 to 24.4)	−1.9 (−8.7 to 5.0)	−9.0% (−41.9 to 24.0)	0.97
TFPI, free, ng/mL	18.9 (11.4)	1,232/594	0.98 (−0.18 to 2.1)	8.6% (−1.6 to 18.8)	391/162/41	0.044 (−2.2 to 2.3)	0.39% (−19.6 to 20.4)	−1.1 (−5.1 to 3.0)	−9.6% (−44.8 to 26.0)	0.73
TG lag-time, min	2.3 (0.56)	1,075/527	0.060 (0.000034 to 0.12)	10.7% (0.0060 to 21.4)	348/141/38	0.058 (−0.042 to 0.16)	10.3% (−7.5 to 28.1)	0.051 (−0.12 to 0.22)	9.1% (−21.7 to 39.9)	0.29
TG ETP, nM.min	709 (174)	1,075/527	0 (−19 to 18)	−0.12% (−10.8 to 10.6)	348/141/38	−23 (−59 to 12)	−13.4% (−33.9 to 7.1)	−20 (−82 to 42)	−11.6% (−47.2 to 23.9)	0.24
TG peak, nM	217 (69)	1,075/527	−1 (−8 to 7)	−1.4% (−12.2 to 9.5)	348/141/38	−10 (−24 to 4)	−14.2% (−34.2 to 5.7)	13 (−11 to 37)	18.5% (−15.9 to 53.0)	0.96

ETP, endogenous thrombin potential; nAPCsr, normalized activated protein C sensitivity ratio; PAI-1, plasminogen activator inhibitor-1 antigen; TFPI, Tissue factor pathway inhibitor; TG, thrombin generation; VMS, vasomotor symptoms; WHI, Women's Health Initiative.

[a]Adjusted for matching variables (age in years [continuous linear], hysterectomy status [dichotomous yes/no], prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent venous thromboembolism at WHI baseline, and case-control status [control, myocardial infarction case, stroke case, venous thromboembolism case]) and potential confounders including any current hormone therapy at WHI baseline, before VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), body mass index (continuous linear in kg/m^2), smoking status (categorical).

[b]$P = 0.009$. All other $P > 0.05$.

© 2016 The North American Menopause Society

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 5. *Adjusted associations between VMS presence and severity and secondary hypothesis group hemostatic parameter levels*

			VMS presence (no VMS as reference)		VMS severity (mild VMS as reference)					
						Moderate		Severe		
Group 2 hemostatic parameters	Mean (SD)	n (no VMS/ VMS)	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	n (mild/ moderate/ severe)	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	Adjusted average difference (95% CI)[a]	Adjusted average difference in %SD units (95% CI)[a]	*P* trend
FVIIIc, %	115 (55)	1,061/551	2 (−4 to 8)	4.2 (−6.6 to 14.9)	357/151/43	3 (−9 to 14)	5.4 (−15.6 to 26.5)	1 (−19 to 20)	1.3 (−34.2 to 36.7)	0.74
Fibrinogen, mg/dL	319 (88)	1,061/551	−4 (−13 to 5)	−4.7 (−15.3 to 5.8)	357/151/43	16 (−1 to 33)	18.3 (−0.76 to 37.3)	18 (−10 to 46)	20.5 (−11.6 to 52.5)	0.051
PAP, nmol/L	4.9 (2.4)	988/511	−0.074 (−0.33 to 0.19)	−3.1 (−14.0 to 7.8)	331/140/40	−0.075 (−0.50 to 0.35)	−3.1 (−21.0 to 14.7)	−0.51 (−1.2 to 2.0)	−21.5 (−51.4 to 8.4)	0.22
Prothrombin, μg/mL	110 (21)	671/327	2 (−1 to 4)	7.5 (−6.7 to 21.6)	226/80/21	0 (−5 to 6)	1.8 (−24.9 to 28.5)	−4 (−14 to 6)	−18.4 (−66.4 to 29.7)	0.66
F1.2, nmol/L	1.8 (1.8)	984/505	0.097 (−0.11 to 0.31)	5.3 (−6.1 to 16.8)	328/139/38	−0.026 (−0.43 to 0.38)	−1.4 (−23.7 to 20.9)	−0.16 (−0.86 to 0.54)	−8.9 (−47.2 to 29.4)	0.69
TAFI, μg/mL	5.1 (1.8)	1,032/539	−0.062 (−0.26 to 0.14)	−3.5 (−14.6 to 7.6)	349/147/43	0.25 (−0.084 to 0.57)	13.6 (−4.7 to 31.9)	−0.39 (−0.94 to 0.16)	−21.6 (−52.2 to 8.8)	0.83
vWF, %	106 (53)	1,059/546	0 (−5 to 6)	0.66 (−10.1 to 11.4)	354/149/43	−2 (−12 to 8)	−3.4 (−22.1 to 15.4)	−9 (−24.5 to 7.8)	−16.8 (−48.3 to 14.6)	0.34

F1.2, prothrombin fragment F1.2; FVIIIc, factor VIII activity; PAP, plasmin-alpha 2-antiplasmin complex; TAFI, thrombin activatable fibrinolysis inhibitor; VMS, vasomotor symptoms; vWF, von Willebrand factor; WHI, Women's Health Initiative.

[a]Adjusted for matching variables (age in years [continuous linear], hysterectomy status [dichotomous yes/no], prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent venous thromboembolism at WHI baseline, and case-control status [control, myocardial infarction case, stroke case, venous thromboembolism case]) and potential confounders including any current hormone therapy at WHI baseline, before VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), body mass index (continuous linear in kg/m^2), smoking status (categorical).

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

It has been previously proposed that VMS occurring during the menopausal transition and in postmenopause may be symptoms of different underlying physiologic processes,[6] with this hypothesis partially stemming from differential responses of VMS to HT treatment dependent on menopause status.[36] In a WHI Observational Study analysis of the relation between VMS and cardiovascular events, the timing of VMS onset seemed to be important, with perimenopausal VMS associated with a lower risk of stroke, total CVD events, and all-cause mortality, but postmenopausal VMS associated with a greater risk of CHD and all-cause mortality.[6] Differences in the etiology of VMS by menopause status are, however, poorly understood. Secondary analyses in our study found no compelling evidence of interaction between age at WHI baseline and VMS presence in relation to hemostatic parameters in hypothesis tier 1, but our study's sample size likely limited our ability to detect significant interaction.

Biologic plausibility

Whether VMS are associated with cardiovascular biomarkers and events is controversial,[37] with some studies suggesting positive associations between VMS and biomarkers associated with greater cardiovascular risk,[3,5,38,39] and others suggesting an association between VMS and markers associated with less cardiovascular risk.[40] One proposed biologic pathway potentially linking VMS to hemostatic parameters is via endothelial dysfunction, as measured by flow-mediated dilation.[5,41] VMS severity among women aged 45 to 58 years[5] and among early postmenopausal women aged 42 to 55 years[41] has been associated with impaired flow-mediated dilation.[5,41] Given that activated endothelial cells may express tissue factor, the extrinsic pathway of the coagulation cascade is activated by the exposure of tissue factor, and by the interaction of tissue factor with activated factor VII, endothelial dysfunction has the potential to impact coagulation.[42,43] The results of our analysis were, however, not supportive of an association of VMS presence or severity with a hemostatic profile in postmenopausal women, even when evaluating endothelial cell-related biomarkers such as TFPI and vWF.[44,45]

Strengths and limitations

A limitation of this cross-sectional study is that the temporality and directionality of the association between VMS and hemostatic parameter levels cannot be determined. In addition, although we adjusted regression analyses for known potential confounders, the possibility of residual confounding by unidentified confounders remains. Samples were stored before the measurement of hemostatic factors. A study of hemostatic parameter measurement stability over time, however, suggested little evidence of measurement degradation or inflation after storage.[5] Furthermore, any possible changes in hemostatic parameter levels measured in samples over time should not be differential by VMS presence or severity.

Our study evaluated a large number of hemostatic parameters in relation to VMS, which increases our possibility of type I error. To increase the threshold for statistical significance due to multiple comparisons, we, however, used a multiple comparisons-corrected *P* value estimated using a P-min procedure and divided hemostatic parameters into two groups a priori. Given the biologic relationships between our hemostatic parameter exposures of interest, the multiple comparisons-adjusted P-min may be overly conservative.

This analysis was conducted using a subset of WHI-HT participants for whom hemostatic parameter levels were already measured within the scope of a nested case-control study of CHD, stroke, and VT. We also conducted sensitivity analyses restricted to controls from the WHI-HT CVD Biomarkers study and results were similar to those conducted among the population including both cases and controls from the nested case-control study. The distribution of VMS severity in our study population is unlikely representative of the general US population of postmenopausal women, however, because the WHI-HT trials excluded women unwilling to discontinue HT use and who reported severe VMS that would make placebo treatment intolerable.[13]

VMS is a self-reported measure, which can be seen as both a weakness and strength. Although self-reported VMS are minimally invasive to collect and can be reported in-clinic, self-reported VMS are dependent on an individual's perception of discomfort. Such misclassification of ''true'' VMS is, however, likely to be nondifferential, which typically biases effect estimates toward the null. VMS presence and severity in the 4 weeks before the second baseline screening visit is unlikely to be impacted by substantial recall bias, due to the recency of the symptoms reported. In contrast, exposures of interest in secondary analyses, VMS duration and menopausal stage at VMS onset, were created using a retrospectively reported measure of age at VMS onset, which is susceptible to recall bias if women do not remember the age at which VMS began. Owing to this potential for recall bias, these are secondary analyses and results should be interpreted cautiously.

HT use at baseline, but at least 3 months before the reporting of VMS and to the collection of blood specimens, should not have impacted reports of VMS presence and severity. To address the possibility that the cessation of HT that occurred as part of this washout may have impacted reports of VMS presence and severity as well as hemostatic factor levels, due to an instability in hormone levels, we conducted a sensitivity analysis restricted to women who reported no current HT use at WHI baseline and results were similar. Secondary analyses of VMS duration and menopausal stage at VMS onset may have been impacted by HT use before baseline, however. Past HT use may have suppressed the onset of VMS symptoms or may have spurred the cessation of VMS, thereby delaying the reported age at first VMS or hurrying the age at last VMS from what it may have been without HT use before baseline.

Strengths of this study include the inclusion of more than 2,000 women with measured hemostatic parameter levels and the measurement and adjustment for important confounders related to both VMS and hemostatic parameter levels. In

© 2016 The North American Menopause Society

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

addition, although VMS data in our study was cross-sectional, primary analyses used VMS presence and severity information from the 4 weeks prior, which is unlikely to be strongly impacted by recall bias. Furthermore, because all women eligible for this study underwent a 3-month HT washout period before their report of VMS if HT had been previously used, women were not using HT at the time of VMS report, which provides VMS information unsuppressed by HT use. Finally, we report novel analyses using measures of hemostasis not previously evaluated in relation to VMS, including global measures such as TG.

CONCLUSIONS

In this cross-sectional analysis, there was no compelling evidence of an association between VMS presence, severity, timing, or duration with hemostatic parameter levels among postmenopausal women. Although results of this study may be limited in generalizability, they may be helpful in guiding hypotheses in future work. Given that VMS have been previously associated with hemostatic parameters among pre- and perimenopausal women, future studies that attempt to characterize potential differences and similarities in the etiology of VMS by menopausal stage and age would improve our understanding of VMS across the menopausal transition and into postmenopause.

Acknowledgments: We thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. Short List of WHI Investigators: Program Office, National Heart, Lung, and Blood Institute, Bethesda, MD: Jacques Rossouw, Shari Ludlam, Dale Burwen, Joan McGowan, Leslie Ford, and Nancy Geller. Clinical Coordinating Center, Fred Hutchinson Cancer Research Center, Seattle, WA: Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg. Investigators and Academic Centers: (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) JoAnn E. Manson; (MedStar Health Research Institute/Howard University, Washington, DC) Barbara V. Howard; (Stanford Prevention Research Center, Stanford, CA) Marcia L. Stefanick; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Arizona, Tucson/Phoenix, AZ) Cynthia A. Thomson; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker. Women's Health Initiative Memory Study: (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker. For a list of all the investigators who have contributed to WHI science, please visit: https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

REFERENCES

1. National Institutes of Health. National Institutes of Health State-of-the-Science Conference statement: management of menopause-related symptoms. *Ann Intern Med* 2005;142(12 pt 1):1003-1013.
2. Bechlioulis A, Naka KK, Kalantaridou SN, et al. Increased vascular inflammation in early menopausal women is associated with hot flush severity. *J Clin Endocrinol Metab* 2012;97:E760-E764.
3. Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, Powell LH, Matthews KA. Hot flashes and carotid intima media thickness among midlife women. *Menopause* 2011;18:352-358.
4. Thurston RC, El Khoudary SR, Sutton-Tyrrell K, et al. Are vasomotor symptoms associated with alterations in hemostatic and inflammatory markers? Findings from the Study of Women's Health Across the Nation. *Menopause* 2011;18:1044-1051.
5. Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, Hess R, Matthews KA. Hot flashes and subclinical cardiovascular disease: findings from the Study of Women's Health Across the Nation Heart study. *Circulation* 2008;118:1234-1240.
6. Szmuilowicz ED, Manson JE, Rossouw JE, et al. Vasomotor symptoms and cardiovascular events in postmenopausal women. *Menopause* 2011;18:603-610.
7. Guthrie JR, Dennerstein L, Hopper JL, Burger HG. Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstet Gynecol* 1996;88:437-442.
8. Randolph JF Jr, Sowers M, Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab* 2005;90:6106-6112.
9. Sowers MR, Matthews KA, Jannausch M, et al. Hemostatic factors and estrogen during the menopausal transition. *J Clin Endocrinol Metab* 2005;90:5942-5948.
10. Sowers MR, Jannausch M, Randolph JF, et al. Androgens are associated with hemostatic and inflammatory factors among women at the mid-life. *J Clin Endocrinol Metab* 2005;90:6064-6071.
11. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288:321-333.
12. Anderson GL, Manson J, Wallace R, et al. Implementation of the Women's Health Initiative study design. *Ann Epidemiol* 2003;13(9 suppl):S5-S17.
13. Hays J, Hunt JR, Hubbell FA, et al. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003;13(9 suppl):S18-S77.
14. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA* 2004;291:1701-1712.
15. Rossouw JE, Prentice RL, Manson JE, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* 2007;297:1465-1477.
16. Rossouw JE, Johnson KC, Pettinger M, et al. Tissue factor pathway inhibitor, activated protein C resistance, and risk of ischemic stroke due to postmenopausal hormone therapy. *Stroke* 2012;43:952-957.
17. Teede HJ, McGrath BP, Smolich JJ, et al. Postmenopausal hormone replacement therapy increases coagulation activity and fibrinolysis. *Arterioscler Thromb Vasc Biol* 2000;20:1404-1409.
18. Sidelmann JJ, Jespersen J, Andersen LF, Skouby SO. Hormone replacement therapy and hypercoagulability. Results from the Prospective Collaborative Danish Climacteric Study. *BJOG* 2003;110:541-547.
19. Post MS, van der Mooren MJ, van Baal WM, et al. Effects of low-dose oral and transdermal estrogen replacement therapy on hemostatic factors in healthy postmenopausal women: a randomized placebo-controlled study. *Am J Obstet Gynecol* 2003;189:1221-1227.
20. Post MS, Rosing J, Van Der Mooren MJ, et al. Increased resistance to activated protein C after short-term oral hormone replacement therapy in healthy post-menopausal women. *Br J Haematol* 2002;119:1017-1023.
21. Post MS, Hendriks DF, Van Der Mooren MJ, et al. Oral oestradiol/trimegestone replacement reduces procarboxypeptidase U (TAFI): a randomized, placebo-controlled, 12-week study in early postmenopausal women. *J Intern Med* 2002;251:245-251.
22. Post MS, Christella M, Thomassen LG, et al. Effect of oral and transdermal estrogen replacement therapy on hemostatic variables associated with venous thrombosis: a randomized, placebo-controlled study in postmenopausal women. *Arterioscler Thromb Vasc Biol* 2003;23:1116-1121.
23. Koh KK, Shin MS, Sakuma I, et al. Effects of conventional or lower doses of hormone replacement therapy in postmenopausal women. *Arterioscler Thromb Vasc Biol* 2004;24:1516-1521.
24. Koh KK, Ahn JY, Jin DK, et al. Significant differential effects of hormone therapy or tibolone on markers of cardiovascular disease in postmenopausal women: a randomized, double-blind, placebo-controlled, crossover study. *Arterioscler Thromb Vasc Biol* 2003;23:1889-1894.

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

25. Douketis JD, Gordon M, Johnston M, Julian JA, Adachi JR, Ginsberg JS. The effects of hormone replacement therapy on thrombin generation, fibrinolysis inhibition, and resistance to activated protein C: prospective cohort study and review of literature. *Thromb Res* 2000;99:25-34.
26. Cushman M, Psaty BM, Meilahn EN, Dobs AS, Kuller LH. Post-menopausal hormone therapy and concentrations of protein C and antithrombin in elderly women. *Br J Haematol* 2001;114:162-168.
27. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation* 1999; 100:717-722.
28. Cushman M. Effects of hormone replacement therapy and estrogen receptor modulators on markers of inflammation and coagulation. *Am J Cardiol* 2002;90:7F-10F.
29. Kooperberg C, Cushman M, Hsia J, et al. Can biomarkers identify women at increased stroke risk? The Women's Health Initiative Hormone Trials. *PLoS Clin Trials* 2007;2:e28.
30. Nicolaes GA, Thomassen MC, Tans G, Rosing J, Hemker HC. Effect of activated protein C on thrombin generation and on the thrombin potential in plasma of normal and APC-resistant individuals. *Blood Coagul Fibrinolysis* 1997;8:28-38.
31. Cushman M, Lemaitre RN, Kuller LH, et al. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol* 1999;19:493-498.
32. StataCorp. *Impute Missing Values Using Chained Equations. Stata Multiple-Imputation Reference Manual, Release 13*. College Station, TX: StataCorp LP; 2013.
33. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? *Int J Methods Psychiatr Res* 2011;20:40-49.
34. StataCorp. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP; 2013.
35. Westfall P, Young S. *Resampling-Based Multiple Testing: Examples and Methods for P-Value Adjustment*. New York, NY: Wiley; 1993.
36. Barnabei VM, Cochrane BB, Aragaki AK, et al. Menopausal symptoms and treatment-related effects of estrogen and progestin in the Women's Health Initiative. *Obstet Gynecol* 2005;105(5 pt 1):1063-1073.
37. Szmuilowicz ED, Manson JE. Menopausal vasomotor symptoms and cardiovascular disease. *Menopause* 2011;18:345-347.
38. Gast GC, Grobbee DE, Pop VJ, et al. Menopausal complaints are associated with cardiovascular risk factors. *Hypertension* 2008;51: 1492-1498.
39. Gerber LM, Sievert LL, Warren K, Pickering TG, Schwartz JE. Hot flashes are associated with increased ambulatory systolic blood pressure. *Menopause* 2007;14:308-315.
40. Allison MA, Manson JE, Aragaki A, et al. Vasomotor symptoms and coronary artery calcium in postmenopausal women. *Menopause* 2010;17:1136-1145.
41. Bechlioulis A, Kalantaridou SN, Naka KK, et al. Endothelial function, but not carotid intima-media thickness, is affected early in menopause and is associated with severity of hot flushes. *J Clin Endocrinol Metab* 2010;95:1199-1206.
42. Mackman N. New insights into the mechanisms of venous thrombosis. *J Clin Invest* 2012;122:2331-2336.
43. van Hinsbergh VW. Endothelium—role in regulation of coagulation and inflammation. *Semin Immunopathol* 2012;34:93-106.
44. Adams M. Tissue factor pathway inhibitor: new insights into an old inhibitor. *Semin Thromb Hemost* 2012;38:129-134.
45. Mannucci PM. von Willebrand factor: a marker of endothelial damage? *Arterioscler Thromb Vasc Biol* 1998;18:1359-1362.

 © 2016 The North American Menopause Society

Copyright @ 2017 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.