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C-Reactive Protein is Associated with Aortic Stiffness in a Cohort of African American and White Women Transitioning through Menopause

Genevieve A. Woodard, M.D, Ph.D.¹, Vinay G. Mehta, Ph.D., M.S.², Rachel H. Mackey, Ph.D.¹, Ping Tepper, Ph.D.¹, Sheryl F. Kelsey, Ph.D.¹, Anne B. Newman, M.D. M.P.H.¹, and Kim Sutton-Tyrrell, Dr.P.H.¹

¹Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA

²Department of Epidemiology, Merck and Co. Inc, Lansdale, PA

Abstract

Objective—Arterial stiffness is a marker of cardiovascular health. Arterial stiffness and C-reactive protein (CRP) are linked to cardiovascular outcomes. Increases in both inflammation and arterial stiffness are known to occur with menopause. The association between CRP and arterial stiffness is well accepted; however, no study has determined whether there are differences in this association by menopause status and race, independent of age.

Methods—The cross-sectional association between CRP and aortic pulse wave velocity (PWV), a validated measure of central arterial stiffening, was evaluated in 307 African American and White women enrolled in an ancillary study to the Study of Women's Health Across the Nation. Women were categorized into premenopausal or early perimenopausal (Pre/EP, n=185) and late perimenopausal or postmenopausal (LP/Post, n=122).

Results—Natural log transformed CRP was not associated with PWV in a linear regression model adjusted for age and cardiovascular risk factors ($\beta=15.9$, $p=0.11$). Moreover, models stratified by menopausal status showed a linear relationship between CRP and PWV among LP/Post women ($\beta=36.2$, $p=0.049$), but not for Pre/EP women ($\beta=5.9$, $p=0.61$). The menopausal status*logCRP and menopausal status*race interactions were significant in their respective models adjusted for age and risk factors ($p=0.03$ for both), however, when combined into one model, the two interactions were slightly attenuated ($p=0.063$ and 0.052 , respectively).

Conclusion—Menopause is strengthening the association between CRP and PWV, independent of age, and this effect seems to be stronger among African American women. This study provides a potential mechanism for the increased risk of cardiovascular disease among postmenopausal women.

Address for Correspondence, Genevieve A. Woodard, 6303 Morrowfield Ave, Pittsburgh, PA 15217, Telephone: 412-417-2810, Fax: 412-624-1431, woodardg@edc.pitt.edu.

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Disclosures

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Keywords

Arterial Stiffness; C-Reactive Protein; Menopause; Cardiovascular Disease; African American

Introduction

Aging of the vascular system involves both structural and functional changes in the arterial wall which leads to stiffening of the vessel ¹. Arterial stiffness is a contributor to the age-associated increase in cardiovascular morbidity and mortality ^{2, 3}. Aortic pulse wave velocity (PWV), as assessed by carotid-femoral PWV, is a simple, noninvasive, and highly reproducible measure of central arterial stiffness ⁴. In clinical and healthy populations, PWV is a predictor of cardiovascular and all-cause mortality ^{5–9}.

Inflammation is a major feature in the initiation and progression of atherosclerosis and subsequent cardiovascular disease (CVD) ^{10, 11}. Several inflammatory markers exist, but high sensitivity C-reactive protein (CRP) is clinically advantageous because it is well-validated, inexpensive, and widely available ¹². In prospective studies among healthy populations, CRP was associated with increased incidence of cardiovascular events: including death due to coronary heart disease, nonfatal myocardial infarction, peripheral vascular disease, and stroke ^{13–18}. Current guidelines recommend the use of CRP to further risk stratify asymptomatic adults, 60 years and older, who are at intermediate-risk based on a Framingham score ¹⁹. On the other hand, among low-risk adults less than 60 years of age, assessing CRP may not improve prediction beyond traditional CVD risk factors, suggesting that the association between CRP and CVD changes with age.

As women age and transition through menopause there is an increased incidence of CVD ²⁰. Reduced estrogen levels that accompany menopause may trigger an inflammatory response by increasing expression and secretion of cytokines ²¹. Decreased levels of estrogen may also increase arterial stiffening by altering the collagen-to-elastin ratio within the arterial wall or by inhibiting smooth muscle cell proliferation ^{22, 23}. Furthermore, these lower levels of estrogen are associated with increased arterial diameters, and leave the vasculature more vulnerable to increasing cardiovascular risk factors ^{24, 25}. Therefore, menopause may act as a trigger that strengthens the relationship between inflammation and arterial stiffness and thus provides a mechanism for the increased CVD seen as women age. Menopause may consequently be the mechanism for varying utility of CRP as a cardiovascular risk factor in adult women ¹⁹.

Several studies have reported a positive association between CRP and arterial stiffness, which may be due to a direct effect of CRP on nitric oxide and endothelial function ^{26–36}. There is also evidence that the relationship between CRP and arterial stiffness may be stronger among older adults, however, given the evidence that both hormones and race influence CRP levels, further evaluation is needed in these populations ^{37–39}. The objective of this study is to examine the effect of the menopausal transition on the age-associated strengthening between CRP and PWV, and the influence of race on this relationship, using data collected among 307 African-American and White women participating in the SWAN Heart Study, an ancillary study to the population-based SWAN study of the menopausal transition. This analysis will help to provide a rationale for utilizing CRP as a CVD marker and targeting middle-aged women to healthier lifestyle patterns aimed at reducing inflammation and arterial stiffening.

Methods

Study Population

The Study of Women's Health Across the Nation (SWAN) is a multi-site, multi-ethnic, longitudinal epidemiological study designed to examine the physical, psychological, and social changes that women experience during the menopausal transition. At baseline, SWAN recruited a total of 3,302 women between the ages of 42 and 52 from seven clinical sites: Boston, MA, Chicago, IL, Detroit, MI, Los Angeles, CA, Newark, NJ, Pittsburgh, PA, and Oakland, CA. In order to be eligible for the study, women were required to have an intact uterus, at least one menstrual period and no use of reproductive hormones in the previous 3 months. The site-specific institutional review boards of the participating institutions approved this study, and all women signed informed consent forms before participation.

Data from 608 women enrolled in SWAN Heart, an ancillary study to SWAN, were considered for this analysis. The ancillary project is a study of the natural history of subclinical atherosclerosis during the menopausal transition, involves 2 out of the 7 SWAN sites (Pittsburgh, PA and Chicago, IL) and included African American and White women. Baseline enrollment occurred between 2001 and 2003 (corresponding to the 4th – 7th annual SWAN visits). Women were excluded if they had a history of CVD (n=2), hysterectomy/oophorectomy (n=16), diabetes (n=1), hormone therapy (n=32) or missing menopausal status (n=17). Among the 356 women with available CRP data, an additional 78 women were excluded from this analysis due to poor waveforms, data transfer errors or outliers (PWV > 2000cm/sec), resulting in a final sample size of 307.

Aortic Pulse Wave Velocity

Aortic PWV was measured as the distance between the carotid and femoral recording sites divided by time delay between the carotid and femoral waveforms. All measurements were done at the University of Pittsburgh Ultrasound Research Laboratory, with high reproducibility, as previously been described in detail ⁴⁰. Briefly, PWV was assessed in the right carotid and femoral arteries by trained technicians using Doppler ultrasound.

Participants rested in a supine position for 30 minutes prior to measurement. The distance between the carotid and femoral recordings was measured using a standard tape measure over the surface of the body. The time delay was calculated as the foot-to-foot time differential between simultaneously collected carotid and femoral waveforms, using the R-wave of the electrocardiogram. Three runs were recorded for each participant and usable runs were averaged.

Covariate Measures

During annual visits, SWAN administered standard questionnaires, collected blood samples and obtained anthropometric and blood pressure measures. Menopausal status was assigned using self-reported bleeding histories. Premenopausal status was defined as having a menstrual period in the past three months with no change in cycle regularity in the past twelve months. Early perimenopausal status was defined as having a menstrual period in the past three months with some change in cycle regularity in the past twelve months. Late perimenopausal status was defined as not having a menstrual period in the past three months but having one in the past twelve months. Postmenopausal status was defined as not having a menstrual period in the past twelve months. Due to the small sample of premenopausal (n=25) and late perimenopausal (n=31) women, premenopausal (Pre) women were combined with early perimenopausal (EP) women and late perimenopausal (LP) women were combined with postmenopausal (Post) women and to form a dichotomous menopausal status variable (Pre/EP = 185 and LP/Post = 122) as previously reported ^{25, 41}. Self reported

smoking and alcohol use were also collected. Smoking status was dichotomized into nonsmoker versus current smoker. Participants were divided into three categories of average alcohol consumption: none to ≤ 1 drink per month, >1 drink per month to 1 drink per week and >2 drink week.

Fasting blood draws were collected for measurements of lipids, glucose, insulin and CRP. Blood samples were maintained at 4° C until separated and then frozen at -80° C and shipped on ice to Medical Research Laboratories, which is certified by the National Heart, Lung and Blood Institute of the Centers for Disease Control ⁴². Total cholesterol and triglycerides were analyzed with the Hitachi 747 analyzer whereas high-density lipoprotein (HDL) cholesterol was isolated using heparin-2M manganese chloride ^{43, 44}. Low-density lipoprotein cholesterol (LDL) was calculated using the Friedwald equation ⁴⁵. Plasma glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim, Indianapolis, In) and plasma insulin was measured using solid-phase radioimmunoassay procedure (DPC Coat-A-Count, Los Angeles, CA). CRP was assessed using the Behring Nephelometer II (hs-CRP on BN 100, Dade-Behring, Marburg, Germany).

Two blood pressure measurements were averaged on the right arm after five minutes of rest in a seated position. Weight was measured using a balance beam, digital scale, or portable scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured over undergarments or light clothing.

Statistical Methods

Descriptive statistics for continuous and categorical variables were calculated for the total group and across the dichotomous menopausal status variable via t-test, Wilcoxon rank sum test and chi-square. To approximate a normal distribution for CRP and glucose, a natural log transformation was performed (logCRP and logGlucose). Multivariable linear regression was used to test the association between PWV and logCRP while adjusting for potential confounders. Assumptions of linear regression (linearity between PWV and logCRP, independence, homoscedasticity, and normality of residuals) were tested to verify the validity of the model. Stepwise regression (slentry=0.99 and slstay= 0.1) was used in the initial development of the most parsimonious model. Variables of interest, such as age, site, race and menopausal status, were forced into the models. Interactions between menopausal status*logCRP, menopausal status*race, logCRP*race and menopausal status*logCRP*race were tested to determine if there were differences in the PWV and logCRP association by menopausal status and race. The association between logCRP tertiles (bottom tertile ≤ 0.10 mg/L, middle tertile >0.10 to 1.33 mg/L, top tertile >1.33 mg/L) and PWV was tested using ANCOVA and the three way interaction between menopausal status*logCRP*race to demonstrate differences in the estimated mean PWV by menopausal status and race, while adjusting for confounders. Statistical significance was assessed using a type one error rate of 0.05. All analyses used SAS 9.2.

Results

Population characteristics were stratified by menopausal status (Table 1). Among the 307 women, 36.8% (n=113) were African American and 39.7% (n=122) were LP/Post. The proportion of African Americans was similar according to menopausal status (p=0.32). The mean age for the total group was 50.5 ± 2.8 years. Total cholesterol, LDL cholesterol, triglyceride and glucose were all significantly greater in LP/Post women when compared to Pre/EP women. PWV was higher in LP/Post women, but was only borderline significant and after adjusting for age, PWV did not vary by menopausal status. CRP was also significantly higher among LP/Post women; however, adjustment for age attenuated this difference. CRP

had similar interquartile ranges for Pre/EP and LP/Post women and the 90th percentile values of CRP were 10.7, in Pre/EP women, and 9.5 mg/L in LP/Post women.

Women missing CRP or PWV data (n=233) were similar to women included in this analysis (n=307) except for the following: they had significantly ($p<0.05$) higher SBP (122.4 vs. 117.7mmHg), DBP (78.1 vs. 74.6mmHg), glucose (95.6 vs. 90.6), and were significantly younger (49.8 vs. 50.5 years).

Cross-sectional univariate and multivariable regression models were estimated between PWV and natural log transformed CRP (logCRP) (Table 2). Notably, for every one unit increase in logCRP there was a higher estimated PWV ($\beta=34.4$ cm/sec, $p<0.0001$), which was attenuated after adjustment for age, site, race, SBP, glucose and waist circumference ($\beta=15.9$ cm/sec, $p=0.11$). In stratified analyses, adjusted models demonstrated a 36.2 cm/sec higher PWV for every one unit of logCRP among LP/Post women ($p=0.049$), whereas Pre/EP women did not have a significant linear relationship between PWV and logCRP ($p=0.61$). Alcohol and smoking were not independent predictors of PWV in multivariable models, and including these two covariates in the model did not change the association between logCRP and PWV in the total or stratified analyses.

Interactions were tested to assess for possible differences in the association between PWV and CRP by menopausal status and race. The unadjusted interactions between menopausal status*logCRP ($p=0.31$), menopausal status*race ($p=0.061$), logCRP*race ($p=0.99$) and menopausal status*logCRP*race ($p=0.70$) were not significant, however, when both the menopausal status*logCRP and menopausal status*race were included in the same model, the race interaction was significant ($p=0.041$). After adjusting for age and other cardiovascular risk factors, the menopausal status*logCRP and menopausal status*race interactions were significant in their respective models ($p=0.033$ for both) and borderline significant when combined into a single model ($p=0.063$ and $p=0.052$, respectively). Unadjusted plots of PWV by logCRP, stratified by race, display the difference in the estimated regression lines for Pre/EP versus LP/Post women, illustrating the mentioned interactions (Figure 1).

To further demonstrate the influence of menopausal status and race on the PWV and CRP association, the fully adjusted regression model was stratified by menopausal status and race. Among African American women, logCRP was a borderline independent predictor of PWV in LP/Post ($\beta=53.9$, $p=0.072$), but not Pre/EP women ($\beta=-3.0$, $p=0.88$). There was not a significant logCRP and PWV association for either Pre/EP or LP/Post White women ($\beta=3.5$, $p=0.36$ and $\beta=16.5$, $p=0.47$, respectively).

The differences in the adjusted mean PWV estimates by menopausal status and race are presented in Figure 2. In general, African American women had higher PWV regardless of menopausal status or logCRP tertile compared to White women; however, this difference was more pronounced in the LP/Post group. Furthermore, LP/Post African American women in the top tertile of logCRP had the highest PWV on average (917 cm/sec).

Discussion

This study demonstrated a significant association between CRP and PWV in a cohort of women undergoing the menopausal transition. This association was significantly stronger in women who had reached late perimenopausal or postmenopausal status compared to those who were premenopausal or early perimenopausal, suggesting that menopause modified the association between CRP and PWV. Moreover, the significant interactions between menopausal status*CRP and menopausal status*race, indicated the PWV by CRP association varied not only by menopausal status, but also by race. The highest PWV values

were among African American women who were in the later stages of menopause and had the highest CRP levels, independent of age and known cardiovascular risk factors. These findings contribute to the current literature in that the effect of inflammation on arterial stiffness is not only age-associated, but also changes with the menopausal transition and varies by race. Specifically, postmenopausal African American women may benefit from CRP testing in a clinical setting for cardiovascular risk factor assessment, given the strong association with arterial stiffening above and beyond traditional risk factors.

Several studies have looked at the association between inflammation and arterial stiffness and found a positive association^{28–35}. Among these, three utilized a population based cohort; however, average ages were 47, 55 and 61 years, respectively, and men and women were not stratified in final models^{29, 30, 35}. Therefore, conclusions regarding associations across the menopausal transition cannot be made. Studies where no association between PWV and CRP was observed, included younger participants and Asian men and women, who are known to have lower levels of CRP^{46–48}. Studies relating CRP to hypertension have also shown the association appears stronger among older versus younger individuals^{49, 50}.

Several potential mechanisms could explain the relationship between inflammation and arterial stiffening. There is evidence of an increase in the expression and secretion of proinflammatory cytokines (IL-1, IL-6, and TNF- α) with a reduction in estradiol²¹. Activation of CRP by these cytokines could inhibit endothelium-dependent vasodilation and synthesis of nitric oxide. Furthermore, this inflammation could adversely impact the collagen-to-elastin ratio of the vessel thereby leading to arterial stiffening^{29, 30}. These inflammatory mediated vascular changes are likely occurring during a decline in estrogen as menopause progresses. Increases in vessel diameter due to menopausal-induced stiffening could lead to an increase in tensile stress which would make the vessel more susceptible to risk factors for increased inflammation^{24, 25}.

This analysis found a significant interaction between CRP and menopausal status in the combined African American and White group, suggesting menopause in strengthening the association between CRP and PWV independent of age. When stratified by race, this pattern remained among African American women, but was lost for White women. Studies exploring racial differences in inflammation have consistently found higher levels of CRP among African American participants compared to Whites^{48, 51–54}. This observation may be related to differences in adipose tissue distribution, mental health, socioeconomic status and discrimination^{51–53, 55}. There is question of whether CRP is a valuable predictor of CVD risk in women less than 60 years of age; however, studies suggest that using inflammatory markers may reclassify African American participants into higher risk categories more often compared to Whites^{19, 56}. Findings from this SWAN Heart analysis suggest inflammation may be related to arterial stiffening more strongly among women later in the menopausal transition and possibly even more so among African American women. This potential difference is important because as women age and transition through menopause the risk of cardiovascular events increases. Furthermore, higher CRP, and the interaction between inflammation, race, hormones and changes in the vasculature as women age may explain racial differences in cardiovascular risk.

Inherent in any cross-sectional design is the inability to determine causality. Thus, it cannot be ascertained whether increased inflammation results in increased arterial stiffening or vice versa. The associations observed in this study were independent of known confounders, such as age and cardiovascular risk factors, including alcohol and smoking^{57, 58}. Additionally, given the similar CRP ranges between Pre/EP and LP/Post women, it is less likely that the findings in this study were simply due to higher CRP levels among women later in the

menopausal transition. Hormone therapy users were excluded from the analyses due the small sample size and lack of power to detect an effect of hormone therapy on the association by menopausal status. Further research will need to explore the effects of hormone therapy on arterial stiffening and inflammation. Finally, the relatively small sample size when stratified by menopausal status and race limited the power to fully investigate the role of race in postmenopausal women (African American women by logCRP tertile respectively, n= 11, 16, 22, Figure 2). Larger sample sizes are needed to fully evaluate the racial differences in inflammation and arterial stiffening.

Conclusion

This study contributes to the current body of literature because it explores a potential mechanism explaining the increased CVD in women who are later in their menopausal transition. It is likely that menopause is associated with an increase in inflammation, which in turn results in increased stiffening of the vasculature and increased risk of cardiovascular outcomes. Moreover, racial differences in this association likely exist and therefore, the utility of CRP predicting CVD risk may need to be tailored to the individual woman based not only on age, but also menopausal status and race. There is evidence that inflammation and arterial stiffening can be tempered with medication, physical activity, and healthy lifestyles, therefore, it is likely that the menopause-associated increase in cardiovascular risk can be reduced through these strategies^{59–63}.

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Figure 1.
Pulse Wave Velocity by C-Reactive Protein Scatter Plots for Pre/EP versus LP/Post Women Stratified by Race ^a

^a Lines are unadjusted regression estimates; grey dots and solid grey line for Pre/EP women; black dots and solid black line for LP/Post women.

Abbreviations: Pre/EP = Premenopausal/Early Perimenopausal, LP/Post = Late Perimenopausal/Postmenopausal

Figure 2.

Adjusted Mean Pulse Wave Velocity Estimates by C-Reactive Protein Tertiles, Menopausal Status and Race a b c

^a logCRP tertiles: Bottom ≤ 0.10 mg/L, Middle >0.10 to 1.33 mg/L, Top tertile >1.33 mg/L

^b Estimated pulse wave velocity means generated using ANCOVA and the three way interaction between menopausal status*logCRP*race

^c Adjusted for site (Pittsburgh vs. Chicago), age (years), race (African American vs. White), SBP (mmHg), natural log transformed glucose (mg/dL) and waist circumference (cm)
Abbreviations: Pre/EP = Premenopausal/Early Perimenopausal, LP/Post = Late Perimenopausal/Postmenopausal

Table 1Baseline Characteristics of SWAN Heart Women by Menopausal Status (Mean $\pm$ Standard Deviation)

	Pre/EP (N=185)	LP/Post (N=122)	p-value ^c	Age Adjusted p-value ^c
Age (years)	49.3 $\pm$ 2.1	52.4 $\pm$ 2.6	<0.0001	--
African American ^b	34.6	40.2	0.32	0.32
Alcohol Consumption ^b			0.80	0.75
None to $\leq$ 1/month	38.2	41.3		
>1/month to 1/week	38.7	34.7		
>2 week	23.1	24.0		
Current Smoker ^b	14.8	14.7	0.98	0.31
BMI (kg/m ²)	29.1 $\pm$ 6.4	29.9 $\pm$ 6.5	0.30	0.81
Waist Circumference (cm)	88.4 $\pm$ 14.9	90.9 $\pm$ 14.0	0.15	0.57
SBP (mmHg)	116.2 $\pm$ 16.3	119.9 $\pm$ 17.6	0.06	0.51
DBP (mmHg)	74.0 $\pm$ 9.9	75.5 $\pm$ 8.6	0.16	0.21
Total Cholesterol (mg/dL)	193.6 $\pm$ 35.6	211.7 $\pm$ 39.6	<0.0001	<0.0001
LDL Cholesterol (mg/dL)	115.5 $\pm$ 30.7	126.7 $\pm$ 37.0	0.005	0.002
HDL Cholesterol (mg/dL)	55.9 $\pm$ 13.5	58.3 $\pm$ 13.9	0.14	0.38
Triglycerides (mg/dL) ^a	94.0 (71, 122)	108.5 (83, 156)	0.001	0.02
Glucose (mg/dL) ^a	86.0 (81, 93)	91.0 (84, 98)	0.003	0.02
C-Reactive Protein (mg/L) ^a	1.7 (0.6, 4.6)	2.8 (1.1, 6.9)	0.004	0.10
Log C-Reactive Protein Tertiles ^b			0.05	0.31
Top (>1.33 mg/L)	30.1	39.7		
Middle (>0.10 to 1.33 mg/L)	33.3	36.4		
Bottom ($\leq$ 0.10 mg/L)	36.6	24.0		
Pulse Wave Velocity (cm/sec)	790.4 $\pm$ 173.3	835.9 $\pm$ 230.9	0.06	0.97

^aValue is presented as median (IQR)^bValue is presented as percentage^cp-value for difference between Pre/EP versus LP/Post women

Abbreviations: Pre/EP = Premenopausal/Early Perimenopausal, LP/Post = Late Perimenopausal/Postmenopausal

Table 2
Estimated Association between Aortic Pulse Wave Velocity (cm/sec) and Natural Log Transformed C-Reactive Protein (mg/L)

Aortic Pulse Wave Velocity (Dependent Variable)	Total			Pre/EP			LP/Post		
	Beta Estimate (95% CI)	Standardized Beta	p-value	Beta Estimate (95% CI)	Standardized Beta	p-value	Beta Estimate (95% CI)	Standardized Beta	p-value
Unadjusted	34.4 (17.3, 51.4)	0.22	< 0.0001	26.1 (7.2, 45.0)	0.20	0.007	44.8 (10.7, 78.9)	0.23	0.011
Adjusted ^a	15.9 (-3.7, 35.4)	0.10	0.11	5.9 (-16.8, 28.8)	0.05	.61	36.2 (0.13, 72.3)	0.19	0.049

^a Multivariable linear regression model adjusted for site (Pittsburgh vs. Chicago), age (years), race (African American vs. White), SBP (mmHg), natural log transformed glucose (mg/dL) and waist circumference (cm).

Menopausal status by natural log transformed C-reactive protein interaction p-value = 0.31 (unadjusted) and **0.03** (adjusted) Standardized beta coefficients reported to ease comparison regardless of the independent variable's underlying scale of units.

Abbreviations: Pre/EP = Premenopausal/Early Perimenopausal, LP/Post = Late Perimenopausal/Postmenopausal