Am J Physiol Heart Circ Physiol 327: H1286–H1295, 2024.
First published October 4, 2024; doi:10.1152/ajpheart.00373.2024

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
HEART AND CIRCULATORY PHYSIOLOGY

RESEARCH ARTICLE

Vascular Contributions to Human Disease

Characterizing vascular and hormonal changes in women across the life span: a cross-sectional analysis

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Abstract

Vascular dysfunction, marked by lower endothelial function and increased aortic stiffness, is a nontraditional risk factor that precedes the development of cardiovascular disease (CVD). However, the age at which these changes in vascular function occur in women and the degree to which reproductive hormones mediate these changes has not been characterized. Women free from major disease were enrolled across the adult life span (aged 18–70 yr, $n = 140$). Endothelial function was assessed as flow-mediated dilation (FMD) of the brachial artery during reactive hyperemia using duplex ultrasound and expressed as percent dilation. Aortic stiffness was measured by carotid-femoral pulse wave velocity (cfPWV). Blood samples were obtained to quantify reproductive hormone concentration. Regression models determined age-related breakpoints and mediating factors between age and vascular outcomes. FMD declined with age with a breakpoint and steeper decline occurring at 47 yr of age. Thereafter, age was independently associated with lower FMD ($B = -0.13$, $P < 0.001$). cfPWV was relatively stable until a breakpoint at age 48, and age was independently associated with higher cfPWV thereafter ($B = 0.10$, $P < 0.001$). Path analysis revealed that the association between age and FMD was partially mediated by follicle-stimulating hormone ($ab_{ind} = 0.051$, $P = 0.01$) and progesterone ($ab_{ind} = 0.513$, $P < 0.001$) but not estradiol ($ab_{ind} = -0.004$, $P = 0.08$). No mediation was present for cfPWV. Age was associated with endothelial dysfunction and aortic stiffness in women beginning at 47 and 48 yr old, respectively, 3 to 4 yr before the average age of menopause. The association between age and endothelial dysfunction was explained in part by elevations in follicle-stimulating hormone and progesterone, but not declining estradiol.

NEW & NOTEWORTHY We demonstrate that the age at which endothelial function declines and aortic stiffness increases in healthy women is 47 and 48, respectively. The inflection point in flow-mediated dilation (FMD) is 6 yr earlier than previously reported, and the association between age and FMD was mediated by follicle-stimulating hormone (FSH) and progesterone (P_4) but not estradiol (E_2).

aging; arterial stiffness; endothelial function; estrogen; sex hormones

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death in women (1). Although the sharp increase in CVD incidence in postmenopausal women is well established, more recent studies show that rates of CVD are increasing in younger women, aged 35–54 yr (2). Moreover, the timeframe leading up to the final menstrual period is associated with an acceleration in CVD risk and represents a critical window for treatment and intervention (3). However, since traditional risk factors for CVD do not fully explain these increased rates of CVD in younger women (4, 5), other contributing factor(s) remain to be elucidated.

The development of vascular dysfunction, characterized by declining endothelial function and increasing aortic stiffness, is a nontraditional risk factor for CVD and biomarkers for cardiovascular health (6, 7). Endothelial dysfunction (8) and increased aortic stiffness (9) precede the development of

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Submitted 4 June 2024 / Revised 16 September 2024 / Accepted 30 September 2024

CVD. Well-established techniques to measure endothelial function and aortic stiffness noninvasively include flow-mediated dilation (FMD) and carotid-femoral pulse wave velocity (cfPWV), respectively (10). Indeed, numerous studies have demonstrated that endothelial function, as determined by FMD, declines with aging and may be one mechanism contributing to the increased risk of CVD (11–14). Seminal findings by Celermajer et al. (15) in 1994 demonstrated that FMD is preserved in women until ~53 yr old and then declines steeply at ~0.5%/yr. However, in the past 30 yr, the imaging quality of ultrasound and technology for analysis has improved. Guidelines to enhance rigor and reproducibility of FMD have recently been established and take into account critical factors for the protocol (fasting, occlusion cuff placement, occlusion duration, hormonal fluctuations, etc.) and analysis (continuous wall tracking) (16). Given advances in both imaging technology and protocol standardization, it is important to understand if the pattern of decline in endothelial function with aging is reproducible. Similarly, prior studies show that cfPWV appears relatively stable until 50 yr of age, after which advancing age is associated with ~2 m/s higher cfPWV per decade and may be exacerbated in women (10). However, stratification at 50 yr of age was determined a priori, and modeling age by decade may obscure a more precise breakpoint with aging (10). Thus, examining age as a continuous variable as opposed to in bins may offer a more precise characterization of the association between chronological and vascular aging in women.

The development of age-associated vascular dysfunction in women has been attributed to the decline in estradiol (E_2). Although changes in reproductive hormones such as E_2 and progesterone (P_4) occur with aging and impact vascular function, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) also change but are relatively understudied with regard to alterations in vascular function with advancing age in women. Accordingly, the purpose of this study was to characterize endothelial function, aortic stiffness, and reproductive hormones in healthy women to determine the age at which declines in these metrics of vascular function begin. We hypothesized that advancing age would be associated with lower FMD and elevated cfPWV beginning in the fifth decade (consistent with aforementioned studies on aging) (10, 15) and would be mediated by reproductive hormones independently of traditional CVD risk factors (i.e., blood pressure).

METHODS

All experimental protocols were approved by the University of Delaware Institutional Review Board, and the study conformed to the standards outlined in the Declaration of Helsinki. Data were pooled from protocols in the laboratory (MMW) where FMD was performed in women aged 18–70 yr (n = 140 women). Verbal and written consent was acquired voluntarily from all women before participation.

Screening

All women completed a standard medical history questionnaire to rule out prior history of disease; women were excluded if there was a previous history of cardiovascular, metabolic, neurologic, or endocrine disease. A menstrual cycle history was obtained to classify women based on the STRAW + 10 criteria (17). Only postmenopausal women who went through natural menopause and had not used hormone therapy for at least 6 mo were included. Height and body mass were measured in all women, and body mass index (BMI) was calculated. Resting seated blood pressure (BP) was measured in duplicate in all women using an automated oscillometric device. Women were excluded if average resting BP was >140/90 mmHg or BMI was >35 kg/m^2. Anyone using tobacco products within the past 6 mo was excluded. Half of the women (n = 71) reported exercising ≥3 days/wk. The majority of women (n = 80) reported using over-the-counter supplements like vitamins (vitamins C, D, and B_{12}) or a multivitamin or seasonal allergy medication. Women reported using prescription medication for the following: anxiety or depression (n = 13), pain or anti-inflammatories (n = 6), migraines (n = 3), low thyroid (n = 7), attention deficit disorder/attention-deficit/hyperactivity disorder (ADD/ADHD) or obsessive compulsive disorder (OCD) (n = 4), acne or skin conditions (n = 2), cholesterol management (statins, n = 2), asthma (n = 2), and irritable bowel syndrome (IBS) (n = 1). A fasted blood sample was obtained in a subset (n = 116; LabCorp) to analyze cholesterol and blood glucose.

Experimental Protocol

Women reported to the laboratory for testing after refraining from exercise for 24 h, alcohol and caffeine for 12 h, and food for at least 4 h. Participants refrained from using prescription medications the morning of the study visit and taking over-the-counter supplements for ~72 h before testing. Premenopausal women were tested during the early follicular phase of the menstrual cycle (n = 61) or the placebo phase of oral contraceptive use (n = 12). Early perimenopausal women (n = 10) were tested during the early follicular phase of the menstrual cycle. Late perimenopausal women (n = 14) and postmenopausal women (n = 43) were tested at a time of their convenience. Triplicate measures of blood pressure were taken with an oscillometric device after 10 min of supine quiet rest. A blood sample was obtained from an antecubital vein for the analysis of sex hormones (see blood analysis later). Approximately 20 min later, endothelial function was assessed via FMD in line with currently published guidelines (16) (with the exception of fasting for 4 h as opposed to 6 h as stated in the guidelines) and described by our laboratory (14, 18). Briefly, a longitudinal image of the brachial artery was acquired via ultrasound (either GE LogiqE or GE P5) and diameters were captured on a continuous beat-by-beat basis. A rapid inflatable cuff (Hokanson) was placed just distal to the olecranon process. After 1 min of baseline, the cuff was inflated to suprasystolic pressure (220 mmHg) for 5 min. Upon cuff release, images continued to be collected for an additional 2 min. Images acquired by GE LogiqE ultrasound were analyzed offline using commercial software (QUIPU); for images acquired by GE P5, custom edge-detection software was used (Labview as previously described by our group (14); 40% of the images). FMD was similar between the two systems used (LogiqE/QUIPU, 5.65 ± 2.77 vs. GE P5/Labview, 5.30 ± 2.46%, P = 0.45). FMD is presented as a

percent change from the baseline diameter and also scaled to the baseline diameter as previously described (19) to account for differences in baseline diameter with age.

We also assessed aortic stiffness via cfPWV after 30 min of supine rest using the SphygmoCor XCEL system (AtCor Medical) in a subset of women ($n = 99$). The device was calibrated annually per manufacturer instructions. A BP cuff was placed on the upper thigh and a tonometer was simultaneously placed on the ipsilateral carotid pulse site to record pressure waveforms at both femoral and carotid sites by the SphygmoCor software. Readings were validated using the software quality control indicators (stable waveform amplitude and foot-to-foot transit time). An average of two readings within 0.5 m/s was used. Pulse transit distance was measured as the distance between the top of the thigh cuff and the sternal notch minus the distance between carotid pulse site and sternal notch. cfPWV was calculated as pulse transit distance over pulse transit time (foot-to-foot time delay between carotid and femoral waveforms).

Blood Sample Analysis

Blood samples that were collected in the morning of the study visit ($n = 126$) were centrifuged, and serum was stored at −80°C until analyzed. Sex hormones were analyzed by RAD Fertility, member of the CCRM Fertility Network, using the ADVIA Centaur CP Immunoassay System (Siemens Healthcare Diagnostics, Tarrytown, NY), which uses chemiluminescent acridinium ester technology with assay ranges as follows: E_2, 10.7–3,000 pg/mL; P_4, 0.21–60 ng/mL; total testosterone, 0.07–15 ng/mL; FSH, 0.3–200 mIU/mL; and LH, 0.07–200 mIU/mL. The machine is calibrated per manufacturer instructions, and trilevel controls are run daily. Concentrations of E_2, P_4, FSH, and LH were log transferred when examining across age as the concentrations are not normally distributed.

Statistical Analyses

Analyses were conducted using IBM SPSS Statistics Ver. 29 (IBM, Armonk, NY) or GraphPad Prism v.10 (GraphPad, Boston, MA). Baseline characteristics were compared across decades using a one-way analysis of variance and post hoc pairwise comparisons were done using Tukey's HSD. Linear regression models were used to evaluate the association between age and FMD or cfPWV while controlling for a priori covariates [FMD ultrasound device/analysis software versions (for FMD models) and mean arterial pressure (for cfPWV models)]. Cardiometabolic indices were evaluated as covariates if they significantly differed among the age groups (Table 1). Covariates were individually added to a regression model with age to test for its influence on the association between age and FMD or cfPWV. If the inclusion of a covariate indicated meaningful confounding (change in estimate $\beta > 10\%$) (20) covariates would be retained. For FMD, covariates identified included total cholesterol, LDL, and glucose (Table 2), and for cfPWV, just total cholesterol (Table 3). Segmented regression was used to determine the location and change in slope that may occur with age for FMD, cfPWV, BP, and reproductive hormones (GraphPad, Prism, and SPSS). Data were fit using least squares regression and no weighting was applied. Constraints were placed on X_0 so that the break point must be greater than 18 (minimum age in the dataset). Data are plotted with 95% confidence intervals using Bootstrapping. Bivariate Pearson's correlation analyses were run to determine associations between sex hormones and cardiovascular outcome measures. A path analysis (MPlus v.8) was used to determine the degree to which reproductive hormone concentrations (log-transformed E_2, P_4, FSH, and LH) mediated the relation between age and vascular function (FMD, cfPWV). As hormone concentrations are log-transformed, path analysis results are expressed as standardized direct (g) and indirect (ab) effects and standard error. Statistical significance was set to $P < 0.05$ for all analyses.

RESULTS

Participant characteristics are shown in Table 1 for the overall sample ($n = 140$ women aged 18–70 yr) as well as for each decade of life. The racial/ethnic breakdown of women in the study was as follows: White ($n = 111$), Black ($n = 7$), Hispanic ($n = 3$), Asian ($n = 13$), Native American ($n = 1$), with three women reported more than one race and two electing not to report race/ethnicity. BMI was highest in the fourth decade of life ($P < 0.01$ vs. 20 s). Systolic BP was significantly greater in the sixth decade of life compared with women in their 20s, 30s, and 40s (all $P \leq 0.01$). Diastolic BP was higher in women in their 50s and 60s compared with women in their 30s (all $P \leq 0.01$). Total cholesterol was elevated across age groups, with significantly higher values starting in the fourth decade of life ($P < 0.05$). LDL cholesterol was higher in women in their 40s and 50s compared with women in their 20s (all $P \leq 0.01$), whereas HDL was higher in women in their 50s compared with women in their 20s and 30s ($P < 0.03$). Triglycerides were not significantly different across ages ($P \geq 0.25$). Plasma glucose was significantly greater in women in their 40s, 50s, and 60s compared with women in their 20s (all $P < 0.01$). E_2 and P_4 were lower in women in their 50s and 60s compared with women in their 40s, whereas FSH and LH were greater in women in their 40s, 50s, and 60s compared with women in their 20s (all $P < 0.05$). The baseline diameter of the brachial artery was greater in women in their 40s, 50s, and 60s compared with women in their 20s (all $P < 0.04$) and peak diameter was greater in women in their 60s compared with women in their 20s ($P < 0.01$). The shear area under the curve and peak shear rate following cuff deflation were not different across the decade ($P > 0.30$).

As shown in Fig. 1*A*, the age at which FMD begins to significantly decline was 47 yr old (95% CI, 37–57; $R^2 = 0.23$). There was a nonsignificant negative association between age and FMD in women before the age of 47 yr ($B = -0.06 \pm 0.04\%$/yr, $P = 0.12$). Thereafter, there was an accelerated decrease in FMD ($B = -0.13 \pm 0.04\%$/yr, $P < 0.001$) through the fifth and sixth decade of life. Similar results were observed when the baseline diameter was considered (i.e., scaled %FMD), thus only results with %FMD are reported. The association between advancing age and lower FMD was independent of total cholesterol, LDL, and fasted glucose (Table 2) suggesting the age-related increase was independent of traditional CVD risk factors. cfPWV was relatively stable until the age of 48 yr [$B = 0.02 \pm 0.01$ (m/s)/yr, $P = 0.12$] and then was greater with advancing age [$B = 0.10 \pm 0.03$ (m/s)/yr, $P < 0.001$] (Fig. 1*B*; 95% CI, 31–61; $R^2 = 0.32$). The

Table 1. *Participant characteristics*

	n	Overall Group [Range]	Age, yr 20s	30s	40s	50s	60s
N		140	38	16	32	36	18
Age, yr		43 ± 15 [18–70]	22 ± 3	34 ± 3	46 ± 3	54 ± 3	64 ± 3
BMI, kg/m^2		24 ± 3 [17–34]	23 ± 3	24 ± 3	26 ± 3*	24 ± 3	24 ± 2
SBP, mmHg		110 ± 11 [87–140]	109 ± 11	104 ± 6	107 ± 10	112 ± 11	119 ± 13*$#
DBP, mmHg		70 ± 8 [53–89]	70 ± 8	66 ± 5	68 ± 8	74 ± 7$	74 ± 8$
Total cholesterol, mg/dL	116	187 ± 36 [109–253]	166 ± 31	172 ± 27	194 ± 34*	208 ± 31*	195 ± 37*
HDL cholesterol, mg/dL	116	69 ± 17 [32–113]	63 ± 12	62 ± 15	68 ± 15	76 ± 17*$	70 ± 23
LDL cholesterol, mg/dL	116	103 ± 29 [46–178]	87 ± 26	96 ± 24	108 ± 30*	116 ± 26*	107 ± 29
Triglycerides, mg/dL	116	78 ± 34 [37–221]	79 ± 40	67 ± 18	82 ± 39	72 ± 22	90 ± 44
Plasma glucose, mg/dL	116	86 ± 9 [64–120]	81 ± 8	86 ± 7	88 ± 9*	89 ± 10*	91 ± 8*
Estradiol, pg/mL	126	43 ± 46 [10–420]	44 ± 19	52 ± 14	65 ± 76	28 ± 41#	19 ± 6#
Progesterone, ng/mL	126	0.58 ± 0.45 [0.14–4.0]	0.65 ± 0.33	0.53 ± 0.17	0.79 ± 0.74	0.47 ± 0.22#	0.26 ± 0.08*#
FSH, mIU/mL	126	41 ± 43 [4–148]	6.63 ± 1.88	6.94 ± 2.24	28.43 ± 33.72*	83.27 ± 40.10*#$	79.10 ± 32.57*#$
LH, mIU/mL	126	19 ± 19 [1.4–70.5]	4.72 ± 2.47	4.73 ± 2.83	17.67 ± 21.95*$	34.35 ± 17.07*#$	28.94 ± 13.09*$
Total testosterone, ng/dL	126	19 ± 8 [5–41]	24 ± 8	18 ± 7	17 ± 7*	18 ± 7*	16 ± 7*
Brachial artery diameter, mm		3.12 ± 0.43 [2.26–4.77]	2.91 ± 0.33	3.00 ± 0.37	3.17 ± 0.33*	3.20 ± 0.45*	3.44 ± 0.58*
Peak brachial artery diameter, mm		3.29 ± 0.43 [2.44–4.94]	3.11 ± 0.35	3.18 ± 0.34	3.35 ± 0.32	3.34 ± 0.47	3.55 ± 0.59*
Peak shear, s^{-1}		1,229 ± 567 [483–4,018]	1,384 ± 696	1,146 ± 290	1,112 ± 389	1,219 ± 702	1,192 ± 344
Shear rate, AUC		21,423 ± 10,922 [1,991–63,620]	20,296 ± 10,341	24,918 ± 8,324	22,669 ± 12,239	20,267 ± 10,789	20,958 ± 12,288

Values are means ± SD [ranges, min–max]. *$P < 0.05$ vs. *20s, $30s, and #40s. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AUC, area under the curve.

Table 2. *Linear regression models evaluating the influence of individual covariates on the association between age and FMD*

Model	B_{age}	b_{age}	*P* Value
Age (unadjusted)	−0.08 ± 0.01	−0.467	<0.001
Age plus FMD analysis software	−0.08 ± 0.01	−0.469	<0.001
Age plus BMI	−0.09 ± 0.01	−0.483	<0.001
Age plus total cholesterol	−0.09 ± 0.02	−0.516	<0.001
Age plus LDL cholesterol	−0.09 ± 0.02	−0.519	<0.001
Age plus glucose	−0.09 ± 0.02	−0.522	<0.001
Age plus MAP	−0.08 ± 0.01	−0.475	<0.001

Values are means ± SE and are model parameters in association between age and flow-mediated dilation (FMD) with individual adjustment for traditional cardiovascular risk factors; $n = 140$. BMI, body mass index; LDL, low-density lipoprotein; MAP, mean arterial pressure; *B*, unstandardized coefficient; *b*, standardized coefficient.

association between advancing age and higher cfPWV was independent of total cholesterol and mean arterial pressure (Table 3) indicating the age-related increase in PWV was independent of traditional CVD risk factors. Systolic (95% CI, 22–54; $R^2 = 0.18$) and diastolic BPs (95% CI, 23–51; $R^2 = 0.13$) were slightly lower until the age of ~44 and then were greater thereafter (Fig. 1, *C* and *D*).

Reproductive hormones across the adult life span are shown in Fig. 2. E_2 was slightly higher until age 44 where after it declines (Fig. 2*A*; 95% CI, 31–48; $R^2 = 0.28$). P_4 was relatively stable until age 48 and declined thereafter (Fig. 2*B*; 95% CI, 45–53; $R^2 = 0.27$). However, the age-related elevation in FSH and LH was observed in women starting at age 33 (FSH, 95% CI, 25–41; $R^2 = 0.69$; LH, 95% CI, 22–42; $R^2 = 0.53$; Fig. 2, *C* and *D*). Total testosterone declined until age 35 (95% CI, 24–50; $R^2 = 0.15$) and remained low thereafter (Fig. 2*E*).

Path analysis revealed that the effect of age on FMD was partially mediated by FSH ($ab_{ind} = 0.051 \pm 0.020$, $P = 0.01$) and P_4 ($ab_{ind} = 0.513 \pm 0.051$, $P < 0.001$) but not E_2 ($ab_{ind} = -0.004 \pm 0.019$, $P = 0.08$) (Fig. 3). The direct effect of age on FMD was significant ($g = 0.276 \pm 0.055$, $P < 0.001$), and reproductive hormones accounted for 67% of the effect. No mediation was present for cfPWV (all *P* indirect effects > 0.05). Table 4 illustrates the bivariate correlations between reproductive hormones and vascular outcomes (FMD, cfPWV, and blood pressure).

DISCUSSION

The primary findings of the current study are that the age at which FMD declines significantly in women is 47 independent of traditional CV risk factors, which is ~6 yr earlier than previous reports (15). Interestingly, FSH and P_4 partially mediate the effect between aging and FMD, whereas E_2 does not. The age at which cfPWV starts to increase in women is 48, similarly independent of traditional CV risk factors including mean arterial pressure, but not mediated by reproductive hormones. Finally, the age at which BP increases in women is ~44 yr old. Collectively, these novel findings demonstrate that nontraditional (FMD and cfPWV) and traditional (BP) CV risk factors begin to change well before the average age of menopause and that reproductive hormones other than E_2 play an important role in mediating the decline in endothelial function.

Endothelial Function

It has been 30 yr since Celermajer et al. (15) published their seminal findings demonstrating that FMD is preserved in women until ~53 yr of age and then rapidly declines. Although sex hormones were not measured, the authors attributed the decline in FMD to reductions in E_2 associated with menopause. Using updated technology for imaging and analyses, as well as adhering to guidelines to improve rigor and reproducibility of FMD (16), we show for the first time that the age at which FMD starts to decline in healthy women is 47 yr old, which is 6 yr earlier than reported by Celermajer et al. (15). After the age of 47 yr, advancing age was associated with 0.13% lower FMD/yr in women. Although this rate of decline is less than that (0.49%) reported by Celermajer et al. (15), over time this may cumulate to a meaningful increase in CVD risk before the average age of menopause (21). Previous findings suggest that for every 1% increase in FMD, there is a 9%–16% reduction in future risk of CV events (22, 23). Interestingly, the association between age and FMD was independent of traditional cardiovascular risk factors. Moreover, we measured reproductive hormones in the current study and demonstrated that while the inflection point for declines in E_2 and P_4 occurs in women in their mid to late 40s, FSH and LH start to become greater at age 33, ~10+ yr earlier than E_2 and P_4. Although E_2 is correlated with FMD, E_2 did not mediate the relation between age and FMD. Importantly, FSH and P_4 partially mediated the relation between age and FMD, suggesting that sex hormones other than E_2 are important in age-related changes in FMD and occur before menopause. It is interesting to note that in a study by Moreau et al. (12), the strongest correlate to FMD in their sample of women of various menopausal stages was FSH ($r = -0.57$; $P < 0.01$). This finding was reproduced in a smaller sample of menopausal women by Serviente and Witkowski (24) and is consistent with our findings in the current paper. Notably, though FSH and P_4 mediated, in part, the relation between advancing age and FMD, the direct independent association between age and FMD persisted. This finding indicates that hormonal milieu does not fully account for age-related changes in FMD across the life span in women. Furthermore, data from the Study of Women Across the Nation (SWAN) show that there are different trajectories of reproductive hormones across the menopause transition, which are associated with increased CVD risk (25, 26). Thus, the absolute concentration of reproductive hormones may have less of an impact

Table 3. *Linear regression models evaluating the influence of individual covariates on the association between age and PWV*

Model	B_{age}	b_{age}	*P* Value
Age (unadjusted)	0.05 ± 0.01	0.515	<0.001
Age plus BMI	0.05 ± 0.01	0.536	<0.001
Age plus total cholesterol	0.06 ± 0.01	0.603	<0.001
Age plus LDL cholesterol	0.06 ± 0.01	0.554	<0.001
Age plus glucose	0.05 ± 0.01	0.538	<0.001
Age plus MAP	0.05 ± 0.01	0.461	<0.001

Values are means ± SE and are model parameters in association between age and pulse wave velocity (PWV) with individual adjustment for traditional cardiovascular risk factors; $n = 99$. BMI, body mass index; LDL, low-density lipoprotein; MAP, mean arterial pressure; *B*, unstandardized coefficient; *b*, standardized coefficient.

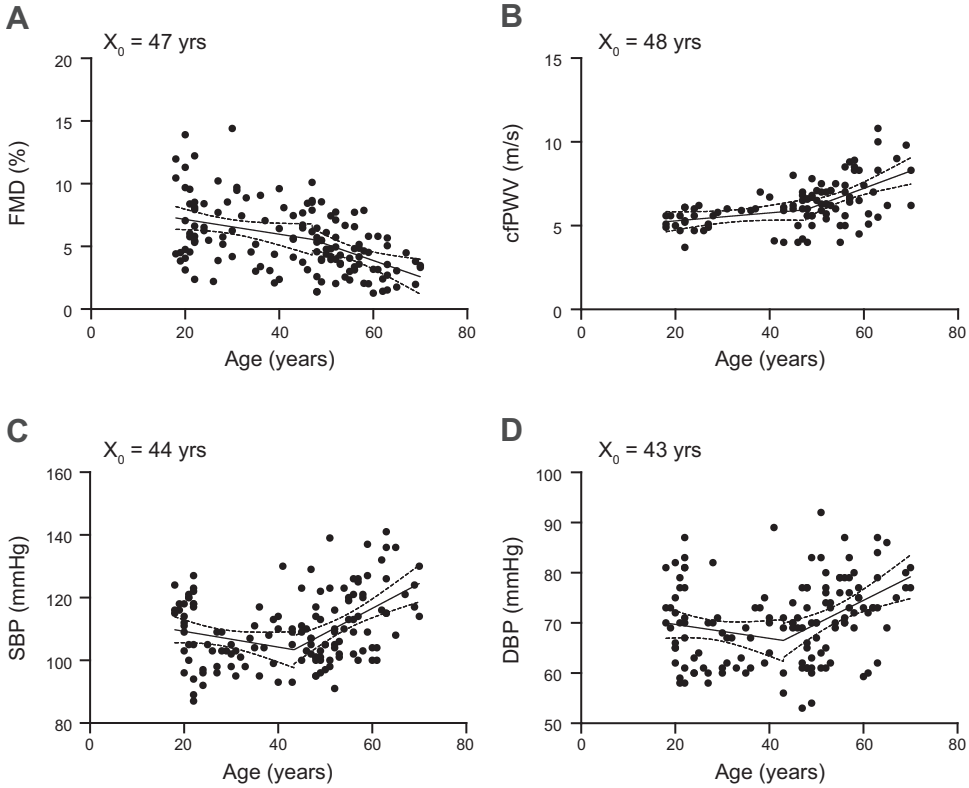

Figure 1. Segmented linear regressions showing association between age and vascular function in women aged 18–70 yr. *A*: flow-mediated dilation (%FMD; n = 140). *B*: carotid-femoral pulse wave velocity (cfPWV; n = 99). *C*: systolic blood pressure (SBP; n = 140). *D*: diastolic blood pressure (DBP; n = 140). n, number of subjects. Data were analyzed using a one-way analysis of variance and post hoc pairwise comparisons were done using Tukey's HSD.

on vascular function than the trajectory of these hormones during the menopausal transition. Nevertheless, together, our findings indicate that age-related declines in endothelial function in women occur earlier in the life span than previously determined, putatively driven in part by changes in FSH and P_4.

The mechanisms by which select sex hormones contribute to declines in FMD are not well understood. To date, the

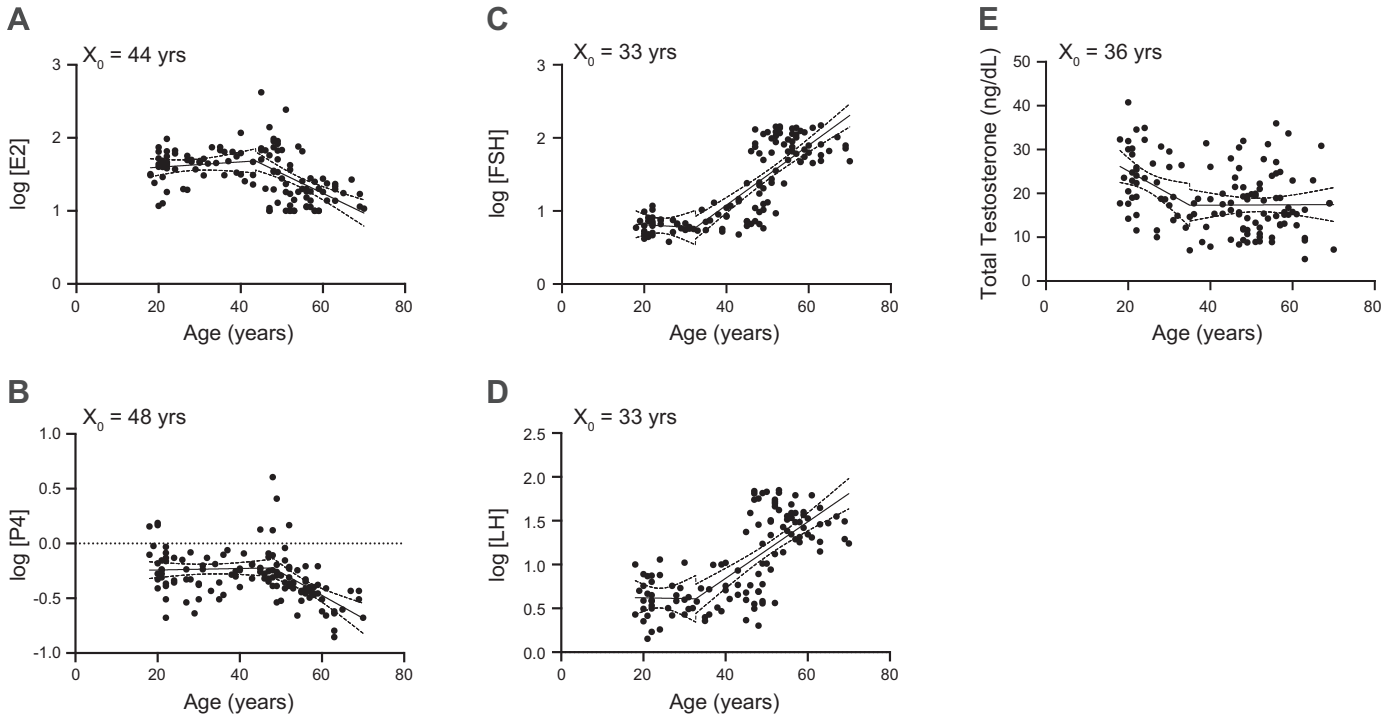

Figure 2. Segmented linear regressions showing the association between age and reproductive hormone concentrations (log transformed) in 126 women aged 18–70 yr. *A–E*: estradiol (E_2; *A*) progesterone (P_4; *B*), follicle-stimulating hormones (FSHs; *C*), luteinizing hormone (LH; *D*), and total testosterone (*E*). n, number of subjects. Data were analyzed using a one-way analysis of variance and post hoc pairwise comparisons were done using Tukey's HSD.

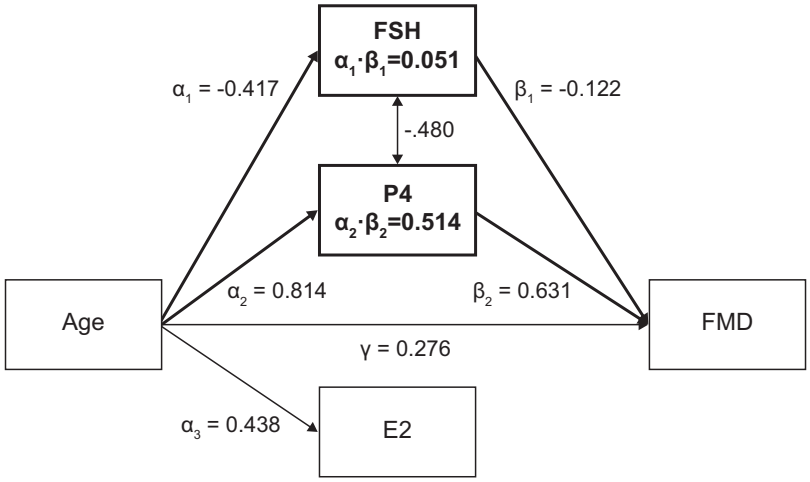

Figure 3. Path analysis evaluating the degree to which reproductive hormone concentrations mediated the association between age and flow-mediated dilation (FMD). Arrows denote significant direct effects. Significant mediation effects (FSH, $a_1b_1 = 0.051$; P_4, $a_2b_2 = 0.513$) are presented in bold. FSHs, follicle-stimulating hormones; P_4, progesterone; E_2, estradiol.

majority of studies have focused on the role of E_2 in contributing to the declines in endothelial function during menopause through various mechanisms such as oxidative stress (27). Data examining the effects of P_4 on vascular function are minimal and have largely been mixed, with some studies showing beneficial effects on nitric oxide production (28) but negative effects on FMD (29). The current research has been summarized in this review (30) but additional work is needed to understand the mechanisms of progesterone on the vasculature. Furthermore, the mechanisms underlying the association between FSH and declines in FMD remain unclear. Recent studies in mice suggest that FSH may stimulate VCAM-1 and NF-KB to promote atherosclerosis (31). This association between FSH and atherosclerotic progression has also been shown in women from SWAN, whereby women with the fastest/greatest rise in FSH during the menopausal transition have the highest carotid intima-media thickness (25). Future research is needed to understand the molecular mechanisms by which FSH contributes to vascular disease in women across the life span.

Aortic Stiffness

In the present study, the inflection point at which advancing age was associated with higher cfPWV in women was 48 yr old, after which cfPWV was higher by 0.1 (m/s)/yr. Although concomitant changes in distending pressure are linked to increasing cfPWV with advancing age in a bidirectional manner, the association between age and cfPWV was independent of mean arterial pressure. This age transition point is consistent with cross-sectional findings from the Framingham Heart Study, in which the slope between age decade and cfPWV was fourfold steeper in men and women ≥50 compared with <50 yr of age (10). Few studies have reported longitudinal cfPWV trajectories in women across this critical age transition. In a study by Asklepios et al., the 10-yr change in cfPWV was 0.95 m/s among women aged 35–40 yr old at enrollment and 1.12 m/s among women aged 50–55 yr at enrollment, suggestive of an age-related acceleration in aortic stiffening in accordance with the present findings (32). However, the study by Asklepios et al. did not account for the role of the menopause transition or associated changes in reproductive hormones, which may further augment increases in cfPWV that occur with chronological aging. Indeed, in the SWAN dataset, the progression of cfPWV was greatest among women who underwent the menopause transition compared with those who stayed premenopausal, or were already postmenopausal, during the 2-yr follow-up period (33). Interestingly, the pronounced increase in cfPWV during the menopause transition in their cohort was not explained by reproductive hormones E_2 and FSH (34). This is aligned with our findings suggesting that reproductive hormones do not statistically mediate the association between age and cfPWV. The potential mechanisms underlying accelerated aortic stiffness around the menopause transition in women may include changes in inflammation, oxidative stress, nitric oxide bioavailability, endothelin-1, and arterial wall structure and diameter (35). Further research is warranted to explicate the direct and indirect associations between chronological aging, reproductive factors, and aortic stiffness in women across the adult life span.

Table 4. *Correlations between sex hormones and vascular outcomes*

	FMD		PWV		SBP		DBP	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
log E_2	0.24	0.01	−0.23	0.03	−0.25	0.01	−0.16	0.07
log FSH	−0.36	<0.001	0.39	<0.001	0.27	<0.01	0.25	0.01
log P_4	0.36	<0.001	−0.22	0.04	−0.19	0.03	−0.07	0.46
log LH	−0.30	<0.01	0.31	<0.01	0.20	0.02	0.20	0.03
log TT	0.26	<0.01	−0.06	0.57	−0.05	0.60	0.05	0.55

Values are bivariate Pearson's correlation coefficients. FMD, flow-mediated dilation; PWV, pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; E_2, estradiol; FSH, follicle-stimulating hormone; P_4, progesterone; LH, luteinizing hormone; TT, total testosterone.

Blood Pressure and Cholesterol

BP increases with age and can rise exponentially during menopause. Cross-sectional studies in women have shown systolic BP to either be stable through the fourth decade and then increase or rise steadily across the life span (36, 37). Our data indicate that BP is relatively stable or decreases slightly until the early to mid-40s and then begins to rise thereafter. Specifically, systolic BP increases by ~1 mmHg (slope 0.81) each year after the age of 44 yr, whereas diastolic BP increases by ~0.5 mmHg/yr after the age of 43 yr. This may be related to activation of the sympathetic nervous system, as BP and muscle sympathetic nerve activity become correlated in women after the age of 40 yr (36, 37). Alternatively, longitudinal data from the SWAN study indicate that midlife BP trajectories are predicted by the age of menopause and FSH but not E_2 (26). Although the SWAN study reported high FSH levels are associated with low SBP, we observed a significant positive correlation between FSH and systolic and diastolic BP in the present cross-sectional study. Additional research is needed to understand the molecular mechanisms underlying this relation between FSH and BP.

The prevalence of dyslipidemia increases with age and menopause, and there is also a rise in fasted plasma glucose. We observed that in the fourth decade of life, women exhibit elevations in total cholesterol, and LDL cholesterol, along with a higher BMI and fasted plasma glucose. These findings are consistent with those of Wu et al. (38) that showed total and LDL cholesterol increase 5 yr before the final menstrual period. Interestingly, higher FSH predicted a greater increase in blood lipids (38). A positive association between FSH and total cholesterol and LDL cholesterol was also observed in a large study of postmenopausal women (39). However, the link between higher FSH, high total and LDL cholesterol, and vascular endothelial dysfunction remains unclear. In the present study, total cholesterol, LDL, BMI, and fasting glucose were not related to FMD and cfPWV, and the associations between age and FMD or cfPWV were independent of these factors. Taken together, these results suggest that age-related prevalence of these traditional cardiovascular risk factors does not fully explain declines in endothelial function or augmented aortic stiffness with aging.

Strengths and Limitations

The present study is strengthened by rigorous characterization of nontraditional vascular risk factors (i.e., FMD and cfPWV) across the adult life span in women and consideration for sex hormones. Nevertheless, results should be interpreted in the context of several limitations. Given the cross-sectional nature of the study, causality cannot be inferred. Longitudinal studies are needed to prospectively assess changes in vascular function and putative mechanisms across the reproductive life span and menopausal transition in women. We are also not able to determine the influence of other factors such as diet or exercise on this earlier inflection point for the age at which FMD and PWV decline. It is interesting to note that recent American Heart Association statistics show that physical inactivity has decreased in the past ~20 yr, and more Americans are reporting meeting aerobic exercise guidelines (40). One could speculate that this would lead to preserved or sustained vascular health across aging and possibly shift the decline in vascular health to an older age. Although our data indicate these age-related declines are occurring earlier, the rate of decline thereafter is also less steep than in previous reports. Other plausible reasons we may have observed an earlier decline in endothelial function may be related to insufficient sleep and the increased prevalence of depression, all of which can contribute to endothelial dysfunction (41–43). Second, we were not powered to characterize the joint effects of menopausal stage and age on declines in endothelial function, as only 17% of our sample were perimenopausal, though the main effect of age persisted with adjustment for menopause stage in our analysis. Previously, Moreau et al. (12) showed endothelial dysfunction occurs early in the menopausal transition, as evident by reductions in FMD in early perimenopausal women. In the current paper, we sought to determine the age at which endothelial function declines using updated technology and methodology compared with previous findings by Celermajer et al. (15) 30 yr ago. Further work is needed to consider the interacting and independent effects of reproductive and biological aging on the vasculature. The women in our study were also majority White, so we are not able to determine the impact of race or ethnicity on the age-related effects on vascular function. Finally, the study sample was free from overt disease by design, to establish the independent effect of age. Future research is warranted to extend these findings in the context of overt cardiovascular, metabolic, or endocrine conditions.

Conclusions

In conclusion, we demonstrate that the age at which endothelial function declines and aortic stiffness increases in healthy women is 47 and 48, respectively. The inflection point in FMD is 6 yr earlier than previously reported (15) and precedes the average age of menopause (51 yr old) (44) by 3 to 4 yr. Furthermore, the association between age and FMD is mediated by FSH and P_4, but not E_2. These novel findings demonstrate that nontraditional (FMD and cfPWV) and traditional (BP) CV risk factors begin to change well before the average age of menopause and not fully explained by changes in reproductive hormones.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

We thank our research nurses Carolyn Haines and Wendy Nichols; the University of Delaware Nurse Managed Primary Care Center for assistance with data collection; RAD Fertility, a member of the CCRM Fertility Network, for reproductive hormone analysis; and Josh Hobson and Katherine Masso for recruiting participants. We are grateful to our participants for their time.

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GRANTS

This research was supported by National Institutes of Health Grants R01-HL146558 (to M.M.W.), P20-GM113125 (to D.G.E.) via the Center of Biomedical Research Excellence in Cardiovascular Health, and U54-AG062319 (to K.L.M.).

DISCLOSURES

Megan M. Wenner is a paid consultant for Orchestra BioMed.

AUTHOR CONTRIBUTIONS

M.M.W., L.S., A.K., S.J.M., and K.L.M. conceived and designed research; M.M.W., N.S., L.S., A.K., K.H., A.D.V., A.S., and S.J.M. performed experiments; M.M.W., R.T.P., V.R.N., L.D., N.S., A.K., A.D.V., and A.S. analyzed data; M.M.W., N.S., L.S., A.K., K.H., A.D.V., A.S., S.J.M., D.G.E., R.T.P., V.R.N., L.D., and K.L.M. interpreted results of experiments; M.M.W., R.T.P., and V.R.N. prepared figures; M.M.W. and V.R.N. drafted manuscript; M.M.W., N.S., L.S., A.K., K.H., A.D.V., A.S., S.J.M., D.G.E., R.T.P., V.R.N., L.D., and K.L.M. edited and revised manuscript; M.M.W., N.S., L.S., A.K., K.H., A.D.V., A.S., S.J.M., D.G.E., R.T.P., V.R.N., L.D., and K.L.M. approved final version of manuscript.

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