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RESEARCH ARTICLE | *Integrative Cardiovascular Physiology and Pathophysiology*

Blood pressure predicts endothelial function and the effects of ethinyl estradiol exposure in young women

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Adler TE, Usselman CW, Takamata A, Stachenfeld NS. Blood pressure predicts endothelial function and the effects of ethinyl estradiol exposure in young women. *Am J Physiol Heart Circ Physiol* 315: H925–H933, 2018. First published June 15, 2018; doi:10.1152/ajpheart.00188.2018.—Hypertension, obesity, and endothelial function predict cardiovascular disease in women, and these factors are interrelated. We hypothesized that hypertension and obesity are associated with endothelial dysfunction in young women and that short-term ethinyl estradiol exposure mitigates this dysfunction. We examined flow-mediated dilation (FMD) responses before and during 7 days of oral ethinyl estradiol (30 μg/day) in 19 women (25 ± 5, 18–35 yr). We divided our sample into two groups based on two criteria: blood pressure and obesity. Women were divided into normal blood pressure (NBP; mean arterial pressure range: 78–91 mmHg, $n = 7$) and high blood pressure (HBP; mean arterial pressure range: 95–113 mmHg, $n = 9$) groups. We also stratified our subjects by body composition (lean: 18–31%, $n = 8$; obese: 38–59%, $n = 9$). We evaluated brachial FMD after two distinct shear stress stimuli: occlusion alone and occlusion with ischemic handgrip exercise. Obesity was unrelated to both FMD responses. Before ethinyl estradiol administration, the HBP group had blunted ischemic exercise responses relative to the NBP group (8.0 ± 3.5 vs. 12.3 ± 3.2%, respectively, $P = 0.05$). However, during ethinyl estradiol administration, ischemic exercise responses increased in the HBP group (12.8 ± 6.1%, $P = 0.04$) but decreased in the NBP group (5.6 ± 2.4%, $P = 0.01$). Standard FMD did not reveal differences between groups. In summary, *1*) moderate HBP predicted endothelial impairment, *2*) ethinyl estradiol administration had divergent effects on FMD in women with NBP versus HBP, and *3*) enhanced FMD (ischemic handgrip exercise) revealed differences in endothelial function, whereas standard FMD (occlusion alone) did not.

NEW & NOTEWORTHY We are the first to show that mild hypertension is a stronger predictor of endothelial dysfunction than obesity in healthy women without overt cardiovascular dysfunction. Importantly, the standard 5-min flow-mediated vasodilation stimulus did not detect endothelial dysfunction in our healthy population; only an enhanced ischemic handgrip exercise shear stress stimulus detected endothelial impairment. Estradiol administration increased flow-mediated dilation in women with high blood pressure, so it may be a therapeutic intervention to improve endothelial function.

blood pressure; ethinyl estradiol; flow-mediated dilation; flow-mediated vasodilation; obesity

INTRODUCTION

The vascular endothelium regulates blood flow by secreting vasoactive agents such as nitric oxide (NO) and prostaglandins in response to stimuli including hormones and physical perturbation such as shear stress (34). Endothelial dysfunction, an early step in the progression of cardiovascular disease, is a state in which the endothelium fails to respond effectively to these stimuli, resulting in loss of vascular homeostasis and excessive vasoconstriction (34). Endothelial function is assessed noninvasively in humans with flow-mediated dilation (FMD) (6). The standard protocol involves determining FMD in response to a 5-min occlusion of the brachial artery (41). Immediately after occlusion, blood flow rapidly increases to the ischemic tissue, eliciting shear stress and stimulating NO release from endothelial cells, causing vasodilation (41). Impaired vasodilation reflects low NO bioavailability and suggests cardiovascular dysfunction (17, 41).

Shear stress stimuli varying in time and intensity are associated with distinct transduction mechanisms and responses (11, 25, 32, 39), so testing responses to diverse shear stress stimuli yields a comprehensive understanding of endothelial function. For instance, FMD responses to a handgrip-induced shear stress stimulus reveal endothelial impairment in young obese men (39) and young smokers (11) relative to control subjects, whereas standard reactive hyperemia (RH) responses are unaffected by smoking (11, 39). Contrasting shear stress stimuli and FMD responses may reflect the functionality of a range of vasodilators beyond NO and therefore provide independent but complementary prognostic information regarding endothelial function (13).

Obesity and hypertension are interrelated risk factors for cardiovascular disease, and their effects are sex specific (9). In premenopausal women, obesity is linked with increased risk for hypertension (26) and may be associated with endothelial dysfunction (4, 8, 27, 39), but studies are inconsistent (3, 23, 37). Although hypertension and FMD are inversely related (16, 29), no study has investigated the direct relationship between mild hypertension and FMD in premenopausal women. The changes to the American Heart Association guidelines for diagnosis of stage 1 hypertension, defined as systolic blood pressure (SBP) between 130 and 139 mmHg or diastolic blood pressure (DBP) between 80 and 89 mmHg, have underlined the importance of understanding the effects of moderate increases in blood pressure on endothelial function as both predict future cardiovascular morbidity (45).

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Estrogen exposure increases endothelium-dependent vasodilation in lean, healthy women (44). Activation of estrogen receptors-α and -β in endothelial cells elicits endothelial NO synthase (eNOS) production and expression of genes encoding eNOS (5). Exposure to exogenous ethinyl estradiol (EE) increases FMD in lean women before (24, 42) and after menopause (15). However, the effects of EE administration on FMD have not been examined in premenopausal women who are obese or hypertensive. This is an especially relevant question given that obese premenopausal women may lose estrogen-associated cardioprotection (9). The goal of the present study was to evaluate the effects of obesity and mild hypertension on endothelial function in premenopausal women. We hypothesized that obesity and mild hypertension are associated with attenuated FMD responses to both standard and enhanced shear stress stimuli. We further hypothesized that short-term low-dose EE exposure would improve FMD responses under standard and enhanced shear stress stimuli.

METHODS

Subjects

Nineteen healthy premenopausal women voluntarily provided written informed consent to participate. All women had regular menstrual cycles (22–32 days), and none were using hormonal contraceptives. Participants were nonsmokers, (25 ± 5 yr, 18–35 yr) with no reported history of cardiovascular, metabolic, or gynecological disease. Subjects were sedentary or recreationally active. Body composition was estimated with a Bod Pod (Cosmed, Rome, Italy) (10). The study conformed with guidelines contained in the Declaration of Helsinki and was approved by the Human Investigation Committee of the Yale School of Medicine.

Experimental Design

Before the first visit, subjects attended a familiarization visit to practice the protocols and experience most aspects of instrumentation. We tested FMD responses twice: before ("pre-EE") and on the last day of 7-day EE exposure ("EE"). Subjects were tested within *days 1–7* of the follicular phase of their menstrual cycle, when endogenous hormone production is low. For 7 days before the second test, subjects administered 30 μg/day oral EE, with the final dose of EE taken the night before the EE test date. Upon arrival, women were well hydrated (urine specific gravity < 1.020) and had abstained from food, alcohol, caffeine, and strenuous exercise for 12 h. Testing was performed in the morning in a dimly lit environmental chamber (25°C).

Experimental Protocols

Before testing, subjects rested supine for at least 15 min and were instrumented for beat-to-beat blood pressure (Finometer Pro, Finapres, Amsterdam, The Netherlands). Baseline blood pressure was obtained using a manual sphygmomanometer; the mean value of three measurements was used to calibrate the Finometer. After baseline measures and at the beginning of each visit, subjects performed three isometric maximal voluntary contractions using a handgrip dynamometer, the highest of which was taken as the maximum voluntary contraction.

Flow-mediated vasodilation. Subjects lay supine with their right arm extended. Continuous recordings of longitudinal brachial artery images and blood flow velocity were obtained simultaneously using the Sonoscope S2 ultrasound imaging system with a 6.0-MHz linear array probe (International Diagnostic Devices, Las Vegas, NV). A probe holder secured the ultrasound transducer in place at a location on the brachial artery distal to the antecubital fossa to ensure that the same vessel segment was measured throughout each test date. The ultrasound display was captured continuously using a VGA to S-Video converter and recorded in AVI format on an external computer (StarTech, London, ON, Canada).

In each protocol, brachial artery measures were recorded at baseline for 3 min. Forearm ischemia was then induced by rapidly inflating a blood pressure cuff to suprasystolic pressure (i.e., ≥160–180 mmHg). Subjects completed two protocols: a standard 5-min occlusion followed by RH (RH-FMD) and an enhanced stimulus involving a 3-min occlusion concomitant with ischemic exercise at 20% maximum voluntary contraction (IE-FMD) (Fig. 1). Brachial artery measures were obtained continuously for 3 min after cuff deflation. The order of the RH-FMD and IE-FMD protocols was randomized, and subjects rested supine for 15 min between trials. The impact of repeated increases in shear stress via reactive hyperemia and handgrip exercise does not induce systematic changes in brachial artery FMD (31). With the use of similar methodology for an enhanced shear stress stimulus, it has been shown that RH-FMD and IE-FMD are highly repeatable within subjects across trials and between days (31).

Data Analysis

Body composition groupings. Women were stratified into lean and obese categories by percent body fat (lean: 18–31%, $n = 8$; obese: 38–59%, $n = 9$) (12). Two overweight women (32% and 34%) were excluded from this subanalysis.

Blood pressure groupings. Women were also stratified based on mean arterial pressure (MAP) measured at their first visit. Nine women were classified as having high blood pressure (HBP; range: 95–113 mmHg) and seven women were classified as having normal blood pressure (NBP; range: 78–91 mmHg). We divided the groups based on median MAP and then dropped three subjects with intermediate MAP (92–94 mmHg) to create distance between groups. All NBP subjects were below the American Heart Association cutoff points for hypertension. One subject in the HBP group was just below the cutoff point for stage 1 hypertension (118/79 mmHg), five of nine subjects in the HBP group qualified as having stage 1 hypertension (SBP $>$ 120 mmHg or DBP $>$ 80 mmHg), and three of nine subjects in the HBP group qualified as having stage 2 hypertension (SBP $>$ 130 mmHg or DBP $>$ 90 mmHg) (45).

Hemodynamics. Heart rate as well as SBP, DBP, and MAP were obtained from beat-by-beat blood pressures. Cardiac output was calculated online using the Modelflow algorithm (Finometer). Total peripheral resistance (TPR) was calculated as MAP/cardiac output.

Flow-mediated vasodilation. Machine-learning software (Brachial Analyzer for Research, Medical Imaging Applications, Coralville, IA) was used to analyze ultrasound screen capture files. Edge detection software was used to measure vessel diameter throughout each protocol and reviewed manually to ensure accurate wall detection. The region of interest was kept constant throughout each protocol to minimize artificial intrasubject variation. Baseline diameter was averaged over 1 min during the initial resting period. Diameters were

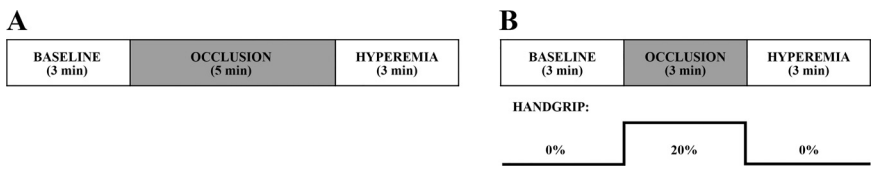

Fig. 1. Protocol timeline for trials. *A*: reactive hyperemia-flow-mediated dilation protocol. *B*: ischemic exercise-flow-mediated dilation protocol.

compiled into 3-s average time bins from which peak diameter and time to peak diameter were identified. FMD was calculated as the percent increase from baseline to the peak diameter bin during RH. Ultrasound analyses were performed by a single trained investigator who was blinded to test identifiers, including group and test condition.

Shear rate. Velocity (in cm/s) was obtained for each frame and compiled into 3-s average time bins. Shear rate was calculated for each bin as mean velocity/mean diameter. The shear rate area under the curve (AUC) was calculated from cuff deflation to the time of peak dilation using the trapezoid rule (41).

Statistical analyses. Two-tailed *t*-tests were used to compare baseline differences between groups. Separate two-way repeated-measures ANOVA tests were used to assess main effects of EE and either body composition or blood pressure on outcome variables, and the Sidak correction was used to correct for multiple post hoc comparisons. Regressions were performed to assess whether FMD responses were predicted by either body composition or MAP. $P < 0.05$ was considered significant. Values are reported as means ± SD. Data were analyzed using GraphPad Prism 7 (La Jolla, CA).

RESULTS

Subjects

Two of nineteen subjects reported mild nausea due to the EE administration, but no subjects left the study due to side effects. By design, obese subjects had a higher body mass index, weight, and percent body fat than lean subjects (Table 1). Blood pressure was similar between obese and lean groups (Table 1). By design, MAP, SBP, and DBP were higher in the HBP group (Table 2). Relative to subjects in the NBP group, subjects in the HBP group had a higher body mass index and weight, although percent body fat was similar between groups (Table 2). Handgrip force did not differ significantly between body composition or blood pressure groups.

Effect of Estrogen Exposure on Cardiovascular Characteristics

Handgrip force did not differ across EE testing days (pre-EE vs. EE: 6.92 ± 2.12 vs. 7.15 ± 1.56 kg). Within each group, heart rate, cardiac output, TPR, MAP, SBP, and DBP were unaffected by EE (Table 3). Because volume overload and cardiac output have been shown to mediate hypertension in young individuals (19), we performed a post hoc analysis to determine whether cardiac output as estimated by the Model-Flow algorithm (Finometer) predicted MAP on either testing date (pre-EE: $r^2 = 0.16$, $P = 0.15$; EE: $r^2 = 0.02$, $P = 0.61$).

Table 1. *Baseline subject characteristics grouped by body composition level*

	Lean Subjects	Obese Subjects	*P* Value
Number of subjects	8	9	NA
Age	22 ± 4	29 ± 4	0.003
Ethnicity (Asian/black/Hispanic/white)	1/2/1/4	3/3/0/3	NA
Percent body fat	23.9 ± 5.4	46.8 ± 6.5	<0.0001
Body mass index, kg/m^2	21.6 ± 2.7	33.4 ± 5.6	0.0001
Total weight, kg	65.6 ± 9.4	87.8 ± 16.15	0.004
Mean arterial pressure, mmHg	93.4 ± 8.7	97.1 ± 9.5	0.42
Systolic blood pressure, mmHg	114.9 ± 2.9	118.8 ± 4.3	0.47
Diastolic blood pressure, mmHg	80.8 ± 3.0	81.3 ± 2.8	0.92
Heart rate, beats/min	57.0 ± 6.7	54.4 ± 7.9	0.47
Cardiac output, l/min	5.2 ± 1.8	6.4 ± 1.8	0.18
Total peripheral resistance, PRU	20.7 ± 8.7	16.0 ± 4.3	0.17

All data are expressed as means ± SD. PRU, peripheral resistance units; NA, not applicable.

Table 2. *Baseline subject characteristics grouped by blood pressure level*

	NBP Group	HBP Group	*P* Value
Number of subjects/group	7	9	NA
Age	25.4 ± 6.3	27 ± 5	0.55
Ethnicity (Asian/Black/White)	2/1/4	2/5/2	NA
Percent body fat	32.3 ± 12.5	41.4 ± 11.7	0.16
Body mass indext, kg/m^2	24.6 ± 5.4	32 ± 6.6	0.03
Total weight, kg	67.4 ± 8.6	87.8 ± 15.7	0.008
Mean arterial pressure, mmHg	86.7 ± 4.6	101.8 ± 6.3	0.001
Systolic blood pressure, mmHg	109.8 ± 7.7	123.1 ± 9.8	0.01
Diastolic blood pressure, mmHg	72.4 ± 4.6	86.6 ± 5.2	<0.001
Heart rate, beats/min	55.2 ± 4.9	58.5 ± 8.2	0.38
Cardiac output, l/min	5.3 ± 1.3	6.7 ± 1.7	0.13
Total peripheral resistance, PRU	17.3 ± 5.6	15.9 ± 3.8	0.58
Cardiac index, l·min^{-1}·m^2	3.0 ± 0.6	3.4 ± 0.7	0.34

All data are expressed as means ± SD. NBP, normal blood pressure; HBP, high blood pressure; PRU, peripheral resistance units; NA, not applicable.

Relationship Between Shear Rate and FMD

Shear rate AUC was higher in IE-FMD trials than RH-FMD trials (pre-EE: IE shear to peak vs. RH shear to peak, 5,707 ± 2,140 vs. 10,321 ± 4,468, $P < 0.001$). Representative time-course data illustrating the distinct nature of the two stimuli are shown in Fig. 2.

We found a weak correlation between shear rate AUC and percent FMD (pre-EE: $r^2 = 0.25$, $P < 0.01$; EE: $r^2 = 0.22$, $P < 0.01$; RH-FMD and IE-FMD data combined). Therefore, to be consistent with current methodological guidelines (41), we did not normalize percent FMD to the shear stress stimulus.

Effect of Body Composition on FMD

The magnitude of vasodilatory responses was independent of percent body fat during RH-FMD or IE-FMD before and during EE (RH-FMD: pre-EE $r^2 = 0.07$ and EE $r^2 = 0.11$, $P = 0.2$; Fig. 3, *A* and *B*). Likewise, body mass index was not associated with the magnitude of RH-FMD or IE-FMD responses before or during EE (RH-FMD: pre-EE $r^2 = 0.08$, $P = 0.2$, and EE $r^2 = 0.07$, $P = 0.3$; IE-FMD: pre-EE $r^2 = 0.08$, $P = 0.2$, and EE $r^2 = 0.11$, $P = 0.2$). When stratified by body composition group, ANOVAs revealed no effects of estrogen treatment or body composition on either FMD response (Fig. 4). When we combined lean and obese groups, we observed an inverse correlation between percent body fat and baseline brachial artery diameter, which was unaffected by EE (pre-EE: $r^2 = 0.38$, $P < 0.01$; EE: $r^2 = 0.35$, $P < 0.01$).

Effect of Blood Pressure on FMD

In RH-FMD, percent FMD was similar between NBP and HBP groups pre-EE (Fig. 5*A*). Furthermore, EE did not affect percent RH-FMD in either group. There were no differences in the shear rate stimulus for RH-FMD trials between or within blood pressure groups during EE (Fig. 5*C*). During the IE-FMD trials, we observed a blood pressure by EE interaction ($P < 0.001$; Fig. 5*B*) such that EE increased percent FMD in the HBP group while having a negative impact in the NBP group (Fig. 5*B*). Pre-EE, IE-FMD responses were higher

Table 3. *Hemodynamics throughout the ischemic exercise-flow-mediated dilation trial*

	NBP Group		HBP Group		*P* Value		
	Pre-EE	EE	Pre-EE	EE	Main effect of EE	Main effect of group	EE × group interaction
Baseline							
Brachial artery diameter, mm	3.4 ± 0.3	3.4 ± 0.4	3.4 ± 0.3	3.3 ± 0.5	0.34	0.74	0.70
MAP, mmHg	86.5 ± 8.0	84.6 ± 9.0	103.2 ± 6.8	106.2 ± 9.5	0.85	<0.01	0.40
SBP, mmHg	108.3 ± 12.9	112.0 ± 15.7	125.6 ± 10.3	135.3 ± 16.4	0.17	<0.01	0.52
DBP, mmHg	72.8 ± 5.3	68.0 ± 8.2	87.9 ± 5.2	85.9 ± 7.0	0.14	<0.01	0.53
HR, beats/min	58.2 ± 7.2	58.5 ± 6.1	58.9 ± 6.5	60.4 ± 4.7	0.61	0.62	0.73
CO, l/min	5.4 ± 1.9	5.0 ± 1.4	6.7 ± 1.4	6.4 ± 1.6	0.55	0.12	0.96
TPR, PRU	17.1 ± 6.3	18.0 ± 5.8	16.0 ± 3.2	17.9 ± 7.0	0.62	0.96	0.58
Occlusion, last minute							
Brachial artery diameter, mm	3.1 ± 0.4	3.3 ± 0.1	3.3 ± 0.3	3.2 ± 0.3	0.20	0.47	0.80
MAP, mmHg	96.7 ± 9.1	94.2 ± 12.8	108.9 ± 7.7	114.4 ± 10.6	0.67	<0.01	0.27
SBP, mmHg	118.3 ± 13.6	120.3 ± 18.2	132.3 ± 11.0	144.6 ± 17.4	0.21	<0.01	0.36
DBP, mmHg	80.7 ± 5.7	75.6 ± 10.4	92.6 ± 6.0	92.7 ± 7.3	0.32	<0.01	0.28
HR, beats/min	63.0 ± 6.0	61.7 ± 9.6	64.1 ± 9.3	67.0 ± 8.9	0.73	0.40	0.39
CO, l/min	6.5 ± 1.9	5.5 ± 1.2	7.2 ± 1.2	7.1 ± 1.9	0.35	0.22	0.41
TPR, PRU	15.4 ± 5.1	17.9 ± 5.2	15.6 ± 3.5	17.8 ± 8.2	0.39	0.81	0.84
Hyperemia							
Peak diameter, mm	3.8 ± 0.3	3.6 ± 0.5	3.7 ± 0.3	3.7 ± 0.4	0.14	0.87	0.09
MAP, mmHg	88.8 ± 8.4	86.5 ± 8.5	104.1 ± 5.7	107.0 ± 10.4	0.90	<0.01	0.35
SBP, mmHg	111.9 ± 13.5	114.2 ± 14.9	127.3 ± 8.8	137.5 ± 18.0	0.20	<0.01	0.42
DBP, mmHg	74.0 ± 5.7	69.2 ± 7.4	88.0 ± 4.4	86.3 ± 7.7	0.10	<0.01	0.42
HR, beats/min	57.1 ± 7.5	58.0 ± 7.3	58.8 ± 4.9	58.9 ± 6.9	0.79	0.65	0.84
CO, l/min	5.8 ± 2.0	5.2 ± 1.2	7.1 ± 1.5	6.5 ± 1.6	0.33	0.14	0.81
TPR, PRU	16.5 ± 6.3	17.5 ± 4.3	15.2 ± 3.2	17.8 ± 7.0	0.49	0.99	0.50
Time to peak dilation, s	61.3 ± 14.6	78.9 ± 20.8	57.9 ± 22.7	78.3 ± 31.8	0.02	0.13	0.12
Shear rate AUC to 30 s	5,294 ± 1,083	5,107 ± 1,019	5,756 ± 2,533	7,132 ± 3,340	0.36	0.31	0.21
Shear rate AUC to 60 s	9,175 ± 1,803	8,627 ± 1,843	9,145 ± 4,367	11,703 ± 5,568	0.35	0.52	0.26
Shear rate AUC to 90 s	11,09 ± 2,417	10,798 ± 25,99	11,001 ± 5,597	14,714 ± 6,947	0.39	0.71	0.30

All data are expressed as means ± SD; $n = 7$ subjects in the normal blood pressure (NBP) group and 9 subjects in the high blood pressure (HBP) group. pre-EE, before 7-day ethinyl estradiol administration; EE, during 7-day ethinyl estradiol administration; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance; PRU, peripheral resistance units; shear rate AUC, shear rate area under the curve.

among subjects in the NBP group compared with HBP group ($P = 0.05$; Fig. 5*B*). However, during EE, IE-FMD increased among subjects in the HBP group ($P = 0.04$; Fig. 5*B*), whereas IE-FMD decreased among subjects in the NBP group ($P = 0.01$; Fig. 5*B*). There was a main effect of EE on shear rate AUC in IE-FMD trials ($P = 0.05$). Post hoc comparisons revealed that this trend was driven by an increase in shear rate AUC in the HBP group ($P = 0.04$; Fig. 5*D*).

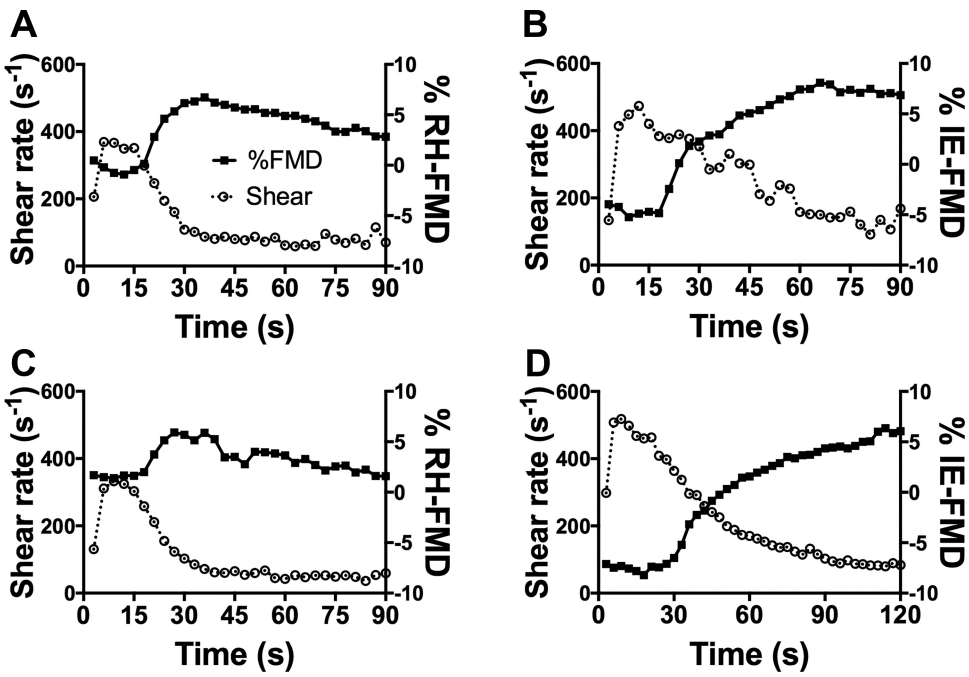

Fig. 2. Representative data for time course of shear stress stimulus and flow-mediated dilation (FMD) in a subject with high blood pressure. *A*: shear rate and percent reactive hyperemia (%RH)-FMD as a function of time before 7-day ethinyl estradiol (EE) administration (pre-EE). *B*: shear rate and percent ischemic exercise (%IE)-FMD as a function of time pre-EE. *C*: shear rate and %RH-FMD as a function of time during EE administration. *D*: shear rate and %IE-FMD as a function of time during EE administration.

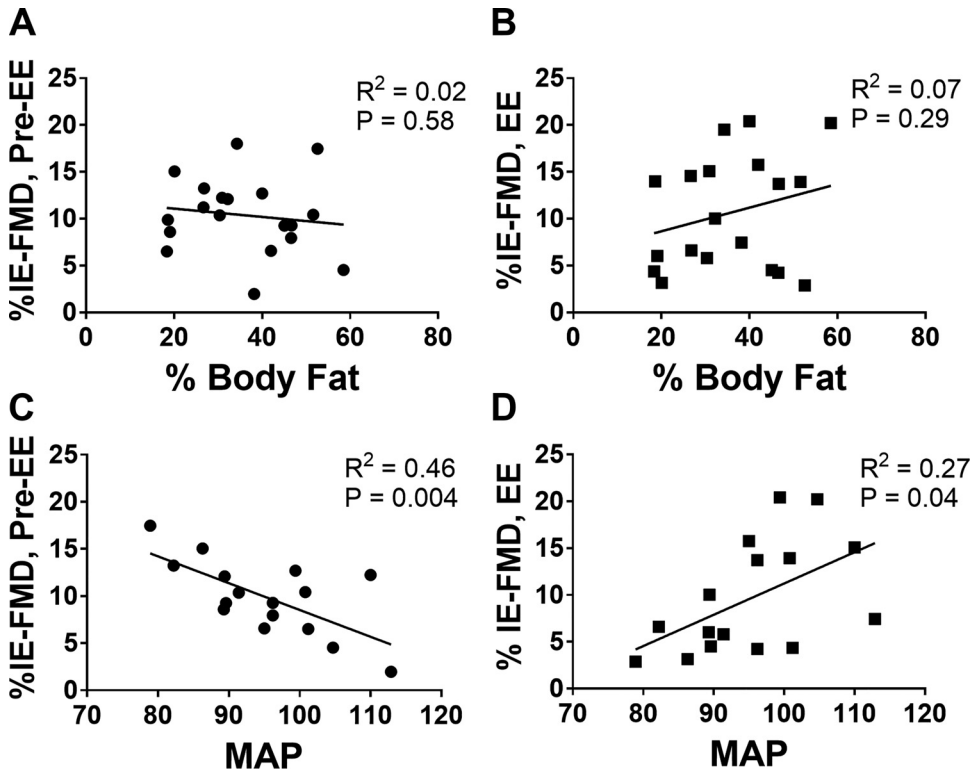

Fig. 3. Relationship between percent ischemic exercise (%IE)-flow-mediated dilation (FMD) and baseline subject characteristics. *A*: %IE-FMD before 7-day ethinyl estradiol (EE) administration (pre-EE) as a function of percent body fat. *B*: %IE-FMD during EE administration as a function of percent body fat. *C*: %IE-FMD pre-EE as a function of baseline mean arterial pressure (MAP). *D*: %IE-FMD during EE administration as a function of baseline MAP.

Blood Pressure Predicted IE-FMD Before and During Estrogen Exposure

Pre-EE, we found an inverse correlation between MAP and IE-FMD (Fig. 3*C*). This trend was reversed with EE and even resulted in a weak positive correlation between MAP and IE-FMD (Fig. 3*D*). MAP also predicted the change in FMD (calculated as the difference between IE-FMD EE and IE-FMD pre-EE) during EE ($r^2 = 0.51$, $P < 0.001$; Fig. 6). In contrast, body composition was not associated with percent IE-FMD either pre-EE or during EE (Fig. 3, *A* and *B*).

Missing Data

Cardiac output (and therefore TPR) was not measured pre-EE for two subjects in the NBP group due to user error. Shear data are missing from 4 of 76 total trials due to loss of the blood velocity signal after cuff release (RH-FMD: 1 subject in the NBP group; IE-FMD: 2 subjects in the NBP group and 1 subject in the HBP group).

DISCUSSION

Ours is the first study to demonstrate that among young women without overt cardiovascular disease or dysfunction, stage 1 hypertension (45) is associated with impaired endothelial function and that EE exposure is effective in improving endothelial function. Furthermore, despite higher body mass index and percent body fat in subjects in the HBP group, we found no direct relationship between body composition and endothelial function, indicating that the effects of blood pressure on FMD are more important than the effects of adiposity. Contrary to our hypothesis, EE exposure decreased IE-FMD in young women with NBP, suggesting distinct EE actions in the presence of NBP versus hypertension. Finally, consistent with recent studies (11, 39), our results support the use of more intense shear stress stimuli in the evaluation of endothelial function in young individuals without overt cardiovascular disease. This more dynamic method exposed differences in endothelial function that were not evident with the standard FMD protocol. It remains unknown whether impairment in nonstandard FMD is predictive of future cardiovascular risk, but evidence suggests that standard and nonstandard FMD responses are vulnerable to distinct vascular insults and may provide complementary information regarding endothelial function (43).

Blood Pressure and FMD

Hypertension, defined classically as SBP $>$ 140 mmHg and DBP $>$ 90 mmHg, is associated with compromised FMD responses, but previous studies that have investigated this relationship in women have largely focused on postmenopausal women (16, 29). Premenopausal women are an understudied population despite data demonstrating that even mild HBP can contribute to conditions such as polycystic ovary syndrome (21) and preeclampsia (38). Our data demonstrating lower FMD responses in our HBP group are the first to show that mild hypertension is associated with endothelial dysfunction among young women without overt cardiovascular morbidity. These data emphasize the importance of recent American Heart Association guidelines demonstrating that moderate HBP (i.e., stage 1 hypertension) constitutes a risk for vascular impairment in young women (45) because endothelial dysfunction is an important predictor of future cardiovascular disease (17). Among our subjects in the HBP group, six subjects presented with stage 1 hypertension and two subjects presented with stage 2 hypertension (45).

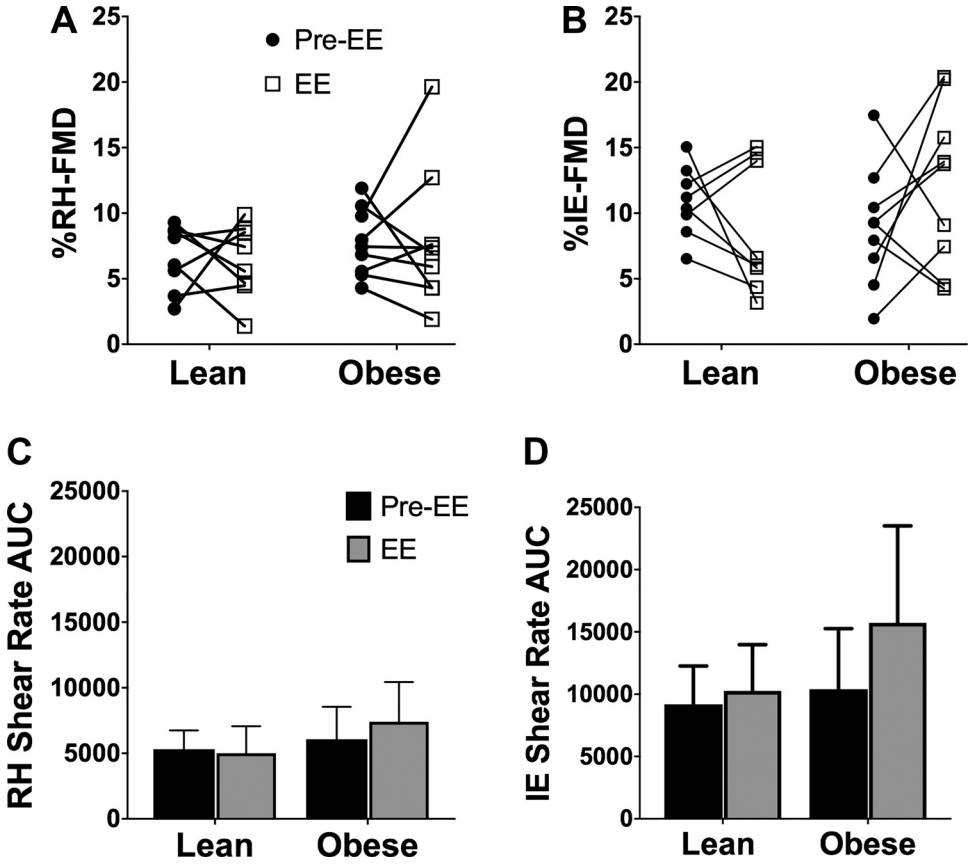

Fig. 4. Flow-mediated dilation (FMD) and shear stress stimulus by body composition (BC) level. *A*: percent reactive hyperemia (%RH)-FMD before 7-day ethinyl estradiol (EE) administration (pre-EE) and during EE testing days; individual data are shown. Effect of BC, $P = 0.33$; effect of EE, $P = 0.95$; BC × EE, $P = 0.87$. *B*: percent ischemic exercise (%IE)-FMD on pre-EE and EE testing days; individual data are shown. Effect of BC, $P = 0.67$; effect of EE, $P = 0.75$; BC × EE, $P = 0.12$. *C*: shear rate area under the curve (AUC) until the time of peak dilation in RH trials. Effect of BC, $P = 0.06$; effect of EE, $P = 0.54$; BC × EE, $P = 0.33$. *D*: shear rate AUC until the time of peak dilation in IE trials. Effect of BC, $P = 0.19$; effect of EE, $P = 0.08$; BC × EE, $P = 0.22$. Shear data are presented as means ± SD. Groups: $n = 8$ lean subjects and $n = 9$ obese subjects.

Estrogens and FMD

In young women with HBP, we found that short-term low-dose exposure to EE was effective in increasing IE-FMD. Some studies have demonstrated increased FMD during the high-estrogen late follicular phase of the menstrual cycle relative to the low-estrogen early follicular phase (1, 14, 46), but these findings are not consistent (7, 22, 35, 36). For example, neither RH-FMD nor handgrip exercise-induced FMD responses were affected by menstrual cycle phase (7). Conversely, estrogen administration in young lean women induced increases in RH-FMD (24). Our study differed in dosage (0.1 vs. 0.03 μg), route of administration (transdermal vs. oral), and type of estrogen (17 β-estradiol vs. EE) (24), and different estrogens do not have the same effects on vasodilator production. All of these factors have important implications for effects of hormones on the cardiovascular system in women (44).

Our data do not elucidate whether hypertension is a cause or a consequence of endothelial impairment, and among young women this relationship is especially unclear (33). However, our data suggest that estrogens play an important role in the relationship between blood pressure and endothelial impairment in women. Among men, blood pressure in adolescence predicts FMD 21 yr later, but this trend does not consistently hold for women (20), as there may be sex differences in clinical manifestations of hypertension even among young people. Premenopausal women with hypertension have increased risk for end-organ damage relative to age-matched men (28). While establishing the mechanism for the relationship between hypertension and endothelial dysfunction is beyond the scope of the present study, we have shown that short-term EE administration can improve endothelial function even while hypertension remains. Thus, the role of estrogens in the mechanistic relationship between blood pressure and endothelial dysfunction in women warrants further study.

An important and unexpected result of our study is that we observed a decrease in IE-FMD after short-term EE administration among women with NBP. This finding was contrary to our hypothesis, as we had anticipated that estrogen exposure would upregulate eNOS through well-established mechanisms that operate downstream of estrogen receptors (5), thereby increasing the response of the endothelium to a given shear stress stimulus. It is possible that the type of estrogen we administered may not upregulate eNOS. An in vitro study found that EE did not upregulate eNOS, in contrast to 17 β-estradiol (2), and may indeed interfere with the actions of other vasodilators (13). Furthermore, the balance of eNOS and other vasodilators may differ in a healthy conduit artery compared with a conduit artery in a hypertensive individual. Thus, this may explain the divergent effects of EE on IE-FMD in the two blood pressure groups. Finally, estrogen may augment sympathoinhibition (30). While blood pressure is unrelated to muscle sympathetic nerve activity in young women (18), it is possible that EE differentially affected neurovascular transduction in normotensive versus hypertensive premenopausal women, thereby resulting in divergent IE-FMD responses.

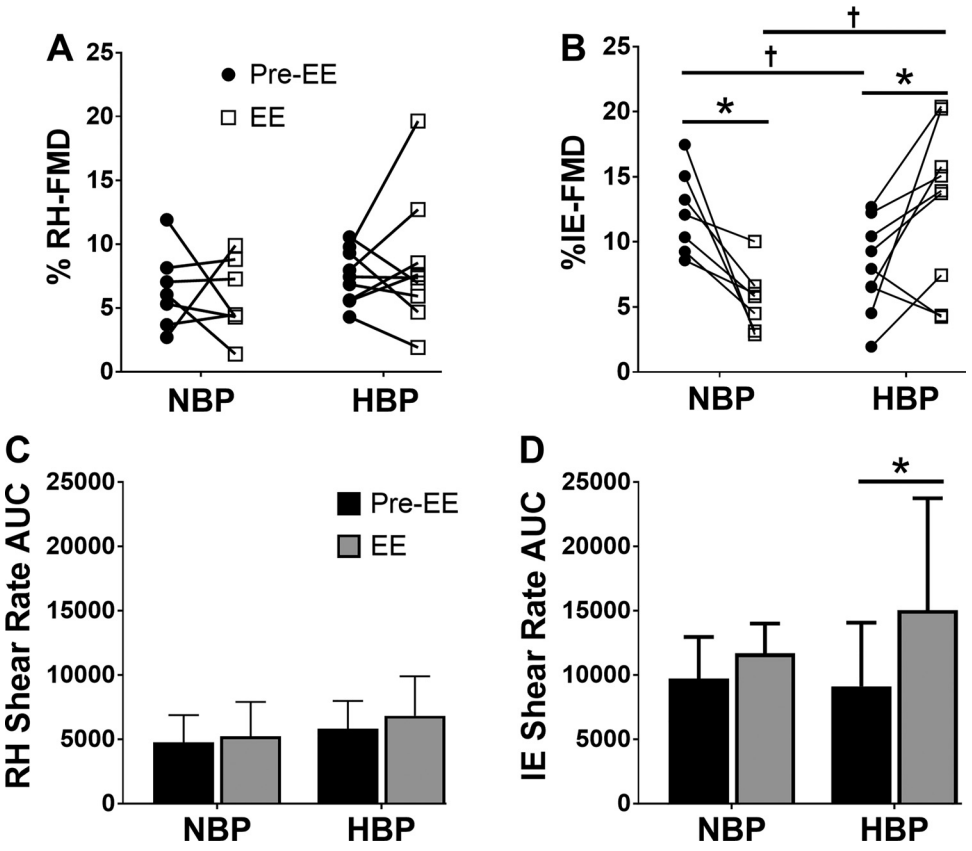

Fig. 5. Flow-mediated dilation (FMD) and shear stress stimulus by blood pressure (BP) level. *A*: percent reactive hyperemia (%RH)-FMD before 7-day ethinyl estradiol (EE) administration (pre-EE) and EE testing days; individual data are shown. Effect of BP, $P = 0.20$; effect of EE, $P = 0.91$; BP × EE, $P = 0.53$. *B*: percent ischemic exercise (%IE)-FMD on pre-EE and EE testing days; individual data are shown. Effect of BP, $P = 0.38$; effect of EE, $P = 0.48$; BP×EE, $P = 0.001$. *C*: shear rate area under the curve (AUC) until the time of peak dilation in RH trials. Effect of BP, $P = 0.27$; effect of EE, $P = 0.30$; BP × EE, $P = 0.71$. *D*: shear rate AUC until time of peak dilation in IE. Effect of BP, $P = 0.64$; effect of EE, $P = 0.05$; BP × EE, $P = 0.29$. Shear data are presented as means ± SD. Groups: $n = 7$ subjects in the normal blood pressure (NBP) group and $n = 9$ subjects in the high blood pressure (HBP) group. *$P < 0.05$, pre-EE vs. EE; †$P < 0.05$, NBP vs. HBP groups.

Obesity and FMD

The fact that women in the HBP group did have higher body mass index and percent body fat indicates there is an important interaction. What is unique about our findings is that we focused on blood pressure and demonstrated that blood pressure is by far the more important predictor of endothelial dysfunction in these women. Some (4, 8, 27, 39) but not all (3, 23, 37) investigations have reported indicators of endothelial dysfunction among obese adults relative to healthy control subjects. Young obese men have similar RH-FMD responses relative to lean control subjects but impaired FMD responses to exercise-induced shear stress (39). Only one study has examined the effects of obesity on FMD in premenopausal women and observed reduced RH-FMD among obese women (27). One explanation for the discrepancy between this earlier study and our study may be that our subjects were over a decade younger than the women in this earlier investigation (25 vs. 37 yr, respectively), suggesting an obesity-associated impairment of RH-FMD may be discernable as women age because of comorbidities in the slightly older population (27). Furthermore, an important caveat in our study is that even though weight and body composition did not predict endothelial function in our subjects, the women in our HBP group had higher total weight and body mass index than those in the NBP group. Regardless, one of our most important findings is that it is blood pressure, not adiposity, that is the primary determinant of endothelial dysfunction in young women.

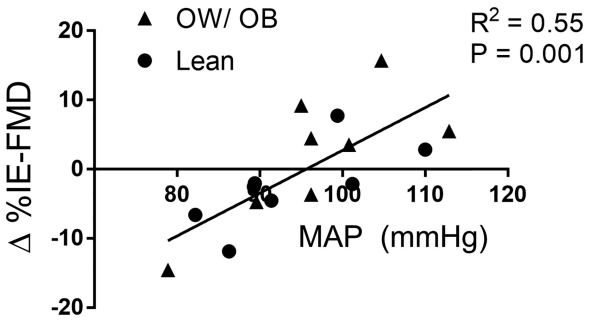

Fig. 6. Change in ischemic exercise (IE)-flow-mediated dilation (FMD) as a function of mean arterial pressure (MAP). Change was calculated as IE-FMD EE – IE-FMD before 7-day ethinyl estradiol administration (pre-EE). OW/OB, overweight/obese.

Limitations

We did not measure blood viscosity or hematocrit, which would have enabled further understanding of the shear stress stimulus and would have been interesting here because EE is associated with plasma volume expansion (40). Plasma volume expansion likely would have reduced blood viscosity and shear stress. Additionally, our subjects administered synthetic EE rather than 17-β estradiol, and, as discussed above, different estrogens impact the cardiovascular system in different ways (44).

Conclusions

Factors that determine cardiovascular health in young women remain incompletely understood. In this study, we examined women without overt cardiovascular disease who fell within a range of body composition and blood pressure.

Our data provide three conclusions. First, responses to the standard 5-min FMD stimulus (RH-FMD), which correlates with a clinically elevated risk for cardiovascular disease, were similar across groups. Therefore, it is unknown whether the observed differences in endothelial function result in increased risk for cardiovascular disease in young women with HBP. Second, when we applied a more intense endothelial stimulus (IE-FMD), which enables a more sensitive assessment of endothelial function (11, 32, 39), we observed responses consistent with endothelial impairment in women presenting with stage 1 hypertension. Thus, elevated blood pressure plays a more prominent role than obesity in endothelial dysfunction in young women. Third, we observed that short-term EE administration increased vasodilatory responses to the IE-FMD test in women with elevated blood pressure, suggesting a potential therapeutic measure to improve endothelial function in women with stage 1 hypertension. Finally, and somewhat surprisingly, we found that short-term EE administration decreased IE-FMD in healthy women with NBP. Given the challenges associated with administering long-term unopposed EE in many young women, future research can evaluate the effectiveness of a combined hormonal contraceptive or other potentially safe estrogen formulations such as combined conjugated estrogens with a selective estrogen receptor modulator to improve endothelial function in women with stage 1 hypertension.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.T. and N.S.S. conceived and designed research; T.E.A. and C.W.U. performed experiments; T.E.A., C.W.U., and N.S.S. analyzed data; T.E.A., C.W.U., and N.S.S. interpreted results of experiments; T.E.A. prepared figures; T.E.A., C.W.U., and N.S.S. drafted manuscript; T.E.A., C.W.U., A.T., and N.S.S. edited and revised manuscript; T.E.A., C.W.U., A.T., and N.S.S. approved final version of manuscript.

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