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RESEARCH ARTICLE

Integrative Cardiovascular Physiology and Pathophysiology

Childhood psychosocial stress is linked with impaired vascular endothelial function, lower SIRT1, and oxidative stress in young adulthood

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Abstract

Adverse childhood experiences (ACEs) are psychosocial stressors that occur during sensitive developmental windows and are associated with increased lifetime cardiovascular disease (CVD) risk in a dose-dependent manner. Vascular endothelial dysfunction is a pathophysiological mechanism that promotes hypertension and CVD and may be a mechanism by which ACEs contribute to lifetime CVD risk. We examined whether exposure to ACEs is associated with reduced vascular endothelial function (VEF) in otherwise healthy, young adult women (20.7 ± 3 yr) with (ACE^+) versus without (ACE^-) ACEs, explored whether differences in circulating sirtuin 1 (SIRT1) or systemic oxidative stress could explain ACEs-related differences in VEF, and examined the ability of a pilot, 8-wk exercise intervention to augment VEF and SIRT1 or reduce oxidized LDL cholesterol (oxLDL) in ACE^+ young adult women. Forty-two otherwise healthy young adults completed this study. Prior to the intervention, VEF (P = 0.002) and SIRT1 (P = 0.004) were lower in the ACE^+ than ACE^- group, but oxLDL concentrations were not different (P = 0.77). There were also significant relationships ($P \leq 0.04$) among flow-mediated dilation (FMD), SIRT1, and oxLDL in the ACE^+, but not ACE^- group. Adjusting for circulating SIRT1 and oxLDL reduced the differences in FMD observed between groups (P = 0.10), but only SIRT1 was a significant adjuster of the means ($P < 0.05$). Finally, the exercise intervention employed was unable to enhance VEF or SIRT1 in the ACE^+ exercise group. Our data suggest that ACEs likely increase susceptibility to hypertension and CVD by causing endothelial dysfunction, perhaps through a SIRT1 pathway-related mechanism.

NEW & NOTEWORTHY Our study provides novel evidence that young adult women with moderate-to-severe adverse childhood experience (ACE) exposure present impaired endothelial function and lower circulating sirtuin 1 (SIRT1) concentrations than age-matched controls. However, an 8-wk exercise intervention was unable to augment endothelial function or SIRT1 concentrations in a subset of those with ACEs. Our data suggest that ACEs-related impairments in endothelial function may be secondary to decreased NO bioavailability via SIRT1 and/or oxidative stress-related mechanisms.

adversity; early life stress; flow-mediated dilation; social determinants of health; vascular function

INTRODUCTION

Adverse childhood experiences (ACEs) are potentially traumatic events experienced during the first 18 years of life that include exposure to emotional, physical, and sexual abuse, neglect, violence, and household and interpersonal dysfunction (1, 2). According to the Centers for Disease Control and Prevention (CDC), approximately two in three adults report that they have experienced at least one ACE, whereas nearly 20% report experiencing four or more (3).

ACEs represent psychosocial stressors that occur during very sensitive developmental windows, causing not only chronic activation of but also developmental modifications to the allostatic systems. Consequently, several groups have reported graded relationships between ACE exposure and chronic disease risk. For example, Dong et al. (4) observed a dose-response relationship between ACEs and ischemic

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heart disease risk. Danese et al. (5) reported independent, graded increases in systemic inflammation, metabolic risk markers, and thus greater age-related disease risk, with increasing ACE exposure. Similarly, Gilbert et al. (6) reported that cardiometabolic disease morbidity and mortality also increase as a function of the number of ACEs experienced. Alarmingly, a 2017 systematic review concluded that ACEs were associated with lifetime cardiovascular outcomes such as myocardial infarction, stroke, and ischemic or coronary heart disease in ~92% of the studies reviewed (7). The CDC has also reported that up to 1.9 million cases of heart disease could be avoided by preventing ACEs (8). Therefore, ACEs are a pervasive, underappreciated, and understudied risk factor for cardiovascular disease (CVD).

Although much work has been done to examine the influence of ACEs on other physiological systems, few studies have examined the effects of ACEs on cardiovascular physiology, despite strong evidence that exposure to ACEs increases cardiovascular risk. Data from the Georgia Stress and Heart study suggest that young adults with ACEs experience earlier and greater age-related increases in systolic and diastolic blood pressure beginning near the age of 30 yr (9). Importantly, this ACEs-related augmentation of systolic blood pressure trajectories remained even after adjusting for negative health behaviors or socioeconomic status (SES) (9). In a study examining the influence of ACEs on hemodynamics in adolescents and young adults (13–29 yr), Su et al. (10) reported greater total peripheral resistance (+12%) and peripheral pulse wave velocity (PWV; +9%) in individuals exposed to two or more ACEs than in those with no exposure, although the PWV values were not adjusted for blood pressure. Taken together, initial evidence suggests that ACEs cause adverse hemodynamic changes that increase hypertensive risk in young adulthood which are independent of behavior or SES, suggesting that the effects of ACEs are biologically embedded.

Vascular endothelial dysfunction has been identified as a pathophysiological mechanism that contributes to increasing hypertensive (11) and CVD risk (12) and is characterized by reduced endothelium-dependent vasodilatory function in conduit arteries (13). However, it is not clear whether endothelial dysfunction may contribute to the increase in blood pressure trajectories or lifetime CVD risk in adults exposed to ACEs. Initial evidence in mice suggests that early life stress may induce endothelial dysfunction via an oxidative stress mechanism (14), but these findings have not yet been translated to humans.

Nitric oxide (NO) bioavailability plays a very important role in vascular endothelial homeostasis, and endothelial dysfunction is typically accompanied by reduced NO bioavailability (15). Endothelial dysfunction is also commonly observed alongside increased production of or reduced degradation of reactive oxygen species (i.e., oxidative stress), which can directly reduce NO bioavailability by reacting with NO to form peroxynitrate or other reactive nitrogen species (16). Sirtuin 1 (SIRT1) is a highly conserved nicotinamide-adenine dinucleotide (NAD^+)-dependent deacetylase that plays an important role in mediating oxidative stress responses and the regulation of vascular function (17–19). In fact, SIRT1 has been shown to directly activate endothelial nitric oxide synthase (eNOS) (20) and inhibit endothelial oxidative stress (21, 22). Accordingly, Rodriguez-Miguelez et al. (23) recently reported that individuals with low circulating SIRT1 during childhood are more likely to exhibit premature vascular dysfunction in young adulthood. Therefore, it is plausible that aberrations in SIRT1 signaling and/or increased oxidative stress may contribute to ACEs-related impairments in vascular endothelial function (VEF). On the other hand, habitual exercise training has been shown to improve VEF (24, 25), increase SIRT1 signaling (26–28), and reduce oxidative stress (29, 30) and could therefore represent an effective strategy to counteract the potential adverse effects of ACE exposure on vascular function in young adulthood.

Therefore, the purposes of this study were to *1*) examine whether exposure to ACEs was associated with VEF in otherwise healthy, young adult women, *2*) explore whether differences in circulating SIRT1 or systemic oxidative stress could explain ACEs-related differences in VEF, and *3*) examine the ability of a pilot exercise intervention to augment VEF and SIRT1, or reduce systemic oxidative stress, as measured by oxidized low-density lipoprotein (oxLDL), in young adult women with ACEs. ACEs are more highly prevalent in women and young adults (13, 49) and appear to exert a greater effect in women than in men. For example, ACEs have been more strongly associated with CVD risk (26), depression, and anxiety (17, 31) in women than men and are also independently associated with psychological outcomes such as hopelessness in women, but not men (14). Therefore, women are not only at greater risk for experiencing ACEs but also at particular risk with regard to detrimental ACEs-related biological and psychological effects. Finally, there are also sex-dependent effects of exercise (19, 50). Consequently, in this pilot study, we chose to examine the effects of ACEs in young adult women only. We hypothesized that moderate-to-severe ACE exposure would be associated with endothelial dysfunction in young adult females, as indicated by lower brachial artery flow-mediated dilation (FMD), and that this difference would be independent of mean arterial pressure (MAP) or body mass index (BMI). We also explored whether moderate-to-severe ACE exposure influenced circulating SIRT1 and oxLDL concentrations, with the hypothesis that circulating SIRT1 levels would be lower and oxLDL levels higher in those with ACEs. We further hypothesized that SIRT1 and oxLDL concentrations would be directly and indirectly related to VEF, respectively. Finally, we hypothesized that an 8-wk progressive exercise training intervention would improve VEF and augment circulating SIRT1 and reduce oxLDL concentrations in young adult females with a history of ACE exposure.

MATERIALS AND METHODS

Participants

Forty-two participants volunteered, qualified, and completed this pilot trial. Prior to enrollment, all participants completed an informed written consent form and health history questionnaire. To be considered eligible, participants must have been an assigned biological female between the ages of 18 and 29 yr (inclusive), must have answered no to all questions on the Physical Activity Readiness Questionnaire

for people aged 15–69 yr, had a BMI between 18.5 and 40.0 kg/m^2 (inclusive), and had an ACE score of either 0 or ≥4, representing moderate-to-severe ACE exposure. Participants were excluded if they had chronic cardiovascular, renal, metabolic, pulmonary, or musculoskeletal disease as determined by a health history questionnaire, were prescribed or taking anti-inflammatory (including habitual NSAID use), antioxidant, or lipid-lowering medications at the time of enrollment, or had been enrolled in another clinical trial within 30 days of enrollment. Participants were recruited by flyers placed on campus, via approved emails to the target population with a university affiliation, by word of mouth, and via the clinicaltrials.gov registry. None of the participants was currently meeting exercise guidelines or had participated in structured exercise training for the previous 6 mo. Physical activity information was collected via the International Physical Activity Questionnaire at baseline and, on average, participants were not meeting United States physical activity guidelines (Table 1). This study was approved by and carried out in accordance with the University's Institutional Review Board for the protection of human subjects.

Experimental Design

This pilot clinical trial is registered on clinicaltrials.gov (Identifier: NCT03521401). Only flow-mediated dilation (FMD), SIRT1, and oxLDL data, as well as baseline ACE, maltreatment and abuse chronology of exposure (MACE), anxiety, depression, blood pressure, and BMI data are reported in this manuscript. This study utilized a repeated-measures design that employed the use of a negative and positive control group. Participants were screened, and those who were eligible and reported ≥4 ACEs (ACE^+) were randomly assigned to either an exercise (ACE^+_{EXT}; n = 14) or control (ACE^+_{CNT}; n = 14) group. Participants who were eligible and reported no ACEs (ACE^-) were assigned to a negative control (ACE^-_{CNT}; n = 14) group. Following enrollment, the participants returned to the laboratory following an overnight fast, provided fasting blood samples, and underwent FMD assessment. All baseline assessments were completed during the early follicular or placebo/withdrawal phase of their menstrual cycle. Participants then completed the 8-wk intervention, with those in the ACE^+_{EXT} group completing 8 wk of exercise training. Those in the CNT groups were instructed to maintain habitual physical activity levels. Following the intervention period, the participants returned to the laboratory to provide a fasting blood sample and have brachial artery FMD assessed.

Table 1. *ACEs-related differences in the participant characteristics at baseline*

	n	ACE^+	ACE^-	*P* Value
ACE score	42	6.0 (1)	0.0 (0)	**<0.0001** MW
$MACE_{TSS}$	41	42.5 (14)	5.5 (9)	**<0.0001** MW
Age, yr	42	20.8 (3)	20.6 (3)	0.94 MW
Race, *n* (%)	42			0.23 χ^2
White		23 (82%)	10 (71%)	
Black		2 (7%)	0 (0%)	
Other		3 (11%)	4 (29%)	
Height, m	42	1.65 (0.1)	1.68 (0.1)	0.15
Weight, kg	42	70.3 (13)	74 (17)	0.50 MW
BMI, kg/m^2	42	26.0 (5)	26.1 (6)	0.95 MW
Physical activity, MET·min/wk	41	393 (441)	405 (383)	0.97 MW
SBP, mmHg	40	108.3 (9)	110.2 (10)	0.57
DBP, mmHg	40	77.3 (7)	77.3 (8)	0.99
MAP, mmHg	40	87.6 (7)	88.3 (8)	0.81
Glucose, mg/dL	41	88.2 (6)	90.1 (7)	0.36
Triglycerides, mg/dL	41	91.1 (56)	73.1 (25)	0.63 MW
HDL C, mg/dL	41	60.4 (15)	58.8 (16)	0.97 MW
LDL C, mg/dL	41	100.7 (32)	98.8 (18)	0.76 MW
Total C, mg/dL	41	179.4 (37)	172 (18)	0.63 MW
Depression	42	17.3 (10)	7.9 (5)	**0.0001** W
Score ≥ 16, *n* (%)*		15 (54%)	1 (7%)	
Anxiety	42	39.6 (9)	29.7 (7)	**<0.0001** MW

Values are means (SD). ACE, adverse childhood experience; BMI, body mass index; MACE, maltreatment and abuse chronology of exposure; $MACE_{TSS}$, MACE total severity score. *Scores ≥ 16 indicate possible depressive symptoms. Bolded *P* values indicate significance ($P < 0.05$). MW, Mann–Whitney test; W, Welch's correction; χ^2, chi-square test were performed to examine distributions between groups.

Adverse Childhood Experiences

The degree of exposure to ACEs was assessed in two ways. First, the ACE questionnaire was used to provide a multiplicity of exposure score. As mentioned previously, only those participants with an ACE score indicating no exposure or four or more exposures (i.e., moderate-to-severe exposure) were eligible for this study. Second, the 52-item MACE questionnaire was used to assess the total severity of exposure to maltreatment during each participant's first 18 years of life, i.e., the MACE total severity score ($MACE_{TSS}$). The $MACE_{TSS}$ has been shown to be highly reliable, displaying a test-retest correlation coefficient of 0.91 and little variability among repeated measures (32) and has been shown to account for approximately twofold more variability in psychiatric symptoms than the ACE score (32).

Depression and Anxiety

Depression and anxiety were assessed using the Center for Epidemiologic Studies Depression Scale Revised (CESD-R) and the Zung Self-Rating Anxiety Scale (S-RAS). The CESD-R was scored on a scale from 0 to 3, and the S-RAS was scored on a scale from 1 to 4. The items, "I talked less than usual" was accidentally omitted from the CESDR and "I am bothered by dizzy spells" from the S-RAS Qualtrics surveys that were used for the final six participants. Consequently, the group average (0 and 2, respectively) was imputed for these items and used in the total depression and anxiety scores for these subjects. Sensitivity analysis revealed that imputing these values, versus omitting the item entirely, did not significantly affect the anxiety outcomes.

Conduit Artery Vascular Function

VEF was assessed using the brachial artery FMD technique. Briefly, the participants were asked to lie supine on a padded medical exam table in a quiet, darkened room for 15 min in a thermoneutral laboratory before assessment. A Doppler ultrasound and 12-MHz probe (Mindray Z5 Ultrasound System, Mindray North America, Mahwah, NJ) were used to view the brachial artery longitudinally, and a high-definition video screen capture device (AV.io HD, Epiphan Systems, Inc., Palo Alto, CA) was used to record the ultrasound screen. After 2 min of baseline recording, a cuff

was rapidly inflated to 240 mmHg and held for 4 min. Following the occlusion period, the cuff was rapidly deflated and the recording continued for an additional 4 min. Semiautomated continuous edge detection wall-tracking software (FMD Studio, Quipu srl, Via Moruzzi, Pisa, Italy) was then used to analyze the vessel diameter from the video recordings, and FMD was calculated as the relative (%) change in brachial artery diameter from baseline to the maximal dilation after the cuff pressure was released. Brachial artery FMD was not normalized to peak shear stimulus because shear rate values were not obtained. Recent coefficients of variation for trial-to-trial reliability for brachial artery baseline diameter, diameter change, and relative FMD in our laboratory are 2.6, 3.9, and 5.3%, respectively.

Serum Sirtuin 1 and Oxidized Low-Density Lipoprotein Concentrations

Blood samples were collected into serum collection tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) in the morning following an overnight fast via a venous blood draw. Samples were inverted, allowed to clot for 45 min, and then centrifuged at 2,500 rpm (700 RCF) for 12 min to separate serum, which was transferred to a 1.7-mL microcentrifuge tube for storage at −20°C. Deidentified samples were transported on dry ice to the Integrative Immunology Center Laboratory at the University of Oklahoma School of Community Medicine for SIRT1 (MyBiosource, Inc., San Diego, CA) and oxLDL (ALPCO, Salem, NH) quantification, which were performed using commercially available, highly sensitive enzyme-linked immunosorbent assay (ELISA) kits. The detection range of the SIRT1 ELISA kit was 15.6–1,000 pg/mL, the sensitivity was 5 pg/mL, the interassay coefficient of variation (CV) was <12% and the average intraassay CV across all duplicates was 2.8%. The detection range of the oxLDL ELISA kit was 9–250 ng/mL, the sensitivity was 9 ng/mL, the interassay coefficient of variation (CV) was <12%, and the average intraassay CV across all duplicates was 4.1%. A natural log transformation was performed on oxLDL values, which were nonnormally distributed. In addition, for both SIRT1 and oxLDL, individual subject data were excluded if the intraassay CV was ≥ 20%. Consequently, oxLDL data points from one subject in the ACE^{+}_{EXT} group at baseline and one subject in the ACE^{-}_{CNT} group at posttesting were excluded from the analysis.

Exercise Intervention

The exercise intervention employed has been detailed elsewhere (33). Briefly, those in the ACE^{+}_{EXT} group completed 8 wk of supervised, progressive resistance, and interval exercise training. Resistance exercise and interval exercise were each performed two times per week (4 total exercise sessions per week). Resistance training sessions were designed to provide a whole body stimulus and included nine different exercises. During the first 4 wk, the participants completed two sets of 15 repetitions at ~60% of maximal load for each exercise, whereas during the last 4 wk, the participants completed three sets of 12 repetitions at ~70% of maximal load. Resistance training loads were also progressed throughout the 8-wk program using the 2 + 2 rule. The interval exercise sessions were completed on a fan bike (Echo Bike, Rogue Fitness, Columbus, OH) that required engagement of the lower and upper body. The interval exercise sessions consisted of a 5-min warm-up and cool-down period with 10 separate, 30-s intervals separated by 90 s of active recovery performed between. The intensities of the interval and active recovery periods were determined using ratings of perceived exertion (RPE) and were set at an RPE of 7 and 3 out of 10, respectively. To confirm that the exercise training program improved fitness, maximal voluntary leg extension strength (MVIC, Nm) and the peak power (PP, W) attained during interval exercise were recorded at the beginning and end of the intervention.

Statistical Analyses

ACEs-related effects.

Independent samples *t* tests were used to examine baseline group differences (ACE^{+} vs. ACE^{-}) except when normality was violated, as indicated by the Shapiro–Wilk test. Between-group differences for nonnormally distributed variables were instead analyzed using Mann–Whitney tests. Welch's correction was applied if between-group variances differed significantly. Analyses of covariance (ANCOVA) were also utilized to examine whether ACEs-related differences in FMD remained after adjusting *1*) for age, MAP, BMI, and depression and anxiety levels and *2*) for circulating SIRT1 and oxLDL concentrations.

Baseline bivariate analyses.

Monotonic relationships among FMD, SIRT1, and oxLDL were examined using one-tailed Spearman's *r* (ρ) correlation coefficients within the ACE^{+} and ACE^{-} groups. In addition, relationships between $MACE_{TSS}$ and FMD, SIRT1, and oxLDL were also examined using one-tailed Spearman's *r* (ρ) correlation coefficients across the entire sample. The $MACE_{TSS}$ was used to examine these relationships because it provides a more holistic assessment of degree of exposure to adverse experiences and is a more heterogeneous and continuous outcome than the simple multiplicity score provided by the ACE scale. Accordingly, the $MACE_{TSS}$ has been shown to account for more than two times the variance in psychiatric symptom ratings than the ACE scale (32) and is likely more appropriate for examining relationships among childhood adversity and psychological and physiological outcomes. One-tailed tests were used because our hypotheses were directional.

Exercise intervention effects.

Due to missing values at one of the time points, the effects of the 8-wk intervention on FMD, oxLDL, and SIRT1 were examined using two-way mixed-effects models with between-group (ACE^{+}_{EXT} vs. ACE^{+}_{CNT} vs. ACE^{-}_{CNT}) and within-group (preintervention vs. postintervention) factors. Depression and anxiety scores were first log-transformed, and then examined using a two-way, group × time analysis of variance and a mixed-effects model, respectively. Exploratory regression analyses were also performed to examine the relationships between absolute change scores in physiological outcomes and ACE exposure in the ACE^{+}_{EXT} group.

Data are reported as means ± SD, except if otherwise denoted. Statistical analyses were performed using Graphpad Prism for macOS (v. 8.4.3) and JASP (JASP Team 2020, v. 0.13.1), and significance was set at $P \leq 0.05$. All figures were made using Graphpad Prism for macOS.

RESULTS

ACEs-Related Effects

Table 1 contains the baseline subject characteristics in the ACE^+ and ACE^- groups. Because of missing data points, not all comparisons included the same number of participants. For example, at baseline, one participant did not complete the MACE questionnaire or the physical activity questionnaire, blood pressure was not obtained in two participants, and a blood sample was not available for one participant. One participant's FMD video was not of sufficient quality for analyses, and the intraassay CV of oxLDL for one participant was outside of the laboratory's tolerable limits (>20%) and was excluded from analyses. Table 1 also indicates the number of participants for which data were available for each comparison.

The participants in the ACE^+ group had greater histories of early life trauma as indicated by both their ACE and $MACE_{TSS}$ scores (Fig. 1). There were no differences in SBP, DBP, or MAP, nor in age, BMI, or fasting metabolic markers between groups (all $P \geq 0.08$, Table 1). Depression and anxiety levels were greater in the ACE^+ group (both, $P < 0.001$).

There were no differences in baseline diameter between the ACE^+ than ACE^- group (3.1 ± 0.7 vs. 2.9 ± 0.6, $P = 0.40$). However, FMD (6.0 ± 3.6% vs. 9.4 ± 3.5%, $P = 0.002$) and the absolute diameter change (0.18 ± 0.1 mm vs. 0.29 ± 0.1 mm, $P = 0.004$) were lower in the ACE^+ than ACE^- group, and this was accompanied by lower circulating SIRT1 concentrations (649.6 ± 140.4 pg/mL vs. 809.5 ± 183.8 pg/mL, $P = 0.003$) in the ACE^+ than ACE^- group (Fig. 2). Adjusting for age, MAP, BMI, and log-transformed depression and anxiety scores had no effect on the observed difference in FMD between the ACE^+ and ACE^- groups [adjusted means ± SE; 5.7 ± 0.7 ($n = 27$, sample size) vs. 10.7 ± 1.1% ($n = 12$, sample size), $P = 0.001$]. However, adjusting for SIRT1 and oxLDL levels reduced the ACEs-related difference in FMD, such that it was no longer significant [adjusted means ± SE; 6.0 ± 0.7 ($n = 25$, sample size) vs. 8.1 ± 1.0% ($n = 13$, sample size), $P = 0.10$], and although oxLDL was not a significant adjuster of the means ($P = 0.96$), circulating SIRT1 was ($P < 0.05$).

Baseline Bivariate Analyses

The relationships among FMD, SIRT1, and oxLDL concentrations are depicted in Fig. 2, *D–F*. Briefly, there were significant relationships among all three variables in the ACE^+ but not in the ACE^- group. When looking across the entire sample, $MACE_{TSS}$ was also inversely related to FMD and circulating SIRT1 concentrations (Fig. 3).

Exercise Intervention Effects

There were group × time interactions for MVIC ($P = 0.0008$) and PP ($P < 0.0001$), indicating that both MVIC (pre vs. post; 121.7 ± 36.3 vs. 145.0 ± 40.8 Nm; $P < 0.0001$) and PP (266.3 ± 70.4 vs. 456.4 ± 130.5 W; $P < 0.0001$) increased in the women in the ACE^+_{EXT} group but not in the control groups (all $P > 0.46$). There was also a group × time interaction for anxiety levels ($P = 0.01$), which improved in the ACE^+_{EXT} group (41.2 ± 7 vs. 36.7 ± 7; $P < 0.01$) but not the ACE^+ or ACE^- control groups. Finally, although there was no group × time interaction for depression ($P = 0.18$), a forced post hoc test indicated that depression scores improved in the ACE^+_{EXT} group (19.2 ± 11 vs. 13.8 ± 9, $P = 0.04$) only. There was no group × time interaction for baseline artery diameter ($P = 0.62$). However, the 8-wk exercise intervention did not significantly improve absolute (mm) or relative (%) FMD ($P = 0.159$ and 0.156, respectively), SIRT1 ($P = 0.94$), or oxLDL ($P = 0.18$) levels in the ACE^+_{EXT} group relative to the ACE^+ or ACE^- control groups. Our exploratory regression analyses indicated that, although nonsignificant ($P \geq 0.07$), there were moderate associations between both the change in FMD and the change in SIRT1 versus ACE score in the ACE^+_{EXT} group (Fig. 4).

DISCUSSION

This is the first study, to our knowledge, to assess the influence of ACE exposure on brachial artery VEF. As expected, those with a history of ACE exposure reported significantly greater total severity of maltreatment and abuse in childhood and greater depression and anxiety levels than those with no history of ACEs. Importantly, the present findings also suggest that moderate-to-severe ACE exposure is associated with impaired endothelial function in young, normotensive females, even after controlling for age, BMI, MAP, depression score, and anxiety levels. Due to their importance related to nitric oxide (NO) bioavailability, we also examined circulating SIRT1 and oxLDL levels. Although oxLDL levels were not different between the ACE^+ and ACE^- groups, circulating SIRT1 was lower in the ACE^+ than the ACE^- control

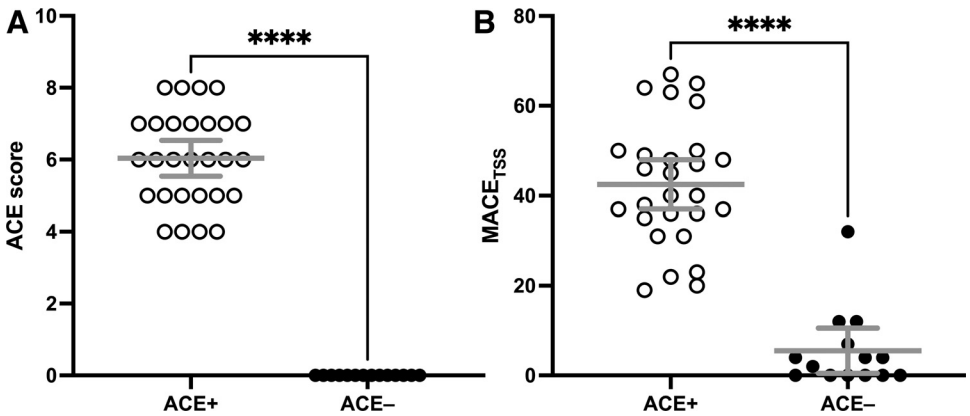

Figure 1. Scores on the ACE (*A*) and MACE (*B*) scales in the young adult females with (ACE^+; open circles) and without (ACE^-; closed circles) moderate-to-severe ACE exposure. ****Significant difference between groups ($P < 0.0001$). ACE, adverse childhood experience; MACE, maltreatment and abuse chronology of exposure. Sample size for comparisons were 28 vs. 14 and 27 vs. 14 for ACE scores and $MACE_{TSS}$, respectively.

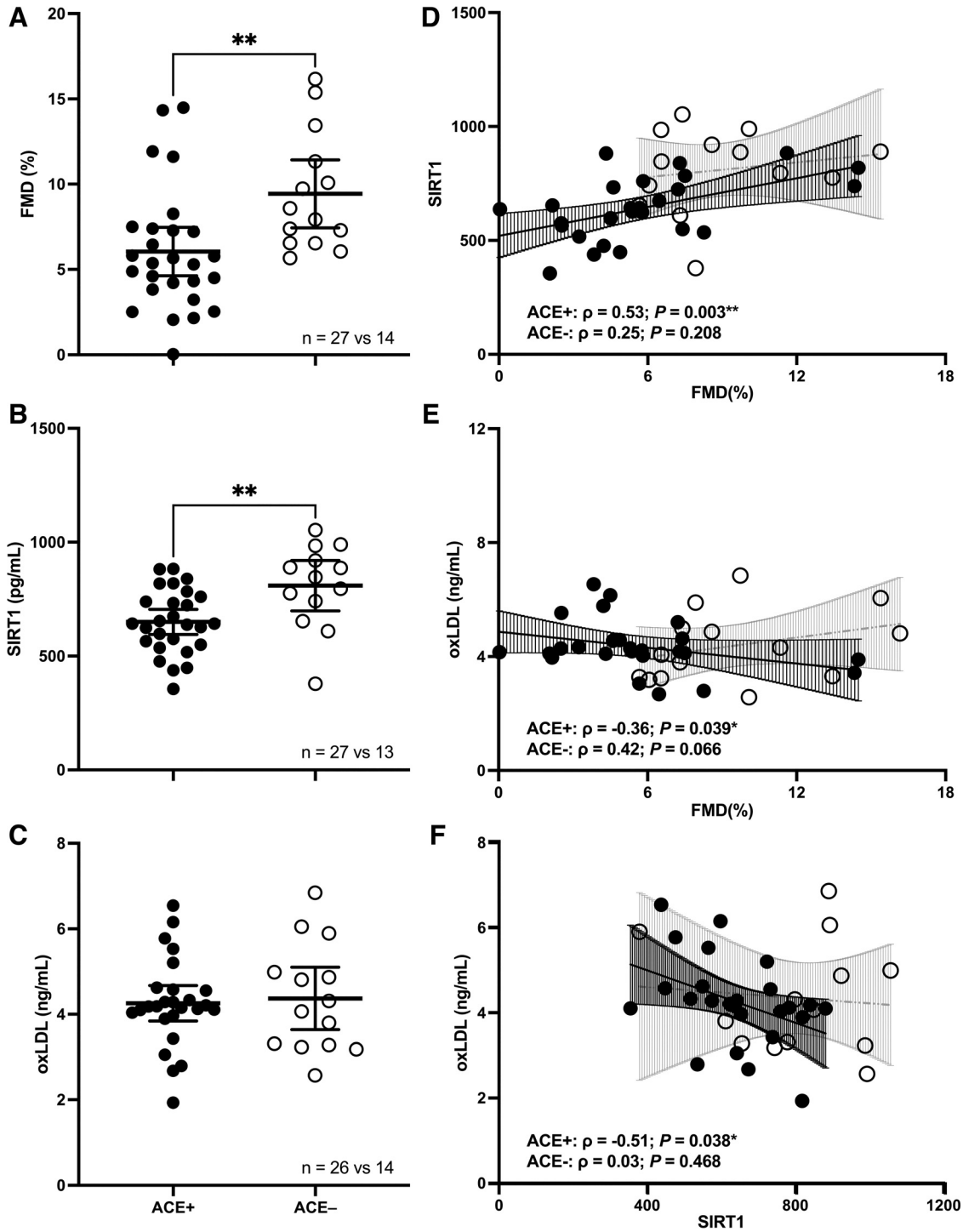

Figure 2. The brachial artery flow-mediated dilation (FMD; *A*), circulating sirtuin 1 concentrations (SIRT1; *B*), circulating, natural-log transformed oxidized low-density lipoprotein (oxLDL; *C*) concentrations in young adult females with (ACE^+; closed circles) and without (ACE^-; open circles) moderate-to-severe ACE exposure. *D–F*: relationships among FMD, SIRT1, and oxLDL in the ACE^+ (closed circles) and ACE^- (open circles) groups. *$P < 0.05$; **$P < 0.01$. ACE, adverse childhood experiences.

group. Furthermore, FMD was inversely related to oxLDL concentrations and positively related to SIRT1 in the ACE^+ group but was not related to either in the ACE^- group. Similarly, SIRT1 and oxLDL were inversely related in the ACE^+ group only (Fig. 2). Consequently, adjusting for circulating SIRT1 and oxLDL tempered the observed ACEs-related difference in brachial artery FMD. Finally, we also examined the ability of an 8-wk exercise training program to improve brachial artery FMD and increase circulating SIRT1 in a subset of women with moderate-to-severe ACE exposure. Although exercise improved fitness indicators and anxiety and depression, our findings suggested that it was insufficient to

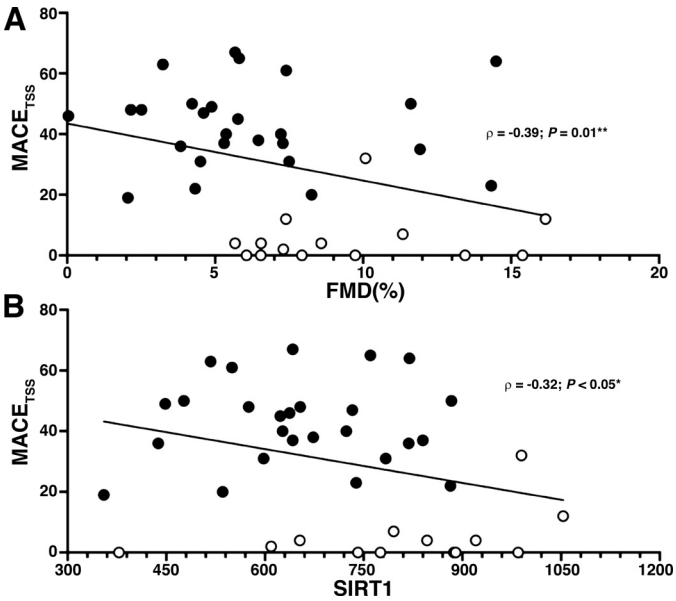

Figure 3. The relationships between the total severity score on the maltreatment and abuse chronology of exposure scale ($MACE_{TSS}$) versus brachial artery flow-mediated dilation (FMD; *A*) and circulating sirtuin 1 concentrations (SIRT1; *B*) across the sample. The closed circles represent those participants with moderate-to-severe ACE exposure (ACE^{+}), whereas the open circles represent the participants with no history of ACE exposure (ACE^{-}). $^{*}P < 0.05$. $^{**}P = 0.01$. ACE, adverse childhood experiences.

augment either FMD or circulating SIRT1 concentrations in this population, with our preliminary evidence suggesting moderate inverse relationships between training-induced improvements in FMD and SIRT1 and ACE exposure (Fig. 4). Overall, our data suggest that ACE exposure is associated with subclinical endothelial dysfunction in otherwise healthy young females and that this dysfunction is likely secondary to decreased NO bioavailability and linked to a SIRT1 and/or oxidative stress-related mechanism.

Endothelial dysfunction precedes and is independently predictive of future cardiovascular disease morbidity and mortality. In an animal model of early life stress, Ho et al. (14) observed that maternal separation induced aortic endothelial dysfunction in adult mice. The same group also reported that young adults with ACE exposure exhibit increased peripheral artery stiffness and total peripheral resistance (10), perhaps indicative of underlying endothelial dysfunction. However, these results have not, to our knowledge, been translated to or verified in humans. In the present study, the ACE^{+} group exhibited lower VEF, as indicated by FMD, compared with the age-matched, ACE^{-} control group (Fig. 2*A*). This ACE-related difference persisted even after adjusting for MAP, BMI, age, and anxiety and depression levels. Therefore, our results indicate that ACE exposure promotes reduced conduit artery vascular function, a likely mechanism by which ACEs may be linked to increased hypertensive and CVD risk.

VEF is highly dependent on NO bioavailability, and SIRT1 and oxidative stress are key effectors of NO. For example, overproduction or decreased degradation of reactive oxygen species directly alters vascular function by reducing NO bioavailability and signaling. SIRT1, by signaling through the NF-κB, FOXOs, NADPH oxidase, and superoxide dismutase pathways, may inhibit oxidative stress (22). SIRT1 also promotes NO production by deacetylating eNOS and stimulating its activity (19, 20). Therefore, greater SIRT1 expression should be associated with enhanced NO bioavailability and, consequently, with greater VEF. Accordingly, endothelial SIRT1 expression has been shown to be positively related to VEF among healthy, aging adults (20). Although the primary origin of circulating SIRT1 and its relationship to tissue

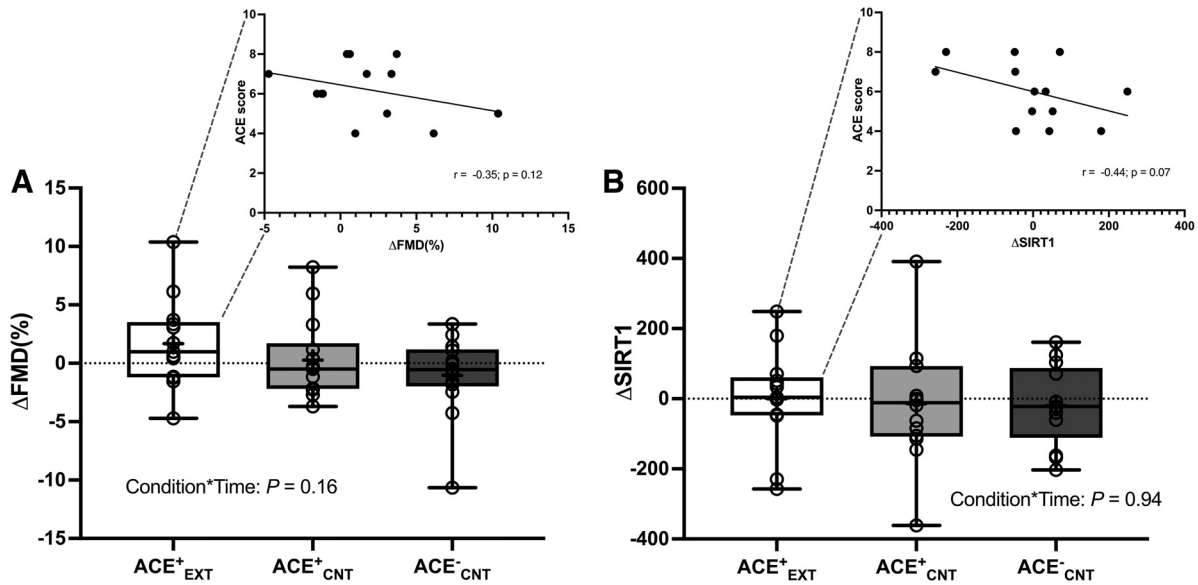

Figure 4. The changes in flow-mediated dilation (FMD; *A*) and circulating sirtuin 1 concentrations (SIRT1; *B*) across the intervention period in the females with moderate-to-severe ACE exposure randomized to the exercise training (ACE^{+}_{EXT}) or control condition (ACE^{+}_{CNT}), and without ACE exposure (ACE^{-}_{CNT}). The boxes extend from the 25th to 75th percentiles, the horizontal line and the "+" in the middle of the boxes represent the median and mean, respectively, and the whiskers represent the minimum and maximum values. The inset figures show the relationship between change in FMD or SIRT1 and ACE exposure (assessed at baseline) in the ACE^{+}_{EXT} group. ACE, adverse childhood experiences.

SIRT1 are not presently clear (36), circulating SIRT1 has also been shown to predict microvascular function (23), supporting the role of SIRT1 in the pathophysiology of endothelial dysfunction and the use of circulating SIRT1 as a novel biomarker to explore this association. In the present study, whereas oxLDL concentrations were similar, circulating SIRT1 was lower in the ACE^+ than ACE^- group (Fig. 2*B*). Strikingly, SIRT1 concentrations were related to both VEF and oxLDL concentrations (Fig. 2, *D* and *E*) in the ACE^+ group, and ACE-related differences in FMD were eliminated when SIRT1 was included as a covariate. It is also worth noting that there were no significant relationships among FMD, SIRT1, or oxLDL in the ACE^- group. We are unable to definitively determine why these relationships were present in ACE^+ but not ACE^-, but we speculate that this difference suggests that those with ACEs may be less able to regulate factors that detrimentally impact vascular function, such as oxidative stress, consistent with allostatic load/overload theory (37). Finally, although we are unable to establish cause and effect in the present study, our data clearly suggest that the SIRT1 pathway contributes to the association between ACEs and endothelial dysfunction, potentially by regulating or buffering oxidative stress. Additional studies are needed to explore these possibilities, as neither circulating SIRT1 nor oxLDL ultimately directly informs the redox status of the endothelium.

Exercise training has robust vascular benefits and has consistently been shown to improve endothelial function in a broad range of populations, especially in those who exhibit some level of dysfunction (38–43). Surprisingly, however, the exercise program employed in the current study did not significantly improve VEF in young females with moderate-to-severe ACE exposure (Fig. 4*A*), despite exhibiting moderate endothelial dysfunction. There are several factors that may explain this result. Acute, exercise induced-increases in vascular shear stress are likely the primary stimulus for training-induced improvements in VEF, as measured by FMD (44). Although both resistance (45) and interval exercise (46) elicit significant acute increases in mean and antegrade shear rates and have also been shown to elicit chronic improvements in vascular function (39, 42, 47, 48), it is plausible that the total exercise dose, and especially the aerobic exercise, was not sufficient to improve FMD in this study. Alternatively, we explored the relationship between ACE exposure and FMD improvements in the training group with the hypothesis that ACEs may blunt exercise adaptations (Fig. 4). Although the correlations were not significant, there were moderate inverse relationships observed between ACE score and change in FMD ($r = -0.35$; $P = 0.12$) and ACE score and change in SIRT1 ($r = -0.44$; $P = 0.07$). Recently, Eller et al. (35) reported that early life stress reduces the therapeutic metabolic benefits of exercise in mice. Taken together, due to the biological embedding of early life trauma, it is plausible that physiological responsiveness to therapeutic interventions may indeed be impaired in those with ACEs, and it would also be realistic to expect that the degree of impairment would be related to the degree of exposure. Unfortunately, the scale of the present study and the lack of an ACE^- exercise group prevent us from drawing a firm conclusion. Importantly, however, the ACE^+_{EXT} group did experience significant improvements in both depression and anxiety levels, suggesting that exercise may improve psychological well-being even in the absence of notable physiological improvements. Additional, larger-scale studies are desperately needed to further explore these possibilities because of the pervasiveness of ACEs and the associated increases in risk and because exercise is perhaps the most ubiquitous and robust, nonpharmacological intervention that can be employed to improve cardiovascular outcomes.

In conclusion, the results of the present study provide strong evidence that ACEs are associated with reduced VEF. Strikingly, this dysfunction is present in otherwise healthy, normotensive young adult women and even after adjusting for age, BMI, MAP, and depression and anxiety levels. We also observed significantly lower circulating SIRT1 concentrations, which were strongly related to VEF, in those with a history of ACEs. Consequently, adjusting for circulating SIRT1 reduced the difference in FMD observed between the ACE^+ and ACE^- groups. Contrary to our hypothesis and although associated with improvements in anxiety and depression, an 8-wk exercise intervention was unable to augment VEF or SIRT1 concentrations in a subset of the ACE^+ women enrolled in this study. Together with initial evidence in rats exposed to early life stress (14), our data suggest that childhood psychosocial stress likely increases susceptibility to hypertension and CVD by promoting endothelial dysfunction in association with a SIRT1 pathway-related mechanism.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

ENDNOTE

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