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Endothelial and inflammatory responses to acute exercise in perimenopausal and late postmenopausal women

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Serviente C, Troy LM, de Jonge M, Shill DD, Jenkins NT, Witkowski S. Endothelial and inflammatory responses to acute exercise in perimenopausal and late postmenopausal women. *Am J Physiol Regul Integr Comp Physiol* 311: R841–R850, 2016. First published August 17, 2016; doi:10.1152/ajpregu.00189.2016.—Endothelial dysfunction and inflammation are characteristics of subclinical atherosclerosis and may increase through progressive menopausal stages. Evaluating endothelial responses to acute exercise can reveal underlying dysfunction not apparent in resting conditions. The purpose of this study was to investigate markers of endothelial function and inflammation before and after acute exercise in healthy low-active perimenopausal (PERI) and late postmenopausal (POST) women. Flow-mediated dilation (FMD), CD31⁺/CD42b⁻ and CD62E⁺ endothelial microparticles (EMPs), and the circulating inflammatory factors monocyte chemoattractant protein 1 (MCP-1), interleukin 8 (IL-8), and tumor necrosis factor- α (TNF- α) were measured before and 30 min after acute exercise. Before exercise, FMD was not different between groups (PERI: $6.4 \pm 0.9\%$ vs. POST: $6.5 \pm 0.8\%$, $P = 0.97$); however, after acute exercise PERI tended to improve FMD ($8.5 \pm 0.9\%$, $P = 0.09$), whereas POST did not ($6.2 \pm 0.8\%$, $P = 0.77$). Independent of exercise, we observed transient endothelial dysfunction in POST with repeated FMD measures. There was a group \times exercise interaction for CD31⁺/CD42b⁻ EMPs ($P = 0.04$), where CD31⁺/CD42b⁻ EMPs were similar before exercise (PERI: 57.0 ± 6.7 EMPs/ μ l vs. POST: 58.5 ± 5.3 EMPs/ μ l, $P = 0.86$) but were higher in POST following exercise (PERI: 48.2 ± 6.7 EMPs/ μ l vs. POST: 69.4 ± 5.3 EMPs/ μ l, $P = 0.023$). CD62E⁺ EMPs were lower in PERI compared with POST before exercise ($P < 0.001$) and increased in PERI ($P = 0.04$) but did not change in POST ($P = 0.68$) in response to acute exercise. After acute exercise, MCP-1 ($P = 0.055$), TNF- α ($P = 0.02$), and IL-8 ($P < 0.001$) were lower in PERI but only IL-8 decreased in POST ($P < 0.001$). Overall, these data suggest that perimenopausal and late postmenopausal women display different endothelial and inflammatory responses to acute exercise.

menopause; endothelial function; inflammation; acute exercise

CARDIOVASCULAR DISEASE (CVD) is the leading cause of death for women in developed countries (40) and 1 in 3 women die from CVD annually (19). Menopause is generally associated with an increase in CVD risk factors, with an acceleration of risk that begins during the perimenopausal years and continues to worsen into postmenopause (38, 39). Ovarian hormones are generally believed to exert protective cardiovascular effects (33, 44). Perimenopausal and postmenopausal women differ in ovarian hormone exposure; perimenopausal women retain intermittent exposure that is absent in postmenopausal women.

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Peri- and postmenopausal women also differ in the length of time ovarian hormone exposure has been reduced. Interestingly, hormone replacement therapy efficacy varies in women at different menopausal stages (31, 37), which may be related to these differences. Because of the accelerated risk that occurs during perimenopause, and ovarian hormone differences between peri- and postmenopausal women, it is important to evaluate mechanisms related to increased CVD risk in women at different menopausal stages. Improved knowledge of CVD risk accumulation during the menopausal transition may advance efforts to monitor and mediate risk in aging women.

The mechanisms behind adverse changes in CVD risk that accompany menopause are still unclear but may be related to changes in ovarian hormones or traditional CVD risk factors such as blood pressure or lipid levels (38–40). These changes, along with changes in inflammatory cytokines such as monocyte chemoattractant protein 1 (MCP-1), interleukin 8 (IL-8), and tumor necrosis factor- α (TNF- α) may adversely impact endothelial function, which itself leads to an increased risk of cardiovascular disease (7). Endothelial cells are a physical barrier between blood and blood vessel walls and have a variety of dynamic properties. Endothelial cells secrete factors that affect vasomodulation, platelet adhesion and aggregation, smooth muscle cell migration and proliferation, and inflammation (69). In response to an increase in shear stress, endothelial cells cause vasodilation via the secretion of nitric oxide (NO) (50) and can release endothelial microparticles (EMP) indicative of endothelial activation (CD62E⁺) and/or apoptosis (CD31⁺/CD42b⁻) (12, 71). Inflammation is associated with oxidation of LDL-cholesterol and expression of cytokines (20, 57). Endothelial function appears to decline through the menopausal transition (46). This decline may be related to changes in inflammatory cytokines such as MCP-1, IL-8, and TNF- α , as these cytokines are involved in the initiation of the inflammatory process and trigger the recruitment of white blood cells to damaged endothelial cells, potentially leading to the initiation of the atherosclerotic process. As such, they have been implicated as potential early markers of cardiovascular disease (3, 41, 61).

Exercise is associated with improved endothelial function. The endothelial response to acute exercise is attributed to increased NO bioavailability and decreased vasoconstrictor factors (i.e., endothelin-1) (20). Acute exercise may also change markers of endothelial apoptosis and endothelial activation (36) and can transiently increase cytokine expression (35, 57, 58). Furthermore, the endothelial response to acute exercise may reveal differences in endothelial function in groups with differing cardiovascular disease risk, despite sim-

ilar preexercise function (16, 18). Therefore, we aimed to assess differences in endothelial function and inflammatory biomarkers before and after an acute bout of exercise in perimenopausal and late postmenopausal women. We hypothesized that markers of endothelial dysfunction and inflammation would be higher in late postmenopausal women compared with perimenopausal women before exercise. After acute exercise, we expected higher levels of inflammation and less of an endothelial response in the late postmenopausal compared with the perimenopausal group.

MATERIALS AND METHODS

The study consisted of three visits. The first and second visits were used for participant screening and to familiarize participants with study protocols. The third visit assessed endothelial and inflammatory markers before and after 30 min of moderate-intensity exercise.

Participants. Data collection was completed on 15 participants. Participants were classified as perimenopausal ($n = 7$) or late postmenopausal ($n = 8$) based on Stages of Reproductive Aging Workshop +10 guidelines (21): early perimenopausal ($n = 1$): variable menstrual cycle length (>7 days different from normal); late perimenopausal ($n = 6$): ≥ 60 days but < 1 yr of amenorrhea; late postmenopausal ($n = 8$): > 5 yr of amenorrhea. Early perimenopausal and late perimenopausal women were combined for this analysis. No early postmenopausal (≥ 1 yr and < 5 yr amenorrhea) women were included.

Participants were screened for cholesterol, triglycerides, fasting plasma glucose, body fat percentage via dual X-ray absorptiometry, and blood pressure. Participants were normotensive, nondiabetic, had normal blood lipid chemistries (LDL-C: ≤ 159 mg/dl, HDL-C > 40 mg/dl, triglycerides < 150 mg/dl), were ≤ 65 yr old, and participated in < 150 min/wk of moderate intensity activity or < 75 min/wk of vigorous intensity activity (accumulated in 10-min bouts). Physical activity was assessed using the International Physical Activity Questionnaire, which has been validated in this age range (11). Participants were excluded if they did not meet inclusion criteria or were taking hormone replacement therapy; were undergoing treatment for menopausal symptoms; had taken oral contraceptives in the past 6 mo; had a history of cardiovascular disease, myocardial infarction, cardiovascular intervention (e.g., pacemaker implant), chronic menstrual irregularities before menopause; breast cancer, vaginal bleeding, abnormal uterine/ovary anatomy, venous thromboembolism, acute liver or gallbladder disease; used medications or vitamin/supplements known to impact endothelial function, such as cholesterol-lowering and/or anti-inflammatory medications; or were current smokers or had smoked in the 6 mo before study enrollment. All experimental protocols were approved by the Institutional Review Board of the University of Massachusetts Amherst.

Acute exercise protocol. An incremental treadmill $\dot{V}O_{2\max}$ test was used to assess peak oxygen consumption (Parvo Medics TrueOne 2400, Sandy, UT) and heart rate using a 12-lead electrocardiogram. On a separate day, participants walked on a treadmill for 30 min at the heart rate that corresponded to 60–64% of peak oxygen uptake. The session began and ended with a 5-min warm up and cool down. A Polar FT1 heart rate monitor (Polar Electro, Lake Success, NY) was used to verify that the participant maintained the prescribed intensity throughout the session. Before and 30 min after the acute bout of exercise, a blood draw and two flow-mediated dilation (FMD) studies, conducted 15 min apart, were completed.

Flow-mediated dilation. All participants underwent an FMD familiarization trial before data collection. FMD protocols were completed in the morning to control for diurnal variation, and menstruating early perimenopausal women were assessed on menstrual cycle days 2–5. Participants followed a 3-day low-nitrate diet before data collection and were instructed to fast for 6 h, to avoid exercise, caffeine,

smoking, and alcohol for 12 h, and to stop taking any vitamins and supplements 72 h before the visit.

FMD was assessed according to published guidelines (66). Briefly, after 10 min of supine rest, an L-12-5 ultrasound and Doppler probe with a 60° insonation angle (Philip's HD11XE Ultrasound System, Bothell, WA) was used to image the brachial artery proximal to the cubital fossa. A rapid inflation cuff (D. E. Hokanson, Bellevue, WA) was placed around the forearm. The brachial artery was continuously imaged during 2 min of rest, 5 min of forearm cuff inflation (200 mmHg), and 4 min following cuff deflation. Blood pressure and heart rate were recorded every minute while artery diameter was digitally captured using FMD Studio Suite Software (FMD Studio, Quipu, Pisa, Italy). For serial FMD trials, participants rested a minimum of 15 min between studies, and the second study began when baseline diameter had returned to prestudy values. Arm position, distance to the cuff, and position of the ultrasound probe were measured and marked to ensure consistency across trials. Digital video files were used to analyze baseline and peak diameter. Percent change in brachial artery diameter was calculated for all studies.

Blood analysis: inflammation, microparticles, and estradiol. Before and 30 min after acute exercise, participants underwent a blood draw to assess CD31⁺/CD42b⁻ and CD62E⁺ EMPs, MCP-1, IL-8, and TNF- α . Failure to obtain blood samples in two of the participants resulted in analysis of five perimenopausal and eight late postmenopausal women. Inflammatory cytokines were assessed using an MSD V-Plex Plus Custom Cytokine & Chemokine Assay (Meso Scale Discovery, Rockville, MD; https://www.mesoscale.com/en/products_and_services/assay_kits/v-plex/v-plex_product_selector). All samples were run in triplicate and fell within the assay detection range. The average coefficients of variation for MCP-1, TNF- α , and IL-8 were 5.6%, 3.8%, and 2.5%, respectively.

EMP concentrations were determined in batch assays as previously described (68). Flow cytometry data were analyzed via FlowJo V10.1r5 (FlowJo, Ashland, OR). EMPs were identified and confirmed using forward and side scatter parameters of 90 nm calibration beads (Polysciences, Warrington, PA), and concentrations were calculated using CountBright Absolute Counting Beads (ThermoFisher Scientific, Waltham, MA). EMPs were defined as CD31⁺/CD42b⁻ (indicative of endothelial apoptosis) and CD62E⁺ (indicative of endothelial activation) events within the microparticle gate.

17- β -Estradiol was assessed using a colorimetric ELISA assay (Invitrogen, Camarillo, CA; <https://www.thermofisher.com/order/catalog/product/KAQ0621>). All samples were run in triplicate according to manufacturer instructions. A standard curve was calculated ($r^2 = 0.99$) and sample values were fit to the curve. The average coefficient of variation for samples was 0.27%.

Statistical analysis. All data are presented as means \pm SE and were analyzed using SigmaStat software (Systat Software, San Jose, CA). Statistical significance in all figures is presented with P values. Interaction and main effects are noted with lines and individual comparisons are noted with brackets. Data were evaluated for the adherence to assumptions for each statistical test proposed. IL-8 data were normalized using a log-transformation, and estradiol data were transformed with a square root transformation for analysis. The nontransformed data are presented for interpretation. Differences between baseline characteristics were evaluated using independent t -tests, or if the data did not meet the equal variance assumption, a Mann-Whitney rank sum test was used. To evaluate differences in FMD (% change in FMD), EMPs, MCP-1, IL-8, and TNF- α concentrations within and between the two menopausal groups before and after exercise, two-way repeated measures ANOVAs (group \times exercise) were used, followed by Holm-Sidak post-hoc testing.

Post-hoc analyses were performed to assess differences in serial FMD measurements and to identify any relationships between inflammatory cytokines, estradiol, FMD values, and baseline characteristics. Serial FMD measurements were assessed with a three-way ANOVA (group \times exercise \times trial) and Holm-Sidak post-hoc testing. Pearson

product moment correlations were used to examine associations between variables, with the most relevant to the study outcomes reported.

Regression analysis was performed as an exploratory aim to determine the best predictors of the FMD response to acute exercise. The dependent variable was the average change in FMD from post- to preexercise. Forwards and backwards stepwise age-adjusted regression analysis was used to determine variables that were most predictive of the FMD response to acute exercise. Baseline characteristics [systolic blood pressure (SBP), diastolic blood pressure (DBP), body fat, $\dot{V}O_{2\text{ peak}}$, HDL-C, LDL-C, TG, and FPG], preexercise endothelial microparticles, preexercise inflammatory cytokines, preexercise FMD, and menopausal status (0: perimenopause; 1: postmenopause) were included in the models. Menopausal status, estradiol, DBP, LDL-C, $\dot{V}O_{2\text{ peak}}$, preexercise CD31⁺/CD42b⁻ and CD62E⁺ EMPs, preexercise MCP-1, and preexercise TNF- α were selected to include in the final models. A separate age-adjusted regression analysis was performed to determine the predictive power of menopausal status on the FMD response to acute exercise.

RESULTS

Participant characteristics. Baseline characteristics were similar for perimenopausal and late postmenopausal women (Table 1). Both groups had statistically similar height, weight, body fat percentage, body mass index (BMI), $\dot{V}O_{2\text{ peak}}$, high-density lipoprotein cholesterol (HDL-C), fasting plasma glucose (FPG), SBP, DBP, and self-reported weekly time spent in moderate-to-vigorous physical activity (MVPA). Late postmenopausal women were significantly older and had higher LDL-C and lower estradiol compared with perimenopausal women. Ten-year risk of developing CVD, calculated using the Framingham Risk Calculator (13), was also significantly higher in the late postmenopausal group; however, both groups had relatively low CVD risk. Baseline characteristics were similar for the subgroups of participants used for EMP and cytokines analyses; however, LDL-C values did not differ between groups.

Flow mediated dilation. There was no effect of menopausal group ($P = 0.234$) or exercise ($P = 0.281$) on FMD. Before

Table 1. Participant characteristics

	Perimenopausal (n = 7)	Postmenopausal (n = 8)
Years since FMP		8.8 ± 1.5
Estradiol, pg/ml	117.3 ± 27.1	16.5 ± 9.0*
Age, yr	47.3 ± 1.5	58.9 ± 1.4*
Height, cm	163.7 ± 3.1	166.5 ± 3.1
Weight, kg	72.9 ± 7.2	69.0 ± 4.3
Body fat, %	40.8 ± 2.6	41.9 ± 1.5
BMI, kg/m ²	27.0 ± 2.3	24.8 ± 1.3
$\dot{V}O_{2\text{ peak}}$, ml·kg ⁻¹ ·min ⁻¹	30.1 ± 1.6	28.3 ± 1.1
HDL-C, mg/dl	70.4 ± 9.7	78.4 ± 4.8
LDL-C, mg/dl	84.7 ± 8.4	116.5 ± 9.5*
FPG, mg/dl	92.4 ± 3.0	99.5 ± 1.7
SBP, mmHg	104.6 ± 5.6	117.3 ± 5.2
DBP, mmHg	64.6 ± 4.5	64.6 ± 2.7
TG, mg/dl	52.0 ± 6.9	46.4 ± 5.7
10-year CVD risk, %	1.9 ± 0.3	3.5 ± 0.6*
MVPA, MET·min/wk	293.1 ± 98	100.3 ± 40.9

Participant characteristics for each group are expressed as means ± SE. FMP, final menstrual period; BMI, body mass index; FPG, fasting plasma glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CVD, cardiovascular disease; MVPA, moderate-to-vigorous physical activity; MET, metabolic equivalent. * $P \leq 0.05$ for between group comparison.

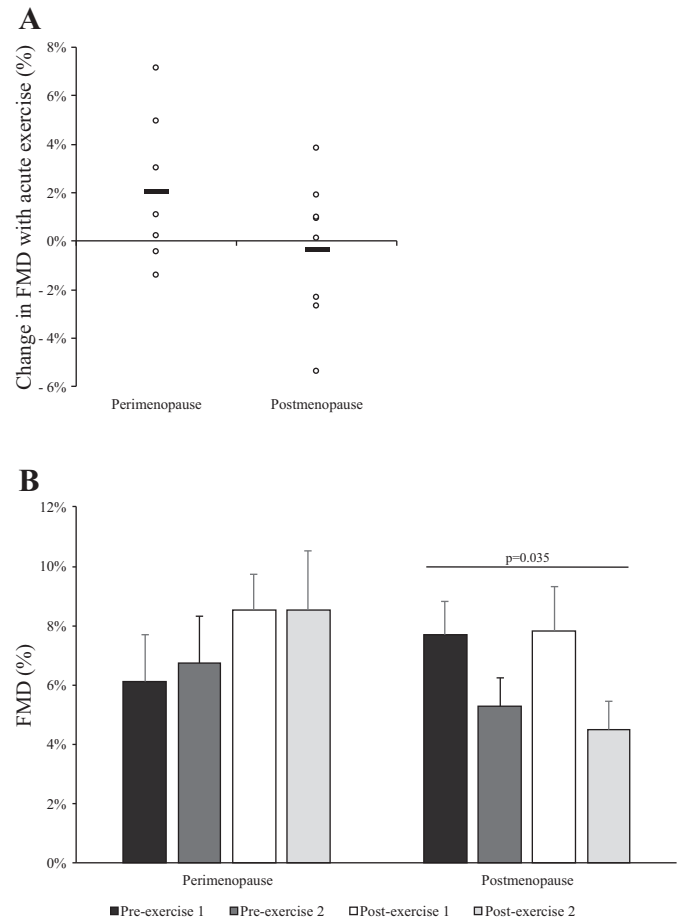


Fig. 1. Before and after exercise, two flow-mediated dilation (FMD) trials were repeated 15 min apart. *A*: average individual response to acute exercise for each group. Open circles, individual changes in measurements, with the group mean represented by the black bar. *B*: average of individual trials for each group. The line represents the main effect of trial.

exercise, there were no differences in FMD between groups (perimenopause: $6.4 \pm 0.9\%$ vs. postmenopause: $6.5 \pm 0.8\%$, $P = 0.97$). After exercise, the perimenopausal group tended to have a higher FMD response compared with preexercise levels ($8.5 \pm 0.9\%$, $P = 0.09$), whereas the late postmenopausal group did not ($6.2 \pm 0.8\%$, $P = 0.77$). Furthermore, perimenopausal women showed a trend for higher FMD after exercise compared with late postmenopausal women ($P = 0.063$). The average change in FMD (Fig. 1A) between the two groups was not statistically different ($P = 0.15$). There were no differences in baseline artery diameter (perimenopause: 3.2 ± 0.1 mm preexercise vs. 3.2 ± 0.1 mm postexercise; postmenopause: 3.3 ± 0.1 mm vs. 3.3 ± 0.1 mm postexercise) across groups ($P = 0.53$) or with exercise ($P = 0.38$).

The effect of serial FMD measurements is controversial in the literature; some studies have shown no effect of serial measures (23, 54), whereas others have shown that the first measurement negatively impacts the second (49). Therefore, we investigated differences between repeated FMD measures (Fig. 1B). In perimenopausal women, there was no difference in FMD between serial measurements ($P = 0.84$). However, the late postmenopausal group had an effect of serial measurements, with a lower FMD response in the second measurement

compared with the first ($P = 0.035$). This effect of serial measurements is further highlighted by a difference between perimenopausal and late postmenopausal women in the second measurement ($P = 0.052$) and the trend for a group \times trial interaction ($P = 0.110$). There were similar baseline diameters between groups ($P = 0.23$), for all trials ($P = 0.45$), before and after exercise ($P = 0.68$), which reveals that the changes were not due to differences in starting diameter.

Pearson correlations were used to determine whether any variables were related to FMD in all participants. There was a trend for a relationship between the FMD response to acute exercise and estradiol ($r = 0.50$, $P = 0.08$).

Endothelial microparticles. For $CD31^+/CD42b^-$ EMPs, there was a group \times exercise interaction ($P = 0.04$). Before exercise, $CD31^+/CD42b^-$ EMPs were similar in the perimenopausal and late postmenopausal group (perimenopause: 57.0 ± 6.7 EMPs/ μ l plasma vs. postmenopause: 58.5 ± 5.3 EMPs/ μ l plasma, $P = 0.86$). After acute exercise, $CD31^+/CD42b^-$ EMPs were higher in the late postmenopausal group compared with the perimenopausal group (perimenopause: 48.2 ± 6.7 EMPs/ μ l plasma vs. postmenopause: 69.4 ± 5.3 EMPs/ μ l plasma, $P = 0.023$). Furthermore, late postmenopausal women had a trend for higher postexercise levels ($P = 0.07$), whereas perimenopausal women did not ($P = 0.22$) (Fig. 2A). Figure 2B shows the individual data for the difference between groups in response to acute exercise ($P = 0.062$).

For $CD62E^+$ EMPs, there was a significant group effect ($P = 0.002$) and a trend for an effect of exercise ($P = 0.06$). Before exercise $CD62E^+$ EMPs were significantly lower in the perimenopausal group (perimenopause: 371.8 ± 61.9 EMPs/ μ l plasma vs. postmenopause: 673.8 ± 48.2 EMPs/ μ l plasma, $P < 0.001$). In response to acute exercise, $CD62E^+$ EMPs increased in the perimenopausal group but did not change in the late postmenopausal group (perimenopause: 562.8 ± 60.9 EMPs/ μ l plasma, $P = 0.04$; postmenopause: 702.4 ± 48.2 EMPs/ μ l plasma, $P = 0.68$) (Fig. 3A), and there was a trend for a difference between groups after exercise ($P = 0.09$). Figure 3B shows the individual data for the difference between groups in response to acute exercise ($P = 0.19$).

Inflammation. For MCP-1 there was a significant group \times exercise interaction ($P = 0.03$). Before exercise, there was no difference in serum MCP-1 between perimenopausal and late postmenopausal women (perimenopause: 341.5 ± 38.4 pg/ml vs. postmenopause: 377.2 ± 30.4 pg/ml, $P = 0.48$). Perimenopausal women tended to decrease MCP-1, whereas MCP-1 in late postmenopausal women did not change in response to acute exercise (perimenopause: 300.2 ± 38.4 pg/ml, $P = 0.055$; postmenopause: 397.4 ± 30.4 pg/ml, $P = 0.21$). Furthermore, there was a trend for a difference between groups following exercise ($P = 0.07$) (Fig. 4A). Figure 4B shows the individual data for the difference between menopausal groups in response to acute exercise ($P = 0.043$).

There was an overall effect of exercise on $TNF-\alpha$ ($P = 0.008$). There was no difference in serum $TNF-\alpha$ between perimenopausal and late postmenopausal women before exercise (perimenopause: 2.3 ± 0.2 pg/ml vs. postmenopause: 2.6 ± 0.2 pg/ml, $P = 0.30$). After exercise, perimenopausal women had a decrease in $TNF-\alpha$, whereas $TNF-\alpha$ did not change in the late postmenopausal group (perimenopause: 1.9 ± 0.2 pg/ml, $P = 0.02$; postmenopause: 2.4 ± 0.2 pg/ml, $P = 0.09$) (Fig. 5A). Figure 5B shows the individual data for

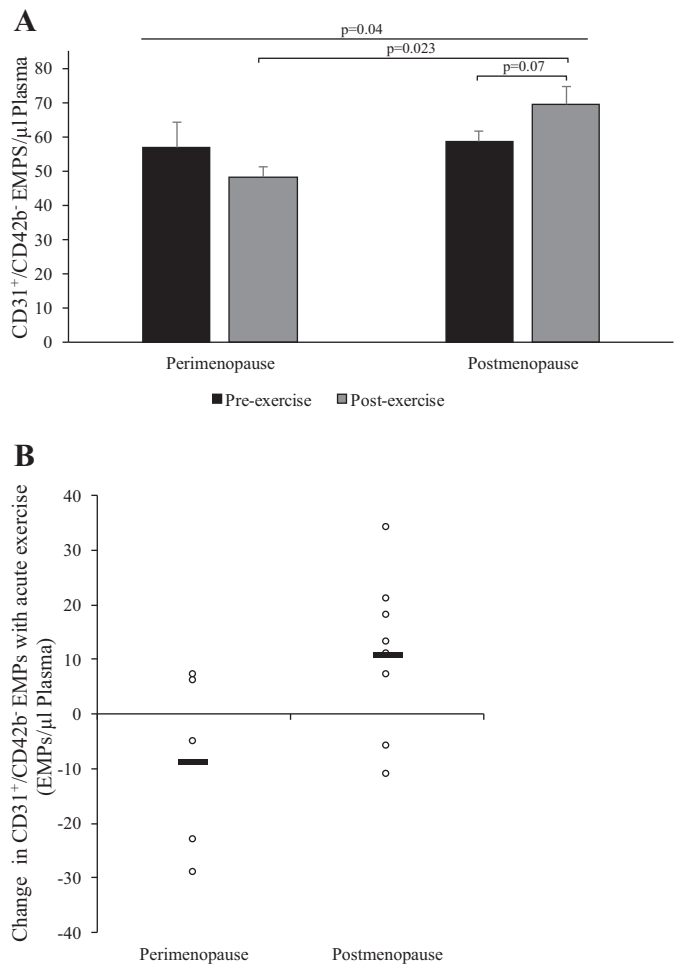


Fig. 2. $CD31^+/CD42b^-$ endothelial microparticles (EMPs) were assessed before and 30 min after acute exercise. Line represents a group \times exercise interaction (A). Individual $CD31^+/CD42b^-$ EMPs responses to acute exercise are represented with open circles. The black bar represents the mean of the group (B).

the difference between groups in response to acute exercise ($P = 0.425$).

For IL-8, there was a group \times exercise interaction ($P = 0.02$) and an overall effect of exercise ($P < 0.001$). Both perimenopausal and late postmenopausal women experienced a decrease in IL-8 compared with preexercise levels (perimenopause: 14.8 ± 3.8 pg/ml, $P < 0.001$; postmenopause: 15.3 ± 4.1 pg/ml, $P < 0.001$). Before exercise, perimenopausal women had a trend for higher serum IL-8 levels compared with late postmenopausal women (perimenopause: 146.1 ± 59.7 pg/ml vs. postmenopause: 57.1 ± 13.3 pg/ml, $P = 0.07$) (Fig. 6A). Figure 6B shows the individual data for the difference between groups in the response to acute exercise ($P = 0.093$). Preexercise IL-8 was significantly related to age ($r = -0.65$, $P = 0.02$).

Regression analysis. Menopausal status alone only explained 15.3% of the variance in the FMD response to acute exercise; however, 70% of the variance in the response was explained when combined with low-density lipoprotein cholesterol, $\dot{V}O_{2\text{ peak}}$, and diastolic blood pressure (Table 2). When DBP was removed from the model, the predictive power decreased by $\sim 10\%$ ($R^2 = 0.604$, adjusted $R^2 = 0.495$,

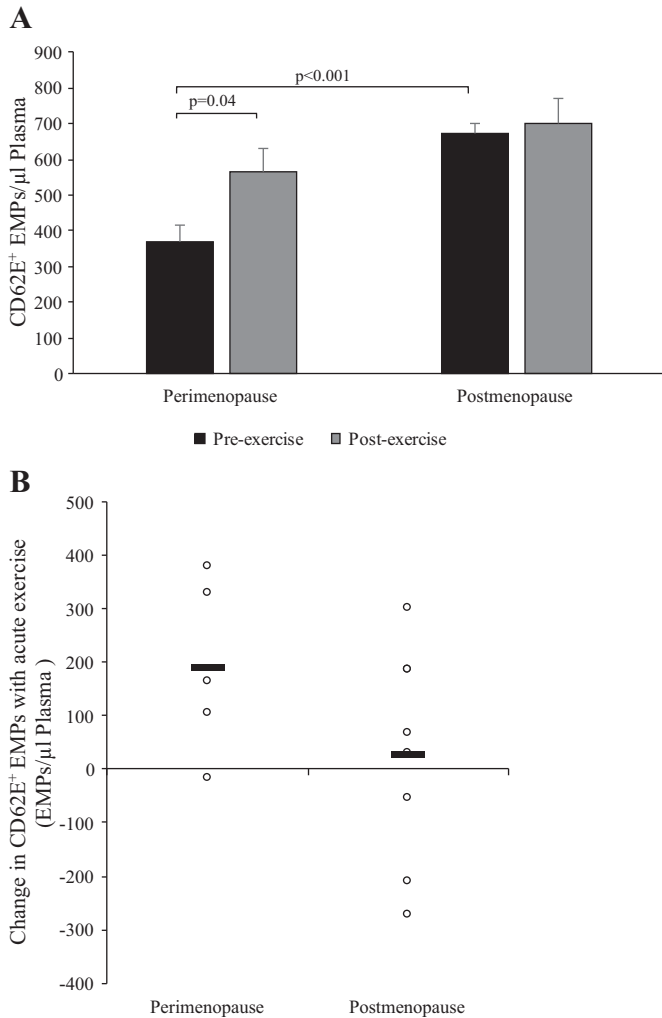


Fig. 3. CD62E⁺ EMPs were assessed before and 30 min after acute exercise (A). Individual responses to acute exercise are represented with open circles. Black bar represents the mean of the group (B).

P-value = 0.014), suggesting that despite its lack of independent statistical significance, DBP has an important influence on the FMD response to acute exercise.

DISCUSSION

Our primary finding was that while there were few baseline differences between groups, healthy low-active perimenopausal and late postmenopausal women had different endothelial and inflammatory responses to acute exercise. Specifically, perimenopausal women responded to acute exercise with an increase in CD62E⁺ EMPs and a trend for an increase in FMD, along with decreases in the inflammatory cytokines MCP-1, TNF-α, and IL-8. Late postmenopausal women had no change in FMD, CD62E⁺ EMPs, MCP-1, or TNF-α and a trend for an increase in CD31⁺/CD42b⁻ EMPs with acute exercise. Interestingly, late postmenopausal women demonstrated transient endothelial dysfunction with repeated FMD measurements. Overall, these results demonstrate that healthy low-active perimenopausal women have more adaptive endothelial and inflammatory responses to acute exercise compared with late postmenopausal women who demonstrate reduced responsiveness.

Endothelial responses. FMD is a preclinical marker for cardiovascular disease and is prognostic of cardiovascular events in postmenopausal women (56). When measured using established guidelines, FMD is largely NO mediated (66) and is related to coronary artery function (2). Most studies in middle-aged and older women have measured FMD after exercise training, with some reporting increased FMD (28) and others reporting no change in FMD (47, 64). Assessment of FMD before and after a single bout of exercise can provide insight into the ability of the endothelium to respond to this acute cardiovascular challenge (14, 52) and may elucidate differences in endothelial function and NO bioavailability that are not observed at rest. Disparate acute exercise FMD responses have been reported in groups with differing CVD risk. In overweight active and sedentary men, FMD did not differ before exercise; however, after acute exercise the sedentary group had a decreased FMD response, whereas the active group had an increased FMD response (22). This differential response to acute exercise has also been shown in smokers compared with nonsmokers (18) and in obese compared with lean premenopausal women (17). In the present study, although most CVD risk factors were similar between groups, late perimenopausal women had higher 10-yr CVD risk due to higher age and LDL-cholesterol. In women at distinct meno-

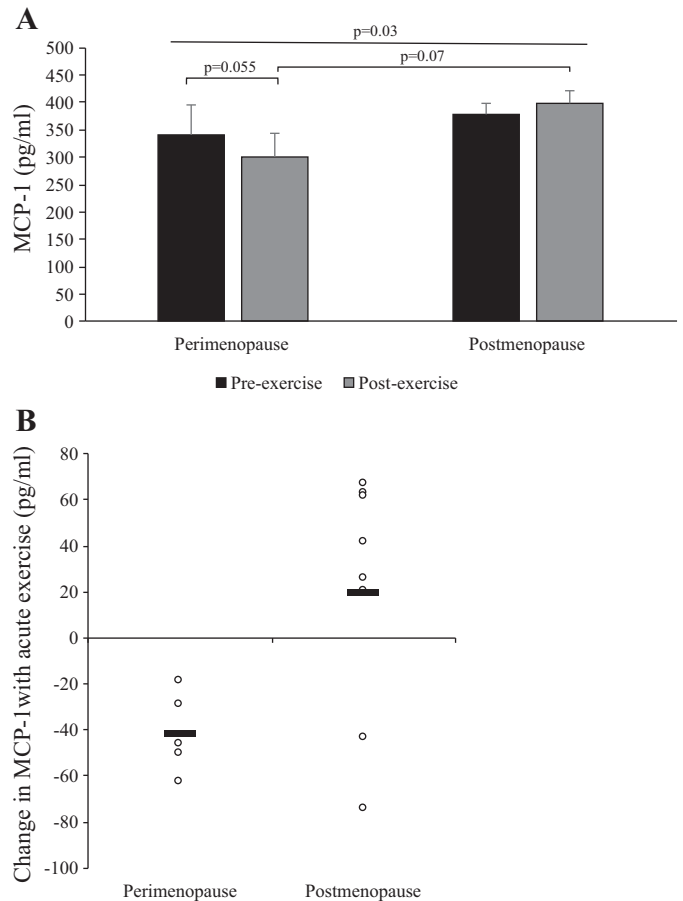


Fig. 4. Monocyte chemoattractant protein 1 (MCP-1) was analyzed from serum samples before and 30 min after acute exercise in both groups. Line represents a group × exercise interaction (A). Individual responses to acute exercise are represented with open circles. Black bar represents the mean of the group (B).

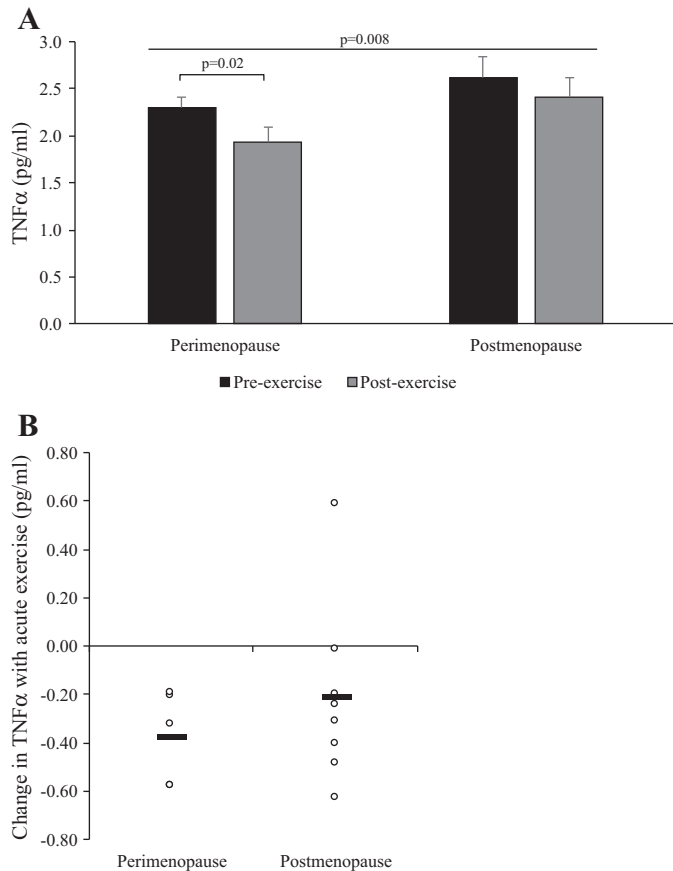


Fig. 5. Tumor necrosis factor- α (TNF- α) was analyzed from serum samples before and 30 min after acute exercise in both groups. Line represents the main effect of exercise (A). Individual responses to acute exercise are represented with open circles. Black bar represents the mean of the group (B).

pausal stages, Moreau et al. (46) demonstrated that FMD generally decreased across the menopausal transition. However, when late perimenopausal and late postmenopausal women were matched for most CVD risk factors, no difference in FMD was found. Therefore, differences in CVD risk factors in our population may have contributed to the trend for a difference in the FMD response to acute exercise and is supported by a regression analysis where we determined that the combination of menopausal status, $\dot{V}O_{2\text{ peak}}$, LDL-C, and DBP predicted 70% of the FMD response to acute exercise.

The FMD response to acute exercise in women at different menopausal stages has not been well characterized. Studies comparing responses between pre- and postmenopausal women reported higher baseline FMD in premenopausal women compared with postmenopausal women and an enhanced FMD response to acute exercise in postmenopausal, but not premenopausal women (24, 25). Differences between these studies and data reported herein could be due to differences in FMD measurement technique. In the current study, FMD was measured with cuff placement distal to the imaged region, which is related to a largely NO-mediated vasodilatory response (66). FMD was measured in the other studies with a cuff placement proximal to the imaging site on the brachial artery. Proximal cuff placement is associated with the release of vasodilatory factors other than NO and/or the arterial myogenic response (66).

We found that in late postmenopausal, but not perimenopausal women, serial FMD measurements were associated with transient endothelial dysfunction that was not caused by differences in artery diameter. A recent review of current literature suggests that there may be a biphasic FMD response after acute exercise characterized by an initial blunted FMD, followed by an enhanced or normalized FMD (14). Furthermore, transient endothelial dysfunction has been reported in response to repeated FMD measurements (15), although it is generally accepted that serial FMD measures do not impact one another (23, 53) as the Brachial Artery Reactivity Task Force recommends completing FMD measurements 15 min apart to assess reproducibility (10). Transient endothelial dysfunction following serial FMD measurements separated by 15 min was previously shown in young healthy men (49). This dysfunction was ameliorated following supplementation with L-arginine, a cofactor necessary for the enzymatic production of NO by endothelial NO synthase, and was associated with elevated asymmetric dimethylarginine, a NO inhibitor (49). It is possible that the difference in postexercise FMD and the response to repeated FMD measurements for peri- and late postmenopausal

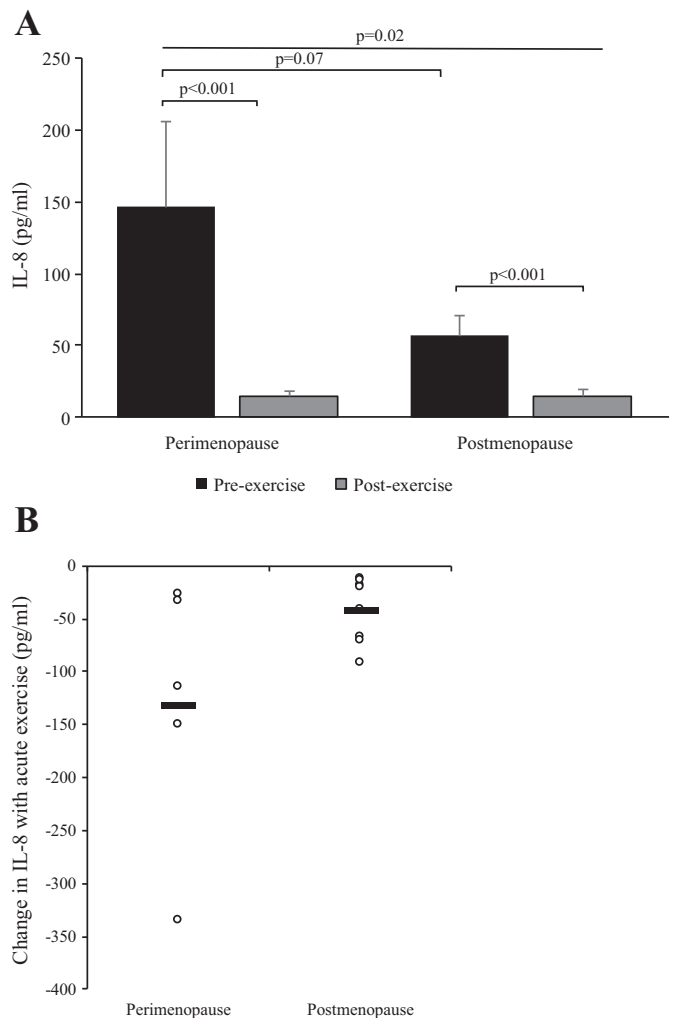


Fig. 6. IL-8 was analyzed from serum samples before and 30 min after acute exercise in both groups. Line represents a group \times exercise interaction (A). Individual responses to acute exercise are represented with open circles. Black bar represents the mean of the group (B).

Table 2. Age-adjusted models of predictors in flow mediated dilation

Model (<i>P</i> Value)	Variables	<i>P</i> Value	<i>R</i> ²	Adjusted <i>R</i> ²
Model 1 (0.15)*	Menopausal status	0.150	0.153	0.088
Model 2 (0.029)*	Menopausal status	0.004	0.705	0.558
	LDL-C	0.009		
	$\dot{V}O_{2peak}$	0.023		
	DBP	0.153		

Menopausal status (0: perimenopause; 1: postmenopause), LDL-C, low-density lipoprotein cholesterol; DBP, diastolic blood pressure. *Model adjusted for age.

women may suggest less NO bioavailability or recovery of NO production capacity in late postmenopausal women.

The higher levels of estradiol may contribute to greater NO bioavailability and brachial artery reactivity in the perimenopausal group. Estrogen acts directly on the vasculature and triggers vasodilation through enhanced NO production, among other mechanisms (27, 32, 43). Chronic estrogen administration enhances FMD in postmenopausal women (25, 47) and NO-dependent vasodilation in perimenopausal women (63). Furthermore, improvements in FMD with exercise training appear to be dependent on the presence of estrogen (47). The relationship between estrogen and FMD is supported by the trend for a positive relationship between estradiol levels and the FMD response to acute exercise in our data.

EMPs are markers of endothelial activation ($CD62E^+$) and apoptosis ($CD31^+/CD42b^-$) that are released when the endothelium undergoes an acute or chronic stress and provide an indication of the overall state of the vasculature (12, 71). Higher circulating levels of EMPs are associated with a variety of disease states (6, 26, 59) and have been suggested as a novel biomarker for CVD (1). We observed no difference in $CD31^+/CD42b^-$ EMPs between perimenopausal and late postmenopausal women before exercise. This is in agreement with previous literature in pre- and postmenopausal women (34). After acute exercise, late postmenopausal women had significantly higher $CD31^+/CD42b^-$ EMPs, indicating greater endothelial apoptosis compared with perimenopausal women. This may suggest that the increased shear stress of unaccustomed acute exercise has an adverse affect on the endothelium of low-active late postmenopausal women. To our knowledge, this is the first study to examine the $CD31^+/CD42b^-$ EMPs response to acute exercise in peri- and late postmenopausal women.

$CD62E^+$ EMPs have not been previously examined in peri-versus late postmenopausal women; however, higher values have been reported in low versus higher estrogen status postmenopausal women (30). Before exercise, $CD62E^+$ EMPs were significantly higher in the late postmenopausal compared with the perimenopausal group, suggesting increased endothelial activation and an endothelium under greater stress in the later menopausal stage. The increase in $CD62E^+$ EMPs and endothelial activation in the perimenopausal group is likely due to the exercise stimulus. Exercise has been previously shown to stimulate the release of $CD62E^+$ EMPs in inactive premenopausal women (16). The lack of a change in $CD62E^+$ EMPs with exercise in late postmenopausal women may indicate a less responsive endothelium to the acute exercise stimulus due to higher activation at rest. Overall, the high levels of activa-

tion before and after exercise and increased apoptosis in response to acute exercise may suggest a stressed endothelium in the late postmenopausal group that may contribute to the lack of FMD response to acute exercise.

Inflammatory responses. MCP-1, TNF- α , and IL-8 are inflammatory cytokines involved in the initiation of atherosclerosis and have been associated with endothelial dysfunction (4, 5, 29, 48, 51). We hypothesized that because of greater cardiovascular disease risk and lower estradiol, late postmenopausal women would have higher circulating cytokines before exercise and would respond to acute exercise with an increase in these factors, when compared with perimenopausal women. We found that while there were no significant preexercise between-group differences, the response to exercise differed by cytokine and menopausal status. To our knowledge, this is the first study to investigate the response to acute exercise in women at different menopausal stages.

Estrogen is both anti-oxidative and anti-inflammatory in nature (32, 42, 62, 63). MCP-1 and TNF- α are inhibited by estrogen through direct and indirect mechanisms (4, 45, 55) and by NO (67, 72). MCP-1 is higher in older compared with younger women (60), and transdermal estrogen administration in postmenopausal women who underwent hysterectomy reduced circulating MCP-1 after 12 mo of therapy (70). In a cross-sectional comparison of women at different reproductive stages, MCP-1 was higher in late perimenopausal and postmenopausal women compared with premenopausal women, but TNF- α was similar among groups (65). Although we anticipated a difference in preexercise MCP-1 and TNF- α between groups, and our groups differed by age and circulating estradiol, they were matched for most other CVD risk factors, including blood pressure, lipid levels, and body fat, which may explain a lack of a difference in preexercise levels of these two cytokines. In fact, Moreau et al. (45) reported no difference in TNF- α between pre- and postmenopausal women who were matched for most CVD risk factors. Conversely, IL-8 has been shown to be higher in postmenopausal compared with early peri- and premenopausal women (65). However, a decrease in IL-8 with age has also been reported (9, 60). Our data supports the hypothesis that aging leads to a decrease in IL-8, as age was negatively associated with preexercise IL-8 in all women.

Generally, MCP-1, TNF- α , and IL-8 have been reported to increase 30–60 min after acute exercise, although this research has largely been conducted in young male populations (8, 35, 57, 58). In our study, TNF- α , MCP-1, and IL-8 decreased with acute exercise in perimenopausal women, but only IL-8 was significantly reduced with acute exercise in late postmenopausal women, revealing that acute exercise reduced inflammation to a lesser degree in late postmenopausal women. It is possible that the decrease in these cytokines in perimenopausal women is beneficial, as preexercise IL-8 and TNF- α were higher than what has been reported in other acute exercise studies (8, 35). Decreased IL-8 following acute exercise has been reported in lean and overweight/obese men and women and was related to increased expression of the anti-inflammatory cytokine interleukin-10 (15). The response to acute exercise may represent an anti-inflammatory response for perimenopausal women that did not occur to the same extent in late postmenopausal women.

Limitations. This pilot study provided novel insight into differences in endothelial and inflammatory responses to acute ex-

ercise in perimenopausal and late postmenopausal but is not without limitations. First, the cross-sectional nature of the study does not allow us to infer causation or an independent evaluation of the effect of age from menopausal status. A sample size estimation was calculated on the primary outcome, FMD, that required 12 participants per group to detect baseline differences and 9 per group to detect differences in response to acute exercise. Calculations were based on baseline FMD responses in women across the menopausal transition (46) and changes in FMD after acute exercise in postmenopausal women (24). Calculations were completed with a one-tailed test, since we hypothesized that the FMD response would decrease with later menopausal stages. Therefore, it is possible that differences between groups may not have been detected due to the limited statistical power. Furthermore, it is likely that other inflammatory factors or reactive oxygen species may be involved in the observed endothelial and inflammatory responses. Future assessment of these markers will allow for a more complete understanding of changes in both endothelial function and inflammation and the interaction of these two factors as women progress through menopause.

Perspectives and Significance

Our findings indicate that perimenopausal women have enhanced endothelial function and activation and decreased inflammation in response to acute exercise. Conversely, the endothelium of late postmenopausal women is less responsive to acute exercise and displays transient endothelial dysfunction with repeated FMD measurements. Overall, these data suggest that factors related to endothelial responsiveness are reduced in later menopausal stages. We speculate that intermittent ovarian hormone exposure in perimenopause is sufficient to reduce subclinical CVD risk. Further development and evaluation of strategies to maintain and improve endothelial responsiveness in women at different menopausal stages are necessary.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

C.S., and S.W. conception and design of research; C.S., M.d.J., and S.W. performed experiments; C.S., M.d.J., D.D.S., and N.T.J. analyzed data; C.S., L.M.T., M.d.J., N.T.J., D.D.S., and S.W. interpreted results of experiments; C.S. prepared figures; C.S. and S.W. drafted manuscript; C.S., L.M.T., M.d.J., D.D.S., N.T.J., and S.W. edited and revised manuscript; C.S., L.M.T., M.d.J., D.D.S., N.T.J., and S.W. approved final version of manuscript.

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