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RESEARCH ARTICLE | *Racial Differences in Cardiovascular and Cerebrovascular Physiology*

Four weeks of vitamin D supplementation improves nitric oxide-mediated microvascular function in college-aged African Americans

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Wolf ST, Jablonski NG, Ferguson SB, Alexander LM, Kenney WL. Four weeks of vitamin D supplementation improves nitric oxide-mediated microvascular function in college-aged African Americans. *Am J Physiol Heart Circ Physiol* 319: H906–H914, 2020. First published August 28, 2020; doi:10.1152/ajpheart.00631.2020.— Reduced nitric oxide (NO)-mediated cutaneous vasodilation, secondary to increased oxidative stress, presents in young African American (AA) compared with European American (EA) adults and may be modulated by vitamin D status. We assessed cutaneous microvascular function in 18 young, healthy (21 ± 2 yr; 9 men, 9 women) subjects before (pre, 8 AA, 10 EA) 4 wk of 2,000 IU/day oral vitamin D supplementation and in 13 subjects after (post, 7 AA, 6 EA) 4 wk of 2,000 IU/day oral vitamin D supplementation. Serum vitamin D concentrations [25(OH)D] were measured at each visit. Three intradermal microdialysis fibers placed in the ventral forearm were randomized for treatment with 10 μM Tempol, 100 μM apocynin, or lactated Ringer's solution (control). Local heating (39°C) induced cutaneous vasodilation; red cell flux was measured at each site (laser-Doppler flowmetry), and cutaneous vascular conductance (CVC = flux/MAP) was expressed as a percentage of maximum (28 mM sodium nitroprusside, +43°C) for each phase of local heating. After stable elevated blood flow was attained, 15 mM N^G-nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor) was perfused at all sites to quantify the NO contribution to cutaneous vasodilation (%NO), calculated as the difference between local heating and L-NAME plateaus. Serum [25(OH)D], the magnitude of the local heating response, and %NO were all lower in AAs versus EAs ($P < 0.01$). Tempol ($P = 0.01$), but not apocynin ($P \geq 0.19$), improved the local heating response and %NO. Four weeks of supplementation improved serum [25(OH)D], the local heating response, and %NO in AAs ($P \leq 0.04$) but not in EAs ($P \geq 0.41$). Vitamin D supplementation mitigated endothelial dysfunction, an antecedent to overt cardiovascular disease (CVD), in otherwise healthy, young AA adults.

NEW & NOTEWORTHY Endothelial dysfunction, an antecedent to overt cardiovascular disease (CVD), is observed earlier and more frequently in otherwise healthy African Americans (AAs) when compared with other ethnic groups. Vitamin D may modulate endothelial function, and darkened skin pigmentation increases risk of vitamin D deficiency. We show that 4 wk of 2,000 IU/day vitamin D supplementation improves microvascular responses to local heating in AAs. Ensuring adequate vitamin D status may mitigate development of cardiovascular dysfunction in this at-risk population.

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microvascular function; nitric oxide; vitamin D

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States, and darkly pigmented African Americans (AAs) experience a disproportionate burden compared with lightly pigmented European Americans (EAs) (3). Furthermore, hypertension, one of the primary risk factors for CVD, is more prevalent and develops at a younger age in the AA population. Although the causes of hypertension are multifaceted, vascular endothelial dysfunction has been implicated in its development (28, 36). Attenuated vascular [flow-mediated dilation (FMD)] and microvascular (local skin heating) responses have been demonstrated in otherwise healthy AA adults (13, 31, 32), and these impairments may be due, at least in part, to elevated oxidative stress in that population.

Vitamin D supplementation has been shown to improve endothelial function in AA adults, but to date, those studies have been limited to overweight/obese adults or patient populations such as those with type 2 diabetes mellitus or chronic kidney disease (11, 43, 45). Vitamin D is produced from 7-dehydrocholesterol in the skin upon ultraviolet (UV)-B light exposure from the sun (27). Melanin in the skin absorbs UV rays from the sun, and higher melanin concentrations can contribute to impaired vitamin D production in adults with darker pigmentation (22, 44). Furthermore, UV-B exposure varies geographically and seasonally, such that exposure is diminished with increasing distance from the earth's equator and during winter months, particularly in areas of greater seasonal variation (16, 33). These factors combine to place adults with darker pigmentation at an elevated risk for vitamin D deficiency (30). Vitamin D may improve endothelial function and health by signaling for the transcription of endothelial nitric oxide synthase (eNOS) and/or by ameliorating inflammation-induced endothelial dysfunction (2, 14). In addition, vitamin D has been shown to increase superoxide dismutase activity (46) and decrease nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase)-induced production of superoxide radicals (8, 21), which may attenuate oxidative stress-mediated endothelial dysfunction. Taken together, these

findings suggest a potential link between vitamin D deficiency, oxidative stress, and reduced nitric oxide (NO)-mediated vasodilation in AAs compared with EAs.

Reduced cutaneous microvascular vasodilation in response to local heating and a reduced nitric oxide (NO) contribution to that response have been demonstrated in otherwise healthy college-aged AA adults compared with in EA adults (13, 18, 31). Both components (i.e., the magnitude of vasodilation and the NO contribution) of the local heating response were improved in AAs, but not in EAs, by inhibition of superoxide radicals with the superoxide dismutase (SOD) mimetic, Tempol, such that there were no longer differences between groups (13). Inhibition of NADPH oxidase with apocynin similarly improved microvascular function during local heating in AA men but not women (31). Together, the results of those studies suggest that attenuated microvascular responses observed in AAs compared with that in EAs are attributed, at least in part, to elevated superoxide generation and/or attenuated superoxide scavenging by SOD. However, the potential role of vitamin D in modulating oxidative stress and microvascular function in AAs has not been examined.

The purpose of the present study was to examine the influence of vitamin D status and supplementation on the cutaneous microvascular vasodilator response to local skin heating, as well as the NO-mediated component of that response, in healthy young AA and EA adults. In keeping with previous research (13, 31), we initially enrolled participants only if they and both of their parents identified as either AA or EA; however, we also performed genetic testing to better characterize subject groups in terms of biological ancestry, as the word "race" is poorly defined and problematic in terms of heterogeneity of genetic variation. Our primary independent variable of interest was skin pigmentation, which directly influences cutaneous vitamin D production (15, 30), and in turn may indirectly impact endothelial function. We hypothesized that serum [25(OH)D] and microvascular responses to local heating would be reduced in AAs compared with in EAs before vitamin D supplementation, 4 wk of 2,000 IU/day vitamin D supplementation would increase serum [25(OH)D] and augment local heating responses and %NO in AAs but not EAs, and differences in microvascular responses between AAs and EAs would be abrogated by direct perfusion of either Tempol or apocynin before, but not after, vitamin D supplementation.

METHODS

Subjects. All experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University. Healthy men and women [EA = 10 (5 men/5 women), AA = 8 (4 men/4 women)] of age between 18 and 30 yr with normal blood pressure [systolic blood pressure (SBP) < 130 and diastolic blood pressure (DBP) < 85 mmHg], low-density lipoprotein cholesterol of <150 mg/dL, and hemoglobin A1C (HbA1C) of <6.5% were included. Subjects were normally active, healthy nonsmokers who were free of cardiovascular disease, kidney disease, skin disease, pigmentation disorders, or skin allergies and were not taking any prescription medications with primary or secondary vascular effects. Women either had regular menstrual cycles or were taking oral contraceptives. Those who were not taking contraceptives were studied during *days 1–3* of their cycle. Five of the nine women included were taking oral contraceptives (EAs = 4, AAs = 1) and were studied during the placebo week of contraceptive use. Subjects were enrolled only if they, and both of their parents, identified as being of AA or EA ancestry. In addition to self-reported ancestry, a subset of subjects (EA = 10, AA = 6) provided saliva samples for DNA ancestry analysis (23andMe, Sunnyvale, CA). All subjects underwent an initial screening that included physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsburgh, PA). Written and verbal consent were obtained voluntarily from all subjects before participation, in accordance with the guidelines set forth by the Declaration of Helsinki.

Based on previously published data (13), we determined a priori ($\alpha = 0.05$; power = 0.8) that eight subjects per group would be sufficient to detect within- and between-group differences in the %NO contribution to the local heating response. Because of COVID-19 considerations, only seven AA and six EA participants completed the postsupplementation visit. However, given the large effect size (see RESULTS) observed in the current study for %NO at the control site from presupplementation to postsupplementation in the AA group, a post hoc power analysis suggested that six subjects would be adequate (power = 0.90) to detect significant differences.

Assessment of skin pigmentation. Skin pigmentation was measured by reflectance spectrophotometry (DermaSpectrometer; Cortex Technology, Hadsund, Denmark), as previously described (41) to determine the melanin index (M-index) of the skin on the subject's inner aspect of the upper arm. The M-index was measured in this region because of its ease of access and because it represents constitutive skin pigmentation due to its relatively low sun exposure (29).

Experimental procedures. All protocols were performed in a thermoneutral laboratory with the subject resting in a semi-supine position and both arms of the subject supported at the heart level. Testing was conducted before and after 4 wk of 2,000 IU/day vitamin D_3 supplementation. This treatment regimen was chosen based on previous data suggesting that supplementation at this dose and timing is safe and adequate to significantly increase circulating [25(OH)D] (38). Three intradermal microdialysis fibers (10-mm, 20-kDa cutoff membrane, MD 2000; Bioanalytical Systems, West Lafayette, IN) were placed into the dermal layer of the ventral aspect of the left forearm for local delivery of pharmacological agents (5). Fibers were randomized for one of the following three treatments: lactated Ringer's solution (control), 10 μM Tempol (superoxide dismutase mimetic; Sigma-Aldrich, St. Louis, MO), or 100 μM apocynin (NADPH oxidase inhibitor; Tocris Bioscience, Bristol, UK). Pharmacological agents were mixed just before use, dissolved in lactated Ringer's solution, sterilized using syringe microfilters (Acrodisc; Pall, Port Washington, NY), and wrapped in foil to prevent degradation due to light exposure. All solutions were perfused through microdialysis fibers at a rate of 2 μL/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems, West Lafayette, IN) (5). Local red blood cell flux was measured directly over each microdialysis site throughout local heating with an integrated laser-Doppler flowmetry probe placed in a local heating unit (Moor Instruments SHO2, Moor Instruments Inc., Wilmington, DE).

After placement of microdialysis fibers, an ~60-min period was allowed for hyperemia associated with fiber placement to resolve. During the hyperemia resolution phase, pharmacological agents were perfused to allow for a drug wash-in phase. Baseline data were then collected (~20 min) before beginning a local heating (39°C) protocol, as described previously (6, 13). This heating protocol elicits an initial axon reflex-mediated peak skin blood flow response, followed by a brief nadir, after which there is a gradual rise and eventual (after ~40 min) blood flow plateau. After observing a stable local heating plateau, 15 mM N^G-nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor) was perfused through all sites, allowing for quantification of NO-dependent vasodilation (%NO) (1, 34). After observing a stable L-NAME plateau, 28 mM sodium nitroprusside (SNP; USP, Rockland, MD) was perfused through all sites, and local temperature was increased to 43°C to elicit maximal vasodilation (17, 26).

Vitamin D analysis. Blood samples were collected in serum separator tubes during pre-vitamin D and post-vitamin D supplementation visits. Serum was isolated by centrifugation and stored at −80°C for later analysis. Serum concentrations of 25(OH)D, the primary circulating metabolite of vitamin D, were quantified in duplicate using an ELISA kit according to the manufacturer's instructions (CrystalChem, Elk Grove Village, IL).

Socioeconomic status. Subjects responded to a 10-item socioeconomic status (SES) questionnaire similar to that which has previously been associated with increased daily stress and negative affect, diabetes, and other chronic diseases (7, 35, 37). Participants' childhood SES was measured using the following four indicators: father's highest level of education (0 = <high school, 1 = high school/GED, 2 = some college and above), mother's highest level of education (0 = <high school, 1 = high school/GED, 2 = some college and above), financial situation growing up (0 = a lot/somewhat/a little worse off than average family, 1 = same as average family, 2 = a lot/somewhat/a little better off than average family), and whether the participant's family received welfare (0 = all the time/most of the time, 1 = some of the time/a little of the time, 2 = never on welfare). Adult SES was measured using the following six indicators: highest level of education (0 = <high school, 1 = high school/GED, 2 = some college and above), the participant's perceived standing within their community (0 = worst, 1 = average, 2 = best), current financial situation (0 = worst, 1 = average, 2 = best), control over financial situation (0 = worst, 1 = average, 2 = best), availability of money to meet basic needs (0 = not enough money, 1 = just enough money, 2 = more money than needed), and difficulty paying bills (0 = very/somewhat difficult, 1 = not very difficult, 2 = not at all difficult). Participant responses were coded and added to provide an index of childhood SES, adulthood SES, and lifetime SES (childhood + adulthood SES).

Data acquisition and analysis. Data were collected using Windaq (DATAQ Instruments, Akron, OH) at a frequency of 40 Hz. Mean arterial pressure (MAP) was calculated for each phase of the protocol using blood pressure taken from an automated blood pressure monitor (CardioCap; GE Healthcare, Chicago, IL). Cutaneous vascular conductance was calculated as red blood cell flux divided by MAP and expressed as a percentage of cutaneous vascular conductance (CVC_{max}) ($\%CVC_{max}$) for each phase of the local heating protocol (24, 26). The NO contribution to cutaneous vasodilation was calculated as the difference between the local heating and L-NAME plateau responses.

Student's unpaired *t* tests were used to compare subject characteristics and SES. Microvascular responses to local heating were analyzed using SAS PROC MIXED (SAS, version 9.4, SAS Institute, Inc., Cary, NC) three-way, repeated-measures ANOVA to evaluate group (AAs vs. EAs), local pharmacological delivery site (lactated Ringer's solution vs. Tempol vs. apocynin), and time (presupplementation vs. postsupplementation) effects. Serum 25(OH)D concentrations were analyzed using two-way repeated-measures ANOVA (group × time). Post hoc comparisons with Bonferroni's corrections were performed for specific planned comparisons. Hedges' *g* effect sizes, a corrected, unbiased measure of effect size for samples <20 (19), were calculated and reported when comparisons were statistically different (small effect = 0.2, medium effect = 0.5, large effect = 0.8). Data are reported as means ± SD, except in Figs. 2 and 3, which are presented as box-and-whisker plots with individual data points. Significance was accepted at $\alpha = 0.05$.

RESULTS

Subject characteristics are presented in Table 1. All characteristics and blood biochemistry values were within normal limits. By design, M-index was significantly higher in AAs compared with that in EAs ($P < 0.001$, $g = 5.92$). There were otherwise no differences between groups for any characteristic. Figure 1 presents results from the ancestry analysis. AA subjects were predominantly of African descent, whereas EA subjects were primarily of European descent. Adult, childhood, and lifetime SES are displayed in Table 2. Adult ($P = 0.03$), childhood ($P = 0.02$), and lifetime ($P = 0.001$) SES were significantly lower in the AA cohort compared with that in the EA cohort.

Table 1. *Subject characteristics*

	European American	African American
n (men/women)	10 (5/5)	8 (4/4)
Age, yr	22 ± 2	20 ± 2
BMI, kg/m^2	24 ± 2	25 ± 3
Systolic BP, mmHg	113 ± 5	113 ± 6
Diastolic BP, mmHg	72 ± 6	68 ± 11
Heart rate, beats/min	64 ± 8	64 ± 5
M-index	33 ± 3	65 ± 7*
Blood biochemistry		
HbA1c, %	5.1 ± 0.2	5.3 ± 0.3
Total cholesterol, mg/dl	168 ± 41	151 ± 24
HDL, mg/dL	63 ± 16	60 ± 12
LDL, mg/dL	94 ± 28	78 ± 12

Values are means ± SD; *n* = number of participants. BMI, body mass index; BP, blood pressure; M-index, a skin-reflectance measure of melanization; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *$P < 0.05$ compared with European American.

Serum 25(OH)D concentrations are depicted in Fig. 2. Concentrations of 25(OH)D were significantly lower in AAs compared with in EAs presupplementation (17.93 ± 5.24 vs. 32.07 ± 9.14 ng/mL, $P = 0.002$, $g = 1.75$). Vitamin D supplementation significantly increased 25(OH)D concentrations in AAs (from 17.93 ± 5.24 to 26.07 ± 3.73 ng/mL, $P = 0.04$; $g = 1.66$) but not in EAs ($P = 0.16$); a smaller but significant difference between groups remained postsupplementation (37.46 ± 8.19 vs. 26.07 ± 3.73 ng/mL, $P = 0.03$; $g = 1.72$).

Table 3 presents baseline $\%CVC_{max}$ and maximal CVC values for both groups across all sites pre-vitamin D supplementation and post-vitamin D supplementation. There were no differences in baseline $\%CVC_{max}$ ($P \geq 0.12$) or in maximal CVC values ($P \geq 0.90$) within or between groups, before or after vitamin D supplementation, or from presupplementation to postsupplementation.

Figure 3, *A* and *B*, depicts the initial axon reflex-mediated peak ($\%CVC_{max}$) for EAs and AAs before and after vitamin D supplementation, respectively. The magnitude of this response was lower in AAs compared with that in EAs in the control (lactated Ringer's solution) site (41.46 ± 12.28 vs. 53.12 ± 14.26%max, $P < 0.03$, $g = 0.83$) before vitamin D supplementation. Neither Tempol ($P = 0.07$) nor apocynin ($P = 0.08$) significantly improved the initial peak response in AAs, although both minimized the difference between groups ($P \geq 0.74$). There were no differences among the three sites in EAs ($P \geq 0.77$). The initial peak response was improved in AAs at the control site postsupplementation, such that there was no longer a difference between groups ($P = 0.08$), although the response was not significantly different from presupplementation ($P = 0.23$). There were no differences between groups at the Tempol ($P = 0.39$) or apocynin ($P = 0.73$) sites, nor were there within-group differences among sites ($P \geq 0.11$), after vitamin D supplementation. Similarly, there were no differences in either group from presupplementation to postsupplementation ($P \geq 0.42$).

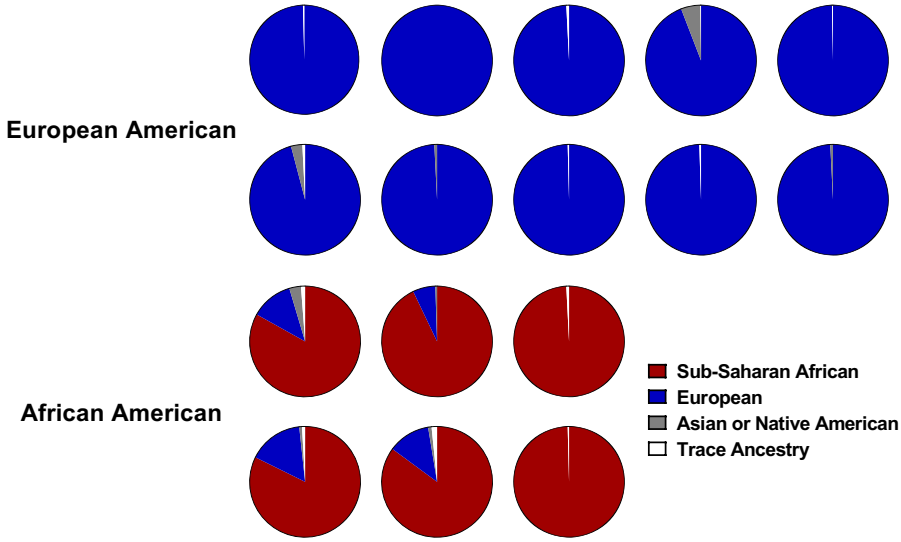

Fig. 1. Individual subject pie charts depicting ancestry analysis for subjects of European American (n = 10) and African American (n = 6) ancestry.

Following a similar format to that of Fig. 3, *A* and *B*, Fig. 3, *C* and *D*, illustrates the local heating plateau responses presupplementation and postsupplementation, respectively. The magnitude of the local heating plateau was attenuated in AAs compared with that in EAs at the control site (36.60 ± 13.71 vs. 54.36 ± 12.71%max, P = 0.007, g = 1.29). In AAs, the magnitude of the plateau was significantly improved by local delivery of Tempol (54.24 ± 23.95 vs. 36.60 ± 13.71%max, P = 0.01, g = 0.86), but not apocynin (P = 0.19), compared with that at the control site. There were no differences between EAs and AAs at the Tempol (P = 0.64) or apocynin (P = 0.50) sites. Vitamin D supplementation improved the local heating response at the control site in AAs (36.60 ± 13.71 vs. 52.33 ± 23.72%max, P = 0.02, g = 0.78), such that there were no longer differences between groups (P = 0.42). There were otherwise no differences within or between groups postsupplementation ($P \geq 0.17$) or from presupplementation to postsupplementation ($P \geq 0.16$).

Figure 3, *E* and *F*, displays the NO contribution to local heating responses for the two groups presupplementation and postsupplementation. The %NO was blunted in AAs compared with that in EAs at the control site (29.83 ± 13.70 vs. 46.41 ± 12.57%max, $P < 0.01$, g = 1.21). Local delivery of Tempol (29.83 ± 13.70 vs. 47.01 ± 23.93%max, P = 0.01, g = 0.83), but not apocynin (P = 0.25), augmented the NO contribution to local heating in AAs, whereas neither treatment influenced the %NO in EAs ($P \geq 0.17$). There was no difference between AAs and EAs at the Tempol (P = 0.19) or apocynin (P = 0.25) sites before supplementation. Vitamin D supplementation improved the NO contribution to local heating at the control site in AAs from presupplementation to postsupplementation (29.83 ± 13.70 vs. 46.79 ± 21.93%max, P = 0.01, g = 0.89), abolishing the difference between groups (P = 0.47). There were otherwise no differences within or between groups postsupplementation ($P \geq 0.17$) or from presupplementation to postsupplementation ($P \geq 0.10$).

Table 2. *Socioeconomic status*

	European American	African American
Adult SES	10 ± 1	8 ± 1*
Childhood SES	7 ± 1	5 ± 2*
Lifelong SES	18 ± 2	13 ± 1*

Values are means ± SD; n = 10 European Americans and 7 African Americans. Participant responses were coded on a 0–2 scale for each question on the 10-item questionnaire. Coded responses were then added up to provide a total childhood, adult, and lifelong socioeconomic status (SES) (lifelong = childhood + adult SES). *$P < 0.05$ compared with European American.

DISCUSSION

The principal findings of this study are as follows: serum 25(OH)D concentrations were lower in AAs compared with that in EAs; the magnitude of the local heating response and the NO-mediated component of that response were likewise lower in AAs compared with that in EAs; 4 wk of 2,000 IU/day oral vitamin D supplementation improved serum 25(OH)D

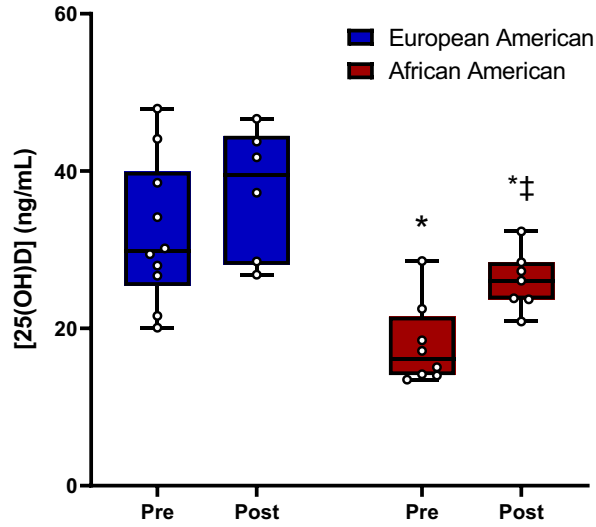

Fig. 2. Serum vitamin D [25(OH)D] concentrations for European American (EA) (blue bars) and African American (AA) (red bars) subjects before (pre: EA, n = 10; AA, n = 8) and after (post: EA, n = 6; AA, n = 7) vitamin D supplementation. *$P < 0.05$ compared to European American at the same time point; ‡$P < 0.05$ compared to pre-vitamin D supplementation.

Table 3. *Baseline and maximal CVC values*

European American	African American
Baseline, %CVC_{max}	
Pre	
Ringer's	10.64 ± 5.79	6.58 ± 2.58
Tempol	9.11 ± 4.22	6.77 ± 3.56
Apocynin	8.45 ± 2.93	6.62 ± 3.07
Post	
Ringer's	7.19 ± 3.09	5.66 ± 2.14
Tempol	9.98 ± 6.40	7.87 ± 3.30
Apocynin	8.46 ± 6.34	8.03 ± 3.28
Maximal CVC, flux/mmHg	
Pre	
Ringer's	2.15 ± 0.55	2.28 ± 0.64
Tempol	2.65 ± 0.83	2.13 ± 0.58
Apocynin	2.46 ± 1.09	2.34 ± 0.99
Post	
Ringer's	2.10 ± 0.38	2.39 ± 0.75
Tempol	2.35 ± 0.91	2.36 ± 0.63
Apocynin	2.66 ± 0.71	2.01 ± 0.83

Values are means ± SD. CVC, cutaneous vascular conductance.

concentrations and mitigated the differences in microvascular responses to local heating between groups; and local delivery of Tempol (a superoxide dismutase mimetic), but not apocynin (an NADPH oxidase inhibitor), improved cutaneous microvascular responses to local heating in AAs before, but not after, vitamin D supplementation. Together, these data suggest that vitamin D supplementation may be an effective intervention to improve cutaneous microvascular endothelial function in the AA population by reducing oxidative stress and/or increasing NO production and bioavailability.

Cardiovascular disease is the primary cause of morbidity and mortality in the United States, and AAs contribute disproportionately to those statistics (3, 31). Hypertension, a primary risk factor for CVD, develops at a younger age in AAs compared with that in EAs, and reduced FMD (32) and local skin heating responses, considered to be strongly implicated in the development of hypertension, are prevalent in healthy young and middle-aged AAs even in the absence of hypertension (13, 18, 31). Attenuated vascular responses in this population, relative to the EA population, are mediated by reduced NO bioavailability, in part due to elevated oxidative stress. Previous work has demonstrated that cutaneous microvascular responses to local heating are improved by direct delivery of the SOD dismutase mimetic, Tempol, in healthy young AA subjects (13). Similarly, suppression of NADPH oxidase with apocynin improved microvascular responses to local heating in AA men but not women (31). Therefore, increased production of superoxide radicals and/or reduced SOD activity appears to play an important role in reduced vascular reactivity in the AA population. Our data are consistent with those of previous studies in that cutaneous microvascular responses to local heating were attenuated in AAs compared with that in EAs and that local delivery of Tempol improves those responses in AAs such that there were no longer differences between groups (13, 18, 31). However, in contrast to previous work (31), local delivery of apocynin did not significantly improve microvascular responses in young AA subjects. It may be that the discrepancy between those results and ours is due to our smaller sample size, which is not powered to detect sex differences.

A novel finding of the current study was that the magnitude of cutaneous vasodilation in response to local heating, as well as the NO-mediated component of that response, was improved in AAs after 4 wk of 2,000 IU/day vitamin D such that there were no longer differences between AAs and EAs at the control site. Furthermore, local heating responses were not significantly improved by local delivery of either Tempol or apocynin after vitamin D supplementation. These results suggest that improving vitamin D status in AAs can effectively ameliorate oxidative stress-induced cutaneous microvascular dysfunction. Previous research demonstrated that administration of calcitriol, the primary bioactive metabolite of vitamin D, increased SOD expression in human umbilical vein endothelial cells (46) and inhibited NADPH oxidase activity in diabetic rats (8) and renal arteries and endothelial cells of hypertensive humans (21). In addition, vitamin D treatment may upregulate eNOS expression (2, 14), thereby increasing NO bioavailability. However, to our knowledge, this is the first study that has demonstrated the efficacy of vitamin D in ameliorating oxidative stress-induced microvascular dysfunction in otherwise healthy, college-aged AA adults.

Consistent with previous studies (31, 42), the axon reflex-mediated initial peak was attenuated in AAs compared with that in EAs before vitamin D supplementation. Vitamin D supplementation mitigated the difference between groups but did not significantly improve the response in AAs. Although NO is implicated in the axon reflex response, its contribution is modest (25, 42). The relatively small contribution of NO to the initial peak may explain why vitamin D supplementation did not significantly improve the response in AAs, despite significant improvements in the local heating response and %NO.

Vitamin D deficiency, insufficiency, and sufficiency are defined as $<$20 ng/mL, 21–29 ng/mL, and $>$30 ng/mL, respectively (4, 12). Before vitamin D supplementation in the current study, five of the EA subjects were vitamin D-sufficient, whereas the other five were insufficient. By contrast, six of the AA subjects were deficient, two were in the insufficient range, and none was vitamin D sufficient. Although average annual UV-B exposure in the northeastern United States is adequate to elicit cutaneous vitamin D synthesis in lightly pigmented skin, it may not be intense enough to catalyze vitamin D synthesis in moderately or darkly pigmented skin (15). As such, it is possible that many AAs living in this region of the United States are chronically vitamin D deficient in the absence of supplementation, whereas EAs may only be at higher risk during the winter months. All data for the present investigation were collected between the months of October and February to eliminate the confounding factor of environmental UV-B-induced cutaneous vitamin D synthesis (39) during vitamin D supplementation. As such, there was a relatively high prevalence of insufficiency and deficiency across all subjects, and it is unclear whether there would be a greater degree of sufficiency in one or both groups had these subjects been studied during other seasons or in different climates.

Based on previous data (38), we chose a vitamin D treatment regimen of 4 wk at 2,000 IU/day. Although vitamin D treatment significantly improved serum 25(OH)D concentrations in AAs, vitamin D status in AAs was still significantly lower than in EAs and clinically insufficient in all but one AA subject. It is likely that a longer regimen and/or higher-dose vitamin D treatment would have further increased circulating 25(OH)D to

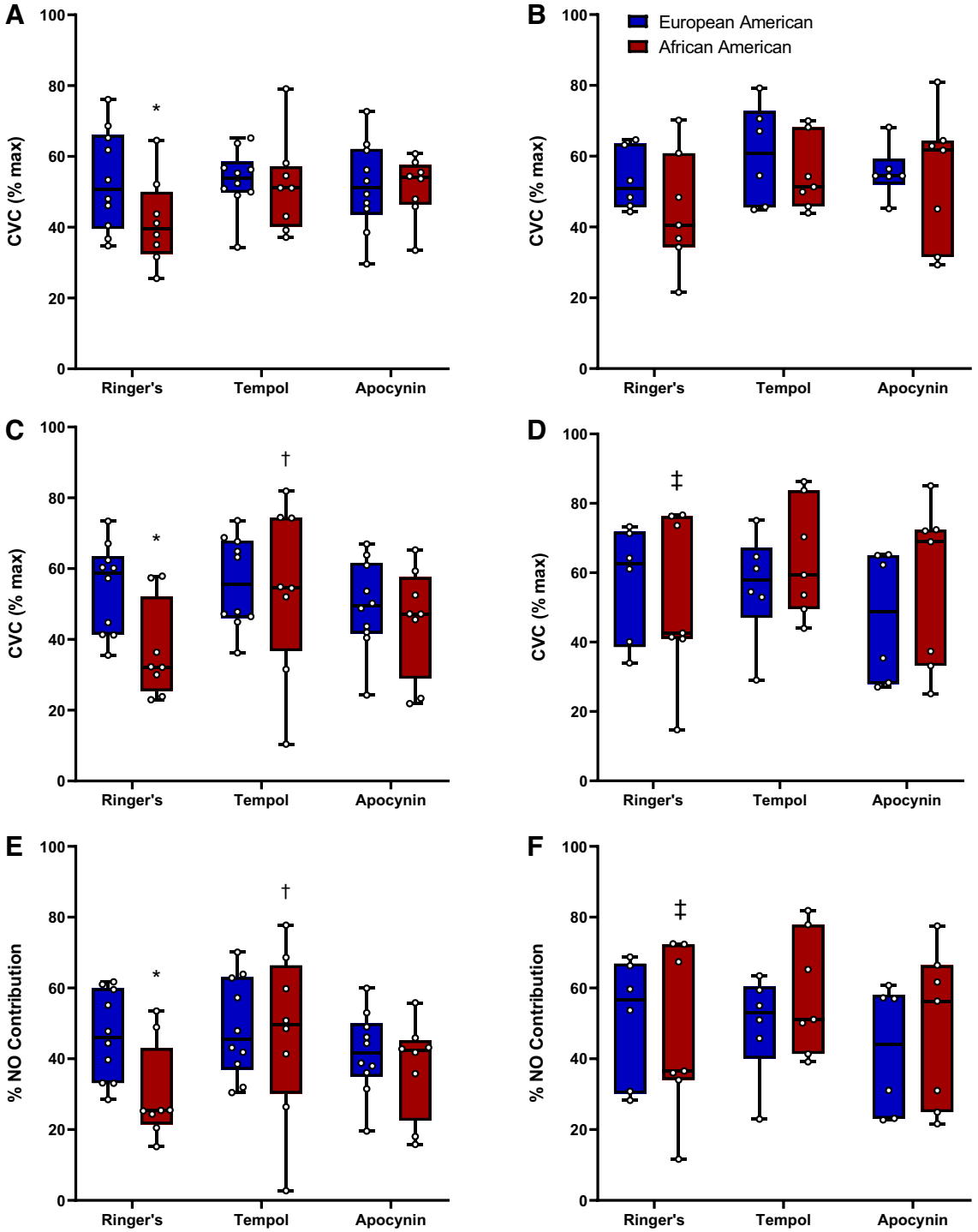

Fig. 3. Cutaneous vascular conductance (%CVC_{max}) responses for the axon reflex-mediated initial peak (*A* and *B*) and subsequent plateau (*C* and *D*) and the percent nitric oxide (%NO) contribution to the plateau response (*E* and *F*) for European American (EA) (blue bars) and African American (AA) (red bars) subjects. Each pair of bars represents responses before (*left*: EA, $n = 10$; AA, $n = 8$) and after (*right*: EA, $n = 6$ AA, $n = 7$) vitamin D supplementation with local delivery of lactated Ringer's (control site), Tempol, or apocynin. Boxes represent first and third quartiles with median values denoted by the horizontal line, and whiskers indicate minimum and maximum observations. *$P < 0.05$ compared to European American; †$P < 0.05$ compared to control site; ‡$P < 0.05$ compared to pre-vitamin D supplementation.

concentrations that are clinically sufficient. Importantly, however, circulating 25(OH)D concentrations in AAs were similar postsupplementation to those in EAs presupplementation (26.07 vs. 32.07 ng/mL, $P = 0.12$), and the group differences in cutaneous microvascular responses to local heating were abolished. The impact of high-dose and/or prolonged vitamin D supplementation on vascular endothelial function in larger cohorts may be a consideration for future research in this area.

An additional novel aspect of this study was enhanced characterization of subject groups with respect to ancestry

and socioeconomic status. DNA analysis confirmed that participants who self-reported being of AA or EA ancestry were indeed primarily of African or European lineage, with little ancestral admixture. Because we did not recruit participants of mixed ethnic/ancestral backgrounds, we are unable to speculate whether differences in microvascular endothelial function would be observed in admixed populations with varying degrees of skin pigmentation or whether vitamin D status would explain the variance in microvascular function. Further research is needed in larger cohorts of more admixed populations to better assess the interaction between skin pigmentation, vitamin D status, and microvascular function.

Associations between SES and various health conditions have been extensively documented (7, 23, 35, 37). In the present study, childhood, adulthood, and lifetime SES were lower in the AA group compared with those in the EA group. However, in this small sample, there was no direct relation between SES and the cutaneous microvascular response to local heating (not depicted; $R^2 = 0.09$, $P = 0.31$). Recent research has also linked daily psychosocial stressors to impairments in microvascular endothelial function (9, 10), presenting one mechanism by which SES may influence vascular health. It is worth noting that although the questionnaire used in the current study has demonstrated relations between SES and increased daily stress and worse cardiometabolic outcomes (7, 35, 37), to our knowledge, no study has been conducted to specifically validate the utilization of this questionnaire in a cohort limited to college-aged adults. However, we believe that such a questionnaire to assess SES provides important insight into the potential underlying causative factors from which disparities in vascular function arise. The influence of SES and associated daily stressors on endothelial function across ethnic groups is a compelling area for future investigation.

There is an urgent need to better understand the physiological underpinnings of "racial" disparities in cardiovascular health, but we must recognize that the term race is poorly defined and constitutes an imprecise method of categorization. A large degree of heterogeneity exists within racial groups; indeed, genetic variation is greater within a population than it is between populations, and individuals are often more genetically similar to members of other racial groups than to members of the group in which they identify (20, 40). The present study, in keeping with previous research (13, 31), initially enrolled participants only if they and both of their parents identified as either AA or EA. However, we also performed genetic testing to better characterize the two groups of subjects in terms of ancestry, a characterization not often performed in physiological studies (Fig. 1). Our primary independent variable of interest (rather than "race," as in previous studies) was skin pigmentation, which directly influences cutaneous vitamin D production (15, 30) and in turn may indirectly impact endothelial function. We found significant correlations (not depicted) between skin pigmentation (M-index) and 25(OH)D concentrations ($R^2 = 0.42$, $P = 0.004$) and the %NO contribution to the local heating response ($R^2 = 0.29$, $P = 0.02$). However, these findings should be taken with caution, as we only included lightly and darkly pigmented subjects, with no intermediate values, in this study. Future research in this area should include a wide range of skin pigmentation to better assess the influence of pigmentation on vitamin D status and endothelial function and consider the role played by dark pigmentation itself, including in people of non-AA ancestry, such as some South Asians and Melanesians, in affecting microvascular responses. This would contribute to an understanding of the roles of underlying causative factors for health disparities in the AA population (and other at-risk populations).

Conclusions. These results suggest that reduced microvascular responses to local heating in young, otherwise healthy AA adults may be, at least in part, attributable to vitamin D deficiency. Our data additionally suggest that improving vitamin D status with daily supplementation in AAs may improve cutaneous microvascular function, essentially correcting the difference between AAs and EAs. These findings provide novel insight into the mechanisms underlying impaired microvascular function, which may precede the development of hypertension and overt CVD, in the AA population. Furthermore, these data suggest that vitamin D supplementation may constitute a simple, effective, and low-cost therapeutic target in this at-risk population. Whether this translates to long-term maintenance of endothelial health and reduced risk of (cardio)vascular dysfunction remains to be determined.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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