Oral sapropterin acutely augments reflex vasodilation in aged human skin through nitric oxide-dependent mechanisms

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Stanhewicz AE, Alexander LM, Kenney WL. Oral sapropterin acutely augments reflex vasodilation in aged human skin through nitric oxide-dependent mechanisms. J Appl Physiol 115: 972–978, 2013. Published June 6, 2013; doi:10.1152/japplphysiol.00481.2013.—Functional constitutive nitric oxide synthase (NOS) and its cofactor tetrahydrobiopterin (BH4) are required for full reflex cutaneous vasodilation and are attenuated in primary aging. Acute, locally administered BH4 increases reflex vasodilation through NO-dependent mechanisms in aged skin. We hypothesized that oral sapropterin (Kuvan, shelf-stable pharmaceutical formulation of BH4) would augment reflex vasodilation in aged human skin during hyperthermia. Nine healthy human subjects (76 ± 1 yr) ingested sapropterin (10 mg/kg) or placebo in a randomized double-blind crossover design. Venous blood samples were collected prior to, and 3 h following, ingestion of sapropterin for measurement of plasma BH4. Three intradermal microdialysis fibers were placed in the forearm skin for local delivery of 1) lactated Ringer’s solution, 2) 10 mM BH4, and 3) 20 mM Nω-nitro-L-arginine methyl ester (t-NNAME) to inhibit NOS. Red cell flux was measured at each site by laser-Doppler flowmetry (LDF) as reflex vasodilation was induced using a water-perfused suit. At 1°C rise in oral temperature, mean body temperature was clamped and 20 mM t-NNAME was perfused at each site. Cutaneous vascular conductance was calculated (CVC = LDF/MAP) and expressed as a percentage of maximum (%CVCmax 28 mM sodium nitropusside and local heat 43°C). Plasma concentrations of BH4 were significantly elevated 3 h after ingestion of sapropterin (0 h: 19.1 ± 2 pmol/ml vs. 3 h: 43.8 ± 3 pmol/ml; P < 0.001). Sapropterin increased NO-dependent vasodilation at control site (placebo: 14 ± 1 %CVCmax vs. sapropterin: 25 ± 4 %CVCmax; P = 0.004). Local BH4 administration increased NO-dependent vasodilation compared with control in placebo trials only (control: 14 ± 1 %CVCmax vs. BH4-treated: 24 ± 3 %CVCmax; P = 0.02). These data suggest oral sapropterin increases bioavailable BH4 in aged skin microvasculature sufficiently to increase NO synthesis through NOS and that sapropterin may be a viable intervention to increase skin blood flow during hyperthermia in healthy aged humans.

SKIN BLOOD FLOW (SkBF) is controlled by dual sympathetic innervation consisting of an adrenergic vasoconstrictor system and a cholinergic active vasodilator system (13,1). With increasing body temperature SkBF is first increased through withdrawal of tonic adrenergic tone followed by activation of the active cholinergic vasodilator system (38). Active vasodilation is mediated by the co-release of acetylcholine and unknown cotransmitter(s) (23) that mediate vasodilation in part through nitric oxide (NO)-dependent mechanisms. NO is required for full expression of reflex cutaneous vasodilation and mediates ~30–40% of the total vasodilator response to whole body heat stress in young, healthy humans (22, 40).

Primary human aging is associated with an attenuated cutaneous vasodilation response to hyperthermia (25) due to decreased cotransmitter and attenuated NO-dependent contributions (17). The decreased NO bioavailability in aged skin results from a decrease in NO production by upregulated vascular arginase and increased nitric oxide synthase (NOS) uncoupling with increased oxidant stress (19, 20). NOS is a dimeric enzyme that requires functional coupling of the oxygenase and reductase domains for NO production (2, 37). In conditions where substrate (L-arginine) availability is reduced through increased arginase activity or oxidative stress, the NOS dimer is uncoupled and produces superoxide rather than functional NO (34, 42). In addition to upregulated arginase activity and increased oxidant stress, we have recently demonstrated that reduced bioavailability of tetrahydrobiopterin (BH4) in aged vasculature also contributes to the attenuated NO-dependent reflex cutaneous vasodilation in aged skin (41). BH4 is an essential cofactor for NOS and is required for optimal NO production through NOS (31, 42). Mechanistically, BH4 stabilizes NOS in the coupled conformation and reduces oxidant stress in and around the NOS molecule (37, 42). In conditions of reduced BH4 bioavailability, NOS uncouples and produces superoxide, which contributes to peroxynitrite formation (37). Furthermore, superoxide produced from uncoupled NOS, as well as peroxynitrite, oxidizes BH4 contributing to increased oxidant stress and vascular dysfunction (26, 30, 42). In vivo human studies demonstrate that administration of exogenous BH4 improves NO-dependent vasodilation in conduit vessels of aged subjects (15, 35). Similarly, we have recently demonstrated that acute, local microperfusion of BH4 augments reflex vasodilation through NO-dependent mechanisms in aged human skin (41). Collectively, these findings suggest that a systemic BH4 intervention may be a clinically relevant therapy for improved NO-dependent reflex cutaneous vasodilation in older adults. In this study, we aimed to specifically address the clinical aspect of systemic exogenous BH4 administration by examining the efficacy of an acute oral dose of sapropterin (pharmaceutical BH4) in improved NO-dependent reflex vasodilation in aged skin.

Sapropterin is a commercially available, shelf-stable, pharmaceutical formulation of R-BH4 that is prescribed clinically for the treatment of BH4-responsive phenylketonuria. Sapropterin is currently not available for the on-label use for the treatment or prevention of vascular dysfunction; however, phase I clinical trials have suggested that it may be efficacious in specific cardiovascular disease states including hypertension, and aging (33, 35, 36). Because sapropterin is commercially available in the US, and oral dosing is more clinically practical than intradermal microdialysis for the delivery of BH4 to the cutaneous vasculature, the purpose of this study was to determine if oral administration of sapropterin

1 This article is the topic of an Invited Editorial by Gary L. Pierce (34a).
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could acutely increase NO-dependent reflex cutaneous vasodilation in healthy aged humans. We hypothesized that oral sapropterin would acutely increase bioavailable BH₄ in aged human vasculature. We further hypothesized that oral sapropterin would acutely augment NO-dependent reflex vasodilation in aged human skin through NOS coupling mechanisms.

**METHODS**

**Subjects.** Experimental protocols were approved by the institutional review board of The Pennsylvania State University. Written and verbal consent was obtained voluntarily from all subjects prior to participation according to the Declaration of Helsinki. Studies were performed on nine healthy subjects (76 ± 1 yr, 4 men and 5 women). Subjects were screened for neurological, cardiovascular, and dermatological diseases and underwent a complete medical screening including resting electrocardiogram, physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsburgh, PA). All subjects were normally active, nonhypertensive, nondiabetic, healthy nonsmokers who were not taking prescription medications with primary or secondary vascular effects (e.g., statins, antihypertensives, anticoagulants, antidepressants, etc.). Women taking hormone replacement therapy or who had recently taken hormone replacement therapy were excluded from the study.

**Instrumentation.** All protocols were performed in a thermoneutral laboratory with the subjects in a semisupine position and the experimental arm supported at heart level. All testing took place in the morning to eliminate diurnal variation in blood flow responses (3). Study days were separated by at least 48 h to ensure adequate washout of sapropterin (11). Subjects entered the laboratory between 0800 and 0900 and were instrumented with an intravenous catheter for blood sampling. A fasted blood sample was obtained, and then subjects ingested 10 mg/kg body wt sapropterin (Kuvan; BioMarin Pharmaceutical, Novato, CA) or placebo with a standardized breakfast meal ingested 10 mg/kg body wt sapropterin (Kuvan; BioMarin Pharmaceutical, Novato, CA) or placebo with a standardized breakfast meal (4.3 kcal/kg body wt). At 10, 20, and 30 min after ingestion of sapropterin or placebo, a second blood sample was obtained for analysis of peak plasma BH₄ concentrations. Pharmacokinetic analysis of sapropterin shows that plasma BH₄ concentrations peak at 3 h following oral administration (11). All blood samples were collected in 4 ml tubes containing EDTA and 0.1% wt/vol dithioerythritol and centrifuged immediately. The plasma was flash-frozen in liquid nitrogen. Plasma BH₄ concentrations of BH₄ and L-NAME were based on previous studies of peak plasma BH₄ concentrations. Pharmacokinetic analysis of sapropterin shows that plasma BH₄ concentrations peak at 3 h following oral administration (11). All blood samples were collected in 4 ml tubes containing EDTA and 0.1% wt/vol dithioerythritol and centrifuged immediately. The plasma was flash-frozen in liquid nitrogen and stored at −80°C until further analysis of BH₄ concentration by HPLC (29). Baseline and posttreatment blood samples from three subjects were not analyzed due to technical difficulties with the samples.

After breakfast, subjects were instrumented with three intradermal microdialysis (MD) fibers (10 mm, 20 kDa cutoff membrane, MD 2000; Bioanalytical Systems, West Lafayette, IN) placed in the ventral forearm skin by sterile technique. Before MD fiber placement, ice packs were applied to the sites for 5 min to temporarily anesthetize the skin (16). MD sites were at least 4 cm apart to ensure no cross-reactivity of the pharmacological agents. For each fiber, a 25-gauge needle was inserted horizontally in the intradermal layers of the skin such that the entry and exit points were ~2.5 cm apart. MD fibers were then threaded through the lumen of the needle, and the needle was removed leaving the membrane of the MD fiber in place. The MD fibers were randomly assigned to deliver 1) lactated Ringer’s solution to serve as control, 2) 10 mM BH₄ (Sigma, St. Louis, MO) or local BH₄ administration, or 3) 20 mM L-NAME (Calbiochem, San Diego, CA) to inhibit NOS. Concentrations of BH₄ and L-NAME were based on previous studies conducted in our laboratory (41). Pharmacological agents were mixed just before use, dissolved in lactated Ringer’s solution, sterilized with syringe microfilters (Acrodisc; Pall, Ann Arbor, MI), and wrapped in foil to prevent degradation due to light exposure. During the trauma resolution period (60–90 min), site-specific pharmacological solutions (10 mM BH₄, 20 mM L-NAME, or lactated Ringer’s) were perfused through the MD fibers at a rate of 2 µl/min (Bee Hive controller and Baby Bee microinfusion pumps, Bioanalytical Systems).

Skin temperature (Tsk) was controlled by a water-perfused suit that covered the entire body except for the head, hands, feet, and forearms. Copper-constantan thermocouples were placed on the surface of the skin at six sites (calf, thigh, abdomen, chest, shoulder, and back) for continuous measurement of Tsk. Each subject’s heart rate was monitored throughout the protocol (Cardiocap, GE Healthcare), and arterial blood pressure was measured by brachial auscultation every 5 min. Oral temperature (Tₚ) was measured as an index of changes in body temperature with a thermistor placed in the sublingual sulcus throughout baseline and whole body heating. Proper placement of the thermistor was checked on the basis of temperature readings and, once verified, was taped in place and closely monitored to ensure that it did not move throughout the protocol. Local Tsk over each MD site was clamped at 33°C throughout baseline and whole body heating (Moor-Lab, Temperature Monitor, SHO2; Moor Instruments, Devon, UK) to ensure that changes in SkBF were reflex in origin.

To obtain an index of SkBF, cutaneous red blood cell flux was calculated as red blood cell flux divided by mean arterial pressure (MAP) and expressed as a percent of site-specific maximal vasodilation [%CVmax; 28 mM sodium nitroprusside (SNP) and local heat to 43°C]. MAP was calculated as diastolic pressure plus one-third pulse pressure. Forearm blood flow (FFB) was measured at baseline and every 0.1°C rise in Tₚ by venous occlusion plethysmography using a mercury-in-silastic strain gauge (ECG Pletysmograph, Ho-kanson, Bellevue, WA) while blood flow to the hand was occluded (43). In contrast to laser-Doppler flowmetry, which images SkBF over a limited area (1 mm²) above the MD fiber, venous occlusion plethysmography provides an index of SkBF over the entire forearm. During whole body heating under resting conditions, increases in FBF are confined to the skin rather than the underlying muscle (7). Forearm vascular conductance (FVC) was calculated as FBF divided by MAP.

**Experimental protocol.** Figure 1 shows a representative tracing of Tₚ and mean Tsk throughout baseline and whole body heating (4.3 kcal/kg body wt). Warm water was perfused through the MD fibers at a rate of 2 µl/min (Bee Hive controller and Baby Bee microinfusion pumps, Bioanalytical Systems).

**Figure 1.** Representative tracing of oral temperature (Tₚ) and mean skin temperature (Tsk) throughout baseline, whole body heating, and clamped Tₚ.
1°C rise in $T_{\text{or}}$, mean body temperature ($T_b$) was clamped by lowering the water temperature in the suit, such that mean $T_{sk}$ and $T_{or}$ ceased to rise. After 5 min of steady laser-Doppler flux values, 20 mM L-NAME was perfused through the control and BH$_4$-perfused MD fibers at a rate of 4 μl/min to inhibit NO synthesis of NO and quantify NO-dependent vasodilation within each site. L-NAME perfusion was discontinued after laser-Doppler flux values decreased to a steady plateau (~40 min). At this time, whole body heat was terminated, the water-perfused suit was perfused with 33°C water, and subjects were returned to thermoneutral.

After completion of the whole body heating protocol, site-specific pharmacological treatments were discontinued and each MD fiber was perfused with 28 mM SNP (Nitropress; Abbott Laboratories, Chicago, IL) at a rate of 4 μl/min. Simultaneously, the local $T_{sk}$ over the experimental sites was increased to 43°C to obtain maximal CVC values within each site.

Data acquisition and analysis. CVC data from the control, BH$_4$-, and L-NAME-perfused sites were acquired at 40 Hz, digitized, and stored on a personal computer until further analysis (WinDaq; Dataq Instruments, Akron, OH). CVC values were averaged over a stable period of laser-Doppler flux at baseline, over a stable period for every 0.1°C rise in $T_{or}$ during whole body heating, and over a stable plateau during L-NAME perfusion. Maximal CVC values were averaged over a stable plateau in laser-Doppler flux during perfusion of 28 mM SNP and local temperature of 43°C. NO-dependent vasodilation within each site was assessed at clamped 1°C rise in $T_{or}$ by quantifying the decrease in CVC observed with complete NOS inhibition (20 mM L-NAME).

A three-way repeated-measures mixed-model ANOVA was conducted to detect oral treatment and local drug treatment differences over the rise in $T_{or}$. A two-way repeated-measures mixed-model ANOVA was used to detect oral treatment and local drug treatment differences in NO-dependent vasodilation, plasma BH$_4$ concentration, and maximal CVC (version 9.1.3; SAS, Cary, NC). Post hoc comparisons with Bonferroni corrections were performed when necessary to determine where differences between oral treatments and local drug treatments occurred. The level of significance was set at $\alpha = 0.05$ for main effects. Values are presented as means ± SE.

RESULTS

Subject characteristics are presented in Table 1. There was no effect of acute sapropterin treatment on systolic pressure, diastolic pressure, or MAP. Table 2 presents plasma BH$_4$ concentration data following oral placebo and sapropterin treatment from six subjects. Plasma BH$_4$ was significantly elevated 3 h after ingestion of sapropterin (0 h: 19.1 ± 2 pmol/ml vs. 3 h: 43.8 ± 3 pmol/ml; $P < 0.001$) but not after ingestion of placebo (0 h: 15.2 ± 1 pmol/ml vs. 3 h: 18.6 ± 4 pmol/ml; $P = 0.40$).

Figure 2 shows SkBF (%CVC$_{\text{max}}$) as a function of increasing core temperature ($\Delta T_{or}$) at baseline ($\Delta T_{or} = 0$) and throughout whole body heating at Ringer’s control, BH$_4$-perfused, and L-NAME-perfused MD sites with placebo and sapropterin treatment. Local BH$_4$ administration increased baseline %CVC$_{\text{max}}$ compared with Ringer’s control (control: 11 ± 2 %CVC$_{\text{max}}$ vs. BH$_4$: 19 ± 3 %CVC$_{\text{max}}$; $P < 0.001$) with placebo treatment only. Oral sapropterin increased baseline %CVC$_{\text{max}}$ at the Ringer’s control site compared with placebo treatment (placebo control: 11 ± 2 %CVC$_{\text{max}}$ vs. sapropterin control: 16 ± 2 %CVC$_{\text{max}}$; $P = 0.01$), L-NAME perfusion did not alter baseline %CVC$_{\text{max}}$ in either group. Oral sapropterin treatment increased vasodilation in the Ringer’s control site compared with placebo treatment during hyperthermia (all $P < 0.05$). Local administration of BH$_4$ increased %CVC$_{\text{max}}$ response compared with Ringer’s control with placebo treatment only (all $P < 0.05$). There was no difference in vasodilation between sapropterin treatment and placebo treatment at the BH$_4$-perfused site. When NOS was inhibited throughout the heating protocol, there was no difference in %CVC$_{\text{max}}$ response between sapropterin treatment and placebo treatment.

Figure 3 shows FVC as a function of increasing core temperature ($\Delta T_{or}$) at baseline and throughout whole body heating with oral sapropterin and placebo treatment. Oral sapropterin treatment increased FVC compared with placebo ($P < 0.01$ main effect of oral treatment).

Figure 4 shows the NO-dependent vasodilation (%CVC$_{\text{max}}$) response at a 1°C rise in $T_{or}$ at Ringer’s control and BH$_4$-perfused MD sites with oral sapropterin and placebo treatments. Oral sapropterin increased NO-dependent vasodilation in the Ringer’s control site (placebo: 14 ± 1 %CVC$_{\text{max}}$ vs. sapropterin: 25 ± 4 %CVC$_{\text{max}}$; $P = 0.004$). Local BH$_4$ perfusion increased NO-dependent vasodilation compared with Ringer’s control site with placebo treatment only (control: 14 ± 1 %CVC$_{\text{max}}$ vs. BH$_4$: 24 ± 3 %CVC$_{\text{max}}$; $P = 0.02$). There was no difference between NO-dependent vasodilation with oral sapropterin or placebo in the BH$_4$-perfused site (placebo: 24 ± 3 %CVC$_{\text{max}}$ vs. sapropterin: 27 ± 2 %CVC$_{\text{max}}$; $P = 0.55$). There were no differences between maximal CVC values across MD sites or oral treatments ($P > 0.05$ for all comparisons).

DISCUSSION

The principal finding of this study was that oral sapropterin acutely (3 h postigestion) increased reflex vasodilation in aged human skin measured by both laser-Doppler flowmetry and venous occlusion plethysmography. Furthermore, it did so through NO-dependent mechanisms. These data agree with our previous conclusions that decreased BH$_4$ contributes to attenuated reflex cutaneous vasodilation in aged humans by limiting

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Sex, M:F</th>
<th>BMI, kg/m²</th>
<th>LDL, mg/dl</th>
<th>HDL, mg/dl</th>
<th>oxLDL, U/ml</th>
<th>Total Cholesterol, mg/dl</th>
<th>HbA1c, %</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>MAP, mmHg</th>
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<tbody>
<tr>
<td>76 ± 1</td>
<td>4.5</td>
<td>25 ± 1</td>
<td>120 ± 5</td>
<td>64 ± 4</td>
<td>48 ± 3</td>
<td>203 ± 7</td>
<td>5.7 ± 0.1</td>
<td>122 ± 3</td>
<td>72 ± 3</td>
<td>91 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

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Table 2. Plasma BH$_4$ concentrations at arrival (0 h) and 3 h after ingestion of placebo or sapropterin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 h, pmol/ml</th>
<th>3 h, pmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>15.2 ± 1</td>
<td>18.6 ± 4</td>
</tr>
<tr>
<td>Sapropterin, 10 mg/kg</td>
<td>19.1 ± 2</td>
<td>43.8 ± 3*</td>
</tr>
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Values are means ± SE, $n = 6$. *$P < 0.05$ significant difference from 0 h.
NO production through uncoupled NOS (41) and suggest that with 10 mg/kg oral dose of sapropterin, BH$_4$ becomes acutely bioavailable in aged skin microvasculature sufficiently to increase NO synthesis through NOS. Oral sapropterin may be a clinically relevant intervention for improved thermoregulatory SkBF in aged adults.

In healthy young subjects, ~30–40% of the total reflex vasodilation response is mediated by NO signaling, with the remaining 60–70% relying on co-released neurotransmitter(s) with downstream vasodilation mediated through other second messenger pathways and activation of cyclo-oxygenase (4, 28, 44). With aging, the cofactor-mediated contribution to the overall expression of reflex vasodilation is attenuated and COX-dependent signaling favors the production of vasoconstrictors (18). Consequently, healthy aged adults rely predominately on a functionally compromised NO-dependent vasodilation to increase SkBF during hyperthermia (17). Because the contributions and identities of the cotransmitters in human skin are unclear and many of these cotransmitters converge on the NO pathway, interventions that target NO production and bioavailability may be capable of increasing reflex vasodilation in aged human skin. We have examined the efficacy of an acute oral sapropterin intervention in aged humans because 1) BH$_4$ bioavailability is decreased with age, 2) this decrease contributes to attenuated endothelial function in older adults, and 3) BH$_4$, as an essential NOS cofactor, is capable of modulating NO synthesis. Our results suggest that oral sapropterin increases the magnitude of reflex cutaneous vasodilation in aged human skin by increasing NOS coupling and subsequent NO synthesis.

NO is synthesized in the cutaneous vasculature by the constitutively expressed NOS isoforms endothelial NOS (eNOS) and neuronal NOS (nNOS). Although specific NOS inhibitors have not been utilized to examine the exact contributions of these NOS isoforms to reflex cutaneous vasodilation in the aged, in healthy young subjects the response appears to be mediated primarily by nNOS (24). BH$_4$ acts as an essential enzymatic cofactor for both eNOS and nNOS isoforms and in the absence of adequate BH$_4$ availability both isoforms uncouple and produce superoxide rather than NO (37). Consequently, NOS is highly dependent on BH$_4$ bioavailability for functional NO synthesis. In agreement with our previous findings, in the present study we show that local BH$_4$ perfusion through
intradermal microdialysis augments full and NO-dependent reflex vasodilation in aged skin following placebo treatment. However, this localized perfusion did not further increase the magnitude of the full or NO-dependent cutaneous vasodilation response observed with oral sapropterin treatment. Furthermore, there was no difference in full or NO-dependent reflex vasodilation between the BH₄-perfused microdialysis sites across oral treatments. These data could indicate that 1) the 10 mg/kg oral sapropterin dose maximized activity through the NOS pathway such that the enzyme was working at or near Vₘₐₓ and/or 2) we had reached a ceiling effect for the ability of the aged cutaneous vessels to vasodilate during hyperthermia. Collectively, these data suggest that 10 mg/kg oral sapropterin increases bioavailable BH₄ sufficiently to increase NO production through NOS.

In contrast to laser-Doppler flowmetry, which measures a limited area of skin (1 mm²) directly over the microdialysis membrane, venous occlusion plethysmography provides an index of SkBF over the entire arm at rest. In this study, oral sapropterin treatment increased FVC during hyperthermia. Given the systemic nature of the oral treatment and the clinical significance of demonstrating changes in SkBF over large areas of skin, these data further support the finding that oral sapropterin acutely increases the magnitude of reflex vasodilation and reiterates the clinically relevant application of sapropterin in improved thermoregulatory SkBF during hyperthermia.

Sapropterin is a shelf-stable, pharmaceutical formulation of R-BH₄ that is commercially available in the EU and the US for the treatment of BH₄-responsive phenylketonuria. In BH₄ deficiency, its mechanism of action is presumed to be secondary to replacement of endogenous cofactor bioavailability (39). Pharmacokinetic analysis of sapropterin shows that it exhibits similar time to peak plasma concentrations (~3 h) and elimination half-life (~4 h) as BH₄ administration, following a single oral dose (11, 12). Prior studies examining oral BH₄ as an intervention for improved vascular function in aging or cardiovascular disease have utilized BH₄ powder or capsules administered orally (35, 36). In the present study we chose to utilize an oral sapropterin intervention because it is commercially available, has superior shelf-stability compared with BH₄ powder or capsules, and has been shown to have a high tolerability among patients (39). Our data suggest that, similar to oral BH₄ administration, a single oral dose of sapropterin increases plasma BH₄ concentrations in older subjects sufficiently to induce a functional increase in NO production through NOS.

Compared with oral placebo control, oral sapropterin and local BH₄ perfusion increased baseline vasodilation measured with laser-Doppler flowmetry. This may indicate that a portion of the increased vasodilation observed in those sites could be due to a baseline shift. Previously we have shown that despite a modest increase in baseline SkBF, the same 10 mM concentration of BH₄ delivered locally through MD does not increase %CVCₘₐₓ response to hyperthermia in healthy young subjects (41). In that study, we concluded that the lack of a continuous upward shift in vasodilation throughout body heating suggested that the differences observed in the aged group were not simply due to a baseline shift. Furthermore, we did not observe a baseline effect of oral sapropterin on FVC, but, using this method, we were still able to detect a significant augmentation of the cutaneous blood flow response from the oral treatment. Considering the putative role of BH₄ in vascular function (6, 10, 35) and the augmentation of %CVCₘₐₓ and FVC with significantly elevated increases in body core temperatures, oral sapropterin is a potential novel pharmaceutical intervention for augmenting thermoregulatory SkBF in aged humans.

One alternate explanation for our results is that the augmented reflex cutaneous vasodilation observed with oral sapropterin and/or local BH₄ perfusion is due to the antioxidant properties of BH₄ independent of its role in NOS coupling. Reducing oxidant stress through local ascorbate perfusion can augment the vasodilator response in aged skin (19), and ascorbate infusion has been utilized to examine the role of oxidant stress in large elastic artery compliance in a number of studies, to mixed results (8, 9, 32). However, ascorbate has been shown to mediate its effects on vasodilation partially through the protection and stabilization of the BH₄ molecule (21). Furthermore, a recent study that aimed to more directly answer the question of antioxidant properties vs. NOS coupling mechanisms by utilizing the stereoisomer S-BH₄, which contains the same antioxidant capacity but lacks the NOS-coupling properties of the cofactor, found that exogenous R-BH₄ predominately restores vasodilation the skin of hypercholesterolemic subjects through its NOS coupling mechanisms (1). Thus, the observed augmentations in full and NO-dependent reflex cutaneous vasodilation seen here are likely due to the NOS-coupling mechanisms of BH₄.

Limitations. We did not examine the effects of an oral sapropterin intervention in a healthy, young subject population. Healthy, young men and women are unlikely to have a reduced BH₄ bioavailability such as that seen in an aged population (6), suggesting that young subjects would be unlikely to benefit from additional supplementation. Furthermore, previously published data from our lab (27, 41) and others (10, 35) suggests that exogenous BH₄ administration has no effect on cutaneous or conduit vascular function in young subjects aged 18–30 yr. However, in previous studies utilizing the same methodology that did include a young subject group, we observed that healthy young subjects exhibit a reflex cutaneous vasodilation response up to ~55% CVCₘₐₓ at ΔT_or = 1°C with ~23% of that dilation being NO dependent (41). In the present study, oral sapropterin increased the magnitude of reflex vasodilation in aged skin and normalized the cutaneous vasodilator response to that observed earlier in healthy, young subjects.

Perspectives. Our results suggest that an acute 10 mg/kg dose of oral sapropterin increases reflex vasodilation in aged human skin through NO-dependent mechanisms and that oral administration of exogenous BH₄ may be a clinically relevant intervention for improved thermoregulatory function in aged adults during hyperthermia. Oral supplementation with BH₄ improves vascular function in animal and human models of vascular disease and dysfunction (14, 36), and taken together these data suggest that the specific NOS-coupling mechanisms of BH₄ may be an emerging therapy for endothelial dysfunction. In contrast to our previous study in which we used a localized microperfusion of BH₄ to explore the role of the cofactor in NOS coupling, in this study we utilized a commercially available, pharmaceutical formulation of BH₄ (sapropterin) to explore the clinically relevant efficacy of an acute oral intervention for improved thermoregulatory SkBF in healthy aged adults exposed to heat stress.

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By design, this study excluded subjects who had overt cardiovascular disease and/or were taking medications. Although these subjects were not elite athletes, they represent a specific subset of the population, and our results may not be generalizable to older adults who are unhealthy and/or taking a variety of medications. However, exogenous BH4 therapy has been shown to effectively increase vascular endothelial function in populations with cardiovascular disease (1, 5, 36). Although the subjects in those studies were younger than the subjects in the present study, their results suggest that BH4 may be efficacious in older adults who do exhibit overt cardiovascular disease. Further research is certainly warranted to investigate the efficacy of this intervention in an older diseased population and other human populations that exhibit attenuated thermoregulatory SkBF and to determine the efficacy of a long-term dosing strategy.

Summary. In summary, acute oral sapropterin treatment increases reflex cutaneous vasodilation in aged humans through NO-dependent mechanisms. In addition, there is no additive effect of local BH4 perfusion, suggesting that the 10 mg/kg dose increases bioavailable BH4 sufficiently to maximally increase NO synthesis through NOS. If one considers the putative role of BH4 in vascular function and the observed increase in the magnitude of reflex cutaneous vasodilation in the present study, oral sapropterin administration is a potential intervention strategy for improved thermoregulatory function in older adults exposed to environmental heat stress.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: A.E.S. performed experiments; A.E.S. analyzed data; A.E.S., L.M.A., and W.L.K. interpreted results of experiments; A.E.S. prepared figures; A.E.S. drafted manuscript; A.E.S., L.M.A., and W.L.K. approved final version of manuscript; L.M.A. and W.L.K. conception and design of research; L.M.A. and W.L.K. edited and revised manuscript.

REFERENCES


