Oral sapropterin augments reflex vasoconstriction in aged human skin through noradrenergic mechanisms

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Sapropterin augments reflex vasoconstriction in aged human skin through noradrenergic mechanisms. J Appl Physiol 115: 1025–1031, 2013. First published July 18, 2013; doi:10.1152/japplphysiol.00626.2013.—Reflex vasoconstriction is attenuated in aged skin due to a functional loss of adrenergic vasoconstriction. Bioavailability of tetrahydrobiopterin (BH4), an essential cofactor for catecholamine synthesis, is reduced with aging. Locally administered BH4 increases vasoconstriction through adrenergic mechanisms in aged human skin. We hypothesized that oral sapropterin (Kuvan, a pharmaceutical BH4) would augment vasoconstriction elicited by whole-body cooling and tyramine perfusion in aged skin. Ten healthy subjects (age 75 ± 2 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. Three intradermal microdialysis fibers were placed in the forearm skin for local delivery of either Ringer or lactated Ringer, or tyramine dose (7). These impairments may be consequent to elevated oxidative stress and reduced substrate availability for norepinephrine synthesis (22), both of which contribute to attenuated reflex vasoconstriction in aged human skin. Collectively, older humans rely entirely on a functionally compromised noradrenergic-mediated vasoconstriction to reduce skin blood flow during cold exposure (4, 19).

In addition to elevated oxidative stress and reduced L-tyrosine availability, our laboratory has recently suggested that reduced bioavailability of tetrahydrobiopterin (BH4) also contributes to the attenuated noradrenergic reflex vasoconstriction in aged human skin (21). BH4 is found throughout the neural and vascular tissue, and is an essential cofactor for nitric oxide synthase (NOS) and tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis (18, 26). Mechanistically, BH4 serves as a reducing agent and is required to maintain TH in its active form (18, 44). Oxidant-induced depletion of intraneuronal BH4 may deplete newly synthesized or stored pools of norepinephrine within the perivascular neurons of aged skin, resulting in a functionally attenuated vasoconstriction during cold-induced sympathetic activation. We recently demonstrated that localized exogenous BH4 administration through an intradermal microdialysis fiber increases vasoconstriction in response to physiological (whole-body cold exposure) and pharmacological (local tyramine perfusion) stimuli through noradrenergic mechanisms with no effect on cotransmitter-mediated vasoconstriction or end organ responsiveness to norepinephrine (21, 23). These findings open the possibility that a systemic BH4 intervention may be a clinically applicable intervention to increase reflex cutaneous vasoconstriction in older adults during cold exposure.

We have previously examined the role of oral sapropterin (pharmaceutical BH4) administration in improved reflex cutaneous vasodilation in aged humans exposed to environmental heat stress (36). Sapropterin is a commercially available, shelf-stable, pharmaceutical formulation of R-BH4, which is prescribed clinically in the United States for the treatment of BH4-responsive phenylketonuria. In that study, an acute oral dose (10 mg/kg) of sapropterin increased bioavailable BH4 sufficiently to increase nitric oxide (NO)-dependent reflex vasodilation in aged human skin, presumably though the coupling cofactor properties of BH4 on NOS. However, the clinical efficacy of a systemic BH4 intervention on the separate...
neural catecholamine synthesis mechanisms mediated by TH and its essential cofactor BH4 has not been examined.

The purpose of the present study was to specifically address the role of oral BH4 administration in improved reflex cutaneous vasoconstriction in aged human skin exposed to cold stress. Because sapropterin is a commercially available drug that has been shown to increase bioavailable BH4 in aged cutaneous vessels, and because oral dosing is more clinically practical than intradermal microdialysis for the delivery of BH4, we chose to utilize an oral sapropterin intervention. We hypothesized that oral sapropterin would acutely augment reflex and pharmacologically induced cutaneous vasoconstriction in aged human skin through noradrenergic mechanisms.

METHODS

Subjects. Experimental protocols were approved by the institutional review board of The Pennsylvania State University. Written and verbal consent were obtained voluntarily from all subjects prior to participation according to the Declaration of Helsinki. Studies were performed on 10 healthy subjects (age, 75 ± 2 yr; 5 men, 5 women). Subjects were screened for neurological, cardiovascular, and dermatological diseases and underwent a complete medical screening including resting electrocardiography, physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsburgh, PA). Subject characteristics are presented in Table 1. All subjects were normally active, nonhypertensive, nondiabetic, healthy nonsmokers who were not taking over-the-counter or prescription medications or supplements 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 (1). Study days were separated by at least 48 h to ensure adequate washout of sapropterin (10). Subjects entered the laboratory between 8:00 and 9:00 A.M. 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 in a double-blind, randomized crossover study design. The 10 mg/kg dose was chosen because it has been shown to increase plasma biopterin concentration approximately 50-fold (11), and improvements in vascular function have been observed at increases as small as approximately 4-fold above baseline (43). A second blood sample was obtained 3 h after ingestion of the treatment for analysis of peak plasma BH4 concentrations. Pharmacokinetic analysis of sapropterin shows that plasma BH4 concentrations peak at 3 h following oral administration (10). All blood samples were collected in 4-ml tubes containing EDTA and 0.1% w/v dithiothreitol and centrifuged immediately. The plasma was flash-frozen in liquid nitrogen and stored at −80°C until further analysis of BH4 concentration by HPLC (25).

The plasma BH4 concentrations were analyzed using a previously described method (27). Plasma BH4 concentrations were measured within 8 h of sample collection. All plasma BH4 concentrations were based on previous studies conducted in our laboratory (21).

Pharmacological agents were mixed just before use, dissolved in lactated Ringer solution, sterilized using 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), pharmacological solutions were perfused through the MD fibers at a rate of 2 μl/min (Bee Hive controller and Baby Bee micropump; Bioanalytical Systems).

To obtain an index of SKBF, cutaneous red blood cell flux was continually measured directly over each MD site with a laser-Doppler flowmetry probe placed in a local heating unit (Moor Instruments 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 continually measured directly over each MD site with a laser-Doppler flowmetry probe placed in a local heating unit (Moor Instruments SHO2). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by mean arterial pressure (MAP). MAP was calculated as diastolic pressure plus one-third pulse pressure. Three to five measures of forearm blood flow (FBF) were collected and averaged at baseline and every 0.5°C decrease in 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. Local skin temperature over each MD site was clamped at 33°C throughout baseline and whole-body cooling (Moor-Lab, Temperature Monitor, SHO2; Moor Instruments, Devon, UK) to ensure that changes in SKBF were reflex in origin.

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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 (μmol/l)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HbA1c (%)</th>
<th>MAP (mmHg)</th>
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<tbody>
<tr>
<td>75 ± 2</td>
<td>5/5</td>
<td>25 ± 1</td>
<td>119 ± 5</td>
<td>64 ± 4</td>
<td>46 ± 4</td>
<td>200 ± 7</td>
<td>5.7 ± 0.1</td>
<td>90 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SE. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; oxLDL, oxidized LDL; MAP, mean arterial pressure. HbA1c, hemoglobin A1C.

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Experimental protocol. After MD fiber placement, the insertion trauma resolution period, and instrumentation, baseline data were collected (~20 min). Throughout baseline, $T_{sk}$ was held at thermoneutral by perfusing 33°C water through the suit. Following baseline data collection, cool water was perfused through the suit to gradually lower $T_{sk}$ from 34°C to 30.5°C over 30 min, followed by ~10 min in which $T_{sk}$ was clamped at 30.5°C. Cooling began exactly 3 h after ingestion of sapropterin or placebo. This timing was chosen to ensure peak plasma concentrations of sapropterin during cooling (10). Following cooling, warm water was perfused through the suit to return $T_{sk}$ to 34°C. Following rewarming, exogenous norepinephrine (1 × 10⁻⁶ M) was perfused at the Y+P-perfused site to test the α- and β-adrenergic blockade at that site. This norepinephrine dose has been used previously to effectively assess noradrenergic vasoconstriction following whole-body cooling in control and pharmacologically treated MD sites (24).

After completion of the cooling protocol, site-specific pharmacological treatments were discontinued and each MD fiber was perfused with 1 mM tyramine to pharmacologically evoke endogenous norepinephrine release. Exogenous norepinephrine (1 × 10⁻⁶ M) was then perfused through each fiber to elicit further noradrenergically mediated vasoconstriction. Full resolution of the robust vasconstrictor responses to tyramine and norepinephrine prevented the randomization of these steps with whole-body cooling. Finally, 28 mM sodium nitroprusside (SNP) was perfused through each fiber at a rate of 4 μl/min while the local temperature of the skin was increased to 43°C to induce a vasodilation response to ensure that vascular responsiveness remained intact postcooling.

Data acquisition and analysis. CVC data from the control, BH₄, and Y+P-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 5-min period of laser-Doppler flux at baseline, over stable periods of flux for every 0.5°C decrease in $T_{sk}$ during whole-body cooling (approximately 2–3 min), and over a stable plateau during tyramine and norepinephrine perfusion.

A three-way repeated-measures mixed-model ANOVA was conducted to detect oral treatment and local drug treatment differences over the decrease in $T_{sk}$. A two-way repeated-measures mixed-model ANOVA was used to detect oral treatment differences in FVC over the decrease in $T_{sk}$. A two-way repeated-measures mixed-model ANOVA was used to detect oral treatment and local drug treatment differences in vasoconstriction during tyramine and norepinephrine perfusion, plasma BH₄ concentration, and baseline 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 mean ± SE.

RESULTS

Table 2 presents plasma BH₄ concentrations following oral placebo and sapropterin treatment from six subjects. Plasma BH₄ was significantly elevated 3 h after ingestion of sapropterin but not after placebo ingestion.

Table 3 presents baseline CVC values for all MD sites across oral placebo and sapropterin treatments. There was a significant main effect of MD treatment ($P < 0.001$) on baseline CVC. Accordingly, we have represented changes in skin blood flow as absolute changes from site-specific baseline (ΔCVC).

Table 4 presents MAP at baseline, and during whole-body cooling with oral placebo and sapropterin treatments. There was no effect of oral treatment on MAP.

Figure 1 shows changes in skin blood flow (ΔCVC) from baseline ($T_{sk} = 34°C$) and throughout whole-body cooling as a function of decreasing $T_{sk}$ at Ringer control, BH₄-perfused, and Y+P-perfused MD sites with placebo and sapropterin treatment. Oral sapropterin increased vasoconstriction at the Ringer control site compared with placebo at $T_{sk} \leq 32.5°C$ ($P < 0.05$ for all comparisons at $T_{sk} \leq 32.5°C$). Local administration of BH₄ increased vasoconstriction at $T_{sk} \leq 31.5°C$ compared with Ringer control with placebo treatment only ($P < 0.05$ for all comparisons at $T_{sk} \leq 31.5°C$). There was no difference in vasoconstriction between sapropterin treatment and placebo treatment at the BH₄-perfused site. When noradrenergic vasoconstriction was inhibited throughout the protocol (Y+P-perfused site) there was no difference in ΔCVC between oral sapropterin and placebo treatment.

Figure 2 shows FVC as a function of decreasing $T_{sk}$ at baseline and throughout whole-body cooling with oral sapropterin and placebo treatments. Oral sapropterin treatment decreased FVC compared with placebo ($P = 0.02$ main effect of oral treatment).

Figure 3 shows the vasoconstrictor (ΔCVC) response to 1 mM tyramine treatment at the Ringer control, BH₄-perfused, and Y+P-perfused MD sites with oral sapropterin and placebo treatments. Oral sapropterin increased vasoconstriction at the Ringer control site (placebo, $-0.08 \pm 0.02$ ΔCVC vs. sapropterin, $-0.19 \pm 0.03$ ΔCVC; $P = 0.01$). There was no differ-

Table 2. Plasma BH₄ concentrations at baseline (0 h) and 3 h after ingestion of placebo or sapropterin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 Hours</th>
<th>3 Hours</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>15.2 ± 1 pmol/ml</td>
<td>18.6 ± 4 pmol/ml</td>
</tr>
<tr>
<td>Sapropterin (10 mg/kg)</td>
<td>19.1 ± 2 pmol/ml</td>
<td>43.8 ± 3 pmol/ml *</td>
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Values are mean ± SE, $n = 6$. *$P < 0.05$ significant difference from 0 h.

Table 3. Baseline CVC at Ringer (control), yohimbine + propranolol-perfused, and BH₄-perfused microdialysis sites with placebo or sapropterin treatments

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>CVC</th>
</tr>
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<tbody>
<tr>
<td>Ringer</td>
<td>0.19 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Yohimbine + Propranolol</td>
<td>0.49 ± 0.12*</td>
<td></td>
</tr>
<tr>
<td>BH₄</td>
<td>0.29 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>Sapropterin</td>
<td></td>
<td>CVC</td>
</tr>
<tr>
<td>Ringer</td>
<td>0.29 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Yohimbine + Propranolol</td>
<td>0.50 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>BH₄</td>
<td>0.29 ± 0.04</td>
<td></td>
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</table>

Values are mean ± SE. *$P < 0.001$ main effect of local drug treatment
ence in vasoconstriction between oral placebo or sapropterin treatments at the BH4-perfused site (placebo, −0.16 ± 0.04 ΔCVC vs. sapropterin, −0.14 ± 0.03 ΔCVC; P = 0.60) or the Y+P-perfused site (placebo, −0.05 ± 0.02 ΔCVC vs. sapropterin, −0.06 ± 0.02 ΔCVC; P = 0.79). There were no differences between ΔCVC values across MD sites or oral treatments following exogenous norepinephrine (1 × 10−2 M) perfusion through each MD fiber (P > 0.05 for all comparisons).

**DISCUSSION**

The principal finding of this study was that oral sapropterin acutely (3-h postingestion) increased reflex vasoconstriction in aged human skin, as measured by both laser-Doppler flowmetry and venous occlusionplethysmography. Further, it did so through alterations in noradrenergic mechanisms. These data substantiate our previous conclusions that decreased BH4 causes cotransmitter-mediated vasoconstriction is absent in aging, cotransmitters such as ATP and neuropeptide Y (39, 41). With aging, cotransmitter-mediated reflex vasoconstriction is functionally absent (41) and, as a result, aged humans rely predominately on a compromised norepinephrine-mediated vasoconstriction to decrease skin blood flow and increase tissue insulation during environmental cold exposure. Because cotransmitter-mediated vasoconstriction is absent in aged skin, noradrenergic mechanisms (such as norepinephrine synthesis at the perivascular nerve terminal) are the most viable target for pharmacological interventions that aim to increase noradrenergic-mediated vasoconstriction in the skin of aged humans during whole-body cold exposure. We examined the efficacy of an acute oral sapropterin intervention in aged humans because 1) BH4 bioavailability is reduced with advanced age; 2) this decrease contributes to the attenuated noradrenergic-mediated vasoconstriction in older adults; and 3) BH4, as an essential cofactor for TH, plays a central role in norepinephrine biosynthesis. Our results suggest that oral sapropterin increases the magnitude of reflex cutaneous vasoconstriction in aged human skin by increasing norepinephrine synthesis at the perivascular nerve terminal.

In healthy young subjects, ~60% of the total reflex vasoconstriction response to whole-body cooling is mediated by norepinephrine, with the remaining ~40% mediated by cotransmitters such as ATP and neuropeptide Y (39, 41). With aging, cotransmitter-mediated reflex vasoconstriction is functionally absent (41) and, as a result, aged humans rely predominately on a compromised norepinephrine-mediated vasoconstriction to decrease skin blood flow and increase tissue insulation during environmental cold exposure. Because cotransmitter-mediated vasoconstriction is absent in aged skin, noradrenergic mechanisms (such as norepinephrine synthesis at the perivascular nerve terminal) are the most viable target for pharmacological interventions that aim to increase axonal release of norepinephrine and reflex vasoconstriction in the skin of aged humans during whole-body cold exposure. We examined the efficacy of an acute oral sapropterin intervention in aged humans because 1) BH4 bioavailability is reduced with advanced age; 2) this decrease contributes to the attenuated noradrenergic-mediated vasoconstriction in older adults; and 3) BH4, as an essential cofactor for TH, plays a central role in norepinephrine biosynthesis. Our results suggest that oral sapropterin increases the magnitude of reflex cutaneous vasoconstriction in aged human skin by increasing norepinephrine synthesis at the perivascular nerve terminal.
Norepinephrine synthesis requires the functional activity of TH, the rate-limiting enzyme in the biosynthesis of catecholamines. BH₄ acts as an essential cofactor for TH, reducing the iron moiety of TH, thereby priming TH for catalytic reaction (20, 42). Consequently, norepinephrine synthesis is reliant on adequate BH₄, and reduced BH₄ bioavailability in the aged population may contribute to attenuated norepinephrine synthesis and release at a given cold stimulus. Presumably, exogenous BH₄ administration enhances functional vasoconstriction by augmenting norepinephrine biosynthesis and storage in the perivascular nerve terminals, allowing for greater norepinephrine release during sympathetic stimulation (22).

In agreement with previous findings from our laboratory, local BH₄ administration through intradermal microdialysis augments reflex (whole-body cold exposure) and pharmacological (tyramine perfusion) vasoconstriction in aged skin following placebo treatment. However, this localized administration did not further increase the magnitude of the vasoconstriction response to either stimulus after oral sapropterin. Furthermore, there was no difference in reflex or pharmacological vasoconstriction between the BH₄-administered microdialysis sites and oral treatments. These data could indicate that 1) the 10 mg/kg dose of oral sapropterin maximized activity through TH such that the enzyme was working at or near Vₘₐₓ, and/or 2) we had reached a ceiling effect for the ability of the cutaneous vasculature to vasoconstrict under these conditions. Exogenous, locally administered BH₄ does not affect the ability of the vessel to respond to noradrenergic vasoconstrictor stimuli (21). Similarly, we found no differences in the vasoconstrictor response to an exogenous norepinephrine perfusion between microdialysis sites or across oral treatments. Collectively, these data suggest that 10 mg/kg oral sapropterin increases bioavailable BH₄ sufficiently to increase norepinephrine synthesis within the perivascular nerve terminal.

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 blood flow over the entire forearm. At rest in thermoneutral to warm environments, changes in blood flow observed with venous occlusion plethysmography are confined to the skin, and do not reflect changes in muscle blood flow (6). During whole-body cold exposure, reductions in FBF may reflect changes in both skin and skeletal muscle blood flow that serve to increase tissue insulation (15, 27). In this study, oral sapropterin treatment decreased FVC during cold exposure. Given the systemic nature of the oral treatment and the clinical significance of demonstrating changes in blood flow, these data further support the finding that oral sapropterin acutely increases the magnitude of reflex vasoconstriction, and reiterate the clinically relevant application of sapropterin in improved vascular control mechanisms in aged humans.

Sapropterin is a shelf-stable, pharmaceutical formulation of R-BH₄ that is commercially available in the European Union and United States 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 (31). 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 (10, 11). Prior studies examining oral BH₄ as an intervention for improved vascular function in aging or cardiovascular disease have focused on endothelial NO, and have utilized BH₄ powder or capsules administered orally (28, 30). Recent results from our laboratory examining the role of sapropterin in NO-dependent vasodilation suggest that oral sapropterin acutely increases NO-dependent reflex vasodilation in aged human skin (36). In the present study we utilized an oral sapropterin intervention because it is commercially available, has superior shelf stability compared with BH₄ powder or capsules, increases bioavailable BH₄ in the microvasculature of aged humans (36), and has a high tolerability among patients (31). Our data suggest that a single, oral dose of sapropterin increases plasma BH₄ concentrations in older subjects sufficiently to increase norepinephrine synthesis though TH and induce a functional increase in the magnitude of reflex vasoconstriction.

It is of clinical relevance to question whether an intervention that increases vasoconstrictor capacity should be recommended in a population at greater risk for cardiovascular disease. With aging, several signaling mechanisms converge on the vasculature that induce vessel remodeling and endothelial dysfunction, promoting a proconstrictor status (3, 13, 33). Reduced BH₄ bioavailability is one proposed contributor to this age-related vascular dysfunction (2, 29), and clinical studies utilizing BH₄ as an intervention in aging have found that restoring bioavailable BH₄ improves measures of endothelial function in aged vessels (28, 37). Along these lines, we have shown that oral sapropterin increases NO-dependent vasodilation in aged human skin (36). Few studies if any have examined the effects of systemic exogenous BH₄ administration on vasoconstrictor mechanisms in vivo. In the present study, we did not observe any evidence that oral sapropterin increased mean arterial pressure or heart rate during thermoneutral or whole-body cooling conditions compared with placebo. Similarly, clinical trials of sapropterin have not reported adverse hemodynamic results (45, 46). In context, the observed restoration of a physiological vasoconstriction response to a whole-body cold stimulus is not maladaptive, and taken together with our previous finding that oral sapropterin increases NO-dependent reflex vasodilation in a similar subject cohort, these data suggest that exogenous BH₄ administration may improve functional vascular control.

Limitations. For research purposes, we utilized an oral sapropterin dose standardized to body weight. Although scientifically sound, this practice is not commonly used in a medical setting and it is possible that this may affect the clinical validity of our results. However, the results of this study suggest that 10 mg/kg sapropterin administered orally increases plasma BH₄ sufficiently to increase functional reflex vasoconstriction in aged skin during whole-body cold exposure. In a clinical setting, these findings could be of assistance when determining dosing strategies on a patient-to-patient basis. Further research is warranted to determine whether a standard dose could be prescribed generally, and to determine the efficacy of a chronic dosing strategy.

It is also unclear when tissues concentrations of BH₄ peak following oral administration. Animal models suggest that tissue concentrations peak at the same time blood concentrations peak (32); however, these time-course data are not available in human models. Despite this uncertainty, our results suggest that 3 h was sufficient to increase tissue BH₄ in the perivascular nerve terminals of aged cutaneous vessels.
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 reduction in BH4 bioavailability such as that exhibited by an aged population (5). This suggests that young subjects would be unlikely to benefit from added BH4 administration. Furthermore, findings from our laboratory (22, 37) and those of others (9, 28) suggest that exogenous BH4 has no effect on cutaneous or conduit vascular function in young subjects aged 18–30 yr.

Aside from its roles as an essential cofactor for TH, BH4 also increases NO-dependent vasodilation in aged human skin through its putative role as an essential cofactor for the constitutively expressed NOS (37). Similarly, oral sapropterin increases NO-dependent vasodilation in aged human skin (36). In the human cutaneous circulation, NO is capable of inhibiting sympathetic adrenergic vasoconstriction; however, the precise mechanism by which this inhibition occurs remains unclear (8, 34, 35). Lang et al. utilized the NOS-inhibitor nitro-l-arginine methyl ester (l-NAME) to demonstrate that locally administered 5 mM BH4 does not induce NO-mediated effects on the expression of cold- or tyramine-induced vasoconstriction in aged skin (21). In the present study we observed a positive effect of the local BH4 and oral sapropterin treatments on absolute and noradrenergic-mediated vasoconstriction despite the role of BH4 in NO synthesis. However, we did not specifically utilize NOS-inhibitors in this study.

Perspectives. Our results suggest that an acute 10 mg/kg dose of oral sapropterin increases reflex vasoconstriction in aged human skin through noradrenergic mechanisms and that oral administration of exogenous BH4 may be a clinically relevant intervention for improving vasoconstrictor function in aged adults exposed to environmental cold. Oral supplementation with BH4 and/or sapropterin improves measures of endothelial function in aging and vascular disease (16, 30, 36). In the present study we observed a positive effect of the local BH4 and oral sapropterin treatments on absolute and noradrenergic-mediated vasoconstriction despite the role of BH4 in NO synthesis. However, we did not specifically utilize NOS-inhibitors in this study.

Summary. In summary, acute oral sapropterin increases reflex and pharmacologically induced cutaneous vasoconstriction in aged humans by influencing the norepinephrine synthesis pathway. There is no additive effect of local BH4 perfusion, suggesting that the 10 mg/kg dose increases bioavailable BH4 sufficiently to maximally increase norepinephrine synthesis through TH in the perivascular nerve terminal. In addition, there is no effect of locally administered exogenous BH4 or oral sapropterin on end-organ responsiveness to norepinephrine. Considering the role of BH4 in norepinephrine synthesis and the observed increase in the magnitude of reflex vasoconstriction in the present study, oral sapropterin is a clinically relevant potential intervention strategy for improving vascular control in older adults.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: L.M.A. and W.L.K. conception and design of research; 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. edited and revised manuscript; A.E.S., L.M.A., and W.L.K. approved final version of manuscript.

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