Oral BH₄: A novel remedy for age-related skin microvascular impairment during heat stress or fool’s elixir?

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5,6,7,8-TETRAHYDROBIOPTERIN (BH₄) is a critical cofactor for the nitric oxide synthases (NOS) because it facilitates the electron transfer between the reductase and oxygenase domains of the NOS dimer and couples the reduction of molecular oxygen to the oxidation of L-arginine and synthesis of nitric oxide (NO). BH₄ biosynthesis is regulated by the de novo pathway in which guanidine triphosphate (GTP) is converted to BH₄ under the control of the rate-limiting enzyme GTP cyclohydrolase I (GTPCHI) and two intermediate enzymes pyruvoyl tetrahydrobiopterin synthase and sepiapterin reductase (SR). BH₄ also is synthesized by a “salvage pathway,” whereby sepiapterin is converted to 7,8-dihydrobiopterin (BH₂) via SR and subsequently reduced to BH₄ by dihydrofolate reductase (DHFR) (Fig. 1). A large body of evidence implicates a reduction in vascular BH₄ bioavailability as a central mechanism for the development of impaired NO-mediated microvascular function in a wide variety of conditions, including diabetes, hypertension, hypercholesterolemia, atherosclerosis, and aging (7). The aforementioned disorders also are associated with elevated vascular oxidative stress, and BH₄ is particularly susceptible to oxidation by peroxynitrite (ONOO⁻), a reactive oxygen species byproduct of the NO and superoxide anion (O₂⁻) reaction (8) (Fig. 1). Oxidation of BH₄ results in uncoupling of the NOS dimer, promoting transfer of electrons to molecular oxygen resulting in production of O₂⁻ rather than NO. Consequently, supplementation of BH₄ has been advanced for more than a decade as a novel remedy for restoring microvascular dysfunction in aged adults (9) and adults at risk for or with cardiovascular disease (CVD) (7).

In this issue of the Journal of Applied Physiology, Staniewicz et al. (10) investigated the effects of acute oral adminis-

![Fig. 1. Intracellular 5,6,7,8-tetrahydrobiopterin (BH₄), synthesis, oxidation, and recycling in endothelial cells. GTP, guanidine triphosphate; GTPCHI, GTP cyclohydrolase I; PTPS, pyruvoyl tetrahydrobiopterin synthase; SR, sepiapterin reductase; DHFR, dihydrofolate reductase; NO, nitric oxide; O₂⁻, superoxide anion radical; ONOO⁻, peroxynitrite; H₂O₂, hydrogen peroxide; BH₄⁻, trihydrobiopterin radical; DHF, dihydrofolate; 5MTHF, 5-methyltetrahydrofolate; eNOS, endothelial nitric oxide synthase; Asc, ascorbate; Asc⁺, ascorbate radical.](http://www.jappl.org)
of L-NAME to "pharmaco-dissect" the contribution of NO to blind, placebo-controlled crossover design; use of coinfusion previously reported values in young subjects (11). Neous vasodilation in the older adults to levels consistent with sapropterin is through antioxidant actions. Moreover, the single completely ruled out that some of the beneficial effect of action to hyperthermia in older adults, although it cannot be cutaneous microcirculation and improve reflex skin vasodilation to hyperthermia in older adults, although it cannot be completely ruled out that some of the beneficial effect of sapropterin is through antioxidant actions. Moreover, the single dose of sapropterin used appeared to fully restore reflex cutaneous vasodilation in the older adults to levels consistent with previously reported values in young subjects (11).

The strengths of the study include the randomized, double-blind, placebo-controlled crossover design; use of coinfusion of l-NAME to "pharmaco-dissect" the contribution of NO to the BH4-mediated improvement in skin vasodilation; and the use of an FDA-approved drug that could have genuine clinical/translational impact. The latter is important because there are no established pharmacological remedies to prevent impaired cutaneous blood flow during heat stress in older adults who are particularly susceptible to hyperthermia-induced injury. However, as with all good studies, the current study raises many new questions that remain to be answered. For example, what is the minimal effective dose of sapropterin required to show the same effect as the 10 mg/kg dose used in this study? The current study design experimentally clamped body temp after a 1°C rise from baseline. Do the beneficial effects of sapropterin on reflex skin vasodilation observed with mild hyperthermia persist with more severe hyperthermia. The study used a single acute dose of sapropterin followed by experimental measurements at 2- to 3-h postingestion. How long does the beneficial effect on cutaneous blood flow last beyond 3 h? Finally, as the authors point out, the current study was in healthy older adults without risk factors for CVD and not on any vasoactive medications. A recent study demonstrated that 2–6 wk of oral sapropterin had no effect on vascular endothelial function in older adults with advanced atherosclerosis (4). Therefore, it remains to be determined whether oral sapropterin would be effective in improving skin vasodilation during hyperthermia in older adults at risk for or with clinical CVD.

One major issue that remains largely unresolved is how exogenously administered BH4 is transported from the circulation into endothelial cells. There is currently no known cellular transporter or receptor for BH4 and plasma concentrations of BH4 do not correlate well with vascular concentrations (1). One hypothesis is that exogenously administered BH4 is oxidized rapidly to BH2 upon entering circulation (4), diffuses into the cell as BH2, and then is recycled back to BH4 via the "salvage" pathway enzyme DHFR (Fig. 1). Indeed, siRNA knockdown or pharmacological inhibition of DHFR with methotrexate in endothelial cells results in a rapid decrease in intracellular BH4 and an increase in BH2 with concomitant eNOS uncoupling (3). Therefore, these data support the idea that DHFR likely plays a significant role in the regulation of vascular BH4 bioavailability.

Sapropterin's clinical indication is for the treatment of BH4-deficient hyperphenylalaninemia, a rare defect in BH4 synthesis that renders phenylalanine hydroxylase unable to metabolize phenylalanine to tyrosine. The benefits of sapropterin are that it has a good safety profile, is associated with minimal side effects, and is shelf stable (e.g., does not get oxidized easily). However, major limitations for widespread use of sapropterin in studies with "off-label" vascular endpoints is that the drug is currently made by only one manufacturer in the world and is very expensive, making feasibility of chronic intervention studies with microvascular function as a primary outcome challenging.

In light of these limitations there is a growing interest in alternative therapeutic approaches to increase intracellular BH4 bioavailability by protecting BH4 from oxidation and/or stimulating de novo BH4 synthesis. For example, intravenous administration of 5-methyltetrahydrofolate (5MTHF), the biologically active form of folic acid, improves NOS coupling-associated endothelial function in older adults with atherosclerosis through the ONOO scavenging effects of 5MTHF or increased BH4 recycling during conversion of folate to 5MTHF (Fig. 1) (2). Similarly, acute local perfusion of ascorbic acid improves reflex skin vasodilation during heat stress in older adults (6), however it is currently unknown whether oral ascorbic acid preparations or other antioxidants can improve cutaneous vasodilation during hyperthermia with aging. Finally, GTPCHI, the rate-limiting enzyme for BH4 biosynthesis, can be modulated by numerous pharmacological and physiological stimuli. HMG CoA reductase inhibitors (statins) (5) and laminar shear stress (e.g., in vitro exercise blood flow mimetic) (12) increase GTPCHI mRNA and activity in endothelial cells, respectively, thus augmenting de novo synthesis of BH4 and NOS coupling (Fig. 1). Taken together, these studies suggest that alternative strategies to preserve or increase vascular BH4 bioavailability in aged humans deserve further investigation.

In summary, acute oral administration of sapropterin, an FDA-approved BH4 analog, is effective in fully restoring the reflex skin vasodilatory response to hyperthermia in older adults largely through a NO-dependent mechanism. However, before sapropterin is labeled the next "elixir" for prevention of the former-related skin microvascular dysfunction in older adults, randomized controlled trials of sapropterin or other synthetic BH4 analogs are essential to determine whether preserving intracellular BH4 bioavailability ultimately reduces heat-related morbidity and mortality in the aged population.

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

AUTHOR CONTRIBUTIONS
Author contributions: G.L.P. conception and design of research; G.L.P. prepared figures; G.L.P. drafted manuscript; G.L.P. edited and revised manuscript; G.L.P. approved final version of manuscript.
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