The Pennsylvania State University

The Graduate School

CUTANEOUS VASCULAR HEALTH: ULTRAVIOLET RADIATION AND VITAMIN D/FOLATE METABOLISM IN DIFFERENTLY PIGMENTED SKIN

A Dissertation in

Kinesiology

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

December 2020

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death worldwide, and vascular endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is a common antecedent. Ultraviolet radiation (UVR) may influence endothelial function via disparate effects on folate and vitamin D. Vitamin D is produced in the skin upon exposure to UVR (specifically UV-B), and may modulate endothelial function by reducing oxidative stress, increasing expression of endothelial NO synthase (eNOS), and/or reducing vascular inflammation. Conversely, UVR exposure may degrade the bioactive folate metabolite and eNOS cofactor, 5-methyltetrahydrofolate (5-MTHF), and/or increase oxidative stress and inflammation. Darkly-pigmented skin (i.e., high eumelanin concentration) may protect against deleterious effects of UVR, but impair cutaneous vitamin D biosynthesis. On the other hand, lightly-pigmented skin (i.e., low eumelanin concentration) promotes adequate vitamin D synthesis, but may be predisposed to harmful effects of UVR.

This dissertation comprises two published literature reviews and three empirical studies investigating the influence of UVR, and the modulating role of skin pigmentation, on cutaneous microvascular endothelial function through its roles in 5-MTHF and vitamin D metabolism. The first study investigated the acute deleterious effects of UV-B exposure on NO-mediated cutaneous microvascular function via 5-MTHF degradation and/or production of oxidative stress. Results indicated that (1) acute UV-B exposure attenuated NO-mediated dilation in the skin microvasculature, (2) local delivery of 5-MTHF or ascorbate (non-specific antioxidant) ameliorated the effect of UV-B exposure, and (3) these effects were independent of skin pigmentation. Together, these findings suggest that UV-B exposure acutely impairs NO-mediated cutaneous microvascular function, independently of skin pigmentation, via direct and/or indirect photodegradation of 5-MTHF. The second study, consisting of two parts, examined the influence

of sunscreen or a simulated sweat solution on the skin in modulating the cutaneous microvascular effects of broad-spectrum UVR (UV-A + UV-B) exposure. Part one concluded that (1) an erythemal dose of UVR resulted in a linear increase in erythema across an 8-hour post-exposure period with a delayed increase in skin blood flow, and (2) broad-spectrum SPF-50 sunscreen blunted skin erythema and blood flow responses post-exposure. Part two determined that (1) acute exposure of the skin to a sub-erythemal dose of broad-spectrum UVR attenuated NO-mediated vasodilation in response to a local skin heating stimulus, but (2) sunscreen or simulated sweat on the skin during exposure was protective against those deleterious effects of UVR exposure. These data suggest that although a sub-erythemal dose of broad-spectrum UVR attenuates NO-mediated vasodilation during local heating, an erythemal dose is necessary to elicit an inflammatory increase in resting skin blood flow, while sunscreen on the skin during exposure is protective of both responses. The third study investigated the efficacy of four weeks of 2,000 IU/day oral vitamin D supplementation for ameliorating oxidative stress-induced cutaneous microvascular dysfunction in college-aged, darkly-pigmented African Americans (AA). That study established that (1) serum 25(OH)D concentrations were lower in AA compared to their lightly-pigmented European American (EA) counterparts, (2) the magnitude of the response to local skin heating, as well as the NO-mediated component of that response, were lower in AA compared to EA, (3) local delivery of tempol (a superoxide dismutase mimetic), but not apocynin (an NADPH oxidase inhibitor), improved cutaneous microvascular responses to local heating in AA before vitamin D supplementation, and (4) vitamin D supplementation improved serum 25(OH)D concentrations and mitigated the differences in microvascular responses to local heating between groups. The findings of this study suggest that ensuring adequate vitamin D status in otherwise healthy, young AA may provide an effective strategy to mitigate the development of oxidative stress-induced cutaneous microvascular dysfunction.

Together, these three studies provide mechanistic insight into both beneficial and deleterious effects of UVR exposure on cutaneous microvascular function, suggesting (1) a balance between healthy and unhealthy amounts of UVR exposure that may differ based on varying degrees of skin pigmentation, and (2) a beneficial role for vitamin D supplementation in young African Americans.

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LIST OF ABBREVIATIONS

1,25-dihydroxyvitamin D_3	$1,25(OH)_2D_3$
25-hydroxyvitamin D	25(OH)D
5-methyltetrahydrofolate	5-MTHF
7-dehydrocholesterol	7-DHC
African American	AA
Analysis of Variance	ANOVA
Ascorbate	ASC
Body Mass Index	BMI
Blood Pressure	BP
Cardiovascular Disease	CVD
Cutaneous Vascular Conductance	CVC
Dihydrobiopterin	BH2
Endothelial Nitric Oxide Synthase	eNOS
European American	EA
Flow-Mediated Dilation	FMD
Hemoglobin A1C	HbA1c
High Density Lipoprotein	HDL
Immediate Pigment Darkening	IPD
Inducible Nitric Oxide Synthase	iNOS
Laser-Doppler Flowmetry	LDF
L^G-Nitro-L-arginine Methyl Ester	L-NAME
Low Density Lipoprotein	LDL
Mean Arterial Pressure	MAP

Melanin Index	M-Index
Methyltetrahydrofolate Reductase	MTHFR
Minimal Erythemal Dose	MED
Muscle Sympathetic Nerve Activity	MSNA
N^G-monomethyl-L-arginine	L-NMMA
Nicotinamide Adenine Dinucleotide Phosphate Oxidase	NADPH Oxidase
Nitric Oxide	NO
Nitric Oxide Synthase	NOS
Peroxynitrite	$ONOO^-$
Reactive Oxygen Species	ROS
Red Cell Folate	RCF
Serum Folate	SF
Socioeconomic Status	SES
Sodium Nitroprusside	SNP
Standard Erythemal Dose	SED
Sun Protection Factor	SPF
Superoxide Dismutase	SOD
Tetrahydrobiopterin	BH4
Ultraviolet Radiation	UVR
Vitamin D Receptor	VDR

ACKNOWLEDGEMENTS

I have been extremely fortunate to be surrounded by many great people who have supported me, both professionally and personally, throughout the course of my doctoral training. The successes I have experienced would not have been possible without every one of them. I would like to express my sincerest gratitude to all of those who have offered their support throughout the doctoral process.

To Dr. Larry Kenney, thank you first and foremost for your mentorship throughout my doctoral studies. Thank you for providing invaluable guidance and a stimulating lab environment that has allowed me to develop the necessary tools to transition into an independent researcher. You have pushed me to develop my critical thinking skills and ensured that I had every opportunity to develop my own research ideas, while guiding me to refine those ideas and develop them into successful, impactful studies. I am beyond grateful for the opportunity to learn from you as a scientist, and for the example that you have set as a mentor.

To Dr. Lacy Alexander, thank you for your guidance and insight throughout my doctoral studies. I am thankful for your willingness to always answer any questions I may have about physiology or study design, and to offer your support in my professional development. I also appreciate your sense of humor, and the positivity and light-heartedness you bring to the lab. Your enthusiasm and success as a scientist is inspiring, and I am thankful to have had the opportunity to learn from you.

To Dr. Nina Jablonski, you have been such an important source of guidance and knowledge. Thank you for always pushing me to develop a more sophisticated understanding of

the topics comprising this dissertation. You have consistently provided valuable insight and encouragement, and the knowledge that I have gained from working with you will serve me well throughout my career.

To Dr. Sara Ferguson and Dr. Marty Sliwinski, thank you for offering unique perspectives and important feedback throughout the dissertation process. Your comments and suggestions have improved the quality of my dissertation and will help guide my research in the future. Thank you for your time and efforts on my behalf during the dissertation process.

To Dr. Anna Stanhewicz and Dr. Jody Greaney, I am very fortunate to have had the opportunity to work with you both during your time here as post-docs. You have both had a profound impact on how I think about science. Thank you for offering your expertise in running experiments, knowledge of physiology and the scientific process, and support in helping me develop as a researcher.

To my fellow graduate students, past and present; Billie Alba, Gabie Dillon, Craig Berry, Megan Clarke, and Sean Shank. Thank you for your assistance and hard work in the lab, and of equal importance, your friendship in and outside of the lab. It has been a pleasure to work with you, and you have helped to make the doctoral process fun. I count myself lucky to have worked with such a great group of students.

To Sue Slimak, thank you for all you do within the lab, from participant sample collections to ordering supplies. Your tireless efforts in helping all of our lab's experiments run smoothly have been invaluable. Thank you for all of your support during my doctoral studies.

To Jane Pierzga, thank you for your guidance in learning to navigate the regulatory processes involved with our research studies, including the IRB process and gaining FDA approval or exemptions for experimental protocols. I am thankful for your time and efforts to support my training in these aspects of the research process.

Finally, thank you to my family who have always provided unwavering support. To my parents - although my path toward gaining an education was quite rocky at times, your confidence that I would find my way never faltered. To my fiancée, Leann - you have been an incredible source of support, and you always help to make sure that life stays fun, no matter how busy or stressful it gets. My success never would have been possible without all of your love and support.

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Chapter 1

INTRODUCTION

Background and Significance

The impact of sun exposure on skin aging and the development of skin cancer are well recognized. The incidence of skin cancer has risen in the United States, with nearly 5 million individuals being treated annually and 9,000 deaths per year being attributable to melanoma (1). Although the impact of ultraviolet radiation (UVR) exposure from the sun, or indoor tanning devices, on the development of skin cancer is well understood, less is known about other, non-carcinogenic effects of UVR on human health. One such knowledge gap is regarding the potential role of UVR exposure on (cardio)vascular health. Ultraviolet radiation may confer cardiovascular benefits associated with cutaneous biosynthesis of vitamin D (2) and release of nitric oxide (NO) (3) upon exposure to UV-B (290-320 nm) and UV-A (320-410 nm) radiation, respectively. Conversely, over-exposure to UVR may result in vascular dysfunction due to photodegradation of the bioactive metabolite of folate, 5-methyltetrahydrofolate (5-MTHF) (4), or by increasing production of oxidative stress (4) and/or inflammation (5). Given the prevalence of cardiovascular disease (CVD) and associated mortality (6), it is important to better understand the cardiovascular impact of modifiable lifestyle factors, including UVR exposure. Furthermore, it is essential to further our understanding of the role of individual characteristics, such as skin pigmentation, in modulating physiological responses to UVR exposure in order to develop better-informed sun safety guidelines.

Folate

Bioavailability of 5-MTHF, the primary circulating and bioactive metabolite of folate, is associated with improved vascular endothelial function through its role in nitric oxide synthase (NOS) coupling and nitric oxide (NO) production (7, 8). *In vitro* studies have demonstrated that 5-MTHF is directly degraded by UV-B(4), although it remains unclear whether this occurs in vivo due to the limited ability of UV-B to penetrate the dermal layer of the skin. Alternatively, 5-MTHF may be degraded *in vivo* by UV-A and/or UV-B due to increased production of reactive oxygen species upon UVR exposure (4, 9). Recent research has demonstrated a negative correlation between accumulated sun exposure and serum folate concentrations (10, 11), suggesting that UVR exposure likely reduces folate/5-MTHF bioavailability in a dose-dependent fashion. More research is needed, however, to better understand the direct effects of UVR exposure on 5-MTHF bioavailability and vascular function.

Vitamin D

The role of UVR exposure on vitamin D production and bioavailability is well-established. Upon UV-B exposure, epidermal and dermal stores of 7-dehydrocholesterol (7-DHC) are converted to previtamin D_3, which then undergoes a three-step conversion process to ultimately produce the primary bioactive metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. The role of vitamin D in cardiovascular health remains unclear. Although associations between vitamin D status and cardiovascular disease have been reported (12, 13), the effects of vitamin D supplementation on clinical cardiovascular outcomes have been equivocal (14, 15). Recent research has suggested that vitamin D may play an important role in vascular health by increasing endothelial nitric oxide expression (eNOS) and NO bioavailability (16), reducing oxidative stress

(17, 18), or suppressing vascular inflammation (19). Together, these findings suggest an important role for vitamin D in promoting vascular health, which may help to mitigate the development of cardiovascular disease.

Skin Pigmentation

The evolution of human skin pigmentation is postulated to have been an adaptation to geographical variation in UVR exposure (20). Dark, eumelanin rich skin is thought to have evolved in early humans living in equatorial African to protect against deleterious effects of UVR, including degradation of bioavailable 5-MTHF. Depigmentation is believed to have occurred in humans who migrated to areas with lower UVR intensity and greater seasonal variation in sunlight to allow for adequate vitamin D_3 synthesis. Strong correlations have been demonstrated between skin pigmentation and the absolute latitude at which a population lives (21), and between skin pigmentation and the rate of cutaneous vitamin D_3 synthesis (22, 23). Thus, skin pigmentation plays an important role in modulating the physiological responses to UVR exposure. As such, it may be that regional and/or seasonal differences in UVR exposure present disparate vascular consequences associated with UVR-induced 5-MTHF and vitamin D metabolism in lightly- and darkly-pigmented populations.

Regional Variation in Ultraviolet Radiation Exposure

The strength, distribution, variation, and bioactivity of UVR is highly variable by geographic location. The amount of UVB that reaches the earth's surface is less than that of UVA due to greater atmospheric scattering (24). This scattering of UVB, in conjunction with variation of the solar angle at different latitudes, results in greater regional variability of UVB compared to UVA

radiation exposure. UVB exposure is most intense and least variable in equatorial regions, with the greatest amount of variability occurring in northern Eurasia and North America (24). Indeed, UVB exposure is insufficient to elicit adequate cutaneous vitamin D_3 production for large parts of the year in much of the northern hemisphere (25, 26). Thus, regional and seasonal differences in UVA, UVB, and total UVR exposure may have important physiological and biomedical implications that likely differ among populations with varying degrees of skin pigmentation.

Cutaneous Microvascular Function

The skin microcirculation provides an easily-accessible, minimally-invasive circulatory bed to interrogate the mechanisms of vascular dysfunction in various populations, including older adults and those with conditions such as hypertension. Importantly, dysfunction of the cutaneous microvasculature may precede the development of vascular dysfunction in other circulatory beds throughout the human body (27). The endothelium-derived vasodilator, NO, is an important component of vascular health, and reduced NO bioavailability is an early indicator of the development of cardiovascular pathogenesis (28). Utilizing physiological or pharmacological stimuli to elicit vasodilation of the cutaneous microvasculature, in conjunction with the technique of intradermal microdialysis, allows for the elucidation of NO production and availability (29, 30). Thus, the cutaneous circulation can be effectively used to examine the efficacy of local or systemic treatments on vascular endothelial function. As such, investigating function and dysfunction of the cutaneous microvasculature may offer important, mechanistic insight into the future development of systemic vascular dysfunction, particularly in populations with greater propensity for cardiovascular disease.

Local Heating

Increases in skin temperature elicit a cutaneous vasodilation response that is mediated primarily by the release of NO (29, 31). Local increases in skin temperature can be applied to the skin using local heating units to induce cutaneous vasodilation, and the dilatory response can be measured with integrated laser-Doppler flowmetry probes placed within the heaters. The local heating response is characterized by an initial, axon reflex-mediated peak (31), followed by a brief nadir and a more gradual rise until a sustained local heating plateau is attained. When the skin is locally heated to 42°C in healthy young adults, approximately 60% of the local heating plateau response is mediated by NO, produced from enzymatic conversion of L-arginine by eNOS (29, 32). A 39°C local heating protocol has been proposed by Choi et al. (33) to more specifically isolate NO-dependent cutaneous vasodilation, suggesting that over 80% of that response is mediated by NO. Regardless of the approach taken, local skin heating provides a well-accepted tool for interrogating NO-mediated microvascular function.

Because impairments in cutaneous microvascular reactivity often precede the development of clinical signs and symptoms of disease (28, 34), local skin heating serves as a valuable tool for assessing endothelial function and dysfunction. Blunted NO-mediated local heating responses have been demonstrated in conditions such as primary aging (35), hypertension (36), hypercholesterolemia (37), and chronic kidney disease (38). Similarly, the magnitude of the local heating response, and the NO contribution to that response, are attenuated in otherwise healthy, young African American adults (39, 40) – a population that experiences a disproportionate risk of developing overt CVD. The cutaneous microvascular responses to local heating can be further utilized to investigate the efficacy of targeted interventions to improve NO bioavailability and

vascular health by improving substrate (41) or cofactor (42) availability, or by reducing oxidative stress (40, 43).

Summary

The three studies included within this dissertation were designed to investigate the influence of UVR exposure on cutaneous microvascular function. The first study investigated the acute deleterious effects of UVR exposure on cutaneous microvascular function via UVR-induced folate degradation and/or production of oxidative stress. The second study extended the findings of the first to examine the influence of sunscreen or sweat on the skin in modulating the cutaneous microvascular effects of UVR exposure. The third study investigated the efficacy of four weeks of vitamin D supplementation for improving cutaneous microvascular function in healthy, college-aged African American adults.

Specific Aims and Hypotheses

Specific Aim 1. The purpose of the study "Acute Ultraviolet Radiation Exposure Attenuates Nitric Oxide-Mediated Vasodilation in the Cutaneous Microvasculature of Healthy Humans" was to determine the impact of acute exposure to UVR (specifically in the UVB region) on NO-mediated vasodilation in the human cutaneous microvasculature, and the role of direct 5-MTHF photodegradation or oxidative stress in that response. Additionally, this study sought to examine whether a darkened skin pigmentation would be protective against the negative effects of UVB on NO-mediated vasodilation.

Hypothesis 1a: Acute UVB exposure will attenuate NO-mediated vasodilation of the skin microvasculature.

Hypothesis 1b: Local delivery of either 5-MTHF or ascorbate will augment NO-mediated vasodilation after UVB exposure.

Hypothesis 1c: The effect of UVB exposure on NO-mediated vasodilation of the cutaneous microvasculature would be smaller in darkly- vs. lightly-pigmented individuals.

Specific Aim 2. The study entitled "Sunscreen or Simulated Sweat Minimizes the Impact of Acute Ultraviolet Radiation on Cutaneous Microvascular Function in Healthy Humans" was comprised of two separate studies to better understand the impacts of UVR on cutaneous microvascular function and health. The aim of *Study 1* was to investigate the separate time courses of skin erythema (reddening) and blood flow responses for 8 hours after UVR exposure, and the efficacy of sunscreen in mitigating these responses.

Hypothesis 2a: Erythema will increase over the 8-hour post-exposure time period, with a corresponding increase in cutaneous blood flow that will track the erythema response.

Hypothesis 2b: The presence of sunscreen during UVR exposure will blunt skin erythema and blood flow responses post-exposure.

The aim of *Study 2* was to examine the impact of acute broad-spectrum UVR exposure on NO-mediated vasodilation of the human cutaneous microvasculature, and the influence of a simulated sweat solution or broad-spectrum sunscreen in modulating that response.

Hypothesis 2c: NO-dependent vasodilation of the cutaneous microvasculature will be reduced after acute broad-spectrum UVR exposure.

Hypothesis 2d: The presence of simulated sweat on the skin during acute exposure to UVR exposure will exacerbate the reduction in NO-mediated cutaneous vasodilation.

Hypothesis 2e: The presence of broad-spectrum sunscreen on the skin during acute exposure to UVR will attenuate the reduction in NO-mediated cutaneous vasodilation.

Specific Aim 3. The purpose of the study "Four Weeks of Vitamin D Supplementation Improves Nitric Oxide-Mediated Microvascular Function in College-Aged African Americans" was to examine the influence of vitamin D, via modulation of oxidative stress, on cutaneous microvascular responses to local heating in healthy young AA and EA adults.

Hypothesis 3a: The magnitude of the cutaneous microvascular dilatory response to local heating, as well as the NO-mediated component of that response, will be attenuated in AA compared to EA.

Hypothesis 3b: Differences in cutaneous microvascular responses to local heating will be abrogated by direct perfusion of tempol (superoxide dismutase mimetic) or apocynin (NADPH oxidase inhibitor).

Hypothesis 3c: Four weeks of 2,000 IU per day vitamin D supplementation will augment local heating responses in AA, but not EA, such that there will no longer be differences between groups.

Chapter 2

REVIEW OF LITERATURE

THE VITAMIN D-FOLATE HYPOTHESIS IN HUMAN VASCULAR HEALTH

INTRODUCTION

The vitamin D-folate hypothesis is a prevailing theory that explains the evolution of human skin coloration, postulating that changes in skin pigmentation occurred as an evolutionary adaptation to geographical variations in UVR exposure (20, 21). Early humans inhabiting equatorial Africa are purported to have adopted a darkened skin pigment as protection from photodegradation of the bioactive metabolite of folate, 5-methyltetrahydrofolate (5-MTHF). 5-MTHF is important for promoting healthy pregnancy and child birth (44) and its evolutionary preservation would therefore enhance reproductive viability. As humans migrated away from the equator, however, depigmentation likely occurred to allow for adequate biosynthesis of previtamin D_3 upon UVR exposure, which is likewise of evolutionary significance due to the importance of vitamin D in pregnant and lactating women and development of the skeletal system during early childhood (45). However, differences in human skin pigmentation may result in susceptibility to photodegradation of 5-MTHF for those with light skin pigments in UVR-rich environments, or inadequate vitamin D_3 synthesis for those with darker pigments in environments of low UVR exposure or high seasonal variation.

In addition to the importance of 5-MTHF and vitamin D in healthy pregnancy and child development, recent evidence has emerged that both 5-MTHF (46) and vitamin D (47) play important roles in vascular health. Vascular endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is a key pathological antecedent to the development of cardiovascular disease. Both 5-MTHF and vitamin D directly and indirectly regulate nitric NO

bioavailability, and as such, both are important in maintaining healthy endothelial function (48) and protecting against the development of cardiovascular disease (49, 50). UVR exposure may have either deleterious or beneficial effects on vascular function, and those disparate effects may be modulated through either 5-MTHF or vitamin D metabolism. These effects of UVR likely differ based on factors such as UVR wavelength, dose, and individual variation in human characteristics, including skin pigmentation, genetics, and age. The purpose of this review is to examine the potential influences of UVR on vascular health via its differential effects on 5-MTHF and vitamin D metabolism, and the role of skin pigmentation and other biological variants in modulating these effects.

DIFFERENTIAL VASCULAR EFFECTS ACROSS THE ULTRAVIOLET SPECTRUM

The UVR spectrum from sunlight is categorized into three primary regions; UV-A (320-400 nm), UV-B (290-320 nm), and UV-C (200-290 nm) and the biological effects of UVR differ by wavelength (51). UV-C is not considered biologically relevant because it is filtered by the ozone layer and does not reach the surface of the earth (52). UV-A constitutes approximately 95% of UVR that reaches the earth's surface, with the remaining 5% comprising UV-B (53). In the skin, UV-A is able to penetrate the dermis and reach the cutaneous circulation whereas UV-B is mostly absorbed in the epidermis and upper dermis due to its shorter wavelengths. As such, UV-A and UV-B elicit their respective biological effects via distinct direct and indirect mechanisms.

Although UV-B constitutes only a small percentage of the total UVR that reaches the skin and its ability to *directly* affect the cutaneous circulation is limited, it is highly energetic and biologically impactful. Indeed, the minimal erythema dose (the minimal dose required to elicit a reddening response in the skin) is 1,000-fold less for UV-B than UV-A radiation (54). Exposure of human skin keratinocytes to UV-B results in production of reactive oxygen species (ROS) (52, 55, 56),

which may be responsible for multiple deleterious effects of UVR including carcinogenesis and skin aging (57). Conversely, UV-B also interacts with 7-dehydrocholesterol (7-DHC) stores in the skin to produce vitamin D (58), which may have vascular health benefits (discussed in more detail in a subsequent section of this review). UV-A is less energetic than UV-B but penetrates deeper into the skin (52). ROS may be produced by UV-A exposure either in the skin or the dermal circulation via interaction with skin chromophores or circulating photosensitizers such as uroporphyrin or riboflavin (9, 59). UV-A may also elicit some beneficial vascular effects via photolysis of cutaneous nitrites to NO (60, 61) (discussed further in a later section of this review). Thus, UV-A and UV-B exposure may differentially influence vascular function and health through distinct, but interrelated mechanisms (**Figure 2-1**).

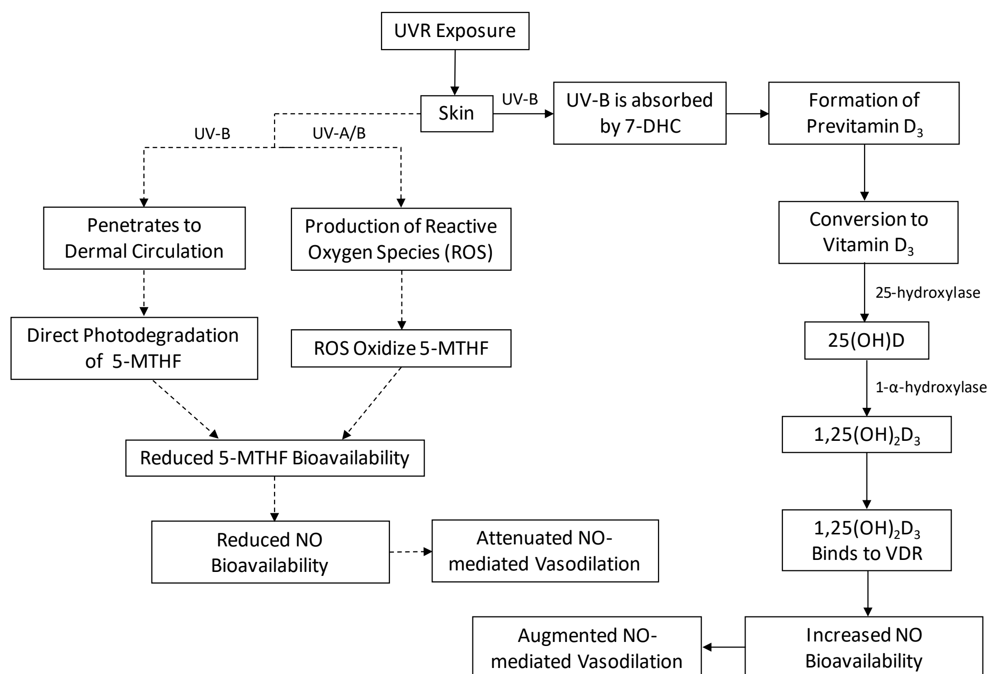

Figure 2-1: Putative mechanistic pathways by which ultraviolet radiation (UVR) exposure may influence nitric oxide (NO) bioavailability and vascular function. Deleterious and beneficial pathways are represented by dotted and solid lines, respectively. Ultraviolet-A (UV-A) and/or B (UV-B) exposure to the skin may reduce 5-methyltetrahydrofolate (5-MTHF) bioavailability, thereby reducing NO production and blunting NO-mediated vasodilation. UV-B is absorbed by 7-dehydrocholesterol (7-DHC) stores in the skin, resulting in formation of previtamin D3, which is rapidly converted to vitamin D3. Vitamin D3 undergoes two hydroxylation steps, first producing 25-hydroxyvitamin D [25(OH)D] in the liver and then producing 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3]. 1,25-(OH)2D3 binds to vitamin D receptors (VDRs), signaling for transcription of endothelial NO synthase (eNOS) and superoxide dismutase (SOD), thereby increasing NO bioavailability and augmenting NO-mediated vasodilation.

ULTRAVIOLET RADIATION, VITAMIN D METABOLISM, AND VASCULAR FUNCTION

The association between vitamin D metabolism and sunlight, or UVR exposure, is well known. Approximately 90% of the human vitamin D requirement (serum 25(OH)D concentration 30 – 60 ng/ml) is met via skin exposure to sunlight (58, 62, 63). Mechanistically, UV-B is absorbed by epidermal and dermal stores of 7-dehydrocholesterol (7-DHC), resulting in formation of previtamin D_3, which is then rapidly converted to vitamin D_3. Subsequently, vitamin D_3

undergoes two hydroxylation steps. The first hydroxylation step forms 25-hydroxyvitamin D [25(OH)D] and is catalyzed within the liver by 25-hydroxylase. The second step forms the predominant biologically active vitamin D metabolite 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], and is catalyzed in the kidney by 1-α-hydroxylase. An independent, local vitamin D metabolism pathway for synthesis of 1,25$(OH)_2D_3$ is found in human skin (64, 65), where it may serve paracrine or autocrine functions. The vitamin D receptor (VDR), found in most human cells, is a transcription factor that controls gene expression for a multitude of tissue-specific responses upon binding with 1,25$(OH)_2D_3$. Although it is most recognized for its role in bone and mineral homeostasis, the VDR serves many physiological functions which may include beneficial effects on vascular function and health (66).

Determinants of Vitamin D Status

An individual's vitamin D status is influenced by a number of exogenous and endogenous factors.

UV exposure. UV-B exposure varies geographically and seasonally, such that exposure is diminished with increasing distance from the earth's equator and during the winter months, particularly in areas of greater seasonal variation (62). Significant variation was observed in vitamin D status between summer and winter months in Boston, with 30% of study participants being vitamin D insufficient at the end of winter compared to only 11% at the end of summer (67). Sufficient production of vitamin D is additionally impacted by lifestyle, including time spent outdoors. Wearing a sunscreen with sun protection factor of 30 or higher reduces skin vitamin D synthesis by more than 95%, and therefore may prevent adequate vitamin D status in at-risk populations (68).

Age. Aging may substantially reduce the skin's ability to produce vitamin D_3 due to a linear age-related decline in epidermal 7-DHC bioavailability after the age of 20 years (69). Adequate vitamin D status can likely be maintained in older adults with regular sun exposure to large areas of the skin (70); otherwise the aged population is at greater risk of insufficient previtamin D_3 production. Further, reduced glomerular filtration rates in the aged kidney may result in attenuated renal hydroxylation of 25(OH)D to $1,25(OH)_2D_3$ (71). Therefore, vitamin D supplementation may be warranted in adults over the age of 60 years to maintain sufficient circulating 25(OH)D concentrations and $1,25(OH)_2D_3$ activity (72).

Skin pigmentation. Melanin within the skin absorbs UV-B radiation, preventing its absorption by cutaneous 7-DHC, and thereby reducing vitamin D_3 biosynthesis (73). Those with greater skin melanization, characterized by a darkened pigmentation, are at a particularly high risk for vitamin D deficiency, especially those living in areas of low UVR exposure or high seasonal variation. In a large cohort of 2,097 darkly pigmented African American and 1,860 lightly pigmented Euro-American women of reproductive age, 41% of African American women were vitamin D deficient at the end of summer, compared to only 4% of Euro-American women (74).

Genetics. Although skin pigmentation is predictive for circulating vitamin D concentration, genes related to vitamin D metabolism also play an important role in UV-B-induced vitamin D production (75, 76). Single nucleotide polymorphisms of four pigment-related genes (*ASIP*, *SLC24A4*, *SLC45A2*, and *MIR196A29*) explain more of the variation in UV-B-induced vitamin D production than skin pigment, per se (76), suggesting that genes related to skin pigmentation have a role in other physiological processes, including vitamin D metabolism, although the mechanisms are currently unclear. Two genome-wide association studies identified single nucleotide polymorphisms associated with 25(OH)D concentrations at three loci; *GC* (vitamin D

binding protein), *DHCR7 (*7-DHC *reductase), and CYP2R1 (25-hydroxylase)* (75, 77). Polymorphisms in *GC* and *CYP2R1* are characterized by alterations in vitamin D binding protein phenotypes (78) and activity of the hepatic enzyme 25-hydroxylase (79), respectively, and therefore may result in variations in vitamin D homeostasis. 7-DHC reductase catalyzes the conversion of 7-DHC to cholesterol, thereby reducing availability of the vitamin D_3 precursor. Thus, variations in *DHCR7* may confer benefits for vitamin D production by conserving cutaneous 7-DHC concentrations. High prevalence of *DHCR7* variants related to reduced 7-DHC reductase activity were demonstrated in European and Northeast Asian populations, but not in African populations, suggesting that selection occurred for these *DHCR7* mutations in populations who migrated away from the equator to more northern latitudes (80).

Vitamin D and Vascular Health

Associations between vitamin D status and hypertension (81), cardiovascular disease (13), and vascular function (82) have been well documented. Adrukhova and colleagues (16) demonstrated that $1,25(OH)_2D_3$ is a direct transcriptional regulator of endothelial nitric oxide synthase (eNOS), and that mice carrying a functionally inactive, mutant VDR were characterized by increased arterial stiffness and reduced eNOS expression and NO bioavailability. Additionally, serum concentrations of 25(OH)D, the primary circulating vitamin D metabolite, were directly related to brachial artery flow mediated dilation (FMD) in otherwise healthy middle-aged and older adults (19). That study further demonstrated that serum concentrations of 25(OH)D were inversely related to vascular endothelial cell expression of interleukin-6 (IL-6), and that suppression of nuclear factor kappa B (NFκB) improved FMD to a greater extent in those with lower compared to higher 25(OH)D status. Lastly, 25(OH)D deficient subjects exhibited lower endothelial cell expression of VDR and 1-α-hydroxylase, the enzyme responsible for conversion of 25(OH)D to $1,25(OH)_2D_3$. Taken together, these studies suggest important roles for vitamin D in vascular

health acting mechanistically to preserve production of NO and suppress inflammation-induced vascular dysfunction.

Data from the National Health and Nutrition Examination Survey (NHANES) demonstrated a high prevalence of vitamin D insufficiency in individuals with a variety of cardiovascular diseases (CVDs) including coronary heart disease, heart failure, stroke, and peripheral arterial disease (83). Importantly, a higher prevalence of hypovitaminosis D was observed in African American (97%) compared to Euro-American (77%) subjects in that survey. These epidemiological data suggest a potential causal link between vitamin D insufficiency and impaired cardiovascular health, which may be reflected in the disproportionate CVD burden in the African American population (84).

Hypertension, a leading risk factor for CVD, develops at a younger age and contributes disproportionately to increased mortality in African Americans (84). Attenuated conduit artery (85) and microvascular (40, 86) function, both of which are implicated in the development of hypertension (87, 88), are observed in otherwise healthy African American, compared to Euro-American, subjects. Brothers and colleagues (40) demonstrated impairments in NO-mediated vasodilation of the cutaneous microvasculature in response to local heating in healthy, college-aged African Americans compared to their Euro-American counterparts. Further, when tempol (a superoxide dismutase mimetic) was locally delivered via intradermal microdialysis, NO-mediated vasodilation was normalized in the African American subjects such that there were no longer differences between groups (40). Thus, microvascular dysfunction observed in African Americans is likely attributed, at least in part, to elevated superoxide generation and/or attenuated superoxide dismutase (SOD) activity in that population.

Superoxide dismutase has been proposed to be a transcriptional target of the vitamin D receptor (**Figure 2-2**), such that SOD expression is increased with vitamin D supplementation or direct cellular treatment with 1,25$(OH)_2D_3$ (17, 89). Additionally, vitamin D may suppress nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase)-induced production of superoxide radicals (90, 91). Together, these data provide potential mechanisms through which vitamin D bioavailability may modulate oxidative stress-induced vascular dysfunction, although this has yet to be explored. Moreover, vitamin D signals for the transcription of eNOS (16) and thus may improve NO bioavailability independent of reductions in oxidative stress. Because UV-B exposure in regions of relatively low sun exposure or high seasonal variation may not offer adequate UVB-induced vitamin D biosynthesis in darkly pigmented populations, either UVB therapy or vitamin D supplementation may be efficacious for upregulating eNOS and SOD expression and/or inhibiting superoxide production by NADPH oxidase, thereby improving vascular function.

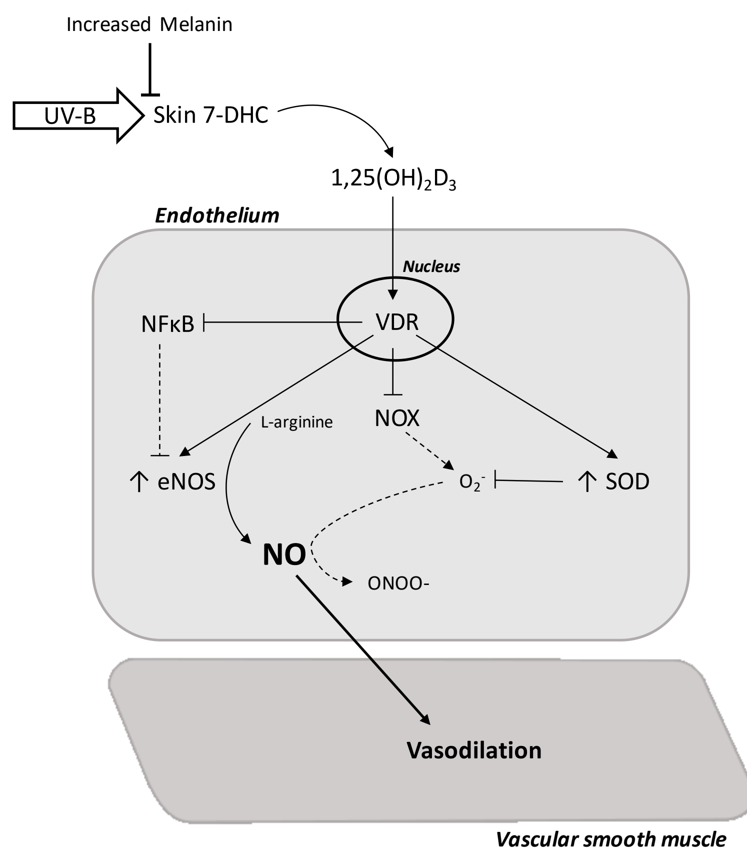

Figure 2-2: Ultraviolet-B radiation interacts with skin 7-dehydrocholesterol (7-DHC), ultimately resulting in production of calcitriol [$1,25(OH)_2D_3$]. Increased circulating $1,25(OH)_2D_3$ binds to nuclear vitamin D receptors (VDR) within the endothelium. Activation of the VDR increases endothelial nitric oxide synthase (eNOS) and superoxide dismutase (SOD) transcription, and suppresses NADPH oxidase (NOX) and nuclear factor kappa B (NFκB) activity. Upregulation of eNOS increases production of nitric oxide (NO) from L-arginine. Elevated SOD activity scavenges superoxide (O_2^-), which may otherwise interact with NO to produce peroxinitrite ($ONOO^-$). Suppression of NOX and NFκB attenuates O_2^- production and inhibition of eNOS activity, respectively. Increased skin melanin absorbs UV-B in the skin, thereby reducing conversion of 7-DHC to bioavailable vitamin D.

Clinical recommendations for the evaluation, treatment, and prevention of vitamin D deficiency suggest screening in individuals at risk of deficiency (68, 92), including older adults (age 60+), those with obesity and/or malabsorption syndromes, or those with darker skin pigmentation. Daily vitamin D supplementation of 800 IU/day is recommended for darkly-pigmented and older

populations, although larger doses may be necessary for deficient individuals to attain sufficient serum 25(OH)D concentrations of at least 30 ng/ml for the maintenance of musculoskeletal and cardiovascular health (92). **Table 2-1** presents a synopsis of studies examining the impact of vitamin D supplementation on vascular outcomes. Those studies have been mostly limited to overweight adults or patient populations such as those with type 2 diabetes mellitus or chronic kidney disease (93-95). In an asymptomatic vitamin D-deficient cohort, however, endothelial function (assessed via FMD) was blunted compared to a vitamin D-sufficient control group (96). In the deficient subjects, 3 months of vitamin D treatment improved FMD such that it no longer differed from the control group. However, that study made no mention of skin pigmentation or genetic characteristics of its participants. To our knowledge there has yet to be any investigation into the potential influence of either vitamin D supplementation or UVB exposure treatment on endothelial function in an otherwise healthy African American population. Therefore, future investigation is needed to elucidate the role of vitamin D in vascular endothelial function in this population and others with darkened skin pigmentation, but varying in genetics and sociocultural environments.

Table 2-1: Studies examining the effects of vitamin D supplementation on endothelial function.

Reference	Participants	No. of Cases	Vitamin D Treatment	Outcome Measure	Results
Sugden et al. (2008)	Type 2 diabetes mellitus patients	17 vitamin D treated 17 placebo	100,000 IU vitamin D_2 or placebo	Brachial artery FMD	↑ [25-OH-D] by 15.3 nmol/L Improved FMD by 2.3% ↓ SBP 7.5 mmHg
Tarcin et al. (2009)	Healthy young vitamin D-deficient subjects	23 vitamin D treated 23 normal controls	300,000 IU vitamin D_3 monthly for 3 months	Brachial artery FMD	↑ in [25-OH-D] of 96.5 nmol/L Improved FMD by 3.4%
Harris et al. (2011)	Overweight African American adults	22 vitamin D treated 23 placebo	60,000 IU monthly oral vitamin D_3 or placebo for 16 weeks	Brachial artery FMD	↑ [25-OH-D] by 66.6 nmol/L Improved FMD by 1.8% ↑ absolute diameter by 0.005 cm Improved FMD/shear by 0.08 $\%\cdot s^{-1}$
Forman et al. (2013)	Black men and women in the United States	283 vitamin D treated	1,000 IU (n=68), 2,000 IU (n=73), or 4,000 IU (n=70) vitamin D_3, or placebo (n=72)	Blood pressure	Dose-dependent ↑ in [25-OH-D] of 13.4, 20.3, and 30.3 ng/mL Dose-dependent ↓ in MAP of 1.93, 2.0, and 2.46 mmHg vs. ↑ MAP of 1.49 mmHg in placebo

Witham et al. (2013)	Patients with history of myocardial infarction	39 vitamin D treated 36 placebo	300,000 IU vitamin D_3 or placebo over 4 months	Reactive hyperemia index	↑ in [25-OH-D] of 13 nmol/L after 6 months No change in reactive hyperemia index
Yiu et al. (2013)	Type 2 diabetes mellitus patients	50 vitamin D treated 50 placebo	5,000 IU daily vitamin D_3 or placebo for 12 weeks	Brachial artery FMD, brachial-ankle PWV	↑ in [25-OH-D] of 37.5 ng/mL No changes in FMD or PWV
Kumar et al. (2017)	Stage 3-4 CKD patients with vitamin D deficiency	58 vitamin D treated 59 placebo	300,000 IU vitamin D_3 or placebo at baseline and after 8 weeks	Brachial artery FMD and NMD	↑ in [25-OH-D] of 24.9 ng/mL Improved FMD by 5.4% Improved NMD by 2.1%
Zhang et al. (2018)	Non-dialysis CKD patients	71 vitamin D treated	50,000 IU vitamin D_3 weekly for 12 weeks	Brachial artery FMD	↑ in [25-OH-D] of 20.5 ng/mL Improved FMD by 0.7%

FMD, flow mediated dilation; NMD, nitroglycerin mediated dilation; PWV, pulse wave velocity; SBP, systolic blood pressure; MAP, mean arterial pressure

ULTRAVIOLET RADIATION, FOLATE METABOLISM, AND VASCULAR FUNCTION

Humans lack the ability to endogenously produce folate (vitamin B9), and rely on dietary sources or supplementation with folic acid (the synthetic form of folate) in order to meet biological requirements. The role of folate/folic acid in vascular function and health (**Figure 2-3**) has been previously reviewed in depth (97), and is therefore beyond the scope of the current review.

Briefly, ingested folate or folic acid is converted into the bioactive folate metabolite, 5-MTHF. 5-MTHF may improve NO bioavailability, and thus vascular function, by promoting stabilization of the eNOS dimer via increased production of the eNOS cofactor tetrahydrobiopterin (BH_4) from its inactive form dihydrobiopterin (BH_2). Additionally, 5-MTHF may act as an antioxidant, directly scavenging ROS which may otherwise reduce NO bioavailability by destabilizing eNOS or directly interacting with NO to produce peroxynitrite ($ONOO^-$). Thus, high-dose folic acid supplementation may improve endothelial function in populations with overt cardiovascular and metabolic disease and endothelial dysfunction, or even protect endothelial function in healthy adults (97).

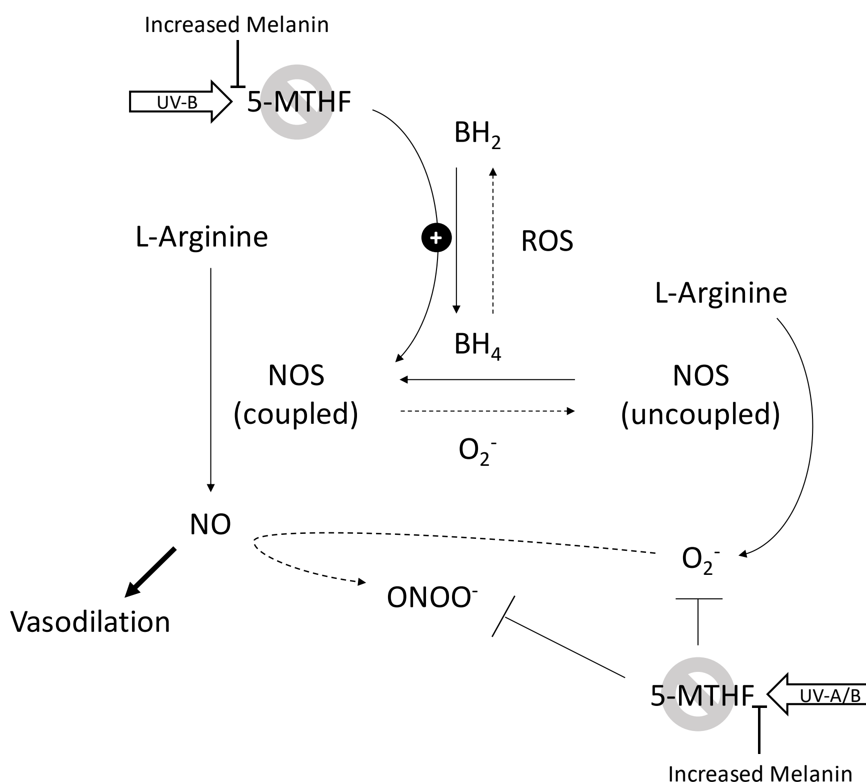

Figure 2-3: 5-methyltetrahydrofolate (5-MTHF) may augment vascular function through increased nitric oxide (NO) production and bioavailability. Ultraviolet-B (UV-B) may reduce 5-MTHF bioavailability via direct photodegradation. Ultraviolet-A (UV-A) and/or UV-B may indirectly reduce 5-MTHF bioavailability by increasing production of reactive oxygen species (ROS) which, in turn, scavenge available 5-MTHF. Increased melanin in the skin may reduce UV-A and/or UV-B-induced photodegradation of 5-MTHF. BH_2 = dihydrobiopterin; BH_4 = tetrahydrobiopterin; NOS = nitric oxide synthase; ROS = reactive oxygen species; O_2^- = superoxide; $ONOO^-$ = peroxinitrite. (Adapted from Stanhewicz & Kenney, 2017).

Exposure of the skin to UVR may deplete bioavailable 5-MTHF. 5-MTHF is directly degraded by UV-B, but not UV-A, *in vitro* (4, 59), although it is unclear whether UV-B directly degrades 5-MTHF *in vivo* due to its limited ability to penetrate the dermis. Alternatively, UV-A and/or UV-B radiation may indirectly degrade 5-MTHF via UVR-induced increases in oxidative stress (4, 9). However, studies examining the impact of UVR exposure on bioavailable folate *in vivo* are equivocal. **Table 2-2** provides a summary of *in vivo* studies examining the impact of UVR

exposure on folate status. Based on the available evidence, it appears that folate degradation in response to UVR exposure is likely dose dependent, requiring relatively large doses and/or prolonged, repeated exposures to observe reductions in serum and/or red blood cell folate.

Table 2-2. Studies examining the effects of UVR exposure on folate bioavailability.

Reference	Participants	No. of Cases	UVR Treatment	Outcome Measure	Results
Branda and Eaton (1978)	Light skinned dermatologic patients and healthy controls	10 UVR exposed patients 64 non-exposed healthy controls	4.5 – 9.5 J/cm^2 UV-A, 30 – 60 min once or twice weekly, minimum of 3 months	SF concentration	Significantly lower SF concentrations in patients compared to controls
Gambichler et al. (2001)	Healthy adults; skin type II	8 UVR exposed 16 control	16 J/cm^2 UV-A, 2x weekly for 3 weeks	SF concentration	No effect on serum folate
Shaheen et al. (2006)	Vitiligo patients and healthy controls; skin types I – III	20 vitiligo patients 20 unexposed healthy controls	Cumulative 76 J/cm^2 nUV-B over 36 sessions	SF concentration	Significantly reduced serum folate in nUV-B treated patients
Rose et al. (2009)	Psoriasis patients with skin types I-III	35	Average of 2.3 J/cm^2 nUV-B per session; minimum of 18 sessions over 6 weeks	SF and RCF concentrations	No effect of nUV-B exposure on either SF or RCF concentrations
Borradale et al. (2014)	Healthy women	45	Personal UVR exposure, measured with UV radiometer	SF concentration	Significant, negative relation between sun exposure and SF
Lucock et al. (2016)	Adults 65-95 years of age	639	Accumulated exposure over 42 and 120 days	RCF concentration	Significant, negative relation between accumulated exposure and RCF
Valencia-Vera et al. (2019)	Primary and secondary health care patients	Study 1: 118,831 samples Study 2: 1,597 patients with repeated measures	Daily environmental UVR exposure	SF concentration	Decreased folate status in summer compared to winter; greater prevalence of deficiency in summer

SF, serum folate; RCF, red cell folate; nUV-B, narrowband UV-B

Potential Determinants of UVR-Induced 5-MTHF Metabolism

Skin pigmentation. A darkened constitutive skin pigmentation appears to play a protective role against photodegradation of 5-MTHF by UVR (20, 98), attributable to absorption of UVR by melanocytes in the skin. Because UV-A penetrates more deeply into the skin, its transmission through the skin is more dependent upon melanin concentration than is UV-B (99, 100). Thus, the ability of UV-A (compared to UV-B) to influence bioavailable 5-MTHF in the cutaneous circulation may be more limited in those with darker skin pigments. This is particularly important in light of the fact that UV-A accounts for approximately 95% of UVR that reaches the earth's surface from the sun (53). Additionally, immediate pigment darkening after an initial UVR exposure protects against photosensitization of 5-MTHF by riboflavin and uroporphyrin during subsequent exposures (59), and development of immediate pigment darkening is greater in those with darker compared to lighter constitutive pigments (101).

Genetics. In addition to the influence of skin pigmentation, variation in the 5-MTHF response to UVR exposure may also be explained, at least in part, by genetic variation (11). Of particular interest is the *MTHFR* gene, responsible for encoding the transcription of the enzyme methylenetetrahydrofolate reductase (MTHFR) which converts 5,10 methylenetetrahydrofolate to 5-MTHF. Lucock and colleagues (11) examined the association between UVR exposure and folate status in carriers of the C677T-*MTHFR* gene polymorphism. They demonstrated a significant negative relation between red cell folate concentrations and accumulated UVR exposure over 42 days for those who carry the T allele (677CT and 677TT genotypes), but not for those with the 677CC genotype. Further, the relation was stronger for carriers of the 677TT compared to the 677CT genotype, suggesting a progressive increase in UVR-induced folate depletion with increasing presence of the polymorphic T allele. Subsequently, the same group

observed associations between skin pigmentation and genes related to folate metabolism, including the *MTHFR* gene, suggesting various folate genotypes may be selected for in different UVR environments (102). It may be that expression of folate-related genes is suppressed for those with darker skin pigments and/or those carrying a genotype that preserves activity of folate metabolism who are living in relatively low UVR environments. Expression of those genes, however, may be upregulated when folate status is reduced. Indeed, DNA methylation was upregulated when plasma or red blood cell folate concentrations fell below 12 nmol/L in individuals with the 677CC, but not the 677TT, genotype (103), suggesting that variants of the *MTHFR* gene elicit differential epigenetic responses to reduced folate status.

UVR Effects on 5-MTHF and Vascular Function

Recent research from our laboratory has demonstrated that exposure of skin of the ventral forearm to 300 mJ/cm^2 UV-B (104) or 450 mJ/cm^2 broad-spectrum UVR (combined UV-A and UV-B) (105) attenuates NO-mediated vasodilation of the cutaneous microvasculature compared to non-exposed skin on the contralateral forearm in healthy, young adults. In the former of those two studies, we demonstrated that direct perfusion of either 5-MTHF or ascorbate (a non-specific antioxidant) to local areas of the UV-B-exposed skin restored post-exposure microvascular function, such that there were no differences in NO-mediated vasodilation between those two sites or compared to the non-exposed control site (**Figure 2-4**). Data from that study suggest that reductions in NO-mediated vasodilation after UV-B exposure were due to photodegradation of 5-MTHF, directly by UV-B exposure and/or indirectly via UV-B-induced increases in ROS. In the latter study, we further demonstrated that application of sunscreen to the skin prior to broad-spectrum UVR exposure protected NO-mediated vasodilation (106). Of note, although the NO contribution to the dilator response during local heating was attenuated after UVR exposure in those studies, the overall magnitude of the dilatory response was unaltered, suggesting

upregulation of compensatory dilatory pathways (e.g. endothelium-derived hyperpolarizing factors) in the face of reduced NO bioavailability. However, reductions in NO bioavailability and NO-mediated microvascular function are associated with increased long-term risk of cardiovascular disease (27, 50); thus, reductions in NO-mediated vasodilation after UVR exposure may suggest risk of future endothelial dysfunction with chronic over-exposure to UVR.

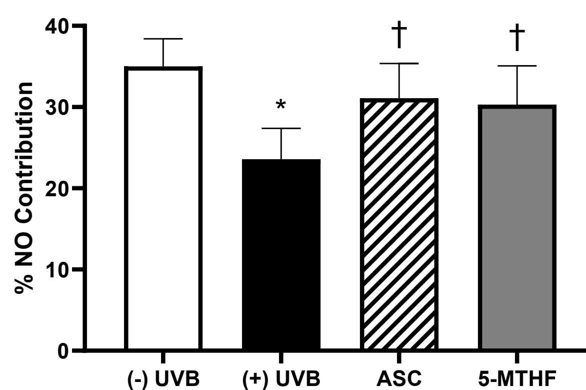

Figure 2-4: The percent nitric oxide (%NO) contribution to the vasodilatory response to local heating in non-exposed skin (white bar), UVB-exposed skin (black bar), and UVB-exposed skin with local perfusion of ascorbate (ASC; hashed bar) or 5-methyltetrahydrofolate (5-MTHF; gray bar). * $P < 0.05$ compared to non-exposed skin; † $P < 0.05$ compared to UVB exposed skin.

We also examined whether differences in skin pigmentation would influence the magnitude of the reduction in NO-mediated vasodilation after UV-B exposure (104). Twenty-two subjects were recruited with a wide range of melanin indices (M-index; a measure of skin pigmentation, as measured by skin spectrophotometry) and there was no relation between M-index and the magnitude of the reduction in NO-dependent dilation after acute UV-B exposure. It may be that those with light and dark skin pigmentation are similarly susceptible to the acute (i.e., single exposure) effects of UVR exposure on NO-mediated vasodilation, but those with a darker pigment are better able to adapt and protect against repeated exposures due to immediate pigment

darkening and/or increased folate metabolism in response to UVR exposure. Future investigation is warranted to elucidate the role skin pigmentation and folate metabolism-related genes in the vascular response to UVR.

Although we demonstrated acute reductions in NO-mediated vasodilation in the cutaneous microvasculature after local UVR exposure (104, 105), it remains unclear whether whole-body UVR exposure may influence systemic vascular function. Oral administration of folic acid has been demonstrated to improve flow-mediated dilation (FMD) (107-109) in patients with coronary artery disease or hypercholesterolemia. However, there has yet to be investigation into whether reductions in bioavailable folate after UVR exposure may impair conduit artery and systemic vascular function. Similarly, our lab previously demonstrated that either direct perfusion of 5-MTHF or oral folic acid supplementation improved microvascular responses to local heating in older adults (65+ y) with impaired microvascular function compared to young adults (18-30 y), such that microvascular responses were no longer different between groups (7). In that study, older and young adults had similar baseline plasma 5-MTHF concentrations, suggesting that elevated 5-MTHF bioavailability promotes healthy vascular function in an older population. Although the effects of UVR on vascular function have yet to be investigated in older adults, those data suggest that UVR-induced 5-MTHF degradation may exacerbate the vascular effects of UVR in that population.

NON-FOLATE, NON-VITAMIN D EFFECTS OF UVR ON SKIN VASCULAR FUNCTION

Ultraviolet radiation exposure may exert effects on vascular health and function independent of those mediated by folate or vitamin D metabolism. Exposure of human skin keratinocytes to UV-A or UV-B radiation elicits significant increases in ROS (110, 111). Furthermore, cells pretreated

with apocynin (110) or diphenylene iodonium (110, 111) suppressed ROS formation such that it did not change from baseline, suggesting that NADPH oxidase is likely a primary source of UV-B-induced ROS production (110). NADPH oxidase generates ROS by catalyzing the removal of an electron from NADPH, of which the final acceptor is molecular oxygen, resulting in the production of superoxide radicals (112). Increased production of superoxide may further promote ROS production by reacting with NO to produce peroxynitrite (113). ROS produced via this cascade of events, particularly peroxynitrite (114) may oxidize the eNOS cofactor BH_4, resulting in uncoupling of the eNOS dimer (115) and subsequent production of superoxide by uncoupled eNOS (116). Thus, UVR may elicit deleterious vascular effects independent of 5-MTHF degradation. However, the impact of such UVR-induced ROS production on vascular function after acute and/or chronic exposures, and the role of skin pigmentation in modulating these responses, remains unclear. Additionally, the interplay between UVR-induced NADPH oxidase activity and vitamin D-induced suppression of NADPH oxidase is unclear.

In addition to its role in ROS production, UVR may also elicit inflammatory responses that influence vascular function and health. In an *ex vivo* model, exposure of human dermal microvascular endothelial cells to 2 – 4 minimal erythema dose of UV-B resulted in upregulation of mRNA expression and production of IL-1β, IL-6, IL-8, and GROα cytokines (117). We and others have demonstrated dose- and time-dependent increases in resting skin blood flow *in vivo* after UV-B or broad-spectrum UVR exposure, beginning in the first 6 hours post-exposure (105) and continuing to develop over 24 hours (5, 118). UV-B-induced skin blood flow responses were blunted by infusion of indomethacin and/or N^G-monomethyl-L-arginine (L-NMMA), suggesting that the increases in skin blood flow were mediated by inflammation-induced upregulation of cyclooxygenase and/or inducible NOS (iNOS) (5). Our recent study demonstrated that the erythema and blood flow responses to an erythemogenic dose of broad-spectrum UVR in light- to

moderately-pigmentated (M-index, 30-49; Fitzpatrick skin type, I-IV) subjects were temporally distinct, suggesting that skin blood flow responses were not simply a function of skin reddening (105). Importantly, vascular inflammation and over-expression of inflammatory cytokines are associated with vascular dysfunction, atherosclerosis, and vascular disease (119). Therefore, chronic over-exposure to UVR could potentially play a role in inflammation-related vascular dysfunction and aging. Although our study did not include darkly-pigmented subjects, darkly-pigmented skin requires substantially larger doses of UVR to elicit an erythema response (120), and it is therefore likely that a darkened pigment would be similarly protective against the vascular inflammatory effects of UVR. Future research is warranted to examine the influence of skin pigmentation on vascular inflammation after UVR exposure.

Exposure of human skin to UV-A radiation has also been proposed to acutely increase NO production (3, 60, 61), but may (60, 61) or may not (3) reduce blood pressure. The NO-producing effect of UV-A appears to be mediated by liberation of NO via photolysis of cutaneous nitrite stores (61), and is independent of NOS (60). Further, because these effects were elicited by exposure of the skin to UV-A and not UV-B, these results suggest a potential role for UVR in vascular health separate from vitamin D. However, in those studies showing reductions in blood pressure, blood pressure and NO concentrations returned to baseline 20 – 60 minutes after UV-A exposure, and it is therefore unclear whether this effect provides any long-term health benefits. Due to absorbance of UV-A by melanocytes in the skin, it is likely that increasingly larger doses of UV-A would be required to observe these effects with increasing skin pigmentation, although this has yet to be explored.

PERSPECTIVES AND SIGNIFICANCE

Exposure to UVR from the sun is associated with both beneficial and deleterious effects on cutaneous vascular health. Both folate and vitamin D play important roles in healthy vascular function, but UVR exposure elicits opposing effects on metabolism and bioavailability of these two compounds. The effects of UVR on folate and vitamin D metabolism appears to be influenced by multiple factors, including skin pigmentation, genetics, geographical location, and age. Beyond the influence of UVR on folate and vitamin D metabolism, UVR exposure may cause oxidative stress and inflammatory responses that impair vascular health in a dose-dependent fashion. As such, under- or over-exposure to UVR may be differentially related to vascular dysfunction and elevated risk of cardiovascular disease in diverse populations. Currently available data are limited to studies examining the acute impact of UVR on vascular health, and it is therefore unclear how long-term, chronic exposure to UVR influences vascular function in various populations. The literature reviewed herein demonstrates the complexity of the interactions between individual characteristics and environment in modulating vitamin D and folate bioavailability and vascular health. The existing data suggest that adequate UVR exposure to maintain normal vitamin D concentrations may be important for cardiovascular health, but that over-exposure may result in reduced folate status and deleterious vascular effects. It is currently unclear, however, how the ideal amount of UVR exposure varies based upon individual characteristics. Further clinical trials are needed to better understand the optimal balance of sun exposure to protect cardiovascular health, and how this balance may differ between and within populations.

ULTRAVIOLET RADIATION EXPOSURE, RISK, AND PROTECTION IN MILITARY AND OUTDOOR ATHLETES

INTRODUCTION

Over-exposure to ultraviolet radiation (UVR) from the sun is associated with multiple health risks including DNA damage, immune suppression, premature skin aging, skin vascular dysfunction, and skin cancer (104, 121-124). Those who regularly exercise or compete outdoors are at particularly high risk of experiencing these deleterious effects of UVR (125). Similarly, tactical athletes such as military personnel frequently spend extended periods of time outdoors, often in high-UVR environments, putting them at elevated risk of experiencing these same potentially harmful effects. Thus, it is crucial to understand the impact of repeated and/or long-term exposures to UVR, as well as sun-safety behaviors and perceptions, in these populations.

The purpose of this review is to examine how UVR exposure experienced by outdoor athletes, regular exercisers, and military personnel elevate UVR-related health risks. The magnitude of UVR exposure in athletes/exercisers and military personnel will be discussed, along with the health risks imposed by such exposures. Lastly, we will address the current state of sun protection practices and barriers to adequate sun protection in these active populations.

MEASURING AND REPORTING ULTRAVIOLET RADIATION EXPOSURE

Personal UVR exposures are typically expressed in a variety of ways, including *multiples of minimal erythema dose* (MED) or *standard erythema dose* (SED). One MED is defined as the minimal UVR dose necessary to elicit a visible erythema (reddening) response with well-defined skin borders 24 hours after exposure (120). MED is highly variable and dependent upon individual factors such as skin pigmentation and regular sun exposure practices.

One SED, on the other hand, is equivalent to an effective erythemogenic radiation exposure of 100 $J \cdot m^{-2}$ (126). SED eliminates individual skin differences in erythema responses and provides a more consistent measure of UVR exposure that can be generalized between individuals and among populations. For individuals with mild to moderate skin pigmentation (Fitzpatrick skin types I-IV), the MED falls within the range of 1.5 – 6 SED (120). Table 1 describes skin sensitivity to UVR exposure for different skin types.

Table 2-3. Fitzpatrick skin types and sensitivity to ultraviolet radiation (UVR) exposure.

Skin Type	Skin Color	Tanning Ability	UVR Sensitivity	Continuous UVR Exposure Resulting in Erythema (SED)
I	Pale white	Always burns, does not tan	Extremely sensitive	2 – 3
II	Fair white	Burns very readily	Very sensitive	2.5 – 3
III	Darker white	May burn with no protection	Moderately sensitive	3 – 5
IV	Light brown	Burns rarely	Relatively tolerant	4.5 – 6
V	Brown	Despite pigmentation, may burn without protection	Very variable	6 – 20
VI	Dark brown or black	Rarely burns, although sunburn is difficult to detect	Relatively insensitive	6 – 20

The UV Index provides an easy-to-use indicator of biologically effective UVR exposure using the time-weighted average effective UV irradiance ($W \cdot m^2$) (127). A UV Index of 0 indicates a low risk of harm from unprotected UVR exposure, whereas an index of 11 or above is associated with extreme risk. One UV Index-hour corresponds to 90 $J \cdot m^{-2}$ (128); thus, at a moderate UV Index of 5, one hour of exposure is equivalent to 450 $J \cdot m^{-2}$ or 4.5 SED. As such, one hour of continuous sun exposure with a UV Index of 5 is likely to elicit a skin-reddening response in those with skin types I – III, and potentially skin type IV, if the skin is unprotected.

OUTDOOR ATHLETES AND ULTRAVIOLET RADIATION EXPOSURE

Those who participate in regular outdoor exercise or sport competition experience high daily doses of UVR exposure. The International Commission on Non-ionizing Radiation Protection (ICNIRP) has established guidelines for protection of outdoor workers against UVR exposure that are equally applicable to exercise occasions (129). According to those guidelines, maximum biologically effective UVR exposure over an eight-hour period should be limited to 30 $J \cdot m^{-2}$, a dose equivalent to approximately 1.0 – 1.3 SED or 0.5 MED for fair skin (129).

Studies using personal UVR dosimetry have allowed for quantification of the magnitude of UVR exposure experienced by those who work, exercise, or compete outdoors. Six professional cyclists competing in the Tour de Suisse wore personal UVR dosimeters attached to the back of their jerseys over the first eight stages of the race, demonstrating daily UVR doses as high as 17.2 MED and average daily UVR exposures of 8.1 MED (130). Three ironman triathletes competing at the World Championships in Kona, Hawaii were exposed to a total of 6.9 to 9.7 MED during the cycling and running portions of the race (131). Alpine ski instructors who wore UVR dosimetry for 26 days were exposed to daily UV-B exposures ranging from 0.5 to 7.6 MED, with more than two-thirds of the ski instructors exposed to greater than 2 MED of UV-B daily for the entire study period (132). Further, 10% of the ski instructors in that study were exposed to more than 1 MED per hour during peak exposure times. In marathon runners competing in the Barcelona and Madrid marathons, UVR exposures of 10 and 4.5 MED were experienced, respectively. These exposures far exceed the ICNIRP guidelines (129), and are likely to result in repeated sun damage and increased risk of UVR-related health issues.

High personal UVR exposures have also been reported in SED for hikers, tennis players, and runners during the summer and autumn months (133). On average, the hiking group spent 6.4

hours per day outdoors and experienced daily UVR exposures ranging from 1.8 to 19.5 SED, with a median daily exposure of 8.1 SED. Daily UVR exposures in tennis players who spent 4 hours per day outdoors ranged from 2.0 to 13.8 SED, and the median daily exposure was 7.5 SED. Runners who wore UVR dosimeters during races and spent 18 hours per day outdoors on average were exposed to 9.3 to 23.8 SED per day, with median exposure of 14.6 SED per day. Importantly, in that study hikers, tennis players, and runners *all* exceeded the UVR dose exposure limit recommended by the ICNIRP (129) by up to 8-fold, and in many cases the UVR exposure limit was reached in less than 20 minutes.

Risk of Adverse Effects from Ultraviolet Radiation Exposure in Athletes and Exercisers

Large doses of UVR exposure in those who exercise and/or compete outdoors may increase their risk of experiencing deleterious health effects. Indeed, the incidence of sunburn was significantly greater in those who self-reported *any* amount of physical activity outdoors compared to those who were inactive (125). Further, for every hour increase in outdoor physical activity there was a 2-4% increase in the likelihood of a sunburn. In a cohort of 290 NCAA athletes across 13 different sports, 84% had experienced one or more sunburns in the previous year and 28% had experienced at least four sunburns (134). These findings support the fact that those who participate in outdoor sports or exercise represent a population that is particularly vulnerable to the deleterious health effects of UVR exposure.

Regular exercisers and athletes who participate in outdoor sports are at increased risk of developing skin cancer. Sun exposure and sunburn may promote skin cancer via DNA damage in the skin, particularly damage to the tumor-suppressor gene *p53* (135, 136), resulting in cellular arrest, apoptosis, and tumorigenesis. The fact that regular physical activity is associated with a reduced risk of most cancers is well established. The exceptions to this relation are prostate and

skin cancer. Examining the relation between physical activity and hazard ratio for 26 types of cancer in 1.44 million participants, those who were in the 90th percentile of physical activity participation demonstrated reduced risk or no change compared to those in the 10th percentile for all types of cancer except prostate cancer and malignant melanoma (137). For malignant melanoma and prostate cancer, however, those in the 90th percentile of physical activity demonstrated *increased* hazard ratios compared to those in the 10th percentile. Furthermore, the association between malignant melanoma and physical activity was strengthened in high-UVR areas, suggesting that physically active people are, indeed, at an elevated risk for malignant melanoma compared to their less-active counterparts. Although that study did not characterize the relative risk of developing malignant melanoma for those who participated in outdoor compared to indoor physical activity, the increased hazard ratio in those who were most physically active can likely be explained by increased time spent outdoors rather than physical activity, per se. Of note, increased risk for prostate cancer was suggested to be a result of screening bias.

Common and atypical melanocytic nevi (moles) and actinic lentigines (lesions on sun exposed skin; also known as liver spots or age spots) are associated with nonmelanoma skin cancer, and may precede the development of malignant melanoma (138). Atypical melanocytic nevi and actinic lentigines are more common in marathon runners compared to control subjects (139). Further, higher training heart rates, training velocity, and physical strain indices were associated with significantly more melanocytic nevi on the left shoulder of marathon runners (140). Thus, development of nevi and lentigines are exaggerated in endurance athletes not only because of increased sun exposure, but also potentially due to immunosuppression during long training bouts. Endurance exercise-induced immune suppression may occur in response to large training loads (141) and diminish DNA repair in the skin after prolonged sun exposures, resulting in gene mutations and tumorigenesis.

Several studies have demonstrated increased incidence of skin cancer development in athletes within a variety of competitive outdoor sports. In a large sample of Swiss residents, the risk of basal cell carcinoma was two-fold greater in those who participated in outdoor sports compared to those who did not (142). Studies examining skin cancer prevalence in surfers in the Texas Gulf Coast (143) and Australia (144) documented non-melanoma and/or melanoma skin cancer in 18% and 14% of subjects, respectively, and the prevalence of skin cancer was greater in competitive surfers (17%) compared to recreational surfers (11%) (144). Precancerous lentigines were significantly more prevalent in a cohort of 283 mountain guides (25%) in Germany, Switzerland, and Austria compared to age-matched controls (7%) (145). Squamous cell carcinoma, basal cell carcinoma, and/or melanoma were diagnosed in approximately 12% of mountain guides, but no skin cancers were found in any of the control subjects. Two of the most important risk factors found in that study were lifetime number of heavy sunburns and lifetime days working as a guide. Taken together, these studies clearly demonstrate an elevated risk of skin cancer for those who regularly participate in outdoor sports or exercise, highlighting the importance of sun-protection strategies in those populations.

Sun Protection in Athletes and Exercisers

Despite being at increased risk for deleterious health effects of sun exposure, regular outdoor exercisers and athletes often do not adhere to adequate sun protection behaviors and practices. In a sample of 290 NCAA collegiate athletes, less than 25% reported regular use of sunscreen despite averaging 4 hours per day training outdoors over 10 months out of the year (134). Similar results were demonstrated in 186 collegiate soccer and cross-country athletes, 85% of whom reported no use of sunscreen over the previous 7 days of practice (146). An analysis of young adult (ages 18-30 years) men and women competing in field hockey, soccer, tennis, and surfing

suggested that there are differences among sports and between men and women in sun protection behavior, with female participants engaging in far greater sunscreen use (Table 2) (147). Similarly, in a cohort of 970 runners in Switzerland, sex and age were predictive of sun-protective behaviors, such that women and adults older than 35 years were more likely to protect themselves from the sun (148). Only 5% of *adolescent* athletes in Buenos Aires reported regular use of sunscreen during training (149); this is particularly important in light of the fact that sun exposure in adolescence is positively associated with risk of melanoma in adulthood (150, 151).

Table 2-4. Prevalence of sunscreen use in men and women competing in four different sports.

Sunscreen Use During Competition (%)		
Sport	**Men**	**Women**
Field Hockey	18	67
Soccer	24	82
Tennis	63	76
Surfing	93	100

Recommended sun safety strategies include: (1) avoiding sun exposure during peak UVR exposure hours, (2) staying in the shade while outdoors, (3) applying sunscreen with sun protection factor (SPF) ≥30 and reapplying sunscreen when sweating or swimming, (4) wearing hats and sunglasses, and (5) wearing protective clothing with long pants or sleeves that are black, navy blue, or green (152). Sunscreen should be properly applied at 2 mg/cm^2 (153) to ensure that the sunscreen is effective at its reported SPF, and reapplied every two hours when spending prolonged periods outdoors. Reasons for inadequate sun safety behavior in athletes include:

- rules of competition (i.e., equipment guidelines),
- lack of sunscreen availability,
- forgetting to apply sunscreen, and

- the belief that using sunscreen will impair their performance (152).

Although the most commonly reported barriers to optimal sun protection are lack of sunscreen availability or forgetting to apply sunscreen (134, 146), many athletes choose not to apply sunscreen, believing that sunscreen might impair performance by making their hands slippery, causing eye irritation (134, 154), or negatively impacting heat dissipation by reducing sweat production and/or evaporation, although studies examining these effects of sunscreen on thermoregulation are equivocal (155-159). Competition guidelines may also pose a substantial barrier to sun protection in many sports. In Ironman triathletes, sunscreen is not allowed on the shoulders or thighs where numbers are marked on the skin (131). Field hockey and soccer players are not permitted to wear hats or sunglasses due to equipment guidelines (147), and volleyball players are required to wear jerseys and shorts that leave a large skin surface area uncovered and exposed to the sun (160). Similarly, traditional sportswear in cycling and running leave large areas of the skin exposed to the sun (139, 161), usually to enhance heat dissipation. Such clothing guidelines and restriction within sport further highlight the importance of sunscreen use for these athlete populations.

MILITARY POPULATION AND ULTRAVIOLET RADIATION EXPOSURE

In addition to those who participate in outdoor recreational exercise or sport, military personnel represent an often-overlooked group with unique exposures to UVR, that likewise place these tactical athletes at higher risk for melanoma. Although rather sparse data are available regarding UVR exposure in the military compared to outdoor exercisers and athletes, active duty personnel are frequently required to be outdoors for prolonged periods of time, often with strict regulations on equipment and clothing coverage. Further, military personnel are often faced with other

immediate safety concerns that may take priority over preventive care such as protection against UVR exposure and associated risks.

Although it is well-known that military personnel, particularly in the deployed environment, may experience large amounts of UVR exposure, no studies have yet been conducted to specifically assess daily doses of UVR in the military population. Over 3 million soldiers were deployed from 2001-2014 in support of Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) in Afghanistan and Iraq, respectively. Due to the relatively equatorial latitudes of these austere deployed environments, UVR exposure is often severe, with minimal seasonal variation, and thus may increase risk of melanoma (162). During deployment in support of OEF and OIF, 84% of respondents worked in a desert climate, with 64% spending three-quarters of their day in bright sun. 77% of respondents reported spending at least four hours per day in bright sun (163). Thus, military personnel deployed in such environments are at increased risk of experiencing deleterious health effects of UVR exposure.

Risks of Adverse Effects from Ultraviolet Radiation Exposure in the Military Population

Several studies have compared the incidence of melanoma in the general population versus specific military populations, with varied conclusions depending upon the specific subpopulation within the military. Increased rates of melanoma and non-melanoma skin cancers have been observed among United States veterans who operated in equatorial climates such as southwest Asia and the Western Pacific (164, 165). Rates of malignant melanoma in active duty personnel compared to the U.S. general population are 62% greater in the military, with the highest incidence observed in the Air Force (166).

Opposing findings, however, have also been reported, showing no differences in the incidence of melanoma between the general U.S. population and the Navy or Air Force branches of the U.S. military (167, 168). A comparison of melanoma incidence rates between active duty and general U.S populations within the age range of 20-59 years revealed *lower* age-adjusted incidence rates in the military (169). However, military age-specific rates were significantly higher in older individuals, with an age-dependent reversal of the incidence rate ratio (IRR) around age 45. Thus, the incidence rates for melanoma are lower in the military compared to the general population among those age 45 or younger, but higher in the military for those older than 45.

These differential skin cancer rates within the military population may be explained, at least in part, by improved education regarding sun safety and better preventative practices in recent years. Incidence rates (per 100,000 person-years) in the military compared to the general U.S. population were 34 vs. 27 among 45-49 year-olds, 50 vs. 32 among 50-54 year-olds, and 178 vs. 39 among 55-59 year-olds. Further, when comparing annual relative increases in the incidence of melanoma, the incidence among men rose by 36% in the military compared to only 7% in the general population from 1990-1994 and 2000-2004, with the greatest rise in incidence occurring in young men (40% in those aged 20-44 compared to 19% in those aged 45-59) in the military. In summary, melanoma rates rose over this decade in both military and civilian populations, but the greatest relative increase in incidence occurred among younger men in the military (169). Although the results of these studies vary, they suggest that the military population represent an at-risk population for adverse health effects of UVR and highlight the need to promote sun-safety behaviors in that population.

Sun Protection in the Military Population

With specific regulations on uniform attire that often requiring soldiers to wear proper long pants and a long-sleeved blouse, sun protection may be an afterthought for most military personnel (170). Though the uniform regulations are strictly enforced for wear in garrison, soldiers are permitted to make uniform adjustments such as rolling shirt sleeves above the elbow or de-blousing during times of high environmental heat loads, as determined by wet-bulb globe temperature (WBGT) (171). Although such adjustments to military uniform wear allow enhanced evaporative and convective cooling to mitigate heat injury, they also result in additional UVR exposures that may go unnoticed. During deployment, military personnel reported that their exposed skin was unprotected at least 70% of the time and fewer than 30% of surveyed soldiers reported regular sunscreen use (172).

The U.S. Army acknowledges the need for sun-safety education and provides sun-specific guidelines as part of their overall environmental casualty prevention guidance (173). The Army also includes written recommendations for sunscreen use with SPF rating of at least 15, and for unit commanders to ensure soldiers maintain their supply and apply when needed (170, 173). However, despite reporting long hours in bright sun, only 13% of military personnel reported routine sunscreen use and less than 30% had routine access to sunscreen while working (163). Thus, although regulations exist to promote sun-safety, further efforts may be warranted to enhance implementation of sun-safety behaviors in the military population.

CONCLUSIONS

Military personnel and outdoor athletes and exercisers represent two populations at elevated risk of over-exposure to UVR from the sun, and consequent negative health impacts from UVR exposure. As such, promoting healthy sun-protection behaviors in these populations is crucial. Risks associated with UVR exposures can be directly mitigated by wearing UVR protective

clothing and sunscreen. However, inadequate sun-protection still often occurs in these athletes due to equipment regulations, lack of readily-available sunscreen, or forgetting to (or consciously choosing not to) apply sunscreen. Some competitive athletes choose not to use sunscreen due to the belief that sunscreen may impair performance or thermoregulatory capacity, although there is not strong evidence to support any impairment. These observations highlight the need for improved education about the negative health consequences of over-exposure to UVR, as well as the importance of practicing appropriate sun-protection behaviors, in outdoor athletes and military populations. Improved efforts should also be made to improve availability and accessibility to sunscreen when no other sun-protection strategies are feasible.

GRANTS

This work is supported by a grant from the National Institutes of Health (NIH T-32 Grant 5T32AG049676-03) and the Marie Underhill Noll Endowment.

DISCLOSURES

The authors declare no conflict of interest and do not have any financial disclosures. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or reflecting the views of the Army or the Department of Defense.

Chapter 3

ACUTE ULTRAVIOLET RADIATION EXPOSURE ATTENUATES NITRIC OXIDE-MEDIATED VASODILATION IN THE CUTANEOUS MICROVASCULATURE OF HEALTHY HUMANS

INTRODUCTION

Endothelium-derived nitric oxide (NO) is an important component of the vasodilatory responses of the cutaneous microvasculature (29, 174) and is associated with a healthy vascular phenotype. 5-methyltetrahydrofolate (5-MTHF), the bioactive metabolite of folate, contributes to the synthesis and vascular bioavailability of endothelial NO (97, 175) by stabilizing endothelial NO synthase (eNOS) via (1) increasing bioavailable tetrahydrobiopterin (BH_4), an essential cofactor in the coupling of the NOS dimer, and (2) direct scavenging of reactive oxygen species (ROS) (97, 175). Under normal conditions, thermally-induced increases in skin blood flow are primarily mediated by NO, which in turn depends on adequate 5-MTHF bioavailability.

In vitro studies have suggested that 5-MTHF is sensitive to photodegradation by ultraviolet (UV) radiation (4, 59, 176), but it remains unclear how cutaneous vascular 5-MTHF is affected by UV *in vivo*. Importantly, 5-MTHF appears to be sensitive to UVB and UVC, but not UVA radiation (4, 59). UVC radiation is unable to penetrate the earth's atmosphere, and UVB may be unable to penetrate the dermis to reach the dermal circulation (4). Alternatively, 5-MTHF may be indirectly degraded *in vivo* by UV exposure-induced increases in ROS. Indeed, 5-MTHF is rapidly degraded by UV in the presence, but not in the absense, of endogenous photosensitizers (9, 59). Such naturally occuring photosensitizers in the skin or blood produce ROS upon exposure to solar radiation (177). It is unknown, however, whether direct and/or indirect photodegradation of 5-MTHF occurs with exposure to UVB *in vivo*.

Differences in skin pigmentation are suggested to result from evolutionary adaptations to geographical variations in UV exposure (21) designed to enhance reproductive viability. Protection of 5-MTHF is of evolutionary importance due to its role in the prevention of macrocytic anemia in pregnant women and the development of neural tube defects in utero (21, 178). Thus, early human populations who inhabited equatorial Africa and were subjected to high levels of UV exposure are thought to have developed a darkened pigment to protect from potentially negative effects of UV including sunburn, skin cancer, and photolysis of bioavailable folate (21, 24, 179). As humans began to move to geographical regions distant from the equator, depigmentation of the skin is reasoned to have occurred to allow for adequate vitamin D biosynthesis under conditions of reduced UV exposure. Lightening of skin pigment, however, may increase susceptibility to UV-induced degradation of bioavailable 5-MTHF.

To date, no studies have determined how cutaneous vascular 5-MTHF is affected by acute UV exposure *in vivo,* and it is unknown whether UV-induced degradation of 5-MTHF affects NO-mediated vasodilation of the human cutaneous microvasculature. Further, whether a darkened skin pigment "protects" the cutaneous microvasculature from the negative effects of acute UV is unknown. Therefore, the purpose of the current study was to elucidate the impact of UV, specifically in the UVB region, on NO-mediated vasodilation in the human cutaneous microvasculature and to examine the role of direct 5-MTHF photodegradation and ROS in these responses. Further, we sought to examine whether there is an association between UVB effects on NO-dependent vasodilation of the skin microvasculature and skin pigmentation. We hypothesized that NO-mediated cutaneous vasodilation would be attenuated after acute UVB exposure, but that this effect would be smaller in darkly- vs. lightly-pigmented individuals. We further hypothesized that local infusion of either 5-MTHF or ascorbate (ASC, a non-specific antioxidant), would

augment NO-mediated vasodilation after UVB exposure, suggesting that one or both mechanisms are involved.

METHODS

Subjects

Experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University. Men and women aged 18-30 who were normally active, healthy, non-smokers, free from cardiovascular disease, and not taking any prescription medications with primary or secondary vascular effects were tested. Additional measures were taken to recruit subjects across a wide range of skin pigmentation. All subjects underwent an initial screening that included physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsuburgh, PA). Subjects with rash, skin disease, disorders of pigmentation, known skin allergies, allergies to folic acid, or kidney disease were excluded. Written and verbal consent were obtained voluntarily from all subjects before participation according to the Declaration of Helsinki.

Assessment of Skin Pigmentation

Skin pigmentation was measured by reflectance spectrophotometry (DermaSpectrometer; Cortex Technology, Hadsund, Denmark) to determine the melanin index (M-Index) of the skin on the subject's inner aspect of the upper arm. The M-Index is routinely measured in this region because of its relatively low sun exposure and ease of access (179). Additional M-Index measurements were taken at the forehead and both forearms. Lower and higher M-Indices are related to lighter and darker skin pigments, respectively.

Instrumentation

All protocols were performed in a thermoneutral laboratory with the subject in a semi-supine position and both arms supported at heart level. Three intradermal microdialysis fibers (10 mm, 55-kDa cut-off membrane; CMA, Holliston, MA) were placed into the dermal layer of the ventral aspect of each forearm (6 sites total) for the local delivery of pharmacological agents (29). Pharmacological agents were dissolved in lactated Ringer's solution just before use, sterilized using syringe microfilters (Acrodisc; Pall, Ann Arbor, MI) and wrapped in foil to prevent degradation due to light exposure. In each forearm, microdialysis sites were randomly assigned for local delivery of either 5mM 5-MTHF (USP, Rockville, MD) (7), 10mM ASC (Sigma, St. Louis, MO) (180), or lactated Ringer's solution alone (control site). All solutions were perfused through their respective microdialysis fibers at a rate of 2 μl/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems, West Lafayette, IN) (29).

Local red blood cell flux was measured directly over each microdialysis site throughout the protocol using an integrated laser-Doppler flowmetry (LDF) probe placed in a local heating unit (moorLab, Temperature Monitor, SHO2; Moor Instruments, Axminster, UK). 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, Millwaukee, WI).

Experimental Protocol

After placement of microdialysis fibers and following a 60-min period for resolution of hyperemia associated with fiber placement, one arm was randomly exposed to 300 mJ/cm^2 (75 sec) UVB (SolRx 500; SolArc Systems, Minesing, ON) while the other served as a non-exposed control. We then collected baseline data and initiated a local heating protocol (local temperature increased to 42°C at a rate of $0.5°C \cdot 5\ sec^{-1}$) as described previously (29, 174). This protocol

elicits an initial peak that is mediated by sensory nerves followed by a plateau (16, 17). After ~40 min of local heating and establishment of a stable local heating plateau, each microdialysis site was perfused with 15mM L-N^G-Nitroarginine methyl ester (L-NAME; Calbiochem, San Diego, CA) at a rate of 4 µl/min to fully inhibit nitric oxide synthase (181, 182). Following a stable L-NAME plateau, 28 mM sodium nitroprusside (SNP; USP, Rockville, MD) was perfused through each site and local temperature was increased to 43°C to elicit maximal cutaneous vascular conductance (CVC_{max}) (174, 183).

Data Acquisition and Analysis

LDF data were collected using Windaq (Windaq; DATAQ Instruments) at a frequency of 40 Hz and stored on a personal computer for subsequent analysis. Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and expressed as a percentage of CVC_{max} for each phase of the local heating protocol (174, 184). The percent of cutaneous vasodilation mediated by NO was calculated from the difference between the local heating and L-NAME plateaus.

Two-way repeated-measures ANOVA were used to detect differences between pharmacological treatment sites in UVB exposed and non-exposed arms at each phase of the local heating protocol (arm x MD site x phase) (SAS v9.4, SAS Institute Inc.). NO-dependent vasodilation was regressed against M-index to ascertain whether skin pigmentation was predictive of the response to UVB. Bonferroni *post hoc* corrections were performed to account for multiple comparisons in the ANOVA when necessary and significance was set *a priori* and accepted at α=0.05. All values are presented as mean ± standard error.

RESULTS

Subject characteristics are presented in **Table 3-1**. All baseline variables and health indicators were normal for this age group, and a broad range of continuous and heterogeneous M-index values were included. Forearm M-Indices are expressed as the average of both forearms.

Table 3-1: Subject Characteristics

	Mean ± S.E.M.	Range
n (M/F)	22 (8/14)	
Age (yrs)	23 ± 1	18-28
BMI ($kg \cdot m^{-2}$)	24 ± 1	19 – 31
Systolic BP (mmHg)	112 ± 2	94 – 120
Diastolic BP (mmHg)	70 ± 2	56 – 88
Heart rate ($beats \cdot min^{-1}$)	65 ± 3	44 – 80
M-Index (a.u.)		
Inner Arm	46 ± 4	30 – 79
Forehead	52 ± 4	32 – 98
Forearm	45 ± 4	31 – 83
Blood biochemistry		
HbA1c (%)	5.0 ± 0.1	4.2 – 5.6
Total cholesterol ($mg \cdot dl^{-1}$)	154 ± 8	83 – 209
HDL ($mg \cdot dl^{-1}$)	60 ± 4	37 – 79
LDL ($mg \cdot dl^{-1}$)	79 ± 6	21 - 118

n = no. of participants. a.u., arbitrary units; 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.

Figure 3-1 illustrates representative tracings of $\%CVC_{max}$ in response to the standardized local heating protocol for one subject, showing the control site in both the UVB-exposed and non-

exposed arm. The percent decrease in $\%CVC_{max}$ with NOS-inhibition by local administration of L-NAME is indicated.

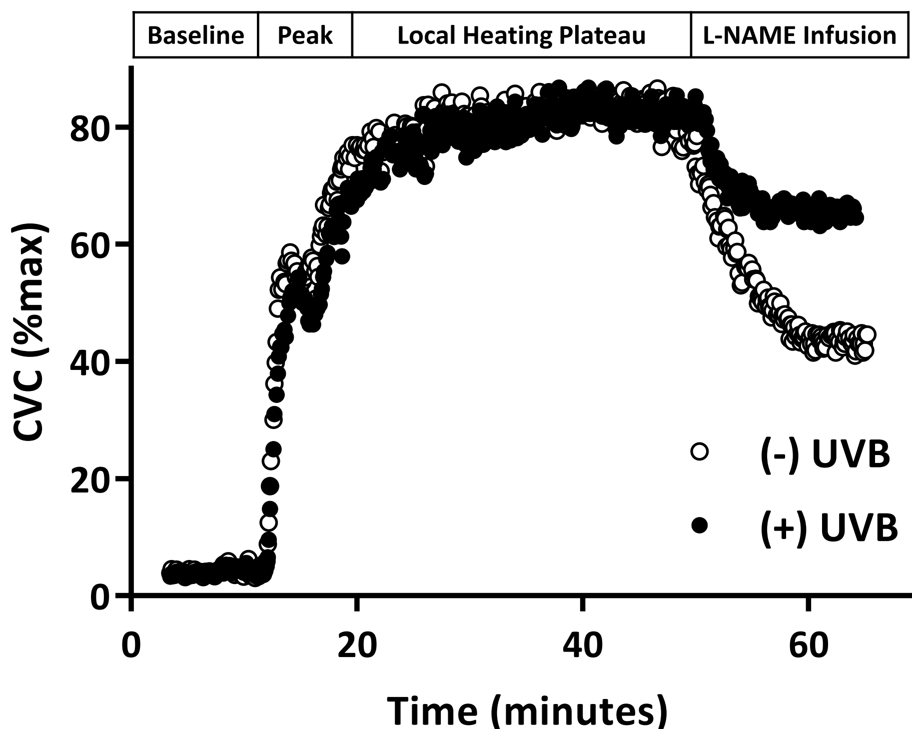

Figure 3-1: Representative tracings from one subject demonstrating the blood flow response [percent maximal cutaneous vascular conductance (%CVCmax)] during the local heating protocol in the control (lactated Ringer's) sites of the ultraviolet B (UVB)-exposed (closed circles) and nonexposed (open circles) arms. The arrow indicates the percentage decrease with nitric oxide synthase (NOS) inhibition [L-NG-nitroarginine methyl ester (L-NAME infusion)].

Figure 3-2 presents $\%CVC_{max}$ for the control site at each phase of the local heating protocol in UVB-exposed and non-exposed arms (left axis). There were no differences in $\%CVC_{max}$ due to acute UVB exposure during baseline, at the initial peak, or during the local heating plateau. As illustrated by the right-most bars and right axis, %NO-mediated vasodilation was attenuated in UVB-exposed, compared to non-exposed, arms (23.1±3.8 vs. 33.9±3.4%; p=0.001). Importantly,

as shown in **Table 3-2**, there were no between-arm or inter-site differences in the maximal heating values.

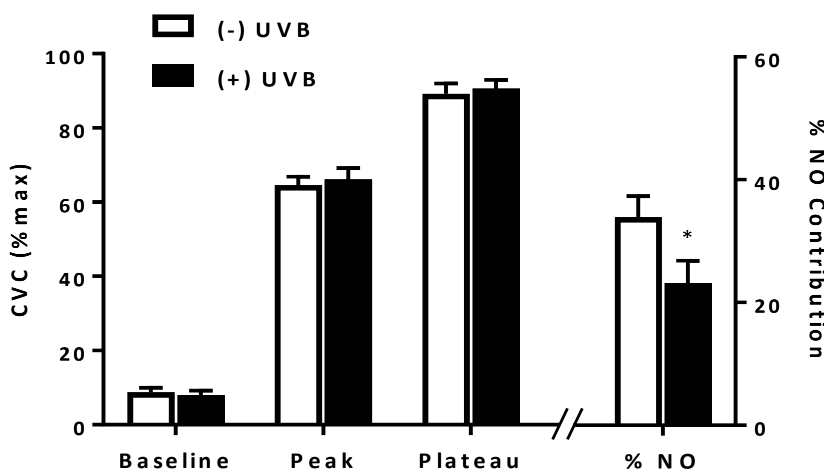

Figure 3-2: Percent maximal cutaneous vascular conductance (%CVCmax) at each phase of the local heating protocol as well as the percent contribution of nitric oxide (NO) to the local heating plateau in the lactated Ringer's sites in ultraviolet B (UVB)-exposed (+UVB; closed bars) and nonexposed (-UVB; open bars) arms. * $P < 0.05$ compared with nonexposed arm; $n = 22$.

Table 3-2: Maximal CVC Values (flux · mmHg^{-1})

	Ringer's	Ascorbate	5-MTHF
Control	2.29 ± 0.25	2.16 ± 0.19	2.34 ± 0.18
UVB	2.34 ± 0.20	1.89 ± 0.17	2.20 ± 0.14

All values are expressed as mean ± S.E.M. in flux/mmHg. 5-MTHF, 5-methyltetrahydrofolate; CVC, cutaneous vascular conductance; UVB, ultraviolet B.

Figure 3-3 depicts CVC responses to the local heating protocol in Ringer's-, ASC-, and 5-MTHF treated sites in the UVB-exposed arm only. There were no differences between sites during baseline, initial peak, or the local heating plateau phases (left axis). The %NO-mediated

vasodilation (right axis) was augmented by local administration of both 5-MTHF (30.1±4.8 vs 23.1±3.8%; p=0.03) and ASC (30.1±4.3 vs 23.1±3.8%; p=0.02) compared to the control site.

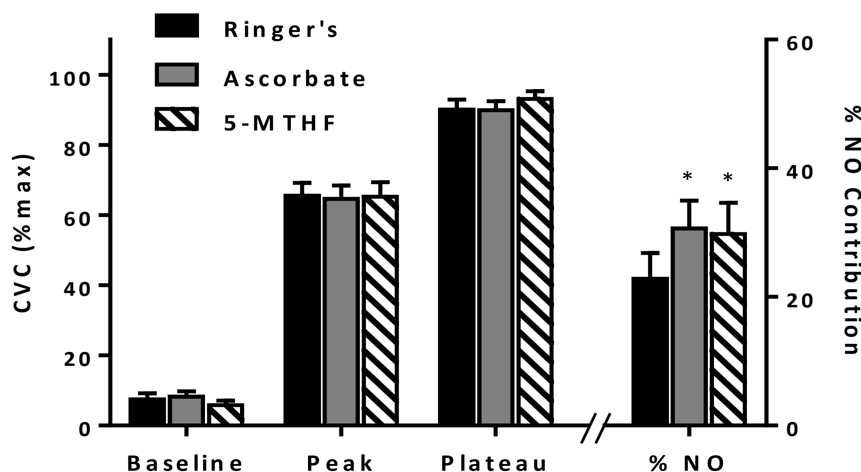

Figure 3-3: Percent maximal cutaneous vascular conductance (%CVCmax) responses at each phase of the local heating protocol as well as the percent contribution of nitric oxide (NO) to the local heating plateau in lactated Ringer's-, ascorbate-, and 5-methyltetrahydrofolate (5-MTHF)-treated sites in the ultraviolet B exposed arm. *$P < 0.05$ compared with Ringer's; $n = 22$.

Neither of the localized treatments (5-MTHF or ASC) had an effect on cutaneous vascular responses to local heating in the non-UVB exposed arm (p≥0.09). In the UVB exposed arm, both 5-MTHF and ASC restored the %NO-mediated vasodilation such that there were no differences between the control site in the non-exposed arm and the treated (5-MTHF: 33.9 ±3.4 vs. 30.1±4.8%, p=0.27; ASC: 33.9 ±3.4 vs. 30.9±4.3%, p=0.32) sites in the UVB exposed arm.

The magnitude of change in %NO-mediated vasodilation after UVB exposure was not associated with M-Index at the inner arm (R^2=0.04, p=0.42), forehead (R^2=0.02, p=0.59), or forearms (R^2=0.05, p=0.34) across the range tested. Further, there was no relation between the responses to 5-MTHF or ASC and skin pigmentation at the inner arm (5-MTHF: R^2=0.02, p=0.53; ASC:

R^2=0.03, p=0.48), forehead (5-MTHF: R^2=0.02, p=0.53; ASC: R^2=0.004, p=0.78), or forearms (5-MTHF: R^2=0.005, p=0.76; ASC: R^2=0.008, p=0.71).

DISCUSSION

The primary finding of this study was that a brief bout of acute UVB exposure attenuated NO-mediated vasodilation in the human cutaneous microvasculature. Additionally, local delivery of either 5-MTHF or ASC augmented the NO-mediated contribution to vasodilation after UVB exposure such that NO-mediated vasodilation in the 5-MTHF and ASC sites of the UVB exposed arm were similar to that of the control site in the non-exposed arm. Taken together, these findings suggest that reductions in the NO-mediated contribution to vasodilation after UVB exposure are mediated, at least in part, by degradation of 5-MTHF -- either directly or via local UVB-induced increases in ROS. Lastly, no relation was found between skin pigmentation and the magnitude of change in %NO-mediated vasodilation consequent to UVB exposure, suggesting that darker skin pigment may not protect against the deleterious effects of acute UVB exposure on cutaneous microvascular function.

In healthy young subjects, ~40-60% of the vasodilatory response to local heating in the cutaneous microcirculation is mediated by NO under control conditions (7, 29, 40). 5-MTHF plays an indirect but essential role in the coupling of NOS by increasing production of tetrahydrobiopterin (BH_4) from its inactive form, dihydrobiopterin (BH_2). 5-MTHF also directly scavenges ROS, which likewise increases NO bioavailability by stabilizing the NOS dimer (97). Thus, full expression of NO-mediated vasodilation is reliant upon adequate bioavailable 5-MTHF. Our results suggested that NO-dependent cutaneous vasodilation is attenuated by acute UVB-exposure due to degradation of 5-MTHF, either by direct photodegradation or via local increases in ROS, or both.

Despite significant reductions in the NO-mediated component of the vasodilatory response following UVB-exposure, which were similar in magnitude to attenuations observed in aging and various diseased states (35, 36, 185), there was no difference in the magnitude of the local heating plateau between UVB-exposed and non-exposed arms. This is likely explained by redundant dilator pathways, such as endothelium-derived hyperpolarizing factors (186), which may compensate for the acute reduction in NO bioavailability in young, healthy subjects (29). This is similar to our previous finding in healthy middle-aged adults, in which the NO-dependent dilation response is attenuated but the magnitude of the overall dilator response to local-heating is preserved by a compensatory increase in other dilator mechanisms (29). The activity of such redundant pathways, however, is diminished in aging adults (35) and those with various pathologies such as hypertension and hypercholesterolemia (36, 185). As such, attenuated production and reductions in NO-dependent dilation after UVB exposure may present an avenue for future risk of cutaneous microvascular dysfunction in otherwise healthy adults.

Although UVB (wavelength = 280 to 315 nm) directly degrades 5-MTHF *in vitro*, the dermis is relatively impenetrable by UVB (4) and has therefore been suggested to be unlikely to directly affect the cutaneous circulation. Conversely, UVA (315 to 400 nm) can penetrate the dermis but 5-MTHF does not appear to be sensitive to UVA (4, 59). Our findings demonstrate that reductions in NO-mediated vasodilation of the cutaneous microvasculature after UVB exposure were improved by local administration of 5-MTHF, suggesting that the reductions were a result of UVB-induced reductions in 5-MTHF bioavailability. Taken together, while it cannot be ruled out based on the present data, it is unlikely that 5-MTHF is *directly* degraded by UVB *in vivo*. Alternatively, UVB may be capable of indirectly degrading 5-MTHF *in vivo* by increasing local production of ROS. Local delivery of ASC augmented NO-mediated vasodilation after UVB

exposure, indicating that local increases in ROS contribute to the reduction in NO-mediated dilation with UVB. Consequently, UVB-induced degradation of 5-MTHF may be mediated by local increases in ROS.

Standard erythema dose (SED) is a standardized measure of the UV dose required to elicit a reddening response of the skin (187). UV exposures in the range of 2-6 SED per hour have been documented in triathletes and cyclists during competition (130, 131). Likewise, hikers and tennis players have been demonstrated to experience 8.1 and 7.5 SED with exposure times of 6.4 and 4.0 hours, respectively (133). UVB exposure in the current study was equivalent to approximately 1.8 SED. As such, the magnitude of UV exposure experienced by individuals participating in outdoor activities can far exceed that of the present study.

The evolution of dark, eumelanin-rich skin is believed to have occurred early in the evolution of the genus *Homo* in equatorial Africa, at the same time as the reduction of somatic body hair, in order to protect from the deleterious effects of UV radiation, including photodegradation of 5-MTHF (21, 24, 179, 188). As humans dispersed to areas with greater seasonal variations in sunlight and lower intensities of UV radiation, depigmentation is thought to have occurred to facilitate adequate UVB-induced vitamin D_3 synthesis. Indeed, strong correlations have been demonstrated between skin pigmentation and the absolute latitude at which a population lives (21), and between skin pigmentation and the rate of cutaneous vitamin D_3 synthesis (22, 23). We therefore hypothesized that individuals with constitutively darker skin would be protected from UVB-induced degradation of 5-MTHF, and resultant reductions in NO-dependent vasodilation of the cutaneous microvasculature. Our data indicate that NO-dependent vasodilation was attenuated in the cutaneous microvasculature after acute UVB exposure, but that this effect was independent

of skin color. Furthermore, local 5-MTHF or ASC treatments had similar effects regardless of the level of skin pigmentation.

It may be that darkly- and lightly-pigmented individuals are equally susceptible to the acute effects of UV radiation, but that those with darker skin are better able to adapt to the effects of UV radiation and are thereby better protected from repeated exposures. Indeed, immediate pigment darkening (IPD) after UV radiation exposure protects 5-MTHF from sensitization to UV radiation by natural photosensitizers such as riboflavin and uroporphyrin (59). Future studies may be warranted to observe the role of IPD in protecting from UV radiation-induced reductions in 5-MTHF and NO-mediated vasodilation in the cutaneous microvasculature. The effects of UVA on 5-MTHF status and NO-mediated vasodilation also warrant study. UVA penetrates the dermis of the skin more deeply than UVB, its passage is more strongly dependent on melanin concentration, and it may have a greater effect on dermal capillaries than UVB (99, 100). UVA is also present at higher levels throughout most of the year at most latitudes (24). It will also be worth examining the effects of multiple UV radiation exposures over the course of days or weeks, a situation that would more closely simulate the conditions of chronic outdoor sun exposure experienced by humans during most of evolution.

Blunted cutaneous vasodilation in response to local heating, as well as an attenuated NO contribution to the response, has been demonstrated in healthy young African American compared to Euro-American subjects (40, 86). The contrary findings in the present study are likely explained by heritage. Subjects were included in the previous studies only if both parents were of African American or Euro-American descent. In contrast, our study included subjects independent of specific heritage and comparisons were made based solely upon skin pigmentation. It is therefore possible that differences in vascular function between African

American and Euro-American subjects demonstrated previously may be explained by genetic factors or mediated by a chemical intermediary and not directly by skin pigment.

Limitations

Data collection for this study occurred in the months from August through April. Thus, there was likely substantial variation in the amount of daily UV radiation from the sun that subjects were exposed to, depending upon the time of year during which they participated. In order to limit this variability, we tested the skin of the ventral forearm, which is exposed to less UV radiation compared to the dorsal aspect of the arm during daily activities. We found that there was no variation (p=0.87) in the response to acute UV radiation exposure as a function of the time of year at which data were collected.

UV radiation exposure in the present study was limited to wavelengths in the UVB region. We chose to utilize only UVB because 5-MTHF may be degraded by UVB either directly or indirectly via production of ROS (4, 59), although the effects of these two mechanisms *in vivo* are unclear. Conversely, UVA is unable to directly affect 5-MTHF. Therefore, the current study sought to elucidate the specific effects of UVB exposure on 5-MTHF bioavailability *in vivo*. Our findings suggest that 5-MTHF is degraded by UVB *in vivo*, at least in part due to UVB-induced production of ROS. Further research is needed to examine how broad-spectrum UV radiation exposure may affect bioavailable 5-MTHF and NO-mediated cutaneous vasodilation.

UVB exposure was limited to the ventral aspect of the forearm, allowing us to observe local effects of UVB on NO-dependent vasodilation and, indirectly, bioavailability of 5-MTHF. Although our results demonstrated reductions in the NO component of cutaneous vasodilation via 5-MTHF-related mechanisms, we are unable to speculate as to how systemic vascular function

and bioavailability of 5-MTHF would be affected by whole-body UV radiation exposure. Recent data have demonstrated a negative relation between blood folate concentration and UV radiation exposure accumulated over 42 days, suggesting that repeated UV radiation exposures may result in diminished systemic 5-MTHF bioavailability (11). However, it remains unclear how accumulated UV radiation exposure may affect cutaneous 5-MTHF bioavailability and microvascular function. Future investigation is warranted to further elucidate of the role of UV radiation on 5-MTHF and cutaneous microvascular function.

Perspectives

The results of the current study suggest that NO-mediated vasodilation is attenuated after acute exposure to UVB. Additionally, the observed reduction in NO-dependent vasodilation is mediated by reductions in bioavailable 5-MTHF, most likely through ROS production. Vasodilation of the cutaneous microvasculature is an important facet of the physiological response to local (174) and whole body heating (189), and proper function of this response is reliant upon adequate bioavailability of NO (189, 190). Although we have demonstrated that UVB exposure acutely diminishes this response to local heat, it is unknown how chronic UV radiation exposure may affect long term cutaneous microvascular function and/or thermoregulatory increases in skin blood flow. The data from the present study exemplify the importance of improving our understanding of the effects of UV radiation on cutaneous microvascular function, as well as how to mitigate these effects.

Summary

In summary, NO-mediated vasodilation in the cutaneous microvasculature was attenuated by acute UVB exposure. This attenuation was due to reductions in bioavailable 5-MTHF and/or UVB-induced increases in ROS. The effects of UVB on NO-mediated vasodilation were

independent of skin pigmentation, suggesting that a dark constitutive skin color does not play a protective role in these outcome measures after acute exposure to UVB. Further investigation is warranted to better understand the role of UV radiation on vascular function, as well as the impact of various interventions on this response.

FUNDING

This research was supported by the PSU Center for Human Evolution and Diversity Research Endowment Grant (WLK) and NIH T-32 Grant #5T32AG049676-03 (STW).

DISCLOSURES

No conflicts of interest are declared by the authors.

ACKNOWLEDGMENTS

We are grateful for the subjects' participation and the assistance of Jane Pierzga, MS, and Susan Slimak, RN.

AUTHOR CONTRIBUTIONS

S.T. Wolf, data collection, analysis, interpretation, and manuscript preparation; A.E. Stanhewicz, research conception and design, data collection, data interpretation, and manuscript preparation; N.G. Jablonski, research conception, anthropological input, and manuscript preparation; W. L. Kenney, research conception and design, data interpretation, manuscript preparation. All authors approved the final version of the manuscript. All laboratory work was conducted at Pennsylvania State University.

Chapter 4

SUNSCREEN OR SIMULATED SWEAT MINIMIZES THE IMPACT OF ACUTE ULTRAVIOLET RADIATION ON CUTANEOUS MICROVASCULAR FUNCTION IN HEALTHY HUMANS

INTRODUCTION

Regular exercise is one of the most effective ways to reduce the risk of cardiovascular (191) and all-cause mortality (192), including many forms of cancer (193); however, for those who choose to perform regular physical activity outdoors, the potential exists for ultraviolet radiation (UVR)-related health risks (193). Indeed, athletes who compete in outdoor sports are at increased risk for melanoma and non-melanoma skin cancers (194). Participation in outdoor physical activities such as tennis, hiking, or running, as well as recreational activities such as beach-going or tourism during summer months, may involve daily UVR exposures of 2 to 14.6 standard erythema doses (SED; a standardized measure of the UV dose associated with reddening of the skin)(133), increasing the risk of sunburn (125). Hourly UVR exposures of 2 to 6 SED have been demonstrated in professional cyclists (130) and ironman triathletes (131) who compete in events that may range from 4 – 10 hours in duration. In addition to the traditional athlete, tactical athletes such as military personnel regularly spend extended periods of time outdoors in the sun during training exercises and deployments, contributing to repeated and/or long-term exposures to potentially harmful UVR. As such, improved understanding of the manifold health effects of UVR exposure and sun safety practices is imperative.

In addition to its well-described carcinogenic effects, UVR may also negatively affect other aspects of skin health, including cutaneous microvascular function. Doses of ultraviolet-B (UV-B; wavelength = 280-315 nm) radiation sufficient to elicit an erythema, or skin reddening

response result in a corresponding inflammatory response in the cutaneous microvasculature, resulting in increased blood flow (5, 118). These two responses are related to time after UVR exposure and intensity of the exposure, and may last 18 hours or longer. However, the time courses of these distinct responses have not been explored after broad-spectrum UVR exposure. We therefore examined the separate time courses of skin erythema and blood flow responses for 8 h after UVR exposure, and the efficacy of sunscreen in mitigating these responses (*study 1*). We hypothesized that erythema would increase over the 8-h post-exposure time period, and that there would be a corresponding increase in cutaneous blood flow that would track the erythema response. Additionally, we hypothesized that the presence of sunscreen during UVR exposure would blunt both responses post-exposure.

Separate from the inflammatory blood flow response after UVR exposure, sub-erythemal doses of UVR may elicit deleterious effects on cutaneous microvascular function. Recent work from our lab suggests that acute exposure to UV-B radiation reduces NO-dependent cutaneous microvascular vasodilation in response to local heating, and that this deleterious effect is related to decreased 5-methyltetrahydrofolate (5-MTHF) bioavailability and/or reactive oxidant species (ROS)-mediated mechanisms (104). Vasodilation of the cutaneous microvasculature is an important component of the physiological response to local (174) and whole body heat stress (189) and, in healthy skin, this response is primarily mediated by nitric oxide (NO) (189, 190). As such, NO-mediated dilation reflects endothelial function and serves as a measure of microvascular health (27). Therefore, we conducted a second study (*study 2*) to examine the acute impact of broad-spectrum UVR on NO-mediated vasodilation in the human cutaneous microvasculature, and the influence of simulated sweat or broad-spectrum sunscreen in modulating this response.

5-MTHF is directly degraded by exposure to UV-B, but not ultraviolet-A (UV-A; wavelength = 315-400 nm), radiation *in vitro* (4). However, UV-B radiation may be unable to penetrate the dermis to reach the dermal circulation. Thus, it may be that degradation of 5-MTHF *in vivo* is mediated by UVR-induced increases in reactive oxygen species (ROS) production (9). UV-A radiation is able to penetrate the dermis (4), and may induce production of ROS in the dermal circulation, resulting in depletion of bioavailable 5-MTHF in the cutaneous vasculature. Although NO-dependent vasodilation was attenuated after exposure to UV-B in our previous study (104), it remains unclear whether this response differs after exposure to broad-spectrum UVR, i.e., that which is experienced during sun exposure (UV-A + UV-B; wavelength = 280-400 nm). Further, it is important to better understand the impact of UVR on this response within the context of conditions common to outdoor exercise, i.e., the presence of sweat on the skin and the prior application of sunscreen.

Erythema, or skin-reddening, effects of UVR exposure are exacerbated by the presence of saline or sweat on the skin (194, 195), as evidenced by a reduction in the minimal dose of UVR required to elicit a reddening-response (minimal erythema dose, MED). Conversely, application of broad-spectrum sunscreen to the exposed skin area increases the MED (196) and reduces the risk of melanoma (197). It is currently unknown, however, whether the negative effects of UVR exposure on cutaneous microvascular function are *(1)* exacerbated by the presence of sweat or *(2)* attenuated by the presence of sunscreen on the skin. Therefore, *study 2* was designed to separately examine NO-mediated vasodilation in response to broad-spectrum UVR exposure, and the influence of simulated sweat or broad-spectrum sunscreen in modulating this response. We hypothesized that NO-dependent vasodilation would be reduced after acute exposure to broad-spectrum UVR similar to that previously seen with UV-B exposure (104). We further hypothesized that the presence of simulated sweat during acute exposure to broad-spectrum UVR

would exacerbate, and broad-spectrum sunscreen would attenuate, the reduction in NO-mediated cutaneous vasodilation.

METHODS

Ethical Approval

All protocols were approved by the Institutional Review Board (No. 9720) of The Pennsylvania State University and complied with the guidelines set forth by the Declaration of Helsinki, except for registration in a database. All subjects provided verbal and written consent prior to participation.

Subjects

Men and women aged 18-35 years who were normally active, healthy, non-smokers, free from cardiovascular disease, and not taking any prescription medications with primary or secondary vascular effects were included for participation. All subjects underwent an initial screening which included physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsuburgh, PA). Subjects with rash, skin disease, disorders of pigmentation, known skin allergies, or kidney disease were excluded. In order to limit variability in the response to UVR exposure which may be explained by variation in skin pigment, only subjects with light to moderate skin pigmentation (M-index of 30-49), corresponding to Fitzpatrick skin types I-IV (198, 199), were recruited. Subjects refrained from alcoholic and caffeinated beverages for 12 h and vigorous physical activity for 24 h before each experiment. All experiments were conducted during the fall and winter months.

Study 1: Time Course Responses

All protocols were performed in a thermoneutral environment with the subject in a semi-supine position and the arms supported at heart level. One arm was randomly chosen for instrumentation with two laser-Doppler flowmetry probes, placed on the ventral aspect of the forearm in local heating units (moorLab, Temperature Monitor, SHO2; Moor Instruments, Axminster, UK) to assess local red blood cell flux before, immediately after, and every 2 h through 8 h post-UVR exposure. Local heaters were set to 33°C at all sites and resting blood flow data were acquired for approximately 5 min at each time point. Mean arterial pressure (MAP) was calculated at each time point using an automated blood pressure monitor (CardioCap; GE Healthcare, Milwaukee, WI) at the end of each collection period. Skin erythema index (a.u.; Range: 5-22) was measured by reflectance spectrophotometry at each site (DermaSpectrometer; Cortex Technology, Hadsund, Denmark) at each time point.

After measurement of baseline blood flow, the two sites were traced with permanent marker to ensure proper placement of the laser-Doppler probes at subsequent time points, and then randomly assigned to either (1) 750 mJ/cm^2 (90 sec) UVR exposure alone or (2) with 2 mg/cm^2 broad-spectrum chemical sunscreen (Equate™ Sport SPF-50, Walmart, Bentonville, AR) applied to the skin. This dose of UVR exposure was utilized after pilot testing established that it would be sufficient to elicit a mild erythema response in subjects with light to moderate skin pigments. Immediately after UVR exposure, sunscreen was gently removed from the skin using alcohol pads before post-exposure measurements were made to allow for placement of laser-Doppler probes and to prevent any potential interference with the signal.

Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP. Change in CVC (ΔCVC) from baseline was calculated for each time point and change in erythema index (Δerythema) was similarly calculated for each time point.

***Study 2*: Local Heating**

Three intradermal microdialysis fibers (10 mm, 55-kDa cut-off membrane; CMA, Holliston, MA) were placed into the dermal layer of the ventral aspect of the left forearm for the local delivery of pharmacological agents (29) while a single fiber was placed in the right forearm. Microdialysis sites in the left forearm were randomly assigned to one of three treatments: (1) UVR exposure (450 mJ/cm^2; UV-A + UV-B) only, (2) UVR exposure with 2 mg/cm^2 broad-spectrum chemical sunscreen (Equate™ Sport SPF-50) applied to the skin (200), or (3) UVR exposure with 0.81±0.07 mL simulated sweat solution applied to the skin. The microdialysis site on the right arm served as a non-exposed control site. The dose of UVR exposure utilized for this study was chosen after pilot testing demonstrated that it would not elicit an erythema response in our subject population. The simulated sweat solution contained 64 mmol/L Na^+, 2.1 mmol/L K^+, and 59 mmol/L Cl^-; concentrations similar to those previously demonstrated in human sweat (201). Sunscreen was measured and applied using a 1 ml syringe (BD Syringe, Franklin Lakes, NJ) and spread over a 3 cm^2 area of the skin (6 mg total) around the microdialysis site. Simulated sweat was sprayed on the skin using a 3 cm^2 stencil cutout to prevent overspray, and was weighed before and after each experiment using a microscale (Sartorius, Goettingen, Germany) to determine the amount used. Both sunscreen and simulated sweat were applied over their respective sites 20 minutes before (and simulated sweat was reapplied immediately before) UVR exposure. Sunscreen and simulated sweat were cleaned from the skin using alcohol pads after UVR exposure, prior to beginning the local heating protocol.

Pharmacological agents were dissolved in lactated Ringer's solution just before use, sterilized using syringe microfilters (Acrodisc; Pall, Ann Arbor, MI), and wrapped in foil to prevent degradation due to light exposure. All solutions were perfused through the microdialysis fibers at

a rate of 2 µl/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems, West Lafayette, IN) (29). Local red blood cell flux was measured throughout the protocol directly over each microdialysis site with an integrated laser-Doppler flowmetry probe placed in a local heating unit after UVR exposure (moorLab, Temperature Monitor, SHO2; Moor Instruments, Axminster, UK).

Figure 4-1 presents a schematic representation of the local heating protocol used to quantify NO-mediated vasodilation. After placement of the microdialysis fibers and following a 60-min period for resolution of hyperemia associated with fiber placement, the left arm was exposed to 450 mJ/cm^2 (75 sec) UVR (SolRx 500; SolArc Systems, Minesing, ON). Baseline data were then collected before beginning a standardized local heating protocol (local temperature increased to 42°C at a rate of $0.5°C \cdot 5\ sec^{-1}$) as described previously (29, 174), with lactated Ringer's solution being perfused through all sites. This protocol elicits an initial peak that is mediated by sensory nerves followed by a plateau (174). After ~40 min of local heating and the clear presence of a stable local heating plateau, all microdialysis sites were perfused with 20 mM L-N^G-Nitroarginine methyl ester (L-NAME) at a rate of 2 µl/min to inhibit NO synthase, allowing for quantification of NO-dependent vasodilation as previously described (%NO) (181, 182). Following a stable post-L-NAME plateau, 28 mM sodium nitroprusside (SNP; USP, Rockville, MD) was perfused through all sites and local temperature was increased to 43°C to elicit maximal cutaneous vascular conductance (CVC_{max}) (174, 183).

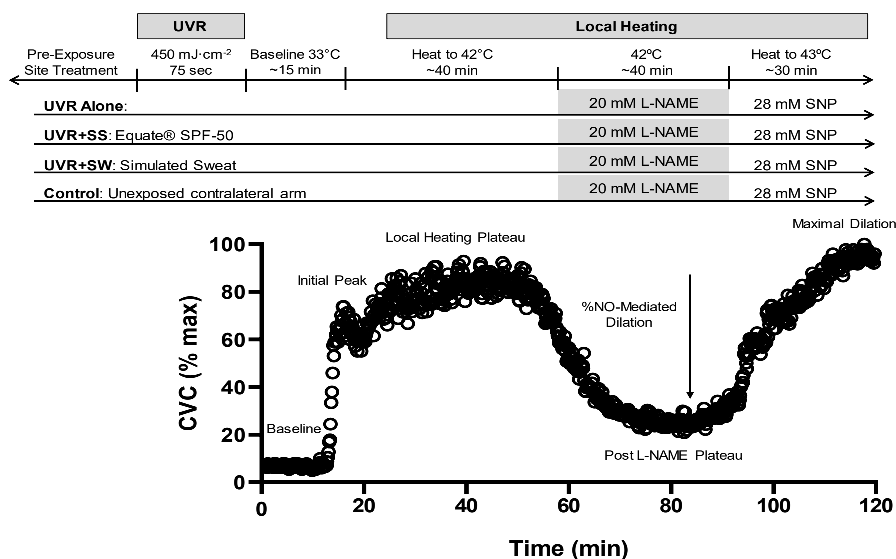

Figure 4-1: Schematic diagram of the local heating protocol (study 2), with a representative trace of cutaneous vascular conductance (CVC; laser-Doppler flux/mean arterial pressure. Before exposure to ultraviolet radiation (UVR), microdialysis sites on the left forearm were randomized for UVR exposure alone, UVR with sunscreen applied to the skin (UVR+SS) or UVR with simulated sweat applied to the skin (UVR+SW). A single site on the right forearm acted as a non-exposed control site. After UVR exposure, each site was locally heated to 42°C to elicit an increase in blood flow. After a local heating plateau was observed, L-NAME was perfused through all sites to inhibit nitric oxide synthase. The arrow indicates the decrease with L-NAME infusion used to calculate the percentage NO-dependent dilation. The experiment concluded with perfusion of sodium nitroprusside (SNP), and local heat was increased to 43°C at all sites to elicit maximal vasodilation.

Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and expressed as a percentage of CVC_{max} for each phase of the local heating protocol ($\%CVC_{max}$) (174, 184). The percent NO (%NO) contribution to cutaneous vasodilation was calculated as the difference between the local heating and L-NAME plateau responses.

Data Acquisition and Statistical Analysis

Data were collected for both studies using Windaq (Windaq; DATAQ Instruments) at a frequency of 40 Hz and stored on a personal computer for subsequent analysis. With an expected effect size

of 10% and standard deviation of 15% for the %NO contribution, based on previous findings (104), we determined *a priori* ($\alpha = 0.05$; power = 0.8) that 14 subjects would be sufficient to detect differences between sites. Two-way repeated-measures ANOVA were used to detect differences between treatment sites at each phase of the local heating protocol (MD site x phase) (SAS v9.4, SAS Institute Inc.). Separate two-way repeated-measures ANOVA (site x time) assessed differences in ΔCVC and Δerythema between sites at each 2-hour time point across the 8 hours after UVR exposure. ΔCVC was regressed against Δerythema for each time point to ascertain whether the two responses are related. Further regression analysis assessed the relation between peak CVC and erythema responses. Segmental regression analyses were performed to compare slopes of the Δerythema and ΔCVC responses after UVR exposure in the UVR only site. Bonferroni *post hoc* corrections were performed to account for multiple comparisons in the ANOVA when necessary and significance was set *a priori* and accepted at α=0.05.

RESULTS

Subject characteristics for each study are presented in **Table 4-1**. All values in the text and table are presented as mean and standard deviation (SD). Baseline characteristics and blood biochemistry values were within normal ranges for this age group. One subject included in *study 1* had elevated cholesterol (TC: 279; LDL: 180), but was otherwise healthy; his responses to UVR exposure did not differ from that of the rest of the sample.

Table 4-1: Subject Characteristics for Each Study

	Study 1		Study 2	
	Mean ± S.D.	Range	Mean ± S.D.	Range
n (M/F)	14 (7/7)		13 (6/7)	
Age (yrs)	24 ± 4	20 – 32	24 ± 4	20-32
BMI (kg·m^{-2})	24 ± 3	20 – 29	25 ± 2	23 – 31
Systolic BP (mmHg)	115 ± 10	96 – 126	113 ± 9	98 – 126
Diastolic BP (mmHg)	73 ± 8	58 – 86	72 ± 9	58 – 86
Heart rate (beats·min^{-1})	63 ± 6	54 – 72	69 ± 11	54 – 84
M-Index (a.u.)	36 ± 7	30 – 49	34 ± 5	27 – 46
Blood biochemistry				
HbA1c (%)	4.9 ± 0.3	4.5 – 5.4	4.8 ± 0.3	4.4 – 5.5
Total cholesterol (mg·dl^{-1})	168 ± 38	127 – 279	152 ± 19	127 – 196
HDL (mg·dl^{-1})	58 ± 13	39 – 81	56 ± 13	34 – 81
LDL (mg·dl^{-1})	91 ± 31	60 – 180	80 ± 19	47 - 115

BMI, body mass index; M-Index, a skin-reflectance measure of melanization; HDL, high density lipoprotein; LDL, low density lipoprotein.

Figure 4-2A depicts Δerythema in both sites across the 8 h post-UVR exposure. In the UVR only site, Δerythema began to rise early, within the first 2 h after UVR exposure, and was significantly elevated compared to baseline at 4 h (1.03 ± 1.49, $p = 0.02$), 6 h (1.27 ± 2.09, $p < 0.01$), and 8 h (2.13 ± 1.69, $p < 0.01$) post-exposure. Sunscreen blunted the Δerythema response such that it did not differ from baseline at any time point ($p \geq 0.06$). Further, Δerythema was significantly lower at the sunscreen site compared to UVR at 8 h post-exposure (2.06 ± 1.74 vs 0.55 ± 0.85, $p < 0.01$). The slope of the increase in Δerythema over time in the UVR only site was linear and significantly different from zero across all time points after UVR exposure ($R^2 \geq 0.20$, p < 0.01).

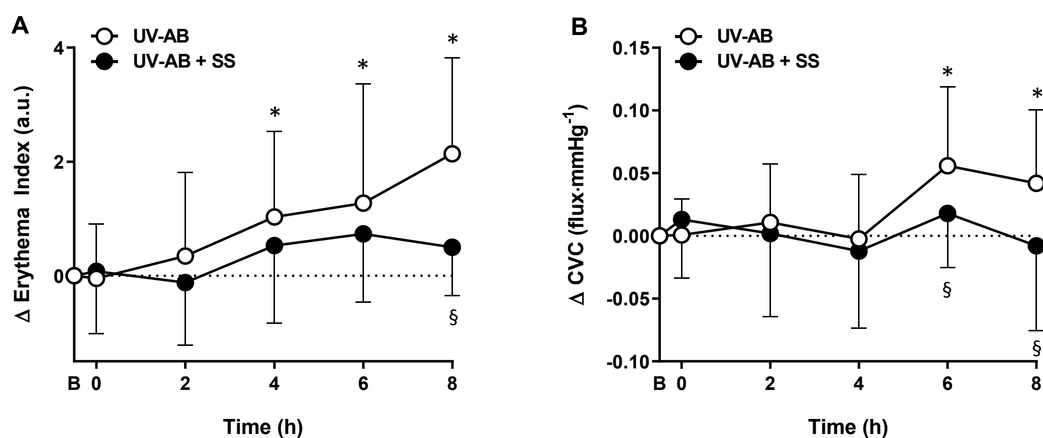

Figure 4-2: Changes (Δ) in the erythema index (left) and cutaneous vascular conductance (CVC; right) after exposure to ultraviolet radiation (UV-AB) only (open circles) or UV-AB with sunscreen applied to the skin (filled circles). Values are means ± SD. *P < 0.05 compared with baseline; §P < 0.05 compared with UV-AB only; n = 14

Figure 4-2B illustrates the ΔCVC responses for both sites across the 8 h post-exposure. In the UVR only site, ΔCVC did not increase over the first 4 h, but increased thereafter (6 h: 0.05 ± 0.06, $p < 0.01$; 8 h: 0.04 ± 0.06, $p = 0.01$) post-exposure. Sunscreen prevented any changes in CVC from baseline ($p \geq 0.29$), and resulted in a significantly lower ΔCVC compared to UVR at 6 h (0.05 ± 0.06 vs 0.01 ± 0.04, $p = 0.02$) and 8 h (0.04 ± 0.06 vs -0.02 ± 0.06, $p < 0.01$) post-exposure. The slope of the increase in ΔCVC over time in the UVR only site was different from zero only between 4 and 6 hours after UVR exposure ($R^2 = 0.22$, p = 0.01).

Importantly, there were no differences between sites in erythema index or CVC at baseline (Erythema: 11.18 ± 5.12 vs. 11.15 ± 4.82, $p = 0.35$; CVC: 0.10 ± 0.05 vs. 0.12 ± 0.02, $p = 0.23$) or immediately after UVR exposure (Erythema: 11.50 ± 5.04 vs. 11.10 ± 4.75, $p = 0.38$; CVC: 0.11 ± 0.07 vs. 0.12 ± 0.02, $p = 0.16$). There was no association between Δerythema and ΔCVC

responses at any time point ($R^2 = 0.002 – 0.07$, $p \geq 0.35$). Similarly, there was no relation between peak erythema and CVC responses ($R^2 = 0.04$, $p = 0.47$).

Figure 4-3 illustrates $\%CVC_{max}$ for all sites at each phase of the local heating protocol in *study 2*. There were no differences between any of the sites during the baseline ($p \geq 0.73$), peak ($p \geq 0.75$), or plateau ($p \geq 0.58$) phases of local heating. UVR exposure resulted in a higher $\%CVC_{max}$ at the L-NAME plateau compared to the non-exposed control site (65.8 ± 22.0 vs 52.9 ± 18.3%, $p = 0.01$), UVR with sunscreen on the skin (65.8 ± 22.0 vs 41.2 ± 19.7%, $p < 0.01$), and UVR with simulated sweat on the skin (65.8 ± 22.0 vs 48.8 ± 19.1%, $p < 0.01$). Further, the $\%CVC_{max}$ during the L-NAME plateau was lower after UVR exposure with sunscreen on the skin compared to the non-exposed control site (41.2 ± 19.7 vs 52.9 ± 18.3%, $p = 0.01$). There were no differences in CVC_{max} across sites ($p \geq 0.97$).

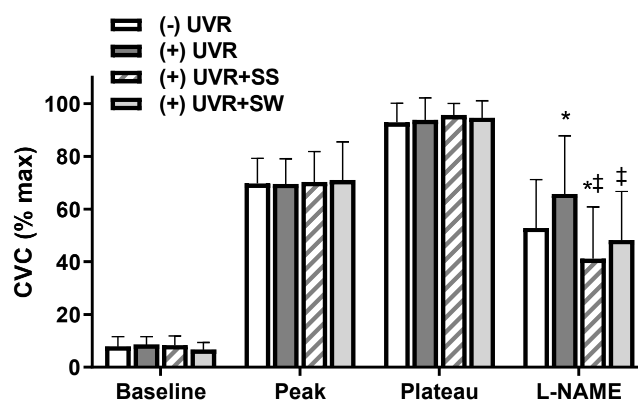

Figure 4-3: Cutaneous vascular conductance (CVC; as a percentage of maximum) at each phase of the local heating protocol in the non-exposed control site (−UVR; white bar) or after UVR exposure alone (+UVR; dark grey bar), with sunscreen on the skin (+UVR+SS; hashed bar) or with simulated sweat on the skin (+UVR+SW; light grey bar). Values are shown as means + SD. *P < 0.05 compared with −UVR; ‡P < 0.05 compared with +UVR; n = 13

The %NO contribution to the dilatory response to local heating for each site is presented in **Figure 4-4**. UVR exposure alone significantly attenuated %NO compared to the non-exposed control site (28.0 ± 23.9 vs 40.8 ± 17.3%, $p = 0.01$) (Panel A). Sunscreen on the skin during UVR prevented the reduction in %NO-mediated vasodilation (28.0 ± 23.9 vs 54.5 ± 20.8%, $p < 0.01$) (Panel B). Simulated sweat on the skin similarly prevented the reduction in %NO-mediated vasodilation after UVR exposure (28.0 ± 23.9 vs 45.8 ± 19.4%, $p < 0.01$) (Panel C), but was not different from the non-exposed control site (40.8 ± 17.3vs 45.8 ± 19.4%, $p = 0.34$). Additionally, when sunscreen was on the skin, UVR augmented %NO-mediated vasodilation such that it was significantly improved compared to the non-exposed control site (54.5 ± 20.8 vs 40.8 ± 17.3%, $p < 0.01$).

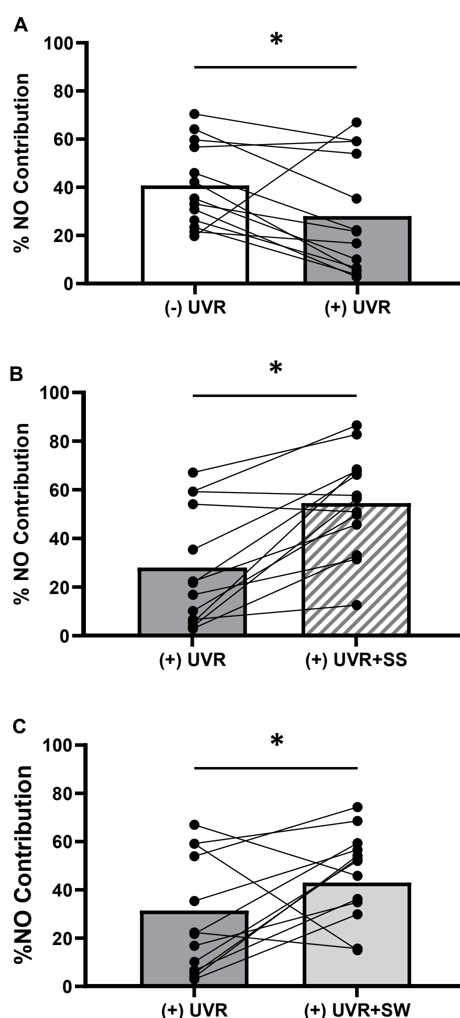

Figure 4-4: Differences in the percentage contribution of nitric oxide (NO) to the local heating plateau in the non-exposed control site (−UVR; white bar) versus UVR exposure alone (+UVR; dark grey bar) (a), UVR exposure alone (+UVR; dark grey bar) versus UVR with sunscreen on the skin (+UVR+SS; hatched bar) (b) or UVR exposure alone (+UVR; dark grey bar) versus UVR with simulated sweat on the skin (+UVR+SW; light grey bar) (c). Data are presented as means, with individual responses illustrated by connecting lines. *P < 0.05 between treatments; n = 13

DISCUSSION

The primary findings of these two studies were that (1) an erythemal dose of UVR elicited a linear increase in erythema beginning in the first 2 hours post-exposure, with a delayed and

unassociated increase in skin blood flow beginning between 4 and 6 hours post-exposure, and (2) acute exposure of the skin to a sub-erythemal dose of broad-spectrum UVR attenuated NO-mediated vasodilation in the cutaneous microvasculature in response to a local heating stimulus, (3) broad-spectrum SPF-50 sunscreen prevented the deleterious effects of UVR in both studies, (4) UVR augmented NO-mediated vasodilation during local heating when sunscreen was applied to the skin prior to exposure, and (5) contrary to our hypothesis, simulated sweat on the skin protected against the acute UVR-induced attenuation in NO-mediated vasodilation.

Investigation of the time-course of skin erythema and skin blood flow responses after exposure to an erythemal dose of UVR (*study 1*) revealed that the erythema response began soon after the exposure and increased linearly over the 8 h of tracking, whereas blood flow did not begin to increase until more than 4-h post-exposure. This descriptive study was performed to clarify that the erythema and blood flow changes were temporally distinct, and that vasodilatory responses did not simply reflect skin surface reddening. This lack of association was confirmed by regression analysis which showed no significant relation between changes in erythema or blood flow at any time point, or between the peak erythema and blood flow responses. An erythemal dose of UV-B exposure was previously reported to result in a five-fold increase in skin blood flow by 18 h post-exposure (5). In that study, the skin blood flow responses were blunted by injection of indomethacin and/or L-NMMA, suggesting that increases in skin blood flow are mediated by inflammation-induced increases in inducible NOS and/or cyclooxygenase (5). Although the current study did not investigate mechanisms mediating the blood flow responses to acute UVR exposure, it is likely that the responses were similar to those demonstrated previously (5, 118). As expected, application of sunscreen prior to UVR exposure blunted both the erythema and blood flow responses. Further research is needed to better characterize the mechanisms which mediate these delayed increases in inflammatory vasodilation after broad spectrum UVR

exposure of varying intensities, and how repeated or chronic exposure to UVR may influence microvascular health and function.

After investigating the time course of the erythema and cutaneous microvascular responses to UVR exposure in *study 1*, we mechanistically examined the impact of a sub-erythemal dose of UVR on cutaneous microvascular function during local skin heating to demonstrate microvascular effects of UVR exposure that are independent of skin erythema. The local heating data after UV-A+UV-B exposure are in qualitative and quantitative agreement with our previous study demonstrating an acute reduction in NO-mediated vasodilation after exposure to UV-B radiation (104). Reductions in NO-dependent vasodilation after acute UVR exposure are mediated either by direct UVR-induced degradation of 5-MTHF or indirect degradation via production of ROS. Previously, we demonstrated that direct perfusion of 5-MTHF or the non-specific antioxidant ascorbate restored NO-dependent vasodilation of the cutaneous microvasculature after UV-B exposure, suggesting that the attenuation in NO-dependent dilation was due, at least in part, to ROS-induced reductions in 5-MTHF bioavailability (104). UV-B radiation directly degrades 5-MTHF *in vitro* while UV-A radiation does not (4), although either UV-A or UV-B radiation may indirectly degrade 5-MTHF by increasing ROS production. In addition to the potential effects of UVR on 5-MTHF, oxidative stress associated with UVR exposure may blunt NO-mediated vasodilation by destabilizing the NOS dimer or via direct quenching of bioavailable NO (97). Together, these findings suggest that the attenuation in NO-mediated vasodilation are predominantly mediated by UV-B or that larger doses of UV-A are required to elicit the same effect.

Although acute broad spectrum UVR exposure attenuated the NO contribution to the dilatory response, no differences were observed in the baseline or initial peak responses to local heating,

implying no effect of acute UVR on baseline blood flow or sensory nerve (axon reflex) function. Likewise, there was no difference in the magnitude of the sustained heating plateau response between radiated and non-exposed sites. This is likely explained by compensatory effects of redundant dilator pathways, such as endothelium-derived hyperpolarizing factors (186), which may be upregulated in response to acute reductions in NO bioavailability in young, healthy subjects (29). Our lab has previously shown similar findings after acute UV-B radiation exposure (104), as well as in middle-aged adults in whom the overall magnitude of the response to local heating is preserved but NO-dependent dilation is attenuated (29). Importantly, redundant dilator pathways are diminished in aging adults (35) or those with pathologies such as hypertension or hypercholesterolemia (36, 185). Because NO is a critical molecule for maintaining vascular health, these effects of UVR exposure on NO bioavailability may pose a risk for future cutaneous microvascular dysfunction. The important role of NO in vascular health is outside the scope of the present study, but has been previously highlighted in several papers (202, 203).

Individuals who work or choose to exercise outdoors may experience UVR exposure at doses similar to that of our study. The UVR exposures used in our studies were equivalent to approximately 4.5 and 2.7 SED, respectively. Previous studies utilizing personal UVR dosimeters carried by exercising individuals demonstrated daily UVR exposures ranging from 2 to 15 SED in runners, hikers, and tennis players (133), and hourly exposures of 2 to 6 SED in triathletes and cyclists during competition (130, 131). Further, risk of sunburn was assessed in 7,802 adults aged 18-74 years in Queensland, Australia, and those who regularly participated in outdoor physical activity were disproportionately more likely to have experienced sunburn over the previous weekend or 12-month period (125). Importantly, and by design, no skin erythema was observed after UVR exposure in any of the subjects during or after *study 2*, suggesting that the deleterious effects of UVR can occur in the cutaneous microvasculature even in the absence of skin

reddening or sunburn. Additionally, the combined results of *study 1* and *study 2* confirm that the effects of UVR on NO-mediated vasodilation during local heating were neither a function of altered baseline cutaneous blood flow nor dependent on skin erythema.

Sun safety is likewise important for the military population as soldiers and sailors spend extended periods of time in the sun during training exercises and deployments. In that regard, sun safety is included as part of overall guidance for preventing environmental casualties in the U.S. Army (TRADOC 350-29) (204). The importance of sun protection may go unnoticed, perhaps in part due to the regulation requiring soldiers to be in proper long sleeves uniform attire (205). However, during times of high environmental heat loads, and based on wet-bulb globe temperature (WBGT) criteria, soldiers are permitted to roll their sleeves above the elbow or de-blouse, enhancing evaporative heat loss but exposing skin to UVR (206). The Army also provides written guidance to unit commanders as part of the general category of preventing environmental casualties to use sunscreen with an SPF rating of at least 15 (204, 205) and to "ensure Soldiers maintain their supply of sunscreen and apply it daily when needed" (204). Those recommendations are particularly relevant with regard to the results presented here, as soldiers often train and continue to deploy in harsh environments with a high UV index, and further highlight the importance of sun protection for this population.

We chose Equate™ Sport broad-spectrum, SPF-50 sunscreen in the current study because it was a top-rated sunscreen by *Consumer Reports* and because additional sun protection provided by sunscreens with SPF >50 is equivocal when applied properly at 2 mg/cm^2 (153). Broad-spectrum SPF-50 sunscreen applied to the skin prior to UVR exposure protected against erythema and blood flow responses in *study 1*, as well as UVR-induced reductions in NO-mediated vasodilation in *study 2*. These findings parallel previous studies that demonstrated that sunscreen increases the

dose of UVR required to elicit an erythema response (196). Ad libitum sunscreen application is often less than the 2 mg/cm^2 required to receive the advertised sun protection factor. In a sample of fifty individuals, ad libitum sunscreen application was approximately 1 mg/cm^2 (207), thus providing only 50% of the protection which would be afforded with proper application. Therefore, it is important that those who are exposed to repeated and/or prolonged sun exposure practice proper application of sunscreen to prevent the deleterious effects of UVR.

Interestingly, NO-mediated vasodilation was not only preserved in the microvasculature after UVR exposure to sunscreen-coated skin, but was enhanced compared to the non-exposed control site. Although speculative, it may be that UVR-exposure with sunscreen on the skin protected against UVR-induced production of ROS, but allowed for the biosynthesis of vitamin D, which may play a role in NO production (19). Importantly, the sunscreen applied to the skin in the present study was a chemical-based sunscreen as opposed to mineral-based. Chemical-based sunscreens absorb UVR, whereas mineral-based sunscreens, such as zinc oxide, reflect UVR (208). As such, absorption of UVB by the sunscreen used in the present study may have allowed for UV-B-induced synthesis of vitamin D while simultaneously protecting from the deleterious effects of UVR. Past studies have suggested that vitamin D may improve endothelial function by signaling for the transcription of endothelial NOS (19) and/or by attenuating inflammation-linked endothelial dysfunction (16). Further, increased vitamin D synthesis has been demonstrated in keratinocytes one hour after exposure to UV-B radiation (209), although the impact of such UVR-induced vitamin D synthesis on vascular function is unknown. These findings necessitate further investigation to better understand the relation between UVR exposure, 5-MTHF bioavailability and vitamin D metabolism, and endothelial function.

We hypothesized that simulated sweat on the skin during UVR exposure would exacerbate the UVR-induced reductions in NO-mediated vasodilation as it does with regard to skin erythema (210-212). A reduction in MED was previously reported in subjects for whom sweating was induced either by sitting in a sauna for 10 min or by jogging for 15 min (210). Other studies have demonstrated a reduction in MED after immersion of the skin in salt water baths (211, 212). The putative mechanism by which the reduction in MED occurs is via increased hydration of the horny layer of the skin, which results in attenuated dispersion of UVR. In an attempt to recapitulate this effect, we applied a simulated sweat solution to the skin 20 min prior to, and again immediately before, UVR exposure. However, we found that NO-mediated vasodilation was preserved in our simulated sweat condition such that it was not different from non-exposed skin. It may be that the amount of simulated sweat applied to the skin was inadequate to elicit erythema effects as shown in previous studies -- there was no erythema of the skin observed in the simulated sweat site for any of the subjects, suggesting that the simulated sweat likely did not have a sensitizing effect -- but rather its presence on the skin allowed for reflection and refraction of UVR, thereby reducing the intensity of UVR reaching the skin and skin vasculature. Future investigation should focus on the influence of UVR on NO-mediated vasodilation in subjects for whom sweating has been initiated by, and maintained during, physiological stimuli such as heat stress or exercise.

Limitations

Our measurements of NO-mediated vasodilation were made immediately after exposure of the forearm to broad spectrum UVR, allowing us to examine the acute effects of UVR exposure on microvascular function. Whether there is a prolonged effect of UVR on NO-dependent dilation, or whether repeated exposures may result in chronic dysfunction, remain to be elucidated. Importantly, we included only subjects who were lightly- to moderately-pigmented in the current

studies in order to better standardize the UVR exposure and limit inherent variability that may be explained by pigmentation. However, our previous study (104) examined the influence of UV-B radiation exposure on a wide range of skin pigments and found no role of pigmentation in modulating the effects of UV-B exposure on NO-mediated vasodilation.

UVR exposure in the present studies were local to the ventral forearm, and therefore we cannot speculate as to how vascular function may be affected by whole-body exposure. Further, the ventral aspect of the forearm receives relatively less sun exposure compared to the lateral surface. As such, the lateral surface of the forearm would likely require a larger dose of UVR to elicit the responses presented herein due to increased absorption of UVR by melanocytes (213), although we did not investigate these effects. Lastly, individuals who exercise outdoors often have both sweat and sunscreen on the skin. We did not examine the effects of combined sweat and sunscreen on the skin, and it is therefore unclear how the observed responses may have differed with a combination of sweat and sunscreen on the skin during UVR exposure.

Conclusions

These results demonstrate differential increases in erythema and skin blood flow in the first eight hours after UVR exposure, characterized by an immediate and linear increase in erythema but a delayed increase in skin blood flow. Additionally, these data affirm our previous findings that acute exposure of the skin to UVR attenuates NO-mediated vasodilation in the cutaneous microvasculature, and extends those findings to broad spectrum (UV-A+UV-B) exposure more comparable to sun exposure. Application of sunscreen to the skin before UVR exposure protected against both erythema and inflammatory vascular effects, and simulated sweat protected against UVR-induced reductions in NO-mediated vasodilation. Unexpectedly, NO-dependent vasodilation was greater after UVR exposure when sunscreen was on the skin compared to the

non-exposed control. These results provide novel insight into the impact of UVR exposure on cutaneous microvascular function, demonstrating detrimental effects of UVR which are separate from skin erythema, and add to an existing body of research demonstrating the importance of sun protection for those who work or exercise outdoors. Future investigation is needed to improve our understanding of the balance between 5-MTHF bioavailability and vitamin D metabolism in response to UVR exposure, as well as the potential role of inflammation, and how these factors may influence vascular function and health.

ACKNOWLEDGEMENTS

We are grateful for the subjects' participation and the expert assistance of Jane Pierzga, M.S. and Susan Slimak, RN.

COMPETING INTERESTS

No conflicts of interest, financial or otherwise, are declared by the authors. The opinions or assertions contained herein are the private views of the authors and should not be construed as official or reflecting the views of the Pennsylvania State University, the Army, the Department of Defense, or the American College of Sports Medicine. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

AUTHOR CONTRIBUTIONS

A.E.S., S.B.F., and W.L.K. conceived and designed research; S.T.W., C.W.B., and A.E.S. performed experiments; S.T.W. and C.W.B. analyzed data; S.T.W., A.E.S., C.W.B. and W.L.K.

interpreted results of experiments; S.T.W. and C.W.B. prepared figures; S.T.W., L.E.K., and W.L.K. drafted manuscript; S.T.W., A.E.S., L.E.K., and W.L.K. edited and revised manuscript; S.T.W., C.W.B., A.E.S., S.B.F., L.E.K., and W.L.K. approved final version of manuscript.

GRANTS

This research was supported by an American College of Sports Medicine Foundation Doctoral Student Research Grant, NIH T-32 Grant #5T-32-AG-049676, and the Penn State Dermatology Marks Endowment Fund.

Chapter 5

FOUR WEEKS OF VITAMIN D SUPPLEMENTATION IMPROVES NITRIC OXIDE-MEDIATED MICROVASCULAR FUNCTION IN COLLEGE-AGED AFRICAN AMERICANS

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States, and darkly-pigmented African Americans (AA) experience a disproportionate burden compared to lightly-pigmented European Americans (EA) (84). Further, 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 (88, 214). Attenuated vascular (flow-mediated dilation; FMD) and microvascular (local skin heating) responses have been demonstrated in otherwise healthy AA adults (39, 40, 215), 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 (93-95). Vitamin D is produced from 7-dehydrocholesterol in the skin upon ultraviolet (UV)-B light exposure from the sun (216). 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 (217, 218). Further, 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 (24, 62). These factors combine to place adults with darker pigmentation at elevated risk for vitamin D deficiency (219). 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 (16, 19). Additionally, vitamin D has been shown to increase superoxide dismutase activity (17) and decrease nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase)-induced production of superoxide radicals (90, 91), which may attenuate oxidative stress-mediated endothelial dysfunction. Taken together, these findings suggest a potential link between vitamin D deficiency, oxidative stress, and reduced NO-mediated vasodilation in AA compared to EA.

Reduced cutaneous microvascular vasodilation in response to local heating, as well as a reduced nitric oxide (NO) contribution to that response, have been demonstrated in otherwise healthy college-aged AA compared to EA adults (39, 40, 86). Both components (i.e., the magnitude of vasodilation and the NO contribution) of the local heating response were improved in AA, but not EA, by inhibition of superoxide radicals with the superoxide dismutase (SOD) mimetic, tempol, such that there were no longer differences between groups (40). Inhibition of NADPH oxidase with apocynin similarly improved microvascular function during local heating in AA men, but not women (39). Together, the results of those studies suggest that attenuated microvascular responses observed in AA compared to EA 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 AA 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 (39, 40), 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, since 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 (21, 219), and in turn may indirectly impact endothelial function. We hypothesized that (1) serum [25(OH)D] and microvascular responses to local heating would be reduced in AA compared to EA prior to vitamin D supplementation, (2) four weeks of 2,000 IU per day vitamin D supplementation would increase serum [25(OH)D] and augment local heating responses and %NO in AA, but not EA, and (3) differences in microvascular responses between AA and EA 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 (5M/5 F), AA = 8 (4 M/4 F)], aged 18-30 years, with normal blood pressure (SBP <130 and DBP <85 mmHg), low density lipoprotein cholesterol <150mg/dl, and HbA1C <6.5% were included. Subjects were normally active, healthy nonsmokers who were free of cardiovascular, kidney, or skin diseases, disorders of pigmentation, 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 the cycle. Five of the nine women included were taking oral contraceptives (EA = 4, AA = 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 (10 EA, 6 AA) provided saliva samples for DNA ancestry analysis (23andMe, Sunnyvale, CA, U.S.A.). All subjects underwent an initial screening that included physical examination, lipid profile, and blood chemistry (Quest Diagnostics, Pittsburgh, PA, U.S.A.). 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 (40), we determined a priori ($\alpha = 0.05$; power = 0.8) that 8 subjects per group would be sufficient to detect within- and between-group differences in the %NO contribution to the local heating response. Due to COVID-19 considerations, only 7 AA and 6 EA participants completed the post-supplementation visit. However, given the large effect size (see results) observed in the current study for %NO at the control site from pre- to post-supplementation in the AA group, a post-hoc power analysis suggested that 6 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 (104), 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 (179).

Experimental Procedures

All protocols were performed in a thermoneutral laboratory with the subject in a semi-supine position and both arms supported at heart level. Testing was conducted before and after 4 weeks 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] (220). Three intradermal microdialysis fibers (10-mm, 20-kDa cut-off membrane, MD 2000; Bioanalytical Systems, West Lafayette, IN, U.S.A.) were placed into the dermal layer of the ventral aspect of the left forearm for local delivery of pharmacological agents (29). Fibers were randomized for one of three treatments; 1) lactated Ringer's (control), 2) 10 μM tempol (superoxide dismutase mimetic; Sigma-Aldrich, St. Louis, MO, U.S.A.), or 3) 100 μM apocynin (NADPH oxidase inhibitor; Tocris Bioscience, Bristol, U.K.). Pharmacological agents were mixed just before use, dissolved in lactated Ringer's solution, sterilized using syringe microfilters (Acrodisc; Pall, Port Washington, NY, U.S.A.), 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) (29). 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, U.S.A.).

After placement of microdialysis fibers, a ~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 (33, 40). 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 of L-N^G-Nitroarginine methyl ester (L-NAME; NO synthase inhibitor) was perfused through all sites, allowing for quantification of NO-dependent vasodilation (%NO) (181, 182). After observing a stable L-NAME plateau, 28 mM

sodium nitroprusside (SNP; USP, Rockland, MD, U.S.A.) was perfused through all sites and local temperature was increased to 43°C to elicit maximal vasodilation (174, 183).

Vitamin D Analysis

Blood samples were collected in serum separator tubes during pre- 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 manufacturer's instructions (CrystalChem, Elk Grove Village, IL, U.S.A.).

Socioeconomic Status

Subjects responded to a ten-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 (221-223). Participants' childhood SES was measured using four indicators: 1) father's highest level of education (0 = < high school, 1 = high school/GED, 2 = some college and above), 2) mother's highest level of education (0 = < high school, 1 = high school/GED, 2 = some college and above), 3) 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 4) 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 in welfare). Adult SES was measured using six indicators: 1) highest level of education (0 = < high school, 1 = high school/GED, 2 = some college and above), 2) the participant's perceived standing within their community (0 = worst, 1 = average, 2 = best), 3) current financial situation (0 = worst, 1 = average, 2 = best) 4) control over financial situation (0 = worst, 1 = average, 2 = best), 5) availability of money to meet

basic needs (0 = not enough money, 1 = just enough money, 2 = more money than need), and 6) difficulty paying bills (0 = very/somewhat difficult, 1 = not very difficult, 2 = not at all difficult). Participant responses 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 (Windaq; DATAQ Instruments, Akron, OH, U.S.A.) 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, U.S.A.). Cutaneous vascular conductance was calculated as red blood cell flux divided by MAP and expressed as a percentage of CVC_{max} ($\%CVC_{max}$) for each phase of the local heating protocol (174, 184). 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, U.S.A.) three-way, repeated-measures ANOVA to evaluate group (AA vs. EA), local pharmacological delivery site (lactated Ringer's vs. tempol vs. apocynin), and time (pre- vs. post-supplementation) effects. Serum 25(OH)D concentrations were analyzed using two-way repeated-measures ANOVA (group x 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 (224), 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 mean ± standard deviation, except in figures 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 5-1**. All characteristics and blood biochemistry values were within normal limits. By design, M-index was significantly higher in AA compared to EA ($p < 0.001$; $g = 5.92$). There were otherwise no differences between groups for any characteristic.

Table 5-1. Participant characteristics (mean ± S.D.)

	European American	African American
n (M/F)	10 (5/5)	8 (4/4)
Age (yrs)	22 ± 2	20 ± 2
BMI ($kg \cdot m^{-2}$)	24 ± 2	25 ± 3
Systolic BP (mmHg)	113 ± 5	113 ± 6
Diastolic BP (mmHg)	72 ± 6	68 ± 11
Heart rate ($beats \cdot min^{-1}$)	64 ± 8	64 ± 5
M-Index	33 ± 3	65 ± 7*
Blood biochemistry		
HbA1c (%)	5.1 ± 0.2	5.3 ± 0.3
Total cholesterol ($mg \cdot dl^{-1}$)	168 ± 41	151 ± 24
HDL ($mg \cdot dl^{-1}$)	63 ± 16	60 ± 12
LDL ($mg \cdot dl^{-1}$)	94 ± 28	78 ± 12

n = no. of participants. a.u., arbitrary units; 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. Values are mean ± S.D. * $P < 0.05$ compared to European American.

Figure 5-1 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 5-2**. Adult (p = 0.03), childhood (p = 0.02), and lifetime (p = 0.001) SES were significantly lower in the AA cohort compared to EA.

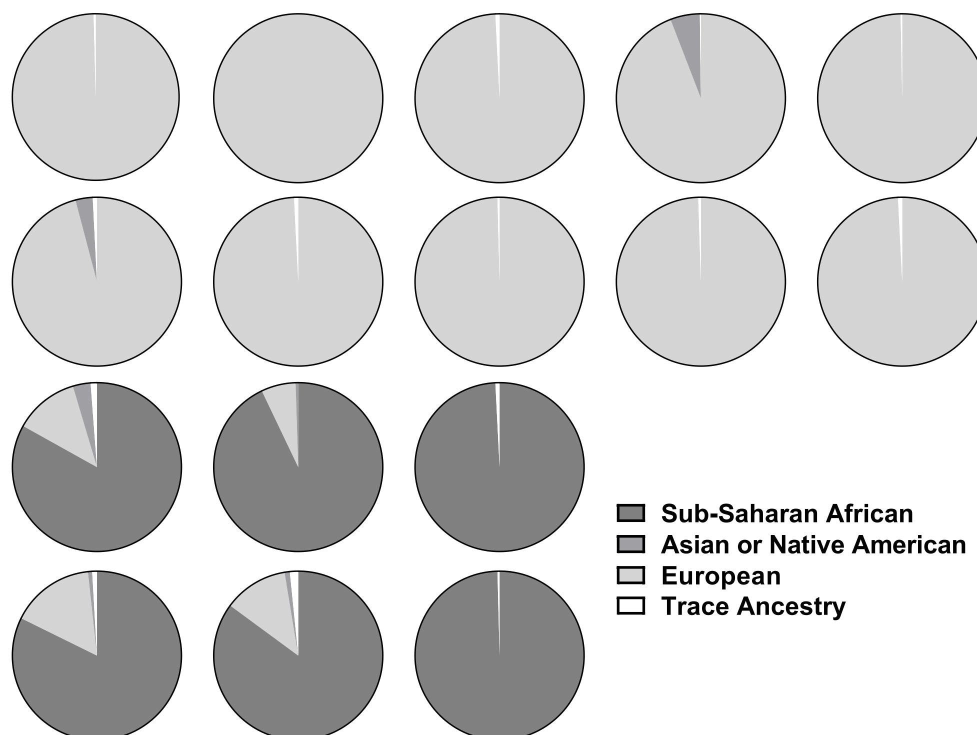

Figure 5-1. Individual subject pie charts depicting ancestry analysis for subjects of European (n = 10) and African (n = 6) American ancestry.

Table 5-2. Socioeconomic status (mean ± S.D.)

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

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

Serum 25(OH)D concentrations are depicted in **Figure 5-2**. Concentrations of 25(OH)D were significantly lower in AA compared to EA pre-supplementation (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 AA (from 17.93 ± 5.24 to 26.07 ± 3.73 ng/mL, $p = 0.04$; $g = 1.66$), but not EA ($p = 0.16$); a smaller but significant difference between groups remained post-supplementation (37.46 ± 8.19 vs. 26.07 ± 3.73 ng/mL, $p = 0.03$; $g = 1.72$).

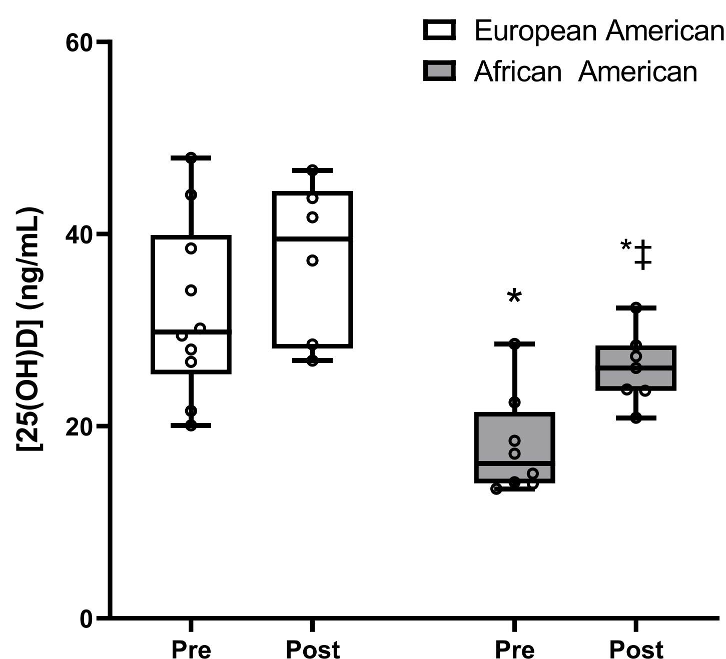

Figure 5-2: Serum vitamin D concentrations for European American (EA; blue bars) and African American (AA; red bars) subjects before (Pre: EA, $n = 10$; AA, $n = 8$) and after (Post: EA, $n = 6$; AA, $n = 7$) vitamin D supplementation. * $P < 0.05$ compared to European American at the same time point; ‡ $P < 0.05$ compared to pre-vitamin D supplementation.

Table 5-3 presents baseline $\%CVC_{max}$ and maximal CVC values for both groups across all sites pre- 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 pre- to post-supplementation.

Table 5-3. Baseline (%CVC_{max}) and maximal CVC (flux · $mmHg^{-1}$) values (mean ± S.D.)

			European American	African American
Baseline				
	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				
	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

CVC, cutaneous vascular conductance.

Figures 5-3A and 5-3B depict the initial axon reflex-mediated peak (%CVC_{max}) for EA and AA before and after vitamin D supplementation, respectively. The magnitude of this response was

lower in AA compared to EA in the control (lactated Ringer's) site (41.46 ± 12.28 vs. 53.12 ± 14.26 %max, $p < 0.03$; $g = 0.83$) prior to vitamin D supplementation. Neither tempol ($p = 0.07$) nor apocynin ($p = 0.08$) significantly improved the initial peak response in AA, although both minimized the difference between groups ($p \geq 0.74$). There were no differences among the 3 sites in EA ($p \geq 0.77$). The initial peak response was improved in AA at the control site post-supplementation such that there was no longer a difference between groups ($p = 0.08$), although the response was not significantly different from pre-supplementation ($p = 0.23$). 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 pre- to post-supplementation ($p \geq 0.42$).

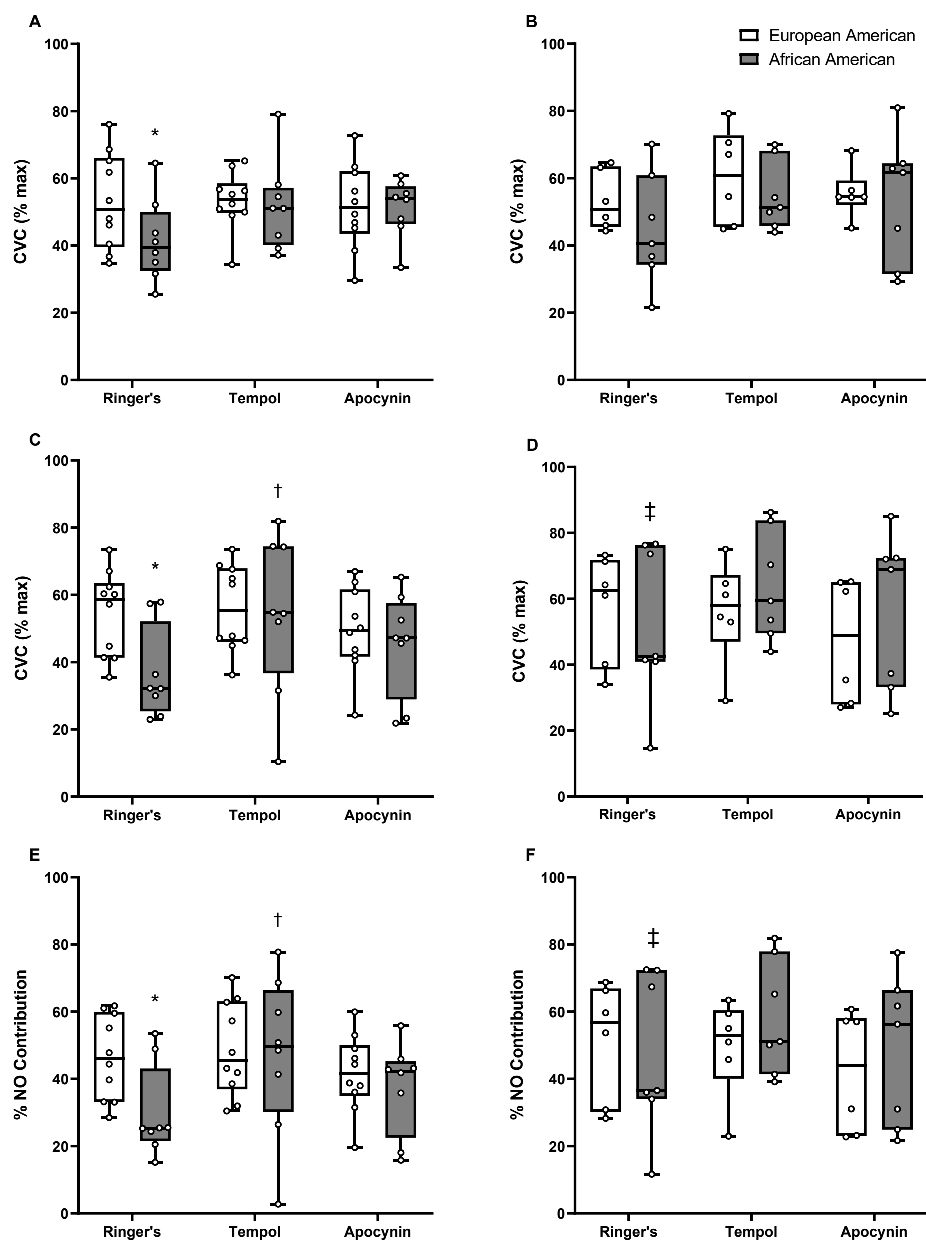

Figure 5-3: Cutaneous vascular conductance (%CVC_{max}) responses for the axon reflex-mediated initial peak (Panels A and B) and subsequent plateau (Panels C and D) and the percent nitric oxide (%NO) contribution to the plateau response (Panels E and F) for European American (EA; blue bars) and African American (AA; red bars) subjects. Each pair of bars represents responses before (left panels; n = 10 EA, n = 8 AA) and after (right panels; n = 6 EA, n = 7 AA) 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.

Following a similar format to figure 5-3 A and B, **figures 5-3C and 5-3D** illustrate the local heating plateau responses pre- and post-supplementation, respectively. The magnitude of the local heating plateau was attenuated in AA compared to EA at the control site (36.60 ± 13.71 vs. 54.36 ± 12.71 %max, $p = 0.007$; $g = 1.29$). In AA, 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 to the control site. There were no differences between EA and AA 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 AA (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 post-supplementation ($p \geq 0.17$), or from pre- to post-supplementation ($p \geq 0.16$).

Figures 5-3E and 5-3F display the NO contribution to local heating responses for the two groups pre- and post-supplementation. The %NO was blunted in AA compared to EA 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 AA while neither treatment influenced the %NO in EA ($p \geq 0.17$). There was no difference between AA and EA 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 AA from pre- to post-supplementation (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 post-supplementation ($p \geq 0.17$), or from pre- to post-supplementation ($p \geq 0.10$).

DISCUSSION

The principle findings of this study are as follows: 1) serum 25(OH)D concentrations were lower in AA compared to EA, 2) the magnitude of the local heating response, as well as the NO-mediated component of that response, were likewise lower in AA compared to EA, 3) four weeks of 2,000 IU/day oral vitamin D supplementation improved serum 25(OH)D concentrations and mitigated the differences in microvascular responses to local heating between groups, and 4) local delivery of tempol (a superoxide dismutase mimetic), but not apocynin (an NADPH oxidase inhibitor), improved cutaneous microvascular responses to local heating in AA 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 AA contribute disproportionately to those statistics (39, 84). Hypertension, a primary risk factor for CVD, develops at a younger age in AA compared to EA, and vascular endothelial dysfunction -- strongly implicated in the development of hypertension -- is prevalent in healthy young and middle-aged AA even in the absence of hypertension, as evidenced by reduced flow-mediated dilation (FMD) (215) and impaired cutaneous microvascular responses to local skin heating (39, 40, 86). Endothelial dysfunction in this population is 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 (40). Similarly, suppression of NADPH oxidase with apocynin improved microvascular responses to local heating in AA men, but not women (39). Therefore, increased production of superoxide radicals and/or reduced SOD activity appears to play an important role in endothelial dysfunction in the AA population. Our data are consistent

with those of previous studies in that cutaneous microvascular responses to local heating were attenuated in AA compared to EA and that local delivery of tempol improves those responses in AA such that there were no longer differences between groups (39, 40, 86). However, in contrast to previous work (39), 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 AA after four weeks of 2,000 IU/day vitamin D such that there were no longer differences between AA and EA 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 AA 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 (17) and inhibited NADPH oxidase activity in diabetic rats (90) and renal arteries and endothelial cells of hypertensive humans (91). Additionally, vitamin D treatment may upregulate eNOS expression (16, 19), 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 (39, 225), the axon reflex-mediated initial peak was attenuated in AA compared to EA prior to vitamin D supplementation. Vitamin D supplementation mitigated the difference between groups, but did not significantly improve the response in AA. Although

NO is implicated in the axon reflex response, its contribution is modest (31, 225). The relatively small contribution of NO to the initial peak may explain why vitamin D supplementation did not significantly improve the response in AA, 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 (226, 227). Prior to vitamin D supplementation in the current study, five of the EA subjects were sufficient, while the other five were insufficient. By contrast, six of the AA subjects were deficient, two were in the insufficient range, and none were vitamin D sufficient. Although average annual UVB 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 (21). As such, it is possible that many AA living in this region of the U.S. are chronically vitamin D deficient in the absence of supplementation, while EA 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 UVB-induced cutaneous vitamin D synthesis (228) 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 (220), we chose a vitamin D treatment regimen of 4 weeks at 2,000 IU/day. Although vitamin D treatment significantly improved serum 25(OH)D concentrations in AA, vitamin D status in AA was still significantly lower than EA 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 concentrations that are clinically sufficient. Importantly, however, circulating 25(OH)D concentrations in AA were similar post-supplementation to those of EA pre-supplementation (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 considerations 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 (221-223, 229). In the present study, childhood, adulthood, and lifetime SES were lower in the AA group compared to 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 (230, 231), 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 (221-223), 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 (232, 233). The present study, in keeping with previous research (39, 40), 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 (21, 219), 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 ($R^2 = 029$, $p = 0.02$). 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 microvascular endothelial dysfunction 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 AA may improve cutaneous microvascular function, essentially correcting the difference between AA and EA. 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. Further, 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.

ACKNOWLEDGEMENTS

We are grateful for the subjects' participation and the expert assistance of Susan Slimak, RN.

GRANTS

This research was supported by the Penn State Center for Human Evolution and Diversity Research Endowment Grant, NIH T-32 Grant #5T32AG049676-03, and the Penn State College of Health and Human Development Limited Endowed Funds for Dissertation Research.

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 and 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.

Chapter 6

Examining the Place of "Race" in Physiology

INTRODUCTION

Differential risks of developing cardiovascular and/or cerebrovascular disease are often ascribed, at least in part, to membership in a particular race (6, 234). The underlying causative factors for ascribing such differences to "race," however, are not clear and the assumption that race membership is itself a risk factor for disease has been uncritically retained and insufficiently examined. Although important strides have been made toward understanding the underlying physiological mechanisms of racial disparities, there is a critical need for research to better understand the physiological and sociocultural underpinnings of apparent racial differences in cardiovascular and cerebrovascular health. To that end, consideration of the imprecise nature of "race" is warranted. This Perspective briefly (1) addresses the origins of categorizing humans by race and why such categorization is problematic in modern physiology research, and (2) makes recommendations for moving away from categorization by race to more directly address underlying social determinants of health (i.e. conditions in the places where people live, learn, work, etc. that affect a wide range of health and quality-of-life risks and outcomes (235)) and environmental and biological factors that result in disparate cardiovascular and cerebrovascular health outcomes.

HISTORICAL PERSPECTIVES IN RACIAL THEORY

The origins of racial categorization began with the Swedish taxonomist Carl Linnaeus, who first categorized humans into four main groups – *Homo Europaeus albescens*, *Homo Americanus rubescens*, *Homo Asiaticus fuscus*, and *Homo Africanus niger* – according to skin color and geography (236). By 1758, Linnaeus had introduced the binomial nomenclature of *homo sapien*,

and his original four classifications were retained as subspecies, with additional details of physical traits and mental and cultural characteristics (237). In 1777, the natural philosopher Immanuel Kant was the first to describe race as an immutable characteristic (238). In contrast to a "variety," an organism that could revert to a common stock over generations, a "race" was fixed (239). Kant postulated that the four races – "first race," very blonde/northern European; "second race," copper red/American; "third race," black/Senegambia; and "fourth race," olive-yellow/Indian - stemmed from the genus "white brunette," which represented the ideal human (240). Any variation away from this ideal was considered a degeneration from the original genus, resulting from less equable climates. Importantly, Kant suggested that such degradation was reflected not only in skin coloration, but in character or "talent" (239, 240). This served as the basis from which dark skin became associated with inferiority, and practices such as segregation and slave trade were justified (239). Race as a source of differences in humanity, morality, and physical well-being became a deeply ingrained construct that has served social purposes for centuries, and continues to persist.

Use of the term race has become ubiquitous in physiological research and medicine, although it is a continuously evolving and ill-defined concept. The five racial categories used in the current United States Census – White, Black or African American, American Indian or Alaska Native, Asian, and Native Hawaiian or Other Pacific Islander - reflect social definitions of race that are "recognized in this country and not an attempt to define race biologically, anthropologically, or genetically" (241). The National Institutes of Health use six "racial and ethnic" categories, adding Hispanic or Latino to the five categories included in the Census, that are based primarily upon geographical origins (242). Further complicating matters is confounding the term race with ethnicity, which is defined by shared factors such as nationality, language, religion, values, and norms, and is considered voluntaristic (243, 244).

The ill-defined nature of race and ethnicity is particularly problematic in physiological research, given that most studies rely on participant self-identification. Racial self-identification embodies myriad social, biological, and morphological factors, including observed race (the race others believe you to be), reflected race (your perception of what race others believe you to be), skin color, ancestry, and hair texture (245). Some of the constructs encompassed under the term race may indirectly influence health status; for example, discrimination and perceived discrimination related to socially-assigned and reflected race are associated with poorer health outcomes (246-249). By categorizing groups based on racial self-identification, we gain insight into how the intersection of the various dimensions of "race" influences health outcomes. However, although research aimed at understanding differences in disease processes between self-identified groups does provide important information, it also falls short by not considering the specific biological, social, and/or environmental foundations from which these differences arise.

"RACE" IN CARDIOVASCULAR RESEARCH

There is an expanding body of research investigating racial differences in cardiovascular and cerebrovascular health. In particular, there is substantial focus on characterizing the mechanisms of vascular dysfunction in the African American, or non-Hispanic black, population – important given that the African American population experiences greater rates of cardio- and cerebrovascular diseases compared to all other groups (6, 250). Dysfunction in this context is generally defined as divergent responses relative to a non-Hispanic white cohort, with the assumption that responses in non-Hispanic white participants represent "normal" function. Extensive reviews on this topic have recently been published (251, 252), therefore this article will provide only a brief synopsis of the currently available literature.

Dysfunction of the peripheral macro- and microvasculature, secondary to increased oxidative stress and reduced nitric oxide (NO) bioavailability, has been documented in otherwise healthy African Americans (40, 85, 86, 253-255). Similar dysfunction has been documented in the cerebral vasculature when examining cerebral vasomotor reactivity in response to hypercapnia (256). Further, exaggerated vasoconstrictor responsiveness to phenylephrine infusion (257) and muscle sympathetic nervous system activity at rest (258) and during static exercise (259) has been observed in young, normotensive black adults compared to their white counterparts. Together, these studies have provided substantial and important insights into the mechanisms underlying the development of cardiovascular and cerebrovascular disease in the African American population. Although important strides have been made to elucidate the mechanisms of cardiovascular and cerebrovascular dysfunction in the African American population, less is known about other minority populations, including Hispanic, Asian, or Native Americans. However, some evidence exists for an increased prevalence of type 2 diabetes and associated cardiovascular dysfunction in the Hispanic and Asian American populations (260, 261). Additionally, there is some evidence of increasing prevalence of cardiovascular disease in the Native American and Alaska Native populations (262-264). Although relatively limited, these findings underscore the need for future research aimed toward better understanding the factors lending to the development of cardiovascular disease across diverse populations in the United States.

An important limitation to consider in much of the currently available literature regarding race differences in cardiovascular and cerebrovascular dysfunction is how participant groups are categorized. For example, there is an abundance of research that identifies participants as black, African American, or non-Hispanic black. Herein lies a significant problem; some individuals may identify as black or non-Hispanic black, but not as African American. An individual who identifies as black may be a first-generation immigrant from Africa, or from other regions such as

the Caribbean or Melanesia. Importantly, differential rates of morbidity have been demonstrated in black adults born in majority white regions, such as the United States or Europe, compared to those who are first-generation immigrants to those regions (265-267). Past research has provided crucial information regarding the physiological mechanisms mediating racial disparities in cardiovascular and cerebrovascular function. However, the development of vascular dysfunction is multifactorial and most likely includes biological, social, and environmental underpinnings (discussed in more detail in the next section). As such, future research in this area should attempt to specifically address the roles of these factors, rather than race per se, in the development of cardiovascular and cerebrovascular diseases.

CORRECTING PERCEPTIONS OF RACE AND IMPROVING STUDY DESIGN IN PHYSIOLOGY

Despite there being no direct biological distinction between one race and another (232, 233), the uncritical categorization of research subjects by race persists. Continued examination of mechanistic differences in cardiovascular and cerebrovascular function between racial groups, rather than addressing the underlying social and environmental factors that engender population health disparities, serves to promulgate the false narrative that there are inherent "racial" differences. Furthermore, the persistent use of race categories may, in and of itself, result in a widening of health disparities by promoting stereotype threat, whereby the perception of worse health in an individual's own racial group triggers physiological and psychological processes that propagate the development of morbidity (268, 269). Accordingly, attempts should be made to directly assess the cardiovascular and cerebrovascular health effects of sociocultural, environmental, and biological factors that are encompassed under, and obscured by, the term "race".

Chronic cardiovascular and cerebrovascular dysfunction may arise from societal conditions that result in increased exposure to daily stressors. Recent research has demonstrated that greater exposure to daily psychosocial stress in healthy adults is associated with cutaneous microvascular dysfunction, characterized by impaired vasodilator and exaggerated vasoconstrictor responsiveness (230, 231). Those who identify as belonging to a minority group are more likely to experience daily psychosocial stress (270-272). Personal experiences of discrimination result in more frequently experienced daily stressors that can, in turn, impact cardiovascular health (273-275). Further, unhealthy behaviors adopted to cope with stress may exacerbate the development of physiological dysfunction (272). In the African American community, particularly in men, John Henryism coping (high-effort coping in the face of adversity, despite limited resources and higher psychosocial stress (276)) in those from low socioeconomic status backgrounds has been associated with worse cardiometabolic outcomes (277-279). However, mechanistic studies investigating the role of daily psychosocial stress in explaining population differences in the development of vascular dysfunction are still needed.

Differences in socioeconomic status can result in impaired cardiovascular and cerebrovascular health via less access to quality nutrition, health care, availability of recreational resources, and quality sleeping conditions. Higher socioeconomic status and availability of favorable food stores and physical activity resources are associated with better cardiovascular health (280). Minority and low-income neighborhoods are less likely to have recreational facilities (281), and concerns about safety and lack of organized, supervised activities may curtail use of available park space for outdoor recreation (282, 283). Lower socioeconomic status and associated neighborhood disadvantage is associated with poorer sleep quality and may contribute to disparities in cardiovascular and cerebrovascular health outcomes (284-286). Unequal health outcomes also reflect inequalities in health care between populations, representing a significant barrier to

bridging the gap in cardiovascular and cerebrovascular health. Future research considering the role of socioeconomic status in mediating the development of vascular dysfunction in at-risk populations is warranted.

Apart from psychosocial determinants of health, skin pigmentation represents a potential source of biological variation which may contribute to disparities in endothelial function by influencing bioavailability of nutrients that are metabolized via sun exposure. The vitamin D-folate hypothesis is the prevailing theory regarding the evolution of human skin pigmentation (21, 24, 287). The potential implications of skin pigmentation in the context of vascular health have recently been reviewed in-depth (288). Briefly, those with darkened skin pigmentation are at increased risk of vitamin D deficiency in relatively low ultraviolet radiation (UVR) environments. Vitamin D may have important roles in endothelial function and health by signaling for the transcription of endothelial nitric oxide synthase and/or by ameliorating inflammation- and oxidative stress-induced endothelial dysfunction (16, 19, 90, 91), suggesting a potential avenue for the development of endothelial dysfunction in darkly-pigmented populations living in relatively low-UVR regions (289). The interaction between skin pigmentation, environmental UVR, and vascular health presents an additional avenue by which disparities in vascular health may arise.

CONCLUSIONS

The purpose of this brief Perspectives article is to encourage researchers of cardiovascular and cerebrovascular physiology to reconsider the practice of investigating health disparities based on race alone. We assert that the term “race” is ill-defined and built on faulty scientific foundations. We do not mean to imply that race is inconsequential; perceptions of race and/or racial discrimination are factors that have significant health impacts. However, we propose that

physiology researchers should directly acknowledge that physiological differences between populations are not due to inherent “racial” differences – rather, they are primarily functions of disparate social and environmental conditions - and design studies to specifically identify the conditions that result in differential health outcomes.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

S.T.W., N.G.J., and W.L.K. conceived manuscript; S.T.W. drafted manuscript; S.T.W., N.G.J., and W.L.K. edited and revised manuscript; S.T.W., N.G.J., and W.L.K. approved final version of manuscript.

Chapter 7

CONCLUSIONS AND FUTURE DIRECTIONS

The studies that comprise this dissertation were designed to examine the influence of UVR on cutaneous microvascular function through folate (5-MTHF) and vitamin D metabolism. Specifically, this dissertation investigated (1) the potential acute, negative impacts of UVR on cutaneous microvascular function via 5-MTHF degradation and/or increased oxidative stress, (2) the role of sunscreen or sweat on the skin in modulating cutaneous microvascular responses to acute UVR exposure, and (3) the role of vitamin D in promoting healthy cutaneous microvascular function in young, darkly-pigmented African American adults. Together, the findings of these studies suggest that UVR exposure may elicit either deleterious or beneficial effects on the cutaneous microvasculature via distinct and opposing effects on 5-MTHF and vitamin D metabolism. Further, these studies suggest that the cutaneous microvascular effects of UVR exposure may be dependent upon factors such as skin pigmentation and sun protection practices (i.e. wearing sunscreen). This chapter summarizes the findings of these studies and discusses future directions for this area of research.

Acute Ultraviolet Radiation Exposure Attenuates Nitric Oxide-Mediated Vasodilation in the Cutaneous Microvasculature of Healthy Humans

The primary finding of this study was that acute UVB exposure attenuated NO-mediated vasodilation of the cutaneous microvasculature of healthy, college-aged humans. Furthermore, this study demonstrated that local 5-MTHF or ascorbate delivery augmented NO-mediated cutaneous vasodilation in UVB-exposed skin, such that the NO contribution to cutaneous vasodilation during local heating was not different between non-exposed skin and 5-MTHF- or ascorbate-treated sites in UVB-exposed skin.

Implications

Together, these data suggest that acute skin exposure to UVB radiation may impair NO-mediated cutaneous microvascular function via direct photodegradation of 5-MTHF and/or increased production of ROS. This study provides evidence to substantiate previous research suggesting that UVR exposure degrades 5-MTHF, and extends those findings to demonstrate that UVR-induced degradation of 5-MTHF may impair NO-mediated vasodilation of the cutaneous microvasculature.

Sunscreen or Simulated Sweat Minimizes the Impact of Acute Ultraviolet Radiation on Cutaneous Microvascular Function in Healthy Humans

The findings of this two-part study were: (1) an erythemogenic dose of broad-spectrum UVR exposure elicited a linear increase in skin erythema beginning in the first 2 hours post-exposure, and a delayed, unrelated increase in skin blood flow that began between 4 and 6 hours post-exposure; (2) acute skin exposure to broad-spectrum UVR blunted NO-mediated vasodilation of the cutaneous microvasculature during local skin heating; (3) broad-spectrum sunscreen prevented the inflammatory increase in skin blood flow in study 1, and the impairment in NO-mediated vasodilation during local heating in study 2; (4) NO-mediated vasodilation was augmented after exposure to broad-spectrum UVR when sunscreen was applied to the skin before exposure; and (5) contrary to our hypothesis, the presence of simulated sweat on the skin during UVR exposure prevented the UVR-induced impairment in NO-mediated vasodilation during local skin heating.

Implications

The results from these two studies confirm our previous findings that acute UVB exposure attenuates NO-mediated vasodilation of the cutaneous microvasculature during local heating, and expand those findings to broad-spectrum UVR, more like that which is experienced from the sun. These studies further extend those findings to demonstrate that a dose of UVR exposure sufficient to elicit an erythema response provokes an inflammatory increase in skin blood flow. Finally, the data from these studies suggest that sunscreen on the skin protects against the deleterious effects of UVR exposure observed in both studies, and that sweat on the skin may protect against UVR-induced reductions in NO-mediated cutaneous vasodilation. Together, these studies provide further support for adverse consequences of overexposure to UVR in the cutaneous microvasculature, and highlight the importance of sun protection for individuals who spend long hours working or exercising in the sun.

Four Weeks of Vitamin D Supplementation Improves Nitric Oxide-Mediated Microvascular Function in College-Aged African Americans

The principal findings of this study were that (1) serum 25(OH)D concentrations were lower in otherwise healthy, young African American adults compared to their European American counterparts (2) cutaneous microvascular responses to local heating were attenuated in African American compared to European American participants, (3) direct delivery of tempol (superoxide dismutase mimetic) or apocynin (NADPH oxidase inhibitor) improved cutaneous microvascular responses to local heating in African American subjects, such that differences between groups were abrogated, and (4) four weeks of vitamin D supplementation increased serum 25(OH)D concentrations and augmented cutaneous microvascular responses to local heating in African American, but not European American, subjects.

Implications

The results of this study confirm previous data suggesting that cutaneous microvascular responses to local heating are impaired in otherwise healthy, young African American adults, and that those impairments are mediated by reduced NO bioavailability secondary to increased oxidative stress. Importantly, this study expands on those findings to demonstrate that increased oxidative stress and/or reduced NO bioavailability observed in college-aged African Americans may be due, at least in part, to vitamin D deficiency/insufficiency, resulting from inadequate UVB-induced vitamin D production in highly melanized skin in a relatively low-UVR environment. Together, these results suggest that ensuring vitamin D sufficiency may provide an effective therapeutic strategy to mitigate the development of (cardio)vascular dysfunction in the African American population.

Future Directions

1) The studies comprising chapters 3 and 4 of this dissertation, examining the deleterious impacts of UVR exposure on NO-mediated vasodilation of the cutaneous microvasculature via 5-MTHF degradation, are limited in that NO-mediated dilation was only quantified acutely (i.e. in the first ~3 hours) after UVR exposure. As such, it remains unclear how long these effects persist, or whether there is a cumulative impact of repeated and/or prolonged exposures. Previous research has demonstrated a negative relation between doses of UVR exposure and serum folate concentrations in an elderly, primarily lightly-pigmented Australian cohort (11), suggesting a cumulative negative effect of UVR in that population. Repeated, prolonged exposures may therefore result in chronic impairments in NO-mediated vasodilation of the cutaneous microvasculature in those with lightly-pigmented skin.

In the study comprising chapter 3, contrary to our hypothesis, the acute effects of UVR exposure were independent of skin pigmentation. It is likely, however, that those with darker skin pigmentation are better protected against repeated, prolonged exposures. Increased melanogenesis upon UVR exposure is amplified in darkly-pigmented compared to lightly-pigmented skin (290), providing further protection against subsequent UVR exposures in darkly-pigmented skin. Thus, darkly-pigmented skin would likely have a protective role against chronic effects of UVR exposure on NO-mediated microvascular function.

Future research may be designed to elucidate the duration of the detrimental effects of UVR exposure on NO-mediated vasodilation of the cutaneous microvasculature, whether repeated exposures exacerbate these effects, whether there is a dose-response effect of increasing exposures to UVR, and the role of skin pigmentation in modulating these effects.

2) Baseline 5-MTHF bioavailability may play a modulating role in the cutaneous microvascular response to acute and/or repeated exposures to UVR. In the study comprising chapter 3 of this dissertation, although we investigated the potential role of skin pigmentation in mitigating the impact of UVR exposure on 5-MTHF and NO-mediated vasodilation, we did not measure baseline serum 5-MTHF concentrations. Because the study was conducted during the fall and early-winter months, daily environmental UVR exposure was likely not sufficient to have a substantial impact on 5-MTHF bioavailability in our participants, regardless of skin pigmentation. Thus, it is possible that the lack of an association between skin pigmentation and the effect of UVR exposure on NO-mediated vasodilation may have been due to the fact baseline serum 5-MTHF concentrations were similar among all subjects. Previous research from our lab has demonstrated sufficient 5-MTHF status in young, college-

aged participants of primarily European descent (7). Because human folate requirements are met entirely through food sources, variation in nutritional status among subjects may influence baseline 5-MTHF status. In subjects with marginal 5-MTHF bioavailability, the acute and prolonged impacts of UVR exposure may be exaggerated compared to those who are 5-MTHF replete. Thus, individuals living in high-UVR environments, particularly those with lighter skin pigmentation, and/or with inadequate access to folate-fortified foods, are likely most susceptible to the deleterious effects of UVR exposure on cutaneous microvascular function. Future studies may elucidate the influence of baseline 5-MTHF availability on the cutaneous microvascular responses to UVR exposure.

3) Genetic polymorphisms related to vitamin D and 5-MTHF metabolism may also influence the impact of UVR exposure on NO-mediated microvascular function. Polymorphisms in the *MTHFR* gene, for example, may play an important role in the maintenance of sufficient 5-MTHF availability after UVR exposure (11, 103). Upregulated DNA methylation when plasma or red blood cell folate concentrations fall below 12 nmol/L in individuals with the 677CC, but not the 677TT, *MTHFR* genotype (103) may improve maintenance of 5-MTHF bioavailability, and thereby protect NO-mediated microvascular function, in those with the 677CC variant.

Single nucleotide polymorphisms at *GC* (vitamin D binding protein), *DHCR7 (*7-DHC *reductase), and CYP2R1 (25-hydroxylase)* loci may influence UVB-induced vitamin D production and bioavailability (75-77). Polymorphisms in GC are related to lower vitamin D binding protein concentrations in Americans of African descent compared to those of European descent (291). Differential prevalence of *DHCR7* variants related to reduced 7-DHC reductase activity have been demonstrated in European and Northeast Asian compared

to African populations (80). Thus, variation in vitamin D bioavailability may be contributable, at least in part, to variation in genes associated with vitamin D biosynthesis and storage.

Although associations have been demonstrated between skin pigmentation and genetic polymorphisms related to 5-MTHF and vitamin D metabolism (76, 102), less is known about how admixture among populations may influence 5-MTHF and vitamin D genotypes independent of skin pigmentation. Future research should be designed to investigate the interactions between UVR exposure, skin pigmentation, geographical ancestry, genotype, and NO-mediated vascular function. Such investigation should include participants with a wide range of skin pigmentation and ancestral backgrounds, allowing for the dissection of the specific influences of pigmentation and genetic factors in mediating NO bioavailability, secondary to variation in 5-MTHF and vitamin D status.

4) Another important question that needs more clarification is how UVR exposure may modulate vascular inflammation. Increased vascular inflammation is associated with reduced NO bioavailability, the development of atherosclerosis, and vascular disease (19, 119). In the study comprising chapter 4 of this dissertation, and in past studies (5, 118), it has been established that an erythemogenic dose of UVR results in an increase in skin blood flow that is driven by increased inflammation. It is unclear, however, whether inflammation produced from UVR exposure manifests in circulatory beds other than the cutaneous microvasculature, or what magnitude of UVR exposure would be required to elicit such a response. Conversely, there is evidence that greater vitamin D bioavailability is associated with reduced vascular inflammation and improved endothelial function (19), suggesting an anti-inflammatory role for UVR in the vasculature through vitamin D production. It may be, therefore, that sub-

erythemogenic doses of UVR have a net anti-inflammatory effect in the vasculature, although this requires further investigation.

5) UVR exposure may elicit effects on vascular function and health independently of folate or vitamin D metabolism. Skin exposure to UVA radiation has been suggested to induce cutaneous NO production independent of NOS (3, 61, 292), potentially causing a reduction in blood pressure (61, 292), although the blood pressure-lowering effect has been disputed (3) and is likely transient. It also remains unclear how these effects of UVA exposure may vary by skin pigmentation. UVA or UVB exposure to the skin may also increase production of ROS, which in turn can directly scavenge bioavailable NO. In chapter 3 of this dissertation we demonstrated that direct delivery of ascorbate restored NO-mediated vasodilation in UVB-exposed skin to a similar degree as 5-MTHF delivery, suggesting that UVB-induced reductions in NO-dependent dilation may be due to increased ROS production. Future research may attempt to elucidate the direct and indirect (i.e. via ROS production) role of UVR on 5-MTHF degradation and NO bioavailability and vascular function.

6) Elevated blood pressure is an independent risk factor for CVD, and may be mediated, in part, by dysfunction of the autonomic nervous system. Exaggerated vasoconstrictor responses to spontaneous bursts of muscle sympathetic nerve activity (MSNA) have been demonstrated in otherwise healthy, young African American adults (258), suggesting a potential mechanism by which that population may develop hypertension. The origins of such augmented responses to MSNA in the African American population remain unclear, but may be partially mediated by reduced NO bioavailability, resulting in an inability of vasodilator pathways to dampen the effects of MSNA on vascular tone. In the study comprising chapter 5 of this dissertation, we demonstrated that improving vitamin D status in college-aged African

American adults may increase NO bioavailability. However, it remains unknown whether increased NO availability after vitamin D supplementation can effectively normalize vasoconstrictor responses to bursts of MSNA in this population. Increased vasoconstrictor responses to bursts of MSNA may also be mediated by autonomic dysfunction, including differences in norepinephrine spillover and/or adrenergic sensitivity. Vitamin D deficiency has been implicated in autonomic dysfunction (293, 294), but more research is needed in this area.

Disclaimer: The opinions or assertions contained within this dissertation are the private views of the authors and should not be construed as official or reflecting the views of the Pennsylvania State University or the National Institutes of Health.

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Appendix

Replace with Appendix Title

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: Effects of UVR on Cutaneous Endothelial Function in Subjects Differing in Skin Pigment (IRB# 6953)

Principal Investigator: W. Larry Kenney, Ph.D.

Address: 102 Noll Laboratory

Telephone Number: 814-867-1781

Subject's Printed Name: ____

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?

When you are exposed to the heat, nerves in your skin make natural chemicals that cause the skin's blood vessels to get bigger. This increases the amount of blood flowing through those vessels and helps to cool your body. Loss of this blood flow can make you more prone to illness in extreme heat. Exposure to ultraviolet light reduces the skin's ability to make blood vessels get bigger. Folic acid (vitamin B9) is a natural substance found in many commons foods. Researchers have shown that folic acid helps your body control blood flow. Ultraviolet light has shown to reduce folic acid in the skin, possibly reducing blood flow in heat. In the summer months and in warmer climates, when people go outside in the sun they are exposed to high temperatures and need more blood flow to cool down. However, they are also exposed to ultraviolet light from the sun which can reduce folate and stop the body from increasing blood flow and cool. Darker skin pigmentation is believed to be protective of folic acid loss by ultraviolet light exposure. This research looks at whether skin pigmentation protects against folic acid and blood flow loss by ultraviolet light exposure, and whether adding folic acid to your skin helps to restore lost blood flow when you are exposed to heat.

Approximately 30 people will take part in this research study at Penn State University. This study has two experiments that look at the role of skin pigmentation and folic acid in the control of skin blood flow when exposed to ultraviolet light.

In this study, the researchers use "microdialysis". This technique involves placing very thin plastic tubing between the layers of the skin. The largest part of the tubing is about 6x the diameter of a human hair. They pump fluid like that found in the body's tissues through the tubing. The tubing acts like very small blood vessels in the skin by allowing some substances to pass between the fluid in the tubing and the fluid in the skin. During the experiment, they will add substances to the fluid in the tubing. The substances can only reach a 2.5 cm^2 (0.4 $inch^2$), nickel-sized area of skin at each tube. Some of these substances are like natural chemicals found in the body. Some of these substances block the actions of natural chemicals found in the body. The substances used for these experiments are:

1. SNP (*sodium nitroprusside*) – causes your blood vessels to dilate as much as they can.
2. Ca-5MTHF *(D calcium L-5-methyltetrahydrofolate)* – the metabolite of folic acid as a calcium salt. This is a natural substance found in your blood after you ingest folic acid.
3. Ascorbate (Vitamin C) – found in many foods such as citrus fruits; is an antioxidant.
4. L-NAME (*NG-nitro-L-arginine methyl ester*) – blocks some proteins that cause your blood vessels to dilate.
5. Lactated Ringer's – like the plain fluid that bathes cells in the body.

The researchers use ultraviolet light on the skin for no more than 3 minutes during each session. Also, the researchers use weak laser light to measure blood flow in small vessels in the skin.

2. What will happen in this research study?

You participate in all of the following procedures. Please read the descriptions of each procedure then write your initials by the line next to the procedure.

We may ask you to repeat a trial, procedure, or test. This could happen for many reasons such as equipment failure, power outage, inconclusive test results, etc. You do not have to repeat a trial, procedure, and/or test if you do not wish to do so.

Note: This study involves the use of drugs that are not approved by the FDA to treat disease. All of the drugs have been used in humans by us or others in the past. The FDA approved the use of the drugs for this study. We dilute some of the drugs in Lactated Ringer's, a type of saline fluid like that found throughout your body.

The drugs are:

- L-Ascorbate *(vitamin C):* found in many foods such as citrus fruits; antioxidant and promotes vasodilation.
- NG-nitro-L-arginine methyl ester *(L-NAME)*: non-specific nitric oxide synthase inhibitor that inhibits production of nitric oxide that causes vasodilation; L-NAME is an analog to the amino acid L-arginine.
- Sodium nitroprusside (*SNP*) – supplies nitric oxide; causes blood vessels to dilate
- Ca-5MTHF *(D calcium L-5-methyltetrahydrofolate)* – the metabolite of folic acid; natural substance found in blood after ingestion of folic acid.

________ initial **A. Screening**

1. You drink only water and do not eat for 12 hours before the screening.

2. The research nurse performs the screening. The staff measures your height and weight, blood pressure (BP), and heart rate (HR). They measure waist circumference and how much your skin reflects light. The research nurse reviews your medical history.
3. The research nurse draws 30 ml (2 Tbsp) of blood from a vein in your arm. We send some of the blood to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. We may test the blood for other substances of interest. The researchers do not perform genetic analyses on the blood nor look for presence of disease (e.g. HIV).
4. If you are a woman who is not post-menopausal, you will have a urine pregnancy test.

________ initial **B. Preparation for Acute Ultraviolet Light Experiment**

1. Before you arrive at the lab
    a. If you have questions, please contact us right away
    b. You do not eat or drink anything containing caffeine (ex. coffee, tea, Coca Cola, chocolate) for 12 hours before the experiment.

2. When you arrive at the laboratory
    a. We measure your blood pressure (BP) and heart rate (HR).
    b. Women who are not post-menopausal submit urine for a pregnancy test if we have not tested them within 2 weeks of the experiment.
    c. Blood pressure: We use two methods. Two methods use a cuff that inflates on the upper arm. In one, we listen with a stethoscope at the inside of the elbow. Another method, CardioCap, also uses an upper arm cuff.
    d. Microdialysis (MD): MD involves placing very thin plastic tubing between the layers of your skin. The largest part of the tubing is about 6 times the diameter of a human hair. We pump fluid like that found in your body's tissues (lactated Ringer's solution) through the thin tubing. The thin tubing acts like the very small blood vessels in your skin. There is an exchange of substances between the fluid in the tubing and the fluid in the surrounding tissue. During the experiment, we add substances to the fluid in the tubing. The substances can only reach a 2.5 cm2 (0.4 inch2), nickel-sized area of skin.
        1) MD Probe Insertion: For each MD site, we make pairs of pen-marks 2.5 cm (1 inch) apart and away from veins. The MD tubing enters and exits your skin at the marks. We clean the MD sites with an orange-colored fluid and alcohol. We place an ice bag on the sites for 5 minutes to numb your skin. Then we insert a thin needle into your skin at each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch). It leaves your skin at the matching exit mark. We thread the MD tubing through the needle. Next, we withdraw the needle leaving the tubing in your skin.
        2) We prepare 3 MD sites on each forearm in this manner.
            a) Lactated Ringer's
            b) Lactated Ringer's + Ca-5MTHF
            c) Lactated Ringer's + L-Ascorbate
        3) Any redness of your skin subsides in about 60 minutes

________ initial **C. Acute Ultraviolet Light Experiment**

1. Ultraviolet Light Phase: After redness of the skin subdues, the experiment begins with an ultraviolet (UV) light phase. You may end the UV light phase at any time.
    a. Protective eyewear will be provided and required while an UV lamp is in use.
    b. An UV lamp is turned on and focused on one of the forearms for no more than 3

minutes.

c. After the UV lamp is turned off, we tape a thin probe and its holder over each MD site. The thin probe measures skin blood flow at the MD site with a weak laser light. We control the temperature of the holders. The holders start at 33°C (91.4°F).
d. We collect skin blood flow and pressure data for 30 minutes.

2. Local Heating Protocol: After we have collected baseline data, we increase the skin temperature at each of the MD sites to 42ºC (107ºF).
   a. We hold the skin temperature at 42ºC until the skin blood flow becomes stable (about 30 minutes).
   b. We add LNAME to the sites until the skin blood flow becomes stable again (about 30 minutes).

3. Maximum Skin Blood Flow: To begin the heating phase, we increase the local temperature of the skin to 43ºC (109.4ºF). you may end the local heating phase at any time.
   a. Local skin temperature is maintained at 43ºC (109.4ºF) for 30 minutes.
   b. Lactated Ringer's + L-NAME is replaced in the fluid for each microdialysis tube for an additional 30 minutes.
   c. We then replace the fluid with Lactated Ringer's + SNP for a final 30 minutes.
   d. The experiment ends. We measure blood pressure and heart rate before you leave. You will protect both forearms from sunlight and ultraviolet light exposure until your next visit.

________ initial **D. Ultraviolet Light Pretreatments**

1. You will arrive at the Noll Lab for 3 consecutive days following the acute ultraviolet light experiment. If you have questions, please contact us right away.

2. When you arrive at the laboratory
   a. We measure your blood pressure (BP) and heart rate (HR).
   b. Protective eyewear will be provided and required while an UV lamp is in use.
   c. An UV lamp is turned on and focused on one of the forearms for no more than 3 minutes.
   d. The experiment ends. You will protect both forearms from sunlight and ultraviolet light exposure until your next visit.

________ initial **E. Preparation for Chronic Ultraviolet Light Experiment**

1. Before you arrive at the lab
   a. If you have questions, please contact us right away
   b. You do not eat or drink anything containing caffeine (ex. coffee, tea, Coca Cola, chocolate) for 12 hours before the experiment.

2. When you arrive at the laboratory
   a. We measure your blood pressure (BP) and heart rate (HR).
   b. Women who are not post-menopausal submit urine for a pregnancy test if we have not tested them within 2 weeks of the experiment.

We place 3 MD probes in the forearm that has been exposed to UV light during the pretreatment. We use the same technique as described for the *Acute Ultraviolet Light Experiment.*

________initial **F. Chronic Ultraviolet Light Experiment**
We repeat the same protocol described for the *Acute Ultraviolet Light Experiment.*

3. What are the risks and possible discomforts from being in this research study?

Microdialysis (MD): The risks are less than that for a blood draw because MD uses only a small, local area of skin. In contrast, a blood draw involves not only skin, but also large blood vessels and blood. MD is likely to cause some pain and bruising like that of a blood draw. However, the researchers use ice to numb the arm before they insert the tubing. Also, the small needle helps to reduce pain. Most people do not feel pain after the tubing is in place. You may feel a little pain when they remove the tubing at the end of the experiment. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Sometimes the tubing can break during removal from the skin. Then the researchers remove the tubing by pulling on the other end of it. This produces no added risk for you. The tubing could break so that a small piece is left under the skin. This has not occurred in any of the studies in this lab. If this happened, they would treat any tubing remaining in the skin like a splinter. In this case, they would cut the thin layer of skin over the tubing to remove the tubing. Mild pressure with sterile gauze stops any slight bleeding that may occur. Aseptic technique and sterile supplies like those used in hospitals keep the risk of infection minimal. Infection has not occurred with MD in this lab or others that the researchers know of. They apply a sterile bandage after the experiment. They tell you how to take care of the site.

Fluid flowing through the tubing: The substances flowing through the tubing only go to a 2.5 cm^2 (0.4 $inch^2$) area of skin at each tubing site. The amount that enters the skin is very small. However, there is a chance of a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, blood pressure change and/or fainting. If a bad reaction should occur, medical help is summoned right away.

Lactated Ringer's Solution: This fluid is similar to the natural fluids in the skin. This fluid contains salt, potassium, lactate, and chloride. The acid content is like that of the body's fluids. A bad reaction to this fluid is highly unlikely.

Ascorbate, 5-MTHF, L-NAME, and SNP: Only minute amounts of these substances enter the nickel-sized area of skin around the MD tubing. These and other researchers have used the substances in human skin before. There have been no reports of bad reactions.

Microdialysis delivers small amounts of the substances to a nickel-sized area of the skin. The small quantities used and the extremely localized administration during microdialysis does not produce systemic effects. To the researchers' knowledge, there are no reports of long or short-term side effects of these substances administered through microdialysis. The chance of adverse reactions to these substances is extremely small given the minute amount delivered to the a very small area of skin, the lack of adverse reactions to similar amounts delivered via microdialysis in many other studies, and lack of adverse effects in human cell cultures. There is a slight chance of allergic reaction to these substances that could produce redness, itching, rash, and/or swelling. A severe reaction (anaphylactic shock) could also cause fever, difficulty in breathing, changes in pulse, convulsions, and/or loss of consciousness.

Blood Draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a

slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Using the same techniques used in hospitals keeps the chance of infection minimal. Do not exercise hard for 24 hours before a blood draw.

Tape and adhesive disks: You could be sensitive to the adhesive of the tape and double-sided adhesive disks used in the study causing redness, rash, tenderness, and/or itching.

Blood Pressure (manual, CardioCap): The researchers measure blood pressure using the method common in a doctor's office or with a machine. A cuff inflates on the upper arm. As the cuff slowly deflates, the researchers listen with a stethoscope at the bend in the elbow or the machine takes a reading. During the short time the researchers inflate the cuff, your arm may feel numb or tingly. The cuff could cause mild bruising.

Medical Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. If you request someone of the same sex to conduct the screening, the researchers will make their best effort to provide one.

Phone screening form: Only the researcher uses this form. They use the form to help decide whether you are a good candidate for the study. You may feel shy about answering questions. You may request someone of the same sex to ask you the questions. They collect the information in a private and professional manner. The completed form is kept confidential and secure.

Thermoregulation Lab Website: You may enter data into the screening form via the Qualtrics website. You may be concerned about the data's security. Qualtrics is a secure website and survey application designed to support data capture for research studies. The data is kept encrypted and your confidentiality and security are protected.

Laser Doppler Flowmetry: The probe attaches to the skin with double-sided tape and measures skin blood flow in a $1mm^3$ volume of skin. Weak lasers can hurt the eye if one should stare into the light for a long time. The red light seen on the surface of the skin is harmless. The researchers have used this technique in their lab with IRB approval for many years without incident.

Local Heating: The researchers measure the temperature of the skin under the holders. The skin feels very warm but does not hurt. The heating makes the skin of the arm under the holders red like when you take a hot bath. The redness will not last more than several hours. Some people may be more sensitive to the heating than others. If your arm feels too hot, you tell the researchers, and they reduce or stop the heating.

Povidone Iodine: Hospitals and researchers use this orange-colored fluid to clean and sterilize the skin. You could have a bad reaction to povidone iodine if you are allergic to iodine. You inform the researchers if you have this allergy so that they use only alcohol instead. A bad reaction could cause redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or blood pressure change and/or fainting.

Latex: Some gloves and medical materials are made of latex rubber. Inform the researchers if you are allergic to latex and decline to participate in the study.

Confidentiality: There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

Ultraviolet light: Although the UV exposure used in this study is unlikely to produce long-term ill effects, the risks of the ultraviolet light exposure directed on the forearm will be similar to those associated with a 3 minute tanning session.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to you?
There is no guarantee that you will benefit from this research. The possible benefits you may experience from this research study include receiving a medical screening that could inform you about your health. You may also gain some knowledge about how your body works.

4b. What are the possible benefits to others?
Skin pigmentation developed from the need to protect the skin from ultraviolet light to keep folic acid and blood flow normal. People are increasingly exposing themselves to higher levels of ultraviolet light. In turn, this may lead to reduced folic acid and control of blood flow in skin. This can lead to skin and cardiovascular disease. The knowledge gained from this study can help us learn how ultraviolet light causes reduced skin blood flow. The results could also help influence policy decisions related to sun safety and exposure of ultraviolet light on human health and disease. The project provides valuable experience, education, and partial fulfillment of degree work for graduate and undergraduate students of Penn State University.

5. What other options are available instead of being in this research study?

You may decide not to participate in this research.

The procedures used in this study are used in many other research labs around the world. The procedures are the best ways to explore the questions and accomplish the goals of this research.

6. How long will you take part in this research study?
If you agree to take part in this study it will take you about 1 week for completion. You will be asked to return to the research site 6 times.

You will need to visit the Noll Lab for the following:

Day 1, Screening: 1.5 hours
Day 2, Acute Ultraviolet Light Experiment: 5 hours
Day 3-5, Ultraviolet Light Pretreatment: 1.5 hours (0.5 hour per visit)
Day 6, Chronic Ultraviolet Light Experiment: 5 hours

Total: 13 hours

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?

Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information.

- We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list.
- We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied.
- We label your research samples with your code number. We keep the samples in a dedicated ultralow freezer in Noll Lab until analysis.

All research specimens sent to outside labs for analysis (e.g. Quest Labs) are identified only by a code number.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies)
- The Office for Research Protections
- The U. S. Food and Drug Administration

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

8. What are the costs of taking part in this research study?

8a. What will you have to pay for if you take part in this research study?

There are no costs for taking part in this research study.

8b. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

Ultraviolet Light Experiments: $15.00 / MD probe inserted + $25.00 completing experiment

Total for acute experiment: $115.00 (6 MD probes + completion)

Total for chronic experiment: $115.00 (6 MD probes + completion)

TOTAL: $230.00 (2 experiments)

For each experiment, you are paid an amount of money equal to the part of the trial that you complete. For instance, if you complete only half of an ultraviolet light experiment, you will be

paid for each probe that was inserted plus $12.50 for that experiment. This is because $12.50 is one half of $25.00. If you agree to repeat an experiment, you will be paid for the repeated experiment as stated above. You are reimbursed for gasoline if you live more than 20 miles from Noll Lab.

Total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. What are your rights if you take part in this research study?
Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you choose to withdraw from the study, any data collected prior to the point of withdrawal will remain in the study database and may not be removed.

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal from the study include if we deem that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

11. If you have questions or concerns about this research study, whom should you call?
Please contact Stephen (Tony) Wolf (W: 814-863-8556) or Susan Slimak (W: 814-863-8556, H: 814-237-4618) with questions, complaints or concerns about this research. You can also call these numbers if you feel this study has harmed you. If there are findings during the research that could relate to you wanting to help with the study, you will be told of the findings. If you have any questions, concerns, or problems about your rights as a research participant or would like to offer input, please contact Penn State University's Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. All questions about research procedures can only be answered by the research team.

You may also contact the Office for Research Protections at (814) 865-1775, ORProtections@psu.edu if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_______________________________ __________ _________________
Signature of person who explained this research Date Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

_____________________________ ___________ _________________
Signature of Subject Date Printed Name

CONSENT FOR RESEARCH
The Pennsylvania State University

The Modulating Effects of Sunscreen and Simulated Sweat on Ultraviolet Radiation-Induced Cutaneous Microvascular Dysfunction (IRB# 9720)

Principal Investigator: W. Larry Kenney, Ph.D.

Address: 102 Noll Laboratory

Telephone Number: 814-867-1781

Subject's Printed Name: ______________________________________

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?

Exercise is an effective way to improve health. However, people are exposed to ultraviolet (UV) light from the sun when they go outside. UV light from the sun may increase some health risks during outdoor exercise. UV light has been shown to reduce the ability of blood vessels in the skin to expand. This reduces skin blood flow, which can have negative health effects.

People also sweat when they are outside in the heat. Sweat might worsen the effects of UV light on skin blood vessels. Putting sunscreen on the skin might protect the blood vessels from these bad effects. This research looks at whether sweat or sunscreen on the skin alter the effects of UV light on skin blood flow.

Approximately 20 people will take part in this research study at Penn State University. This study has one experiment that will look at the role of sweat and sunscreen in the effects of UV light on skin blood flow.

We use "microdialysis" (MD) in this study. MD allows us to perfuse research drugs through nickel-sized areas of skin on your arm. The drugs remain in the small areas and do not go into the rest of your body. The research drugs are not approved by the FDA to treat disease. However, the FDA has approved our using the drugs in this study. We and others have used these drugs in people in research studies for many years without problem. We dilute some of the drugs in Lactated Ringer's, a type of saline fluid like that found throughout your body. The substances used with MD for this experiment are:

6. Lactated Ringer's – like the plain fluid that bathes cells in the body.
7. SNP (*sodium nitroprusside*) – causes your blood vessels to fully expand.
8. L-NAME (*NG-nitro-L-arginine methyl ester*) – blocks some proteins that cause your blood vessels to expand.

2. What will happen in this research study?
You participate in all of the following procedures. Please read the descriptions of each procedure then write your initials by the line next to the procedure.

We may ask you to repeat a trial, procedure, or test. This could happen for many reasons such as equipment failure, power outage, inconclusive test results, etc. You do not have to repeat a trial, procedure, and/or test if you do not wish to do so.

________ initial **A. Screening**

1. The research staff measures the following:
   a. Height;
   b. Weight;
   c. Waist circumference;
   d. Blood pressure;
   e. Heart rate;
   f. Skin reflectance
2. You complete a health history questionnaire.
3. Women of childbearing age have a urine pregnancy test.
4. The research staff will draw 30 mL (2 Tbsp) of blood from a vein in your arm. Some of the blood will be sent to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. Your blood may be tested for other substances of interest. We do not perform genetic tests on the blood or look for the presence of disease (e.g. HIV). All of the blood tests are common tests to determine your health status.

________ initial **B. Microdialysis Experiment**

1. Preparation for the MD experiment:
   a. Do not eat or drink anything with caffeine (ex. Coffee, tea, Coca Cola, chocolate) for 12 hours before the MD experiment.
   b. When you arrive at the lab, a staff member measures your heart rate, blood pressure, and oral temperature.
   c. Women of childbearing age have a urine pregnancy test if they have not had one within 2 weeks.

2. Microdialysis probe insertion:
   a. A tight band is placed around your arm to visualize veins.
   b. For each MD site, a pair of pen-marks is made on the arm 2.5 cm (1 inch) apart and away from veins. The tight band is removed.
   c. Your forearm is cleaned with an orange fluid called povidone iodine and alcohol.
   d. An ice bag is placed on your arm for 5 minutes to numb the skin.
   e. A thin needle is then inserted into the skin at each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch) and exits the skin at the matching exit mark.
   f. The MD tubing is threaded through the needle and then the needle is taken out, leaving the tubing in the skin.
   g. Three MD sites are placed in the left arm. One MD site is placed in the right arm.
   h. Any redness of the skin caused by the needle insertion fades in about 60 minutes.
   i. During this time, lactated Ringer's is perfused through the MD tubing.

3. Ultraviolet Light Treatment:
    a. After redness of the skin subsides, the skin over each of the MD sites is prepared with one of the following treatments.
        - **UV only**: No treatment is applied to this site before UV exposure.
        - **UV + Sunscreen**: 0.5 mL of SPF-50 sunscreen is spread on the skin 15 minutes before UV exposure. The sunscreen is applied using a 1 mL syringe and then spread on the skin over the MD site.
        - **UV + lactated Ringer's**: 0.5 mL of Ringer's fluid is applied just before UV exposure. The Ringer's mimics sweat on the skin. Ringer's is applied using a 1 mL syringe and then spread around the skin over the MD site.
    b. We provide eyewear that must be worn to protect your eyes while the UV light is on.
    c. We turn the UV lamp on and focus the light on the forearm for no more than 2 minutes. The lamp is then turned off.
    d. The sunscreen, Ringer's, and foil which are applied to the skin are removed after UV exposure.
    e. We then tape a thin probe and its holder over each MD site. The thin probe measures skin blood flow at the MD site with a weak laser light. We control the temperature of the holders. The holders start at 33°C (91.4°F).
    f. We place a blood pressure cuff on your upper arm. Blood pressure is measured every 5 minutes for the rest of the protocol. We tape 3 ECG pads to your skin to measure heart rate.
    g. We collect baseline skin blood flow and pressure data for ~20 minutes.

4. Local Heating Protocol: We increase the temperature of the skin to 42ºC (107.6ºF). You may end the local heating phase at any time.
    a. We hold the skin temperature at 42ºC until skin blood flow becomes stable (~40 minutes).
    b. We add L-NAME to the MD sites until skin blood flow becomes stable again (~40 minutes).

5. Maximum Skin Blood Flow: We increase the temperature of the skin to 43ºC (109.4ºF). You may end the maximal blood flow phase at any time.
    a. We change the fluid in each MD tube to lactated Ringer's.
    b. Local skin temperature is maintained at 43ºC (109.4ºF) for 30 minutes.
    c. We then replace the fluid with Lactated Ringer's + SNP for a final 10 minutes.
    d. The experiment ends. We remove the MD tubing and place bandages over the sites.
    e. We measure blood pressure and heart rate before you leave.

________ initial **C. Follow-Up Measurements**

1. We give you the option to return to the lab to see how blood flow changes 4, 6, and 8 hours after the MD experiment.
    a. We make tracings around the probe holders after the MD experiment.
    b. We measure skin reflectance when you return to the lab.
    c. We tape the probe holders and probes inside their tracings.
    d. Resting skin blood flow is measured for ~10 minutes.
    e. We place a blood pressure cuff on your upper arm. Blood pressure is measured at the end of the data collection period.
    f. The collection period ends. The probes and their holders are removed.

3. What are the risks and possible discomforts from being in this research study?

Microdialysis (MD): The risks are less than that for a blood draw because MD uses only a small, local area of skin. In contrast, a blood draw involves not only skin, but also large blood vessels and blood. MD is likely to cause some pain and bruising like that of a blood draw. However, we use ice to numb the arm before they insert the tubing. Also, the small needle helps to reduce pain. Most people do not feel pain after the tubing is in place. You may feel a little pain when they remove the tubing at the end of the experiment. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Sometimes the tubing can break during removal from the skin. Then we remove the tubing by pulling on the other end of it. This produces no added risk for you. The tubing could break so that a small piece is left under the skin. This has not occurred in any of the studies in this lab. If this happened, they would treat any tubing remaining in the skin like a splinter. In this case, they would cut the thin layer of skin over the tubing to remove the tubing. Mild pressure with sterile gauze stops any slight bleeding that may occur. Sterile technique and supplies like those used in hospitals keep the risk of infection minimal. Infection has not occurred with MD in this lab or others that we know of. They apply a sterile bandage after the experiment. They tell you how to take care of the site.

Fluid flowing through the tubing: The substances flowing through the tubing only go to a 2.5 cm^2 (0.4 $inch^2$) area of skin at each tubing site. The amount that enters the skin is very small. However, there is a chance of a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, seizures, blood pressure change and/or fainting. If a bad reaction should occur, the experiment is stopped and medical help is summoned right away. It is possible to feel lightheadedness, flushing, nausea, "pounding heart", and/or to vomit. We stop the fluids flowing through the tubes in the event of these signs and symptoms.

Lactated Ringer's Solution: This fluid is similar to the natural fluids in the skin. This fluid contains salt, potassium, lactate, and chloride. The acid content is like that of the body's fluids. A bad reaction to this fluid is highly unlikely.

L-NAME and SNP: Only minute amounts of these substances enter the nickel-sized area of skin around the MD tubing. We and other researchers have used the substances in human skin before. There have been no reports of bad reactions.

Blood Draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Using the same techniques used in hospitals keeps the chance of infection minimal. Do not exercise hard for 24 hours before a blood draw.

Tape and adhesive disks: We use tape and sticky disks. You could be sensitive to the adhesive of the tape or disks. This could cause redness, rash, tenderness, and/or itching.

Blood Pressure (manual, critical care monitor): We measure blood pressure using the method common in a doctor's office or with a machine. A cuff inflates on the upper arm. As the cuff slowly deflates, we listen with a stethoscope at the bend in the elbow or the machine takes a reading. During the short time we inflate the cuff, your arm may feel numb or tingly. The cuff could cause

mild bruising.

Medical Screening: You may feel shy about giving health information. We collect the information in a private and professional manner. You may feel shy about being measured. If you request someone of the same sex to conduct the screening, we will make our best effort to provide one.

Laser Doppler Flowmetry: The probe attaches to the skin with double-sided tape and measures skin blood flow in a $1mm^3$ volume of skin. Weak lasers can hurt the eye if one should stare into the light for a long time. The red light seen on the surface of the skin is harmless. We have used this technique in their lab with IRB approval for many years without incident.

Local Heating: We measure the temperature of the skin under the holders. The skin feels very warm but does not hurt. The heating makes the skin of the arm under the holders red like when you take a hot bath. The redness will not last more than several hours. Some people may be more sensitive to the heating than others. If your arm feels too hot, you tell us, and we reduce or stop the heating.

Povidone Iodine: Hospitals use this orange-colored fluid to clean the skin. You could have a bad reaction if you are allergic to iodine. Tell us if you have this allergy so we use only alcohol. A bad reaction could cause redness, itching, rash, and/or swelling. A worse reaction could cause fever, breathing problems, changes in pulse, seizure, blood pressure change, and/or fainting.

Latex: Some gloves and medical materials are made of latex rubber. Inform us if you are allergic to latex and decline to participate in the study.

Confidentiality: There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by us will be maintained to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

Ultraviolet light: UV light can redden the skin. Prolonged exposure can cause pain, swelling, blistering, and peeling of the skin. Long term UV exposure is linked to the development of skin cancer. The UV exposure in this study is like a 3-minute tanning session. The UV exposure in this study is unlikely to produce long-term ill effects. UV light can cause conditions of the eyes later in life. These conditions can impair vision and even lead to blindness. It is unclear how much UV exposure it takes to damage to the eye. While the UV lamp is on, you wear special protective glasses that block UVA/UVB light.

SPF-50 Sunscreen: If you are allergic to sunscreen, you could get an itchy red rash. An allergic reaction could include pain, swelling, burning, and/or blistering of the skin. Tell us if you are allergic or sensitive to sunscreen.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to you?

There is no guarantee that you will benefit from this research. You receive a medical screening that could inform you about your health. You may also gain some knowledge about how your body works.

4b. What are the possible benefits to others?
This study can help us learn how skin blood flow is affected by UV light when sweat or sunscreen is on the skin. The results could also help affect policies for sun safety. The project provides experience and partial fulfillment of degree work for students of Penn State.

5. What other options are available instead of being in this research study?

You may decide not to participate in this research.

The procedures used in this study are used in many other research labs around the world. The procedures are the best ways to explore the questions and accomplish the goals of this research.

4. **How long will you take part in this research study?**
If you agree to take part in this study you will be asked to come to the lab two separate times.

You will need to visit the Noll Lab for the following:
Day 1, Screening: 1.5 hours
Day 2, Ultraviolet Light Experiment: 5 hours
Follow-up Blood Flow Measurements: 3 x ~20 minutes = ~60 minutes
Total: 7.5 hours

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?

Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information.

- We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list.
- We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied.
- We label your research samples with your code number. We keep the samples in a dedicated ultralow freezer in Noll Lab until analysis.

All research specimens sent to outside labs for analysis (e.g. Quest Labs) are identified only by a code number.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies)
- The Office for Research Protections
- The U. S. Food and Drug Administration

Some of these records could contain information that personally identifies you. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

8. What are the costs of taking part in this research study?

8a. What will you have to pay for if you take part in this research study?

There are no costs for taking part in this research study.

8b. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

10. Will you be paid or receive credit to take part in this research study?

UV Light Experiment: $15.00 / MD probe inserted + $25.00 completing experiment
Total for experiment: $85.00 (4 MD probes + completion)
Follow-up Blood Flow Measurements: $10 / time point
Total for follow-up: $30 (3 time points)
Total for UV Light Experiment and Follow-up: $115

For each experiment, you are paid an amount of money equal to the part of the trial that you complete. For instance, if you complete only half of an experiment, you will be paid for each probe that was inserted plus $12.50 for that experiment. This is because $12.50 is one half of $25.00. If you agree to repeat an experiment, you will be paid for the repeated experiment as stated above. You are reimbursed for gasoline if you live more than 20 miles from Noll Lab.

10. Who is paying for this research study?

The American College of Sports Medicine (ACSM) is paying for this study.

11. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you choose to withdraw from the study, any data collected prior to the point of withdrawal will remain in the study database and may not be removed.

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal from the study include if we deem that your

health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

12. If you have questions or concerns about this research study, whom should you call?
If you have any questions, complaints or concerns about this research, you may call any of the phone numbers below. You can also call these numbers if you feel this study has harmed you. If there are findings during the research that could relate to you wanting to help with the study, you will be told of the findings.

- Stephen (Tony) Wolf (W: 814-863-8556, C: 559-269-5198)
- Susan Slimak (W: 814-863-8556, C: 814-880-4396)
- Jane Pierzga (W: 814-865-1236, H: 814-692-4720)

If you have any questions, concerns, or problems about your rights as a research participant or would like to offer input, please contact Penn State University's Office for Research Protections (ORP). The phone number and email for the ORP are below. The ORP cannot answer questions about research procedures. All questions about research procedures can only be answered by the research team.

- Phone: (814) 865-1775
- Email: ORProtections@psu.edu

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

_______________________________ __________ _________________
Signature of person who explained this research Date Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

______________________________	__________	________________
Signature of Subject	Date	Printed Name

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: Modulating Role of Vitamin D in Oxidative Stress-Induced Vascular Dysfunction (IRB# 12598)

Principal Investigator: W. Larry Kenney

Address: 102 Noll Laboratory

Telephone Number: 814-867-1781

Subject's Printed Name: ____________________________

We are asking you to be in a research study. This form gives you information about the research.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you and there will be no penalty or loss of benefits to which you are entitled.

Please ask questions about anything that is unclear to you and take your time to make your choice.

KEY INFORMATION

The following is a short summary of this study to help you decide whether or not to be a part of this research. More detailed information is listed later in this form. If you have any questions, be sure to ask the study team.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because we are looking for healthy 18 – 35 year old adults. We think that you may be a good fit for this study.

What is the purpose of this research study?

This research is being done to find out if vitamin D improves blood vessel health.

How long will the research study last?

If you agree to take part, it will take you about 1 month to complete this research study. You will be asked to return to Noll Lab 3 times. Vitamin D treatment will be 4 weeks between visits 2 and 3.

You will need to visit the Noll Lab for the following:

Day 1, Screening: 1.5 hours
Day 2, Experiments: 6 hours
Day 3, Experiments: 6 hours

Total time for study visits: 13.5 hours

What will you need to do?

For this study, you will be asked to take vitamin D tablets every day for four weeks. You will be asked to come into the lab for testing on two days; once before vitamin D treatment and once after.

What are the main risks of taking part in the study?

For this study, the main risks to know about are: discomfort with needles during blood draws and microdialysis (MD); allergies from some fluids used in the study; allergies to tape or latex; infection from blood draws, MD, or skin biopsies. More information regarding risks can be found in the section labeled "What are the risks and possible discomforts from being in this research study?"

What are the possible benefits to you that may reasonably be expected from being in the research?

We cannot promise any benefits to you from your taking part in this study. You receive a screening that informs you about your health such as your blood pressure and blood cholesterol levels. You could gain knowledge about how your body works. The study may benefit other people in the future by helping us learn more about how vitamin D may improve blood vessel health.

What happens if you do not want to be in this research?

Participation in research is completely voluntary. You may choose not to take part in this research study.

DETAILED INFORMATION

The following is more detailed information about this study in addition to the information provided above.

1. Why is this research study being done?

Cardiovascular disease (CVD) is a leading cause of sickness and death. Studies have shown higher risk of CVD in African Americans (AA). The reasons for this higher risk are not well understood. Vitamin D may play a role in blood vessel health and reduce risk of CVD. Darker skin absorbs light from the sun, and reduces the amount of vitamin D made by the body. We think that lower vitamin D in AA may lead to reduced blood vessel health and increased risk of CVD. This study will look at differences in nitric oxide that helps blood vessels relax. We will also look at "oxidant stress," which can reduce nitric oxide.

In this study, we will examine the function of blood vessels in the skin. The blood vessels in the skin are a model for blood vessels in other organs in the body. We will also look at the function of other blood vessels in the body.

This research is being done to find out why blood vessel function is reduced in young AA adults who are healthy. The research is also being done to find out if vitamin D will improve blood vessel function. Approximately 24 people will take part in this study at the Noll Lab.

2. What will happen in this research study?

Note: This study involves the use of drugs that are not approved by the FDA to treat disease. All of the drugs have been used in humans by us or others in the past. The FDA approved the use of the drugs for this study. We dilute some of the drugs in Lactated Ringer's, a type of saline fluid like that found throughout your body. The drugs are:

- Apocynin - antioxidant
- Tempol – antioxidant
- L-NAME – blocks the production of nitric oxide
- Sodium nitroprusside (SNP) – supplies nitric oxide; causes blood vessels to dilate

This project involves taking a daily vitamin D treatment by mouth for 4 weeks. The vitamin D treatment will take place between study visits.

________ initial **A. Screening**

You come to the lab for a screening visit to see if you are eligible for this study.

1. You drink only water and do not eat for 12 hours before the screening.
2. The research staff measures the following:
   g. Height;
   h. Weight;
   i. Waist circumference;
   j. Blood pressure;
   k. Heart rate;
   l. Skin reflectance
3. You complete a health history questionnaire.
4. Women of childbearing age have a urine pregnancy test.
5. The research staff will draw 30 mL (2 Tbsp) of blood from a vein in your arm. Some of the blood will be sent to a lab to see if the proteins, blood cells, electrolytes, etc. are within normal levels. We do not look for the presence of disease (e.g. HIV). All of the blood tests are common tests to determine your health status.
6. If you are eligible, we invite you back to the lab for the study visits.

If you agree to take part in the study, you provide a saliva sample for ancestry analysis. You also answer questions about socioeconomic status.

________ initial **B. Microdialysis Experiment**

6. <u>Preparation for the MD experiment:</u>
   d. Do not eat or drink caffeine (ex. Coffee, tea, Coca Cola, chocolate) or alcohol for 12 hours before the MD study.
   e. When you arrive at the lab, a staff member measures your heart rate, blood pressure, and oral temperature.
   f. Women of childbearing age have a urine pregnancy test if they have not had one within 2 weeks.

7. Microdialysis probe insertion:
   j. A tight band is placed around your arm to visualize veins.
   k. For each MD site, a pair of pen-marks is made on the arm 2.5 cm (1 inch) apart and away from veins. The tight band is removed.
   l. Your forearm is cleaned with an orange fluid called povidone iodine and alcohol.
   m. An ice bag is placed on your arm for 5 minutes to numb the skin.
   n. A thin needle is then inserted into the skin at each entry mark. The needle's tip travels between the layers of skin for 2.5 cm (1 inch) and exits the skin at the matching exit mark.
   o. The MD tubing is threaded through the needle and then the needle is taken out, leaving the tubing in the skin.
   p. Three MD sites are placed in the left arm.
   q. Any redness of the skin caused by the needle insertion fades in about 60 minutes.
   r. During this time, lactated Ringer's is perfused through the MD tubing.

8. Skin Blood Flow (SkBF):
   a. We tape a thin fiber optic laser Doppler flowmeter probe and its holder over the MD sites.
   b. The thin probe measures skin blood flow with a weak laser light. We measure skin blood flow throughout the experiment.

9. Local Heating Protocol: We add test substances to each MD site. The test substances are: (1) lactated Ringer's, (2) lactated Ringer's + tempol, and (3) lactated Ringer's + apocynin.
   c. We collect data for 15 minutes.
   d. We increase the temperature of the skin to 39ºC (102.2ºF). You may end the local heating phase at any time.
   e. We hold the skin temperature at 39ºC until skin blood flow becomes stable (~40 minutes).
   f. We add L-NAME to the MD sites until skin blood flow becomes stable again (~40 minutes).

10. Maximum Skin Blood Flow: We increase the temperature of the skin to 43ºC (109.4ºF). You may end the maximal blood flow phase at any time.
   f. We change the fluid in each MD tube to lactated Ringer's.
   g. Local skin temperature is maintained at 43ºC (109.4ºF) for 30 minutes.
   h. We then replace the fluid with Lactated Ringer's + SNP for a final 10 minutes.
   i. The experiment ends. We remove the MD tubing and place bandages over the sites.
   j. We measure blood pressure and heart rate before you leave.

11. Blood Sample:
   a. The research nurse repeats the blood draw (30 ml, 2 Tbsp) to test blood vitamin D levels.

________ initial **C. Macrovascular Function Assessment**

1. Flow-Mediated Dilation (FMD): FMD measures the health of blood vessels.
   a. We place a blood pressure cuff around your forearm.
   b. We place gel on your upper arm just above the elbow.

c. We place a Doppler ultrasound probe on the gel. The ultrasound makes sound waves to measure the size of blood vessels and the speed of the blood.
d. We make a "resting" measurement before we inflate the cuff.
e. The cuff inflates for 5 minutes to stop blood flow to and from the forearm.
f. We deflate the cuff and perform a second reading for 3 minutes.

2. Sublingual nitroglycerin: This test also measures the health of blood vessels. Nitroglycerin causes blood vessels to dilate.
a. The nurse is present throughout the procedure.
b. You lie on a bed or recliner.
c. We apply a blood pressure cuff on your upper arm.
d. As with FMD, we use an ultrasound probe during the test. We place the probe on an artery near your elbow.
e. A nurse places a 0.4 mg nitroglycerin tablet under your tongue. Then you close your mouth right away. The tablet breaks down in 15-90 seconds. Do not swallow until the tablet dissolves. The effect lasts for 5-10 minutes.
f. You lie still for 20 minutes after you received the nitroglycerin. You remain in the lab at least 20 minutes after you receive the nitroglycerin.
g. You stay in the lab for up to 60 minutes after the nitroglycerin if you have a bad or very strong reaction (e.g. drop in blood pressure that lasts longer than usual). We monitor you during this time.

_________ initial **D. Arterial Stiffness Assessment**

1. Pulse Wave Velocity (PWV): PWV measures the stiffness of blood vessels.
a. The testing room is set to a comfortable temperature with the lights dimmed.
b. You relax on the bed for approximately 10 minutes.
c. Blood pressure cuffs are placed on your upper arm and upper leg.
d. We feel for the strongest pulse from an artery in your neck. Once the spot with the strongest pulse is found, it is marked with a pen.
e. We measure the distance from the mark to the cuff on the leg and to the top of your chest with a tape measure.
f. We measure your blood pressure pulse, and the cuff on the arm inflates and then deflates. This measurement takes about 2 minutes.
g. We then hold a probe on the dot on the neck. The cuff on the leg inflates and deflates. This measurement takes about 2 minutes to complete.

_________ initial **E. Skin Biopsy**

1. We take two small pieces of skin from your arm (skin biopsy) using standard techniques.
2. First, you wash the site with soap and warm water. Then you sit in a recliner
3. We clean the top of the lidocaine-vial with alcohol. We clean the skin with alcohol. An approved clinician injects lidocaine into the skin at the biopsy sites to numb them. We wait a few minutes after injecting the lidocaine to give the drug time to work.
4. We clean the biopsy site 3 times with an alcohol pad.
5. We gently touch the site with the tip of a needle to see if you can feel anything. You may feel the slight pain of the pin-prick or only pressure. If you can feel pain, we wait a little longer. If needed, the approved clinician may add more lidocaine into the skin.

6. We use a punch-tool that looks like a screwdriver that has a round, hollow tip. The tip is 3mm (0.12 in) in diameter. The hollow tip acts like a cookie cutter. We place the tip of the punch against the skin at the biopsy site and apply mild pressure. You feel the pressure. The tip of the punch goes about 3 mm (0.12 in) into the skin. The punch collects a small piece of skin about 3mm x 2mm (0.12 in x 0.08 in).
7. We apply pressure with sterile dressing to the site to stop any bleeding.
8. We place the piece of skin into a small container.
9. We take another small piece of skin in the same way.
10. We apply a sterile bandage to the site.
11. We give you instructions about caring for the biopsy site.

________ initial **F. Vitamin D Treatment**

1. After completing the first study visit, you start to take a daily vitamin D pill. After 4 weeks of taking vitamin D, you repeat the study visit.
    a. The dose is 2000 IU/day vitamin D for four weeks.
    b. Vitamin D pills are given to you at the end of the first study visit.
    c. You return left over vitamin D pills at the final study visit.

3. What are the risks and possible discomforts from being in this research study?

Microdialysis: The risks are less than that for a blood draw because microdialysis uses only a small, local area of skin. In contrast, a blood draw involves not only skin, but also large blood vessels and blood. You are likely to have some pain and bruising like that from a blood draw. However, we use ice to numb your arm when we insert the tubing. Also, the small needle reduces pain when we insert the tubing. You are not likely to have pain after the tubing is in place. You may feel a little pain when we remove the tubing from your skin. Needles make some people feel sick to their stomach, lightheaded, or may cause them to faint. Although rare, the tubing could break as we remove it from the skin. Then we remove the tubing still in your skin by pulling on the other end of it. This presents no added risk for you. Even rarer, the tubing could break so that a piece of the tubing is left under your skin. In this case, we treat any tubing still in your skin like a splinter. We stop any mild bleeding with mild pressure and sterile gauze. Infection is possible. We keep the risk of infection very small by using sterile techniques and supplies like those used with blood draws. We apply a sterile bandage to the site after the experiment. We tell you how to take care of the site.

Fluid flowing through the tubing: The substances flowing through the tubing only go to a 2.5 cm2 (0.4 inch2) area of skin at each tubing site. The amount that enters the skin is very small. However, there is a chance of having a bad reaction to the substances. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting. We and other researchers have used these substances with microdialysis in skin. There have been no reports that these substances caused bad reactions. If a bad reaction should occur, we summon medical help.

Lactated Ringer's Solution and Normal Saline: These fluids are like the natural fluids in your skin. The fluids contain salt, potassium, lactate (Ringer's only), and chloride. The acid content is like that your body's natural fluids. A bad reaction to these fluids is highly unlikely.

Apocynin, Tempol, LNAME, and SNP. These substances stop or mimic the action of your body's natural chemicals upon the blood vessels in the skin. A small amount of these substances enter the skin around the tubing. This only affects the blood flow in the vessels in that nickel-sized area of skin. The effect of these substances is gone within an hour after the experiment.

Laser Doppler Flowmetry: Weak lasers can hurt your eye if you stare into the light for a long time. We do not turn on the laser until the probes are taped to a surface. The tape may irritate your skin.

Blood Pressure (manual, critical care monitor): We measure blood pressure using the method common in a doctor's office or with a machine. A cuff inflates on the upper arm. As the cuff slowly deflates, we listen with a stethoscope at the bend in the elbow or the machine takes a reading. During the short time we inflate the cuff, your arm may feel numb or tingly. The cuff could cause mild bruising.

Povidone Iodine: Researchers and hospitals use this orange-colored fluid to clean the skin. You could have a bad reaction to the fluid if you are allergic to iodine. You inform us if you have this allergy. In this case, we use only alcohol instead. A bad reaction could cause redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting.

Blood Draw: Blood draws often cause mild pain, bruising, swelling, or bleeding. There is also a slight chance of infection or a small clot. If you are nervous about needles, blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. Using the same techniques used in hospitals keeps the chance of infection minimal. Do not exercise hard for 24 hours before a blood draw.

Tape and sticky disks: The tape or sticky disks could cause a rash. During screening, you tell us if you are sensitive to tape. If a disk sticks very strongly, removing the disk could cause an abrasion like a rug-burn on your skin. An abrasion can feel tender or slightly painful, and can increase risk of infection. If you are sensitive to tape, you may have an increased chance for abrasion. An abrasion has occurred only twice during the years that the disks have been used in similar studies in our lab. We may use an adhesive remover like that used in a doctor's office to remove the disks. If you get an abrasion a nurse checks the site. Antibiotic ointment and a sterile bandage are applied. We tell you how to take care of the site. You could have an allergic reaction to the adhesive remover. The reaction could include rash, itching, fever, or breathing problems. Also, it could include changes in pulse, and/or blood pressure, convulsions, shock, and/or fainting. If a bad reaction should occur, we summon medical help right away.

Medical Screening: You may feel shy about giving health information. The staff collects the information in a private and professional manner. You may feel shy about being measured. You may request someone of the same sex to conduct parts of the screening.

Initial screening form: Only members of our lab group use this form. We use the form to help decide whether you are a good candidate for the study. You may feel shy about answering questions. You may request someone of the same sex to ask you the questions. We collect the

information in a private and professional manner. We keep the completed form confidential and secure.

Local heating: We measure the temperature of your skin under the holders. During heating, the skin feels very warm but does not hurt. The heating makes the skin under the holder red like when you take a hot bath. The redness goes away within several hours. Some people may be more sensitive to heating. If your arm feels too hot, tell us, and we reduce or stop the heating.

Skin Biopsy: You may stop the procedure at any time. Trained staff performs the biopsy. You may lie back in the reclining chair during the biopsy, if you wish. We make sure that you are informed and ready. You may still be nervous about needles or the procedure. If so, your blood pressure and heart rate may increase for a little while. You may also feel lightheaded, sick to your stomach, or may faint. The lidocaine numbs the site so that you feel very little or no pain during the biopsy. You feel the pressure of the biopsy tool on your skin. As with any event that breaks the skin, you could get an infection. Trained staff uses sterile techniques to keep the risk of infection very small. The skin biopsy may cause some pain, swelling, bleeding, and bruising. Gauze pressed onto the site stops bleeding. We place a sterile bandage on the site. We give you instructions about caring for the biopsy site. The biopsy is likely leave a small scar. The skin of some people overreacts to injury. If you are one of these, your skin may produce a scar that is larger and easier to see. There may be some minor pain for a couple of days when the lidocaine wears off. The pain would be like that felt after some blood draws.

Lidocaine: You may feel brief pain from the needle. You may feel brief burning when we first inject the lidocaine into the skin. Although unlikely you could have a bad reaction to the lidocaine. This reaction could produce redness, itching, rash, and/or swelling. A worse reaction could also cause fever, breathing problems, changes in pulse, convulsions, and/or fainting. If a bad reaction should occur, we summon medical help right away. If you know that you are allergic to lidocaine, we can reduce pain two other ways. We could inject sterile saline and/or use ice on the site.

FMD Test / Doppler Ultrasound: There is a small chance the probe could irritate the skin. Minor redness may occur where the researchers place the probe against the arm. This is temporary. While the researchers inflate the cuffs, the arms and feet may feel numb or tingly, and the color of the skin may change slightly. The cuffs could cause mild bruising. The gel is the same as that used with medical ultrasound tests. The gel may feel cool or cold on the skin. A bad reaction to the gel is highly unlikely.

Sublingual Nitroglycerine: The research-use of nitroglycerin for artery measurements is not an FDA-approved use of this drug. However, nitroglycerin has been used in this way in many research studies without problem. Nitroglycerin is FDA approved for the treatment of angina (heart pain). The drug is often prescribed for heart patients who have, or are at risk for, angina.
You may have some of the following reactions to the nitroglycerine: headache, lightheadedness, dry mouth, flushing, irregular heartbeat, weakness, nausea, vomiting, 5-10 minute drop in blood pressure, fainting, dizziness, sweating.
You may also notice a sweet taste and/or tingling in your mouth while the tablet dissolves. All these effects are usually short-lived. We can reduce some of them by having you lie down for 20 minutes after you receive the tablet. If your blood pressure drops, it is likely to return to within 10 mmHg of your starting level by the time the test ends. We monitor you for up to an hour after you

receive the nitroglycerin if you have a strong or bad reaction. If your blood pressure does not return to baseline, and you have related symptoms (e.g. dizziness) we advise you to see your doctor. You could have a mild or severe allergic response to the drug. This response could include rash, itching, difficulty breathing, and swelling of your face, lips, tongue, or throat. If you have a severe reaction (e.g. severe allergic response) we call 911.
The effects of nitroglycerin on pregnant or nursing women are unknown. You are not to be in the study if you are pregnant or nursing.

Latex: Some gloves and medical materials are made of latex rubber. Some people may be sensitive to latex. Screening finds and excludes subjects that have a known latex allergy.

Vitamin D_3: Vitamin D may become toxic if blood levels are too high. You will take 2,000 IU vitamin D per day. It is highly unlikely that this amount of vitamin D will cause blood levels to become toxic. There is a small chance of having a bad reaction to the vitamin D tablets.

There is a risk of loss of confidentiality if your information or your identity is obtained by someone other than the investigators, but precautions will be taken to prevent this from happening. The confidentiality of your electronic data created by you or by the researchers will be maintained as required by applicable law and to the degree permitted by the technology used. Absolute confidentiality cannot be guaranteed.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to you?

There is no guarantee that you will benefit from this research. You receive a screening that informs you about your health such as your current blood pressure and blood cholesterol levels. You could gain knowledge about how your body works.

4b. What are the possible benefits to others?

The results of the research may help scientists better understand why risk of CVD is higher for those of African American descent. The results may also help to better understand how to reduce CVD in the African American population. The project provides valuable experience and education for graduate and undergraduate students of The Pennsylvania State University.

5. What other options are available instead of being in this research study?

You may decide not to participate in this research study.

6. How long will you take part in this research study?

If you agree to take part, it will take you about 1 month to complete this research study. You will be asked to return to Noll Lab 3 times. Vitamin D treatment will be 4 weeks between visits 2 and 3.

You will need to visit the Noll Lab for the following:

Day 1, Screening: 1.5 hours

Day 2, Experiments: 6 hours
Day 3, Experiments: 6 hours

Total time for study visits: 13.5 hours

7. How will your privacy and confidentiality be protected if you decide to take part in this research study?

7a. What happens to the information collected for the research?

Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information.

- We keep the list that matches your name with your code number in a locked file or password protected file on a computer in a room that is locked when unoccupied. Only authorized members of the lab have access to the list.
- We label your research records with your code number and keep them in a locked file or password protected computer in a room that is locked when unoccupied.
- We label your research samples with your code number. We keep the samples in a dedicated ultralow freezer in Noll Lab until analysis.

All research specimens sent to outside labs for analysis (e.g. Quest Labs) are identified only by a code number.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

We will do our best to keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people may find out about your participation in this research study. For example, the following people/groups may check and copy records about this research.

- The Office for Human Research Protections in the U. S. Department of Health and Human Services
- The Institutional Review Board (a committee that reviews and approves research studies)
- The Office for Research Protections
- The U. S. Food and Drug Administration

Some of these records could contain information that personally identifies you. Efforts will be made to limit the use and sharing of your personal research information to people who have a need to review this information. Reasonable efforts will be made to keep the personal information in your research record private. However, absolute confidentiality cannot be guaranteed.

7b. What will happen to my research information and/or samples after the study is completed?

Your information or samples that are collected as part of this research will not be used or distributed for future research studies, even if all of your identifiers are removed.

Most tests done in research studies are only for research and have no clear meaning for health care. The screening includes some standard medical tests that may yield results that do have meaning for your health. You will receive copies of the results from the standard blood tests performed in the screening. The researchers inform you of test results about which you may wish tell your own doctor. If this happens, then you may want to get a second test from a certified clinical laboratory and consult your doctor. You will have to pay for those additional services yourself.

8. What are the costs of taking part in this research study?
8a. What happens if you are injured as a result of taking part in this research study?

In the unlikely event you become injured as a result of your participation in this study, medical care is available. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. By signing this document, you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

9. Will you be paid or receive credit to take part in this research study?

If you are eligible for this study, you will receive payment as follows. There is no payment for the screening visit.

MD Experiments: $15.00 / MD probe inserted + $20.00 completing MD experiment
FMD + Nitroglycerin Experiment: $35
PWV Experiment: $15
Skin Biopsies: $50 per biopsy

Experimental Visit 1: $215.00 (3 MD probes; FMD + Nitroglycerin; PWV; 2 skin biopsies)
Experimental Visit 2: $215.00 (3 MD probes; FMD + PWV; 2 skin biopsies)

Total: $430

You can receive payment for experiments not completed. We pay an amount of money equal to the part completed. For instance, if you complete half of Experiment 1, you receive $15.00 for each probe inserted plus $10.00. ($10.0 is one-half of $20.00). We may ask you to repeat a trial. If you agree to repeat a trial, you receive payment for the repeated trial as stated above. You are reimbursed for gasoline if you live more than 20 miles from Noll Lab.

Total payments within one calendar year that exceed $600 will require the University to report these payments to the IRS annually. This may require you to claim the compensation that you receive for participation in this study as taxable income. You will need to provide your social security number and address to receive a check for payment and for tax reporting purposes.

10. What are your rights if you take part in this research study?

Taking part in this research study is voluntary.

- You do not have to be in this research.
- If you choose to be in this research, you have the right to stop at any time.
- If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.
- If you choose to withdraw from the study, all data collected up to the point of withdrawal will remain part of the study and may not be removed.

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal from the study include if we deem that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You may decline to answer certain questions. You may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

11. If you have questions or concerns about this research study, whom should you call?

If you have any questions, complaints or concerns about this research, you may call any of the phone numbers below. You can also call these numbers if you feel this study has harmed you. If there are findings during the research that could relate to you wanting to help with the study, you will be told of the findings.

- Stephen (Tony) Wolf (W: 814-863-8556, C: 559-269-5198)
- Susan Slimak (W: 814-863-8556, C: 814-880-4396)

You may also contact the Office for Research Protections at (814) 865-1775, IRB-ORP@psu.edu if you:

- Have questions regarding your rights as a person in a research study.
- Have concerns, complaints, or general questions about the research.
- You may also call this number if you cannot reach the research team or wish to offer input or to talk to someone else about any concerns related to the research.

You may visit the Office for Research Protections' website at https://www.research.psu.edu/irb/participants for:

- Information about your rights when you are in a research study;
- Information about the Institutional Review Board (IRB), a group of people who review the research to protect your rights; and
- Links to the federal regulations and information about the protection of people who are in research studies. If you do not have access to the internet, copies of these federal regulations are available by calling the ORP at (814) 865-1775.

INFORMED CONSENT TO TAKE PART IN RESEARCH

Signature of Person Obtaining Informed Consent

Your signature below means that you have explained the research to the subject or subject representative, provided the subject or subject representative an opportunity to discuss and consider whether or not to participate in the research, and have answered any questions the subject or subject representative has about the research.

______________________________	__________	________________
Signature of person who explained this research	Date	Printed Name

(Only approved investigators for this research may explain the research and obtain informed consent.)

Signature of Person Giving Informed Consent

Before making the decision about being in this research you should have:

- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Signature of Subject

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

___________________________	__________	________________
Signature of Subject	Date	Printed Name

VITA
S. Tony Wolf

Education

Ph.D.	The Pennsylvania State University, Department of Kinesiology	2020
M.A.	California State University, Department of Kinesiology	2016
B.S.	California State University, Fresno, Department of Kinesiology	2012

Fellowships

NIH T32 Predoctoral Training Fellowship, The Pennsylvania State University 2018
University Graduate Fellowship, The Pennsylvania State University 2017

Grants & Awards

Penn State College of Health and Human Development Limited Endowment Fund for Dissertation Research 2019
Penn State Center for Human Evolution and Diversity Seed Grant 2019
Penn State Dermatology Marks Endowment Fund 2018
American College of Sports Medicine Foundation Doctoral Student Research Grant 2018

Publications

1. **Wolf ST**, Jablonski NG, & Kenney WL. Examining the place of "race" in physiology. *Am J Physiol Heart Circ Physiol*, In Press, 2020.
2. Greaney JL, Stanhewicz AE, **Wolf ST**, & Kenney WL. Thermoregulatory reflex control of cutaneous vasodilation in healthy aging. *Temperature*, In Press, 2020.
3. **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*, 2020.
4. Berry CW, **Wolf ST**, Murray B, & Kenney WL. Hydration efficacy of a milk permeate-based oral hydration solution. *Nutrients*, 2020.
5. **Wolf ST**, Berry CW, & Dillon GD. A role for endothelin-A receptors in altered blood flow and pressor responses during exercise in hypertensive adults. *J Physiol*, 2020.
6. **Wolf ST**, Kenney LE, & Kenney WL. Ultraviolet radiation exposure, risk, and protection in military and outdoor athletes. *Curr Sports Med Rep*, 2020.
7. **Wolf ST** & Kenney WL. The vitamin D-folate hypothesis in human vascular health. *Am J Physiol Regul Integr Comp Physiol*, 2019.
8. **Wolf ST**, Berry CW, Stanhewicz AE, Kenney LE, Ferguson SB, & Kenney WL. Sunscreen or simulated sweat minimizes the impact of acute ultraviolet radiation on cutaneous microvascular function in healthy humans. *Exp Phys*, 2019.
9. **Wolf ST**, Stanhewicz AE, Clarke MM, Cheuvront SN, Kenefick RW, & Kenney WL. Age-related differences in water and sodium handling following commercial hydration beverage ingestion. *J Appl Physiol (1985)*, 2019.
10. Clark MM, Stanhewicz AE, **Wolf ST**, Cheuvront SN, Kenefick RW, & Kenney WL. A randomized trial to assess beverage hydration index in healthy older adults. *Am J Clin Nutr*, 2019.
11. **Wolf ST**, Stanhewicz AE, Jablonski NG, & Kenney WL. Acute ultraviolet radiation exposure attenuates nitric oxide-mediated vasodilation in the cutaneous microvasculature of healthy humans. *J Appl Physiol (1985)*, 2018.