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CUTANEOUS CARDIOVASCULAR AND THERMOREGULATORY RESPONSES TO ULTRAVIOLET RADIATION EXPOSURE WITH SUNSCREEN APPLICATION

A Thesis in

Kinesiology

by

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ABSTRACT

Exposure to UVR is associated with deleterious health effects such as cutaneous vascular dysfunction, DNA damage, and skin cancer. Outdoor athletes routinely eschew using sunscreen as protection from UVR due to perceptions that sunscreen may impair thermoregulatory heat loss, although, past studies examining the impact of sunscreen application on thermoregulatory responses have been equivocal. Additionally, while acute exposure to UVR has been found to attenuate nitric oxide (NO)-dependent vasodilation, it is unknown how seasonal UVR exposure impacts cutaneous microvascular function. Therefore, it is necessary to understand the impacts of UVR and sunscreen application on vascular health and thermoregulatory responses, respectively, so that outdoor recreationists can make informed decisions regarding sun safety.

This thesis comprises two empirical studies investigating (1) the impact of within-limb variation in skin pigmentation on cutaneous microvascular function and (2) the impact of sunscreen application on integrative thermoregulatory responses. The findings of the first study conclude that within-limb differences in skin pigmentation secondary to UVR exposure do not alter NO-dependent cutaneous vasodilation. The findings of the second study suggest that the combinations of temperature and humidity at which heat stress becomes uncompensable are not altered by the application of either chemical or mineral sunscreen. At the highest level of compensable heat stress, sunscreen application did not alter any thermoregulatory responses of interest (body temperatures, heart rate, sweating, sweating efficiency, evaporative efficiency, etc.). Therefore, perceived adverse thermoregulatory effects should not dissuade outdoor athletes from utilizing sunscreen. Together these studies can provide information to outdoor athletes and recreationists so that informed decision about adequate sun protection can be made.

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Chapter 2

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Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Outdoor athletes are often exposed to levels of ultraviolet radiaion (UVR) from the sun that far exceed guildlines given by international commissions for radiation protection. UVR exposure is associated with many deleterious health effects. However, athletes will routinely refrain from using sunscreen as protection from UVR due to perceptions that it may impair thermoregulation. It is imparative to understand the adverse heatlh effects of UVR exposure as well as the impact of intervention and protection practices on human heat balanace.

Adverse Effects of Ultraviolet Radiation Exposure

UVR exposure is a pivotal component in the development of skin cancer due to its role in the production of DNA-damaging reactive oxygen species (ROS) [1, 2]. Both acute, intermittent and cumulative exposure to UVR leads to skin cancer formation [3, 4]. Additionally, UVR may degrade the folate metabolite 5-methyltetrahydrofolate (5-MTHF) which 1) promotes endothelial nitric oxide synthase (eNOS) stability and bioavailability, and 2) directly scavenges ROS [5, 6]. Previously, it was demonstrated that acute UVR exposure reduced NO-mediated vasodilation of the cutaneous microvasculature via direct and/or indirect photodegredation of 5-MTHF [7, 8]. Locally derived NO is a critical component of vascular function and characteristic of a healthy vascular phenotype such that reduction of eNOS negatively impacts microvascular function [9-11]. While, it is established that acute exposure to UVR attenuates NO-dependent cutaneous

vasodilation, it is unknown if intermittent seasonal UVR exposure resulting in tanning causes similar reductions.

Outdoor athletes and recreationists are at greater risk of adverse UVR exposure effects due to experiencing large doses of exposure [12, 13]. Several studies utilizing personal UVR dosimetry for the quantification of UVR exposure experienced by an individual determined that many professional outdoor cyclists [14], triathletes [15], and alpine skiers [16] are exposed to UVR doses that far exceed the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines [17]. Additionally, even among non-professional outdoor athletes and recreationist, UVR exposure frequently exceeds the dose exposure limit recommended by ICNIRP [17]. In particular, the increased incidence of sunburn among outdoor athletes may promote adverse effect of UVR exposure such as DNA damage in the skin increasing the risk of skin cancer [12, 13, 18].

Sun Protection, Performance, and Thermoregulation

Despite it being well established that UVR exposure can put outdoor athletes and recreationist as increased risk of adverse effects such as skin cancer and microvascular dysfunction, many do not participate in adequate sun protection practices, such as wearing sunscreen [18, 19]. Common reasons for refraining from applying sunscreen include competition rules, lack of availability, forgetting to apply, and the belief that sunscreen will impair their performance [18, 20]. One perceived potential performance impairment is that sunscreen will negatively impact heat dissipation and sweating, however, studies examining such effects of sunscreen are inconclusive [21-24].

Studies investigating the application of various sunscreens on small areas of skin or over the whole body have produced equivocal results regarding the impact on heat dissipation and

sweating during exercise in the heat. For example, whole body application of chemical-based sunscreen during exercise in a hot-dry environment potentially elevated mean skin temperature and hindered sweat evaporation [21]. Alternatively, another study determined whole body application of chemical-based sunscreen enhanced heat dissipation through increasing the core-to-skin temperature gradient [22]. Other have reported that mineral-based sunscreen hindered sweat production [23] while chemical-based sunscreen had no effect on sweating [24]. In order for outdoor athletes and recreationist to partake in adequate sun protective practices clear insight into the impact of sunscreen on thermoregulation must be provided.

PSU HEAT Project

The ongoing PSU HEAT project is designed to determine the environmental conditions (i.e. combinations of temperature and humidity) beyond which human heat balance is unattainable. The project utilizes a progressive heat stress protocol for the determination of critical environmental limits above which heat balance within an environment at a given metabolic rate is not possible. This protocol allows for the examination of integrated thermoregulatory responses for determination of the safe upper limits of heat and humidity tolerable. Environmental conditions above critical environmental limits are deemed uncompensable as there is a lack of heat balance, while those below critical environmental limits are compensable and heat balance is maintained. Progressive heat stress protocols have been used to determine the safe upper limits of heat a humidity for a variety of clothing ensembles, age groups, environmental conditions, and metabolic rates [25-29]. The protocol is useful in that integrative thermoregulatory responses can be understood for a wide range of environments in various clothing/outerwear conditions and at various metabolic rates.

Summary

Outdoor athletes experience elevated exposure to UVR compared to their less active counterparts. Adverse cutaneous health effects such as development of skin cancer and microvascular dysfunction are associated with exposure to UVR from the sun. Acute UVR exposure is associated with attenuated NO-dependent cutaneous vasodilation. However, it is unknown whether seasonal UVR exposure resulting in tanning causes similar reductions. Despite being at increased risk of deleterious health effects of UVR, outdoor athletes routinely eschew using sunscreen as protection. This is in part due to perceptions that sunscreen may impair thermoregulatory heat loss. Although, past studies examining the impact of sunscreen application on thermoregulatory responses, such as sweating, have been equivocal. Therefore, the need remains to understand the impact of 1) seasonal UVR exposure on cutaneous microvascular health and 2) sunscreen application and thermoregulatory responses during exercise in the heat, so that outdoor athletes can make informed decisions regarding sun safety.

Chapter 2

WITHIN-LIMB VARIATION IN SKIN PIGMENTATION DOES NOT INFLUENCE CUTANEOUS VASODILATION

INTRODUCTION

Locally-derived endothelial nitric oxide (NO) is a critical contributor to the full expression of cutaneous vasodilation responses [9, 10]. Consequently, greater NO-mediated vasodilation is associated with a healthy vascular phenotype [11]. NO production and bioavailability are influenced by numerous mechanisms including the expression and coupling of the endothelial NO synthase (eNOS) dimer, oxidative stress, and inflammation [5, 30]. As such, any physiological stressor that reduces eNOS activity and/or increases oxidative stress or inflammation may negatively impact microvascular endothelial function.

The folate metabolite, 5-methyltetrahydrofolate (5-MTHF), promotes the stabilization of endothelial NO synthase (NOS) and bioavailability of NO by (1) increasing production of tetrahydrobiopterin (BH_4), an essential cofactor in the coupling of eNOS, and (2) acting as a potent antioxidant, directly scavenging reactive oxygen species (ROS) [5, 6]. Ultraviolet radiation (UVR) may degrade 5-MTHF directly and/or indirectly through increased production of reactive ROS [31-33]. Our laboratory has previously demonstrated reduced NO-mediated vasodilation in the cutaneous microvasculature after an acute exposure to UV-B or broad-spectrum UVR [8, 34]. However, local perfusion of ascorbate (a non-specific antioxidant) or 5-MTHF improved NO-mediated vasodilation after UV-B exposure [34], such that there was no difference between non-exposed control skin and UV-B exposed skin treated with ascorbate or 5-MTHF. Together, our data suggest that UVR exposure acutely reduces NO-mediated cutaneous vasodilation through direct and/or indirect photodegradation of 5-MTHF.

In contrast to the impact of UVR exposure on 5-MTHF, UVB radiation exposure catalyzes the production of vitamin D from epidermal and dermal stores of 7-dehydrocholesterol [35, 36]. UV-B-induced cutaneous vitamin D synthesis is decreased, however, when skin melanin concentrations are greater (i.e., a darker skin pigmentation), due to increased absorption of UV-B by skin melanocytes [37, 38]. Importantly, vitamin D may promote endothelial health by (1) increasing eNOS expression, (2) suppressing ROS production and/or increasing ROS scavenging, and/or (3) reducing inflammation [39, 40]. In this context, our laboratory has demonstrated that constitutive skin pigmentation is negatively associated with serum vitamin D concentrations [25(OH)D] and the NO contribution to cutaneous vasodilation during local heating in healthy young adults [37, 41]. Further, serum [25(OH)D] was directly related to the NO contribution to cutaneous vasodilation, suggesting that microvascular endothelial function was greatest in those who were vitamin D sufficient [37]. Thus, vitamin D concentrations and the NO contribution to cutaneous vasodilation responses were typically highest in those with lightly-pigmented skin. Together, those findings suggest that lower vitamin D concentrations contribute to lesser NO bioavailability in otherwise healthy, darkly-pigmented young adults.

In sum, our laboratory has demonstrated (1) acute UVR-induced reductions in NO-mediated cutaneous vasodilation, secondary to direct and/or indirect degradation of 5-MTHF, and (2) persistently reduced NO-mediated cutaneous vasodilation in those with constitutively moderate to dark skin pigmentation, secondary to vitamin D insufficiency/deficiency. However, it remains unclear whether within-individual variation in skin melanin concentrations, secondary to seasonal UVR exposure, influence NO-dependent cutaneous vasodilation in individuals with constitutively light skin pigmentation. Therefore, the present study aimed to compare NO-dependent cutaneous vasodilation in areas of tanned skin that was regularly exposed to UVR to that of relatively unexposed/untanned skin on the same limb in individuals with light constitutive skin pigmentation. We hypothesized that (1) NO-dependent cutaneous vasodilation would be

reduced in tanned skin relative to untanned skin, and (2) NO-dependent cutaneous vasodilation would be negatively associated with increases in tanning-induced skin pigmentation.

METHODS

Subjects

Experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University. Written and verbal consent were obtained voluntarily from all subjects before participation, according to the Declaration of Helsinki. Seven healthy subjects (4 men and 3 women) underwent an initial screening that included physical examination (i.e., height, weight, blood pressure, and heart rate). Subjects with Fitzpatrick skin type I and II were enrolled [42]. Because this was a strictly within-subject study design, participants were included without regard to age, blood pressure, BMI, or blood biochemistry. Subjects with rash, skin disease, disorders of pigmentation, known skin allergies, allergies to folic acid, or kidney disease were excluded. Subjects were regularly active outdoors (cycling, running, golfing, etc.) according to self-report. Female subjects were premenopausal and menstrual cycle phase was not considered or recorded.

Based on previously published data [41] demonstrating the difference in NO-mediated vasodilation in subjects with lightly- compared to darkly-pigmented skin, we determined a priori using an effect size of 1.21 ($\alpha = 0.05$; power = 0.8) that six subjects would be sufficient to detect within- and between-group differences in the %NO contribution to the local heating response.

Assessment of skin pigmentation

Skin pigmentation was measured by skin reflectance spectrophotometry (DermaSpectrometer; Cortex Technology, Hadsund, Denmark) to determine the melanin index (M-index) of the skin on the subject's dorsal and ventral aspects of the forearm and inner aspect

of the upper arm. These sites were chosen as they provide a range of high (dorsal forearm), moderate (ventral forearm), and low (inner-upper arm) sun exposure. Lower and higher M-indices are related to lighter and darker skin pigmentation, respectively.

Experimental protocol

Data were collected during late summer and early fall (August-October) in order to maximize the potential effect of seasonal exposure to UVR. All protocols were performed on a single day in a thermoneutral laboratory with the subject in a semi-supine position with the experimental arm supported at heart level. Intradermal microdialysis fibers (10 mm, 55-kDa cut-off membrane; CMA, Holliston, MA) were simultaneously placed into the dermal layer of the ventral and dorsal aspects of the forearm and the inner aspect of the upper arm for a total of three fibers. 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 because of light exposure. 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) [9]. 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, Milwaukee, WI).

Each fiber was initially perfused with lactated Ringers solution during the first ~60-min to allow for resolution of hyperemia associated with fiber placement. Following this period, baseline data were collected (~20 min) before beginning a local heating (42°C) protocol, as described previously [9, 10]. This heating protocol elicits an initial peak mediated by the axon reflex, after which there is a gradual rise and eventual plateau in blood flow [30, 43]. After

observing a stable local heating plateau (~40-min), 15 mM N^G-nitro-L-arginine methyl ester (L-NAME; NOS inhibitor) was perfused through all sites, allowing for quantification of NO-dependent vasodilation (%NO) [5, 30]. Following a stable L-NAME plateau, 28 mM sodium nitroprusside (SNP; USP, Rockland, MD) was perfused through all sites, and local temperature was increased to 43°C to induce maximal vasodilation [10, 43].

Data acquisition and analysis

LDF data were recorded at 40 Hz and stored for offline analysis (PowerLab/LabChart, ADInstruments, Colorado Springs, CO). 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 [10, 44]. The percentage of cutaneous vasodilation mediated by NO (%NO) was calculated from the difference between the local heating and L-NAME plateaus.

Two-way repeated-measures ANOVA was used to detect differences among the three experimental sites at each phase of the local heating protocol and the NO-contribution to vasodilation (GraphPad Prism, v. 9.2, GraphPad Software, San Diego, CA). One-way repeated measures ANOVA was used to detect differences in M-index. Data were checked for normality for all statistical analyses. A non-normal distribution was found for the local heating plateau; therefore, a nonparametric test was conducted for that phase only. To determine whether the magnitude of increase in skin pigmentation associated with seasonal tanning influences the potential change in NO-mediated vasodilation, simple linear regression analysis was used to assess the relation between M-index and NO-dependent vasodilation, with all three sites from each subject being entered into the analysis. Significance was set a priori and accepted at $\alpha = 0.05$. All values are presented as mean ± standard deviation.

RESULTS

Subject characteristics are presented in Table 2-1. Subjects who were >40 years of age, had BP >130/80 mmHg, or BMI ≥30 did not quantitatively influence the results. M-index was higher (i.e., pigmentation was darkest) on the dorsal aspect of the forearm compared to the ventral aspect and inner aspect of the upper arm (both $p<0.05$); however, M-index was not significantly different between the ventral aspect of the forearm and the inner-upper arm ($p=0.27$).

Table 2-1. Subject Characteristics

	Mean ± SD	Range
Age, yr	33 ± 14	20-65
Systolic BP, mmHg	126 ± 12	101-141
Diastolic BP, mmHg	75 ± 10	65-90
BMI, kg/m^2	23 ± 3	21-30
M-Index, AU		
Dorsal Forearm	50.5 ± 11.8	39.1-72.7
Ventral Forearm	37.5 ± 7.4*	27.7-47.6
Upper Arm	30.0 ± 4.0*	23.8-35.0

Data are presented as means ± SD and ranges; n = 7 (4 men, 3 women). AU, arbitrary units; BP, blood pressure; M-Index, a skin-reflectance measure of melanization. *P < 0.05 compared with dorsal forearm.

Figure 2-1 illustrates a representative tracing of red cell flux in response to the standardized local heating protocol for one subject. The decrease in red cell flux by NOS inhibition via local administration of L-NAME is indicated.

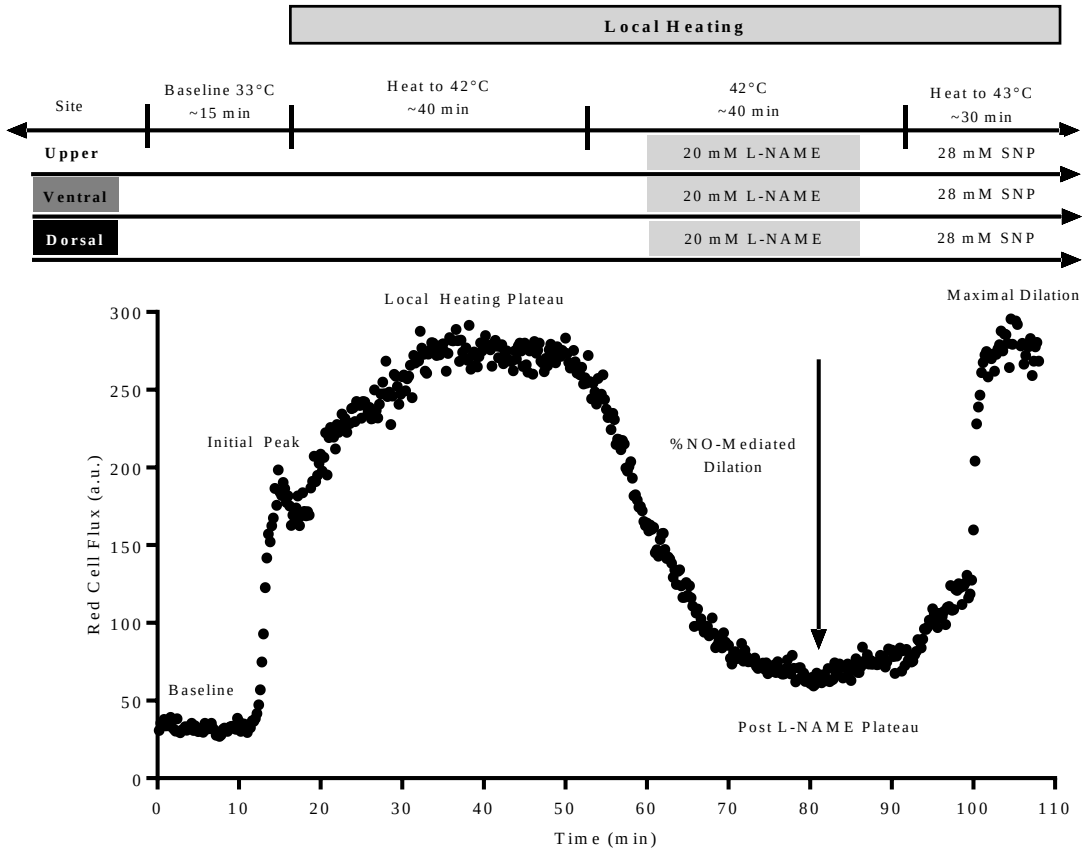

Figure 2-1. A representative tracing of red cell flux in response to the standardized local heating protocol for one subject. The percent decrease in red cell flux with NOS inhibition by local administration of L-NAME (l-N^G-nitroarginine methyl ester) is indicated by the arrow.

Presented in Figure 2-2 is $\%CVC_{max}$ for each phase of the local heating protocol at each of the three sites. There were no differences in $\%CVC_{max}$ among the three sites at any phase of the local heating protocol. Importantly, CVC_{max} was similar among the sites (dorsal: 1.73±0.75 flux/mmHg; ventral: 1.80±0.53 flux/mmHg; upper: 2.04±0.86 mmHg; $P \geq 0.23$).

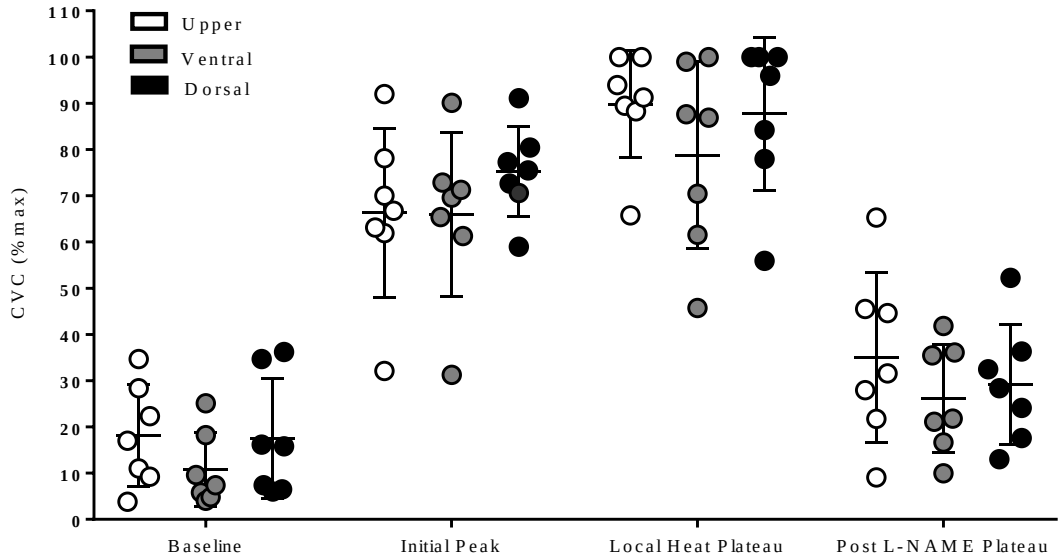

Figure 2-2. Comparisons in $\%CVC_{max}$ among the three sites at each phase of the local heating protocol (n = 7). There were no differences in $\%CVC_{max}$ due to tanning-induced increases in skin pigmentation during baseline, at the initial peak, or during the local heating or post-l-NAME plateaus. Data were analyzed using two-way repeated-measures ANOVA and are presented as means ± SD with individual data points. $\%CVC_{max}$, percent maximal cutaneous vascular conductance.

Figure 2-3 depicts the %NO contribution to cutaneous vasodilation in response to local heating at each site. There were no differences in %NO-mediated vasodilation among the sites. Further, there was no association between %NO-mediated vasodilation and M-index ($r = 0.24$, $P = 0.30$).

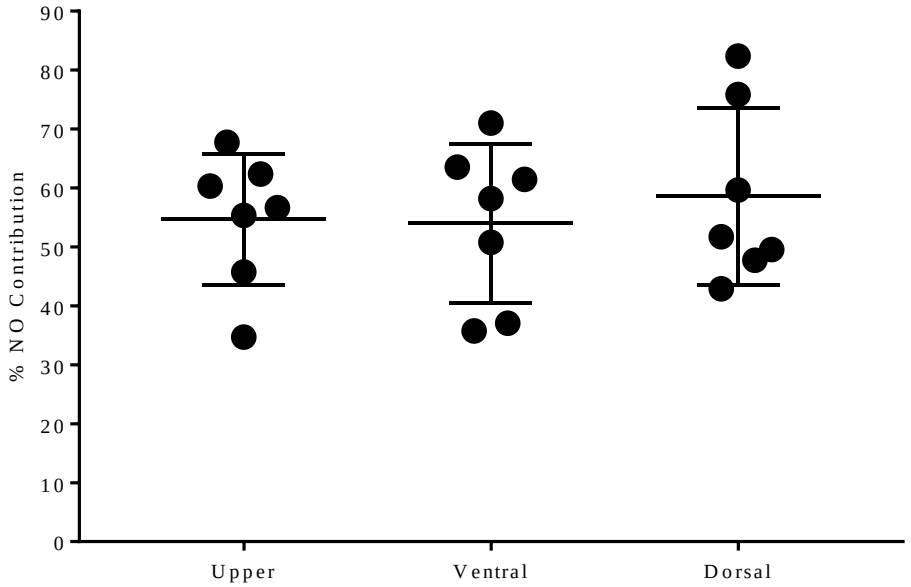

Figure 2-3. Comparisons in the percent contribution of nitric oxide (%NO Contribution) to the local heating plateau among the three sites (n = 7). Data were analyzed using two-way repeated-measures ANOVA and are presented as means ± SD with individual data points.

DISCUSSION

The primary finding of this study was that within-limb variation in M-index due to seasonal UVR exposure – unlike inter-individual differences in constitutive skin pigmentation – did not alter the magnitude of the cutaneous vasodilation response to local heating nor the NO-mediated component of that response. Additionally, there was no association between seasonal tanning-induced increases in skin pigmentation and NO-mediated vasodilation of the cutaneous microvasculature. These data suggest that seasonal tanning-induced increases in skin melanin concentrations do not impact NO-dependent cutaneous vasodilation.

Nitric oxide is a critical component of the vasodilatory response to local heating in healthy adults [9, 11]. Adequate bioavailability of 5-MTHF contributes to the full expression of NO-mediated cutaneous vasodilation responses through its role as an eNOS cofactor and by

acting as a potent antioxidant [5]. In this context, we previously demonstrated [8, 34] that acute UVR exposure attenuated NO-mediated cutaneous vasodilation via photodegredation of 5-MTHF, directly and/or indirectly by increasing local production of ROS [34]. Recovery time between UVR exposure and return to normal NO-mediated cutaneous vasodilation remains unclear.

In contrast to the potential deleterious effects of acute UVR exposure on NO-mediated cutaneous vasodilation observed by our laboratory, chronic UVR exposure, particularly in the UV-B spectrum, promotes adequate vitamin D bioavailability [45]. In turn, vitamin D may promote healthy endothelial function by increasing eNOS expression and/or by reducing oxidative stress and inflammation [39, 40]. Indeed, our laboratory has shown that vitamin D status is directly related to NO-mediated cutaneous vasodilation, such that those who are vitamin D sufficient have a greater NO contribution to cutaneous vasodilation responses relative to those who are insufficient or deficient [37]. Melanin within the skin absorbs UVB such that darker skin pigmentation impairs vitamin D synthesis [46]. As such, individuals with constitutively dark skin pigmentation who are living in areas with relatively low UVR exposure and/or high seasonality are at greatest risk of vitamin D deficiency [38, 47].

The findings of the present study are in contrast with previous studies from our laboratory demonstrating (1) acute UVR-induced reductions in NO-mediated cutaneous vasodilation and (2) persistently reduced NO-mediated cutaneous vasodilation in those with constitutively darkly-pigmented skin [7, 8, 37, 41]. One possible explanation for this difference may be the duration/frequency of UVR exposure. UVR exposure that would typically degrade folate results in rapid generation of protective immediate pigment darkening and, subsequently, delayed tanning [31]. Therefore, acute and local impacts of UVR exposure on folate and NO bioavailability may be transient (resolve after a few hours) and there may be a protective effect of tanning-induced increases in skin melanin concentrations on subsequent exposures.

However, the contrast between the present findings and our previous data demonstrating differences in NO-mediated cutaneous vasodilation between darkly- and lightly-pigmented young adults [41, 48] are more likely explained by the difference between constitutive and tanning-induced skin pigmentation in terms of vitamin D status. Vitamin D status is a mediator in the relation between constitutive skin pigmentation and NO-dependent microvascular function; that is, vitamin D insufficiency or deficiency may contribute to persistent microvascular dysfunction in otherwise healthy, darkly-pigmented adults [37]. The cohort in the present study consisted of individuals with light constitutive skin pigmentation, with seasonal changes in UVR resulting in within-limb variation in M-index. Therefore, the potential mechanisms of function/dysfunction (i.e., vitamin D deficiency vs. UV-induced 5-MTHF degradation) differ from those of a cohort with constitutively dark skin pigmentation. Although we did not measure vitamin D status in this study, our laboratory has demonstrated that those with constitutively light skin pigmentation are typically vitamin D sufficient, and that vitamin D supplementation does not augment microvascular function in those individuals [41]. Furthermore, tanning-induced increases in M-index in lightly-pigmented individuals suggest likely increases in vitamin D bioavailability via regular sun exposure.

An important consideration in the present study is that although participants were regularly active outdoors (cycling, running, golfing, etc.), no data were collected regarding frequency/duration of outdoor activities in each subject, or the temporality of the most recent exposure in relation to the experimental visit. Thus, there is likely variation in the daily amount of UVR that subjects were exposed to, and the proximity of the most recent exposure, that was unaccounted for. For this reason we looked at the relation between M-index and %NO-mediated vasodilation and found no association between tanning-induced increases in skin pigmentation and %NO-mediated vasodilation. However, we cannot rule out the possibility that a greater

magnitude of exposure in a region with more intense and less seasonal UVR may result in cutaneous microvascular dysfunction in lightly-pigmented individuals.

In summary, the present study demonstrated that in constitutively lightly-pigmented skin, seasonal UVR exposure induced variation in M-index does not alter the NO contribution to cutaneous vasodilation responses to local heating. These data suggest that repeated, seasonal UVR exposure does not impact NO-mediated cutaneous microvascular function.

AUTHOR CONTRIBUTIONS

The author's responsibilities were as follows: WLK and STW designed research; KGF and STW conducted research; KGF, STW, and WLK interpreted results of experiments; KGF and STW analyzed data; KGF drafted manuscript; KGF, STW, and WLK revised and edited manuscript; KGF, STW, and WLK approved final version of manuscript.

Chapter 3

SUNSCREEN DOES NOT ALTER THERMOREGULATORY RESPONSES OF CRITICAL ENVIRONMENTAL LIMITS IN YOUNG ADULTS (PSU HEAT)

INTRODUCTION

Exposure to ultraviolet radiation (UVR) from the sun is associated with health risks including DNA damage, cutaneous vascular dysfunction, and skin cancer. Individuals and athletes who routinely participate in outdoor activities are at particularly high risk of experiencing these deleterious effects due to increased exposure to UVR (1). Further, many cyclists and runners will purposefully leave large areas of the skin exposed to UVR to enhance heat dissipation (2). However, outdoor athletes routinely eschew using sunscreen. Despite averaging 4 hours per day of outdoor training 10 months out of the year, less than 25% of a sample of 290 NCAA collegiate athletes across 13 different sports reported regular use of sunscreen (3). Additionally, a survey of NCAA soccer and cross country teams reported only 6% of athletes used sunscreen during the past seven days of outdoor practice (4). This may be due to lack of availability to sunscreen, forgetting to apply, and the belief that it may impair performance, or competition guidelines (3, 5, 6). For example, Ironman triathletes are prohibited from applying sunscreen to the shoulders or thighs where numbers are marked on the skin (7). Further, athletes often perceive sunscreen to impair heat loss by reducing sweat production and/or evaporation, and therefore, may refrain from using sunscreen. However, studies examining the effects of sunscreen on thermoregulation are equivocal (2, 8-11).

Common sunscreens are produced with either mineral- or chemical-based formulations. Mineral-based lotions act as a physical block and reflect UVR off the skin, whereas chemical-based lotions absorb UVR and convert it into heat at the surface of the skin. The impact of these two sunscreen formulations on skin temperature and sweat processes are inconclusive and contradictory (8-11). Wells et al. suggested use of a chemical-based sunscreen during exercise in the heat at a low humidity resulted in elevated mean skin temperature (Tsk), which potentially resulted in greater production of sweat that was not evaporated efficiently (9). Alternatively, Connolly and Wilcox showed that application of sunscreen decreased mean skin temperature and increased the core-to-skin thermal gradient and therefore may have enhanced heat dissipation (10). Two other studies concluded that mineral-based sunscreen hindered sweat production (11) while chemical-block sunscreen did not alter sweating responses (8). These equivocal findings reveal the need for research on the impact of sunscreen on thermoregulatory heat loss so that individuals and athletes can make informed decisions regarding sun protection practices. The ongoing PSU HEAT (Pennsylvania State University-Human Environmental Age Thresholds) project aims to determine the combinations of temperature and humidity above which heat related morbidity and mortality increases. This progressive heat stress protocol allows for an integrated examination of thermoregulatory responses to environments associated with heat balance or lack thereof [25, 49]. This reliable and valid [50] has been used to establish critical environmental limits (i.e., temperature/humidity thresholds above which heat balance cannot be maintained) in young adults across a wide range of environmental conditions [25]. However, the impact of sunscreen on integrative thermoregulatory responses has not been determined.

Therefore, the purpose of this investigation was to determine the effects of mineral and chemical based sunscreens on sweating responses and critical environmental limits in hot-dry and warm-humid environments. We hypothesized that (1) critical environmental limits will be

lowered in conditions when sunscreen is applied relative to no sunscreen conditions, (2) sweating and sweat evaporation will be decreased in conditions when sunscreen is applied relative to no sunscreen conditions.

METHODS

Subjects

All experimental procedures received ethics approval from the Institutional Review Board at The Pennsylvania State University and conformed to the guidelines set forth by the Declaration of Helsinki. After all aspects of the experiment were explained, oral and written informed consent was obtained, 11 healthy young adults (3 men, 8 women) aged 25 ± 2 yr were tested following application of two kinds of sunscreen and a control (no sunscreen) condition in two environmental conditions, in random order. Not all subjects completed trials in both environmental conditions. However, subjects were only included if they completed both sunscreen and the control condition in at least one environment.

All subjects were healthy, normotensive, nonsmokers, and not taking any medications that might affect the physiological variables of interest in this study. No attempt was made to control for menstrual status or contraceptive use. Acclimatization status was not accounted for. Maximal aerobic capacity ($\dot{V}o_{2max}$) was determined with the use of open-circuit spirometry (Parvo Medics TrueOne 2400, Parvo, UT) during a maximal graded exercise test performed on a motor-driven treadmill. During the experiments, clothing was standardized with subject's male subjects wearing only shorts, socks, and walking/running shoes and female subjects wearing a sports bra, shorts, socks, and walking/running shoes.

Testing Procedures

Experimental trials were conducted on separate days with at least 72 h between visits. Before each experimental session, subjects were instructed to abstain from alcoholic beverages and vigorous exercise for 24 h and from caffeine for 12 h. Upon arrival, participants provided a urine sample to ensure euhydration, defined as urine specific gravity ≤ 1.020 (USG; PAL-S, Atago, Bellevue, WA) [51]. Prior to entering the environmental chamber, subject self-applied over all exposed body surface area either a mineral or chemical-based sunscreen lotion. In order to emulate real world scenarios, subjects were instructed to apply the sunscreen as they normally would as long as it covered all exposed skin. Sunscreen bottles were weighed pre and post application with the average amount of sunscreen applied being 5 ± 1 g. Upon entering the chamber, subjects walked on a motor-driven treadmill at a speed of 3.5 mi/h and grade of 4% until a clear rise in gastrointestinal temperature (T_{gi}) was observed. Experimental trials lasted approximately 90 – 120 min.

Critical environmental limits were identified as previously described [25, 27] using a controllable environmental chamber at one constant T_{db} of 34 ºC and one constant P_a of 12 mmHg. Following a 30-min equilibration period, either P_a (during constant T_{db}, P_{crit} trials) or T_{db} (during constant P_a, T_{crit} trials) in the environmental chamber was increased in a stepwise fashion (1 mmHg or 1 ºC) every 5 min. We have previously reported excellent reliability and validity of this protocol to identify critical environmental limits [50]. During each experiment, chamber data (i.e., T_{db}, P_a, and relative humidity (rh)), T_{gi}, and heart rate were continuously monitored as subjects walked until a clear rise in T_{gi} was observed. A representative tracing of the time course of T_{gi} and the environmental conditions for an individual T_{crit} trial is presented in Figure 3-1.

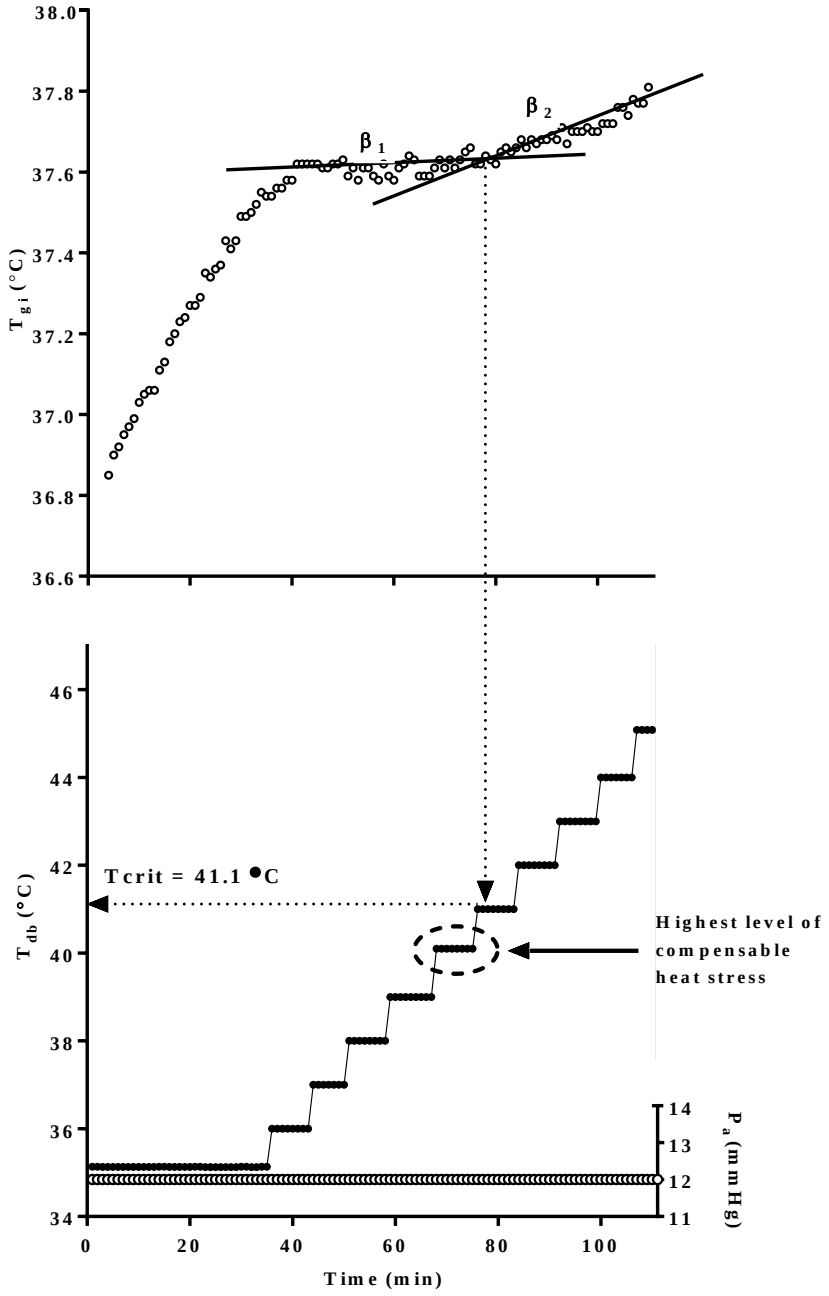

Figure 3-1. Representative tracing of the time course of gastrointestinal temperature (T_{gi}), dry-bulb temperature (T_{db}), and ambient water vapor pressure (P_a) for a hot-dry trial with increasing T_{db}. The dashed vertical line denotes the T_{db} at which T_{gi} begins to continuously rise (i.e. heat stress becomes uncompensable). ß$_1$ represents the rate of change of T_{gi} during compensable heat stress while ß$_2$ represents the rate of change of T_{gi} during uncompensable heat stress.

Measurements

Gastrointestinal temperature telemetry capsules (VitalSense, Philips Respironics, Bend, OR) were provided for subjects to ingest 1-2 h before reporting to the laboratory in accordance with previously published data demonstrating that ingestion times from 1-12 h before use do not influence the precision of T_{gi} data [52]. T_{gi} data were continuously transmitted to a PowerLab data acquisition system and LabChart signal processing software (AD Instruments, Colorado Springs, CO) using an Equivital wireless physiological monitoring system (Equivital Inc., New York, NY). Skin temperature was measured continuously (iButton, Whitewater, WI) at four sites: chest (T_{ch}), arm (T_{arm}), thigh (T_{th}), and lower leg (T_{leg}). Weighted mean skin temperature was calculated [53] as

$$\bar{T}_{sk} = 0.3 \cdot T_{ch} + 0.3 \cdot T_{th} + 0.2 \cdot T_{arm} + 0.2 \cdot T_{leg.} \quad \text{Eq. 1}$$

Oxygen consumption ($\dot{V}O_2$; L/min) and respiratory exchange ratio (RER; unitless) were determined at two time points (5 and 60 min after the onset of exercise) using indirect calorimetry (Parvo Medics TrueOne 2400, Parvo, UT). Metabolic rate [M; Watts (W)], normalized to body surface area, was calculated from $\dot{V}o_2$ and RER [54] as

$$M = \dot{V}o_2 \cdot \frac{\left[\left(\left(\frac{RER-0.7}{0.3}\right)\cdot 21.13\right)+\left(\left(\frac{1.0-RER}{0.3}\right)\cdot 19.62\right)\right]}{60} \cdot 1{,}000 \cdot {A_D}^{-1} \quad \text{Eq. 2}$$

where A_D is the Dubois surface area (m^2). For treadmill walking trials, external work (W; W/m^2) was calculated as

$$W = 9.81 \cdot m_b \cdot v_w \cdot F_g \cdot {A_D}^{-1} \quad \text{Eq. 3}$$

where m_b is body mass (kg), V_w is walking velocity (m/min), and F_g is the fractional grade of the treadmill [54]. Net metabolic heat production (M_{net}) was calculated as $M - W$.

METs were calculated using $\dot{V}O_2$ in $mL \cdot kg^{-1} \cdot min^{-1}$, assuming a resting $\dot{V}O_2$ of 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ [55], as

$$METs = \frac{\dot{V}O_2}{3.5} \quad \text{Eq. 4}$$

Dry heat exchange via radiation and convection ($W \cdot m^{-2}$) was determined as

$$(R + C) = h_{r+c} \cdot (T_{db} - T_{sk}) \quad \text{Eq. 5}$$

where h_{r+c} (in $W \cdot m^{-2} \cdot °C^{-1}$) is the combined radiative and convective heat transfer coefficient and T_{db} - $\overline{T}_{sk}$ represents the temperature gradient between the ambient air and the skin. In this study, h_{r+c} was determined for each subject by using the formula

$$h_{r+c} = 6.5 \cdot (\text{treadmill speed in m} \cdot s^{-1})^{0.39} + 4.7 \quad \text{Eq. 6}$$

where $6.5 \cdot (\text{treadmill speed in m} \cdot s^{-1})^{0.39}$ is the convective coefficient for treadmill walking and 4.7 is the radiative coefficient for indoor environments.

Heat storage (S; $W \cdot m^{-2}$) was calculated as

$$S = (\Delta T_b \cdot \Delta t^{-1}) \cdot (0.97\ W \cdot h \cdot kg^{-1} \cdot °C^{-1}) \cdot (m_b \cdot A_D^{-1}) \quad \text{Eq. 7}$$

where 0.97 $W \cdot h \cdot kg^{-1} \cdot °C^{-1}$ is the specific heat of the body and $\Delta\overline{T}_b$ represents the change in mean body temperature measured over the time period (Δt in h) between 30 min and the time at which the critical T_c inflection point was observed. The equation for $\Delta\overline{T}_b$ (°C), which is a function of the change in T_c and $\overline{T}_{sk}$, was

$$\Delta\overline{T}_b = (0.9 \cdot T_c + 0.1 \cdot \overline{T}_{sk})_{\text{critical point}} - (0.9 \cdot T_c + 0.1 \cdot \overline{T}_{sk})_{\min 30} \quad \text{Eq. 8}$$

Maximal evaporative capacity of the environment (E_{max}; $W \cdot m^{-2}$) was calculated as

$$E_{max} = 14.3 \cdot (\text{treadmill speed in m} \cdot s^{-1})^{0.39} \cdot (P_{s,sk} - P_a) \quad \text{Eq. 9}$$

where treadmill speed was 1.6 m/s. The evaporative cooling required to maintain thermal balance (E; $W \cdot m^{-2}$) was calculated from the heat balance equation as

$$E = M_{net} \pm (R + C) + C_{res} - E_{res} - S \quad \text{Eq. 10}$$

Skin wettedness (ω; unit-less) was calculated from E and E_{max} as

$$\omega = \frac{E_{max}}{E} \quad \text{Eq. 11}$$

Sweating efficiency (η_{sw}; $W \cdot m^{-2}$) was calculated as

$$\eta_{sw} = \frac{E}{E_{total}} \quad \text{Eq. 12}$$

Where E_{total} ($W \cdot m^{-2}$) is calculated as

$$E_{total} = \frac{SR}{A_D} \cdot 0.68\ W \cdot h \cdot kg^{-1} \quad \text{Eq. 13}$$

where $0.68\ W \cdot h \cdot kg^{-1}$ is the latent heat of vaporization.

Total sweat rate (SR) and percent body mass loss (%BML) were determined during each experiment from the loss of nude body mass on a scale accurate to ±10 g. Fluid intake was prohibited between the initial and final measurements of nude body mass.

Perceptual ratings of the environments in sunscreen and control conditions were taken periodically throughout trials. This included environmental perceptual ratings during equilibration, at the inflection, and at the end of experimental trials. Subjects were presented with scales periodically throughout experimental trials and instructed to rate their thermal perception, humidity perception, and thermal comfort perception of the environment.

Statistical Analysis

An a priori power analysis using an effect size of 1.2, based on previously published T_{crit} and P_{crit} data [25], suggested that six subjects would yield sufficient statistical power (power ≥ 0.8, $\alpha = 0.05$) to detect significant differences in T_{crit} and P_{crit} between sunscreen conditions. Repeated-measures ANOVA was conducted to compare T_{crit}, P_{crit}, and slopes of core temperature responses to compensable and uncompensable heat stress between sunscreen conditions. To detect differences in environmental conditions and heat storage at T_c inflection between warm-humid and hot-dry trials, paired samples t-tests were performed. Repeated measures ANOVA was used to detected differences in T_c, $\overline{T}_{sk}$, T_{mb}, and HR among the sunscreen and control trials. Repeated measures ANOVA was used to detect differences in evaporative heat loss, skin wettedness, sweating efficiency, and sweat rate among the sunscreen and control trials. Repeated measures ANOVA was used to detect differences in perceptual rating of the environments reported by subjects between sunscreen and control trials. Data are reported as means ± SD.

RESULTS

Subject characteristics are presented in Table 1. Subjects were recruited without regard to body size or aerobic fitness to be representative of the population in this age group. In all trials subjects walked at 4.9 ± 0.5 METS. M_{net} in HD was not different between control (219 ± 22 $W \cdot m^{-2}$) and chemical (224 ± 17 $W \cdot m^{-2}$, $P = 0.97$) or mineral (210 ± 17 $W \cdot m^{-2}$, $P = 0.97$) sunscreen trials. M_{net} in WH was not different between control (206 ± 16 $W \cdot m^{-2}$) and chemical (207 ± 22 $W \cdot m^{-2}$, $P = 0.95$) or mineral (222 ± 13 $W \cdot m^{-2}$, $P = 0.82$) sunscreen trials.

Table 3-1. Subject Characteristics.

	Mean ± SD	Range
Age, yr	25 ± 2	21–29
Height, m	1.7 ± 0.1	1.6–1.9
Weight, kg	71 ± 12	55–90
A_D, m^2	1.8 ± 0.2	2.19–1.69
$A_D \cdot kg^{-1}$, $m^2 \cdot kg^{-1}$	0.026 ± 0.002	0.023–0.031
Vo_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$	48 ± 9	26.9–58.3

n = 11 (8 women/3 men). A_D, DuBois body surface area; $A_D \cdot kg^{-1}$, body surface area-to-mass ratio; $\dot{V}o_{2max}$, maximal oxygen consumption.

Presented in Table 3-2 are the environmental conditions at the critical environmental limit (i.e., core temperature inflection) in each experimental condition and environment. T_{db} at core temperature inflection was greater in the hot-dry environment in all sunscreen conditions ($P < 0.001$). P_a and T_{wb} at core temperature inflection were greater in the warm-humid environment in all sunscreen conditions ($P < 0.001$). There was no effect of sunscreen in any condition.

Table 3-2. Critical environmental limits and heat storage in compensable environments.

	Warm-Humid	Hot-Dry
$T_{crit,}$ ^{0}C		
No Sunscreen		39.7 ± 3.1
Mineral		39.2 ± 3.5
Chemical		39.6 ± 3.0
$P_{crit,}$ mmHg		
No Sunscreen	18.8 ± 4.0	
Mineral	18.9 ± 4.8	
Chemical	19.5 ± 4.6	
Critical $T_{wb,}$ ^{0}C		
No Sunscreen	24.8 ± 2.0	22.8 ± 0.9
Mineral	24.8 ± 2.5	22.9 ± 1.1
Chemical	25.2 ± 2.5	23.1 ± 1.4
S, $W \cdot m^{-2}$		
No Sunscreen	13 ± 11	23 ± 10
Mineral	13 ± 6	19 ± 8
Chemical	14 ± 6	17 ± 5

Data are presented as means ± SD. $T_{db,}$ dry-bulb temperature; $P_{a,}$ water vapor pressure; $T_{wb,}$ wet-bulb temperature; S, heat storage.

Figures 3-2, 3-3, and 3-4 depict individual critical environmental limits for mineral (panel A) and chemical (panel B) sunscreen compared to control conditions in both environments. Compared to control, critical T_{db} was not different in mineral (P = 0.39) and chemical (P = 0.98) sunscreen trials in the hot-dry environment (Figure 3-2). Compared to control, critical P_a was not different in mineral (P = 0.81) and chemical (P = 0.81) sunscreen trials in the warm-humid environment (Figure 3-3). Compared to control, critical T_{wb} was not different in mineral (P = 0.73) and chemical (P = 0.72) sunscreen trials (Figure 3-4).

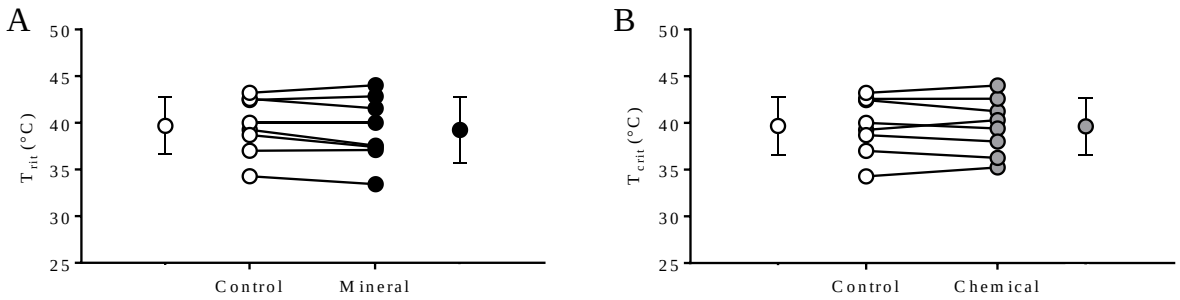

Figure 3-2. Comparisons of critical dry-bulb temperatures for mineral (panel A) and chemical (panel B) sunscreen compared to control (no sunscreen) conditions in the hot-dry environment (n=8). Data are presented as connected, individual data points and the mean point with SD bars.

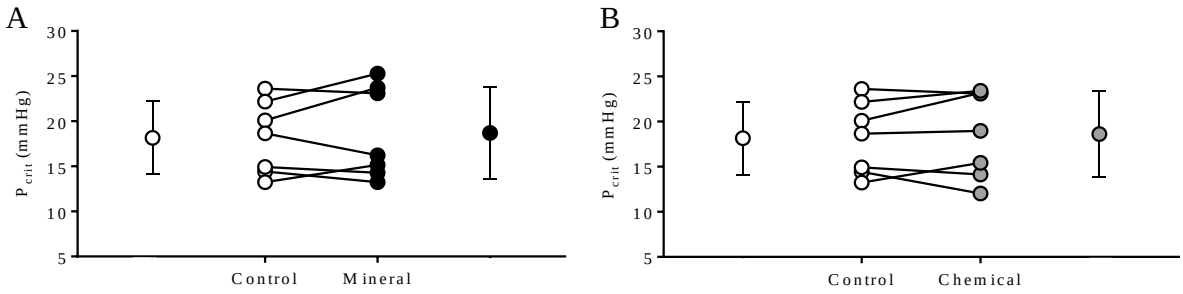

Figure 3-3. Comparisons of critical vapor pressures for mineral (panel A) and chemical (panel B) sunscreen compared to control (no sunscreen) conditions in the warm-humid environment (n=7). Data are presented as connected, individual data points and the mean point with SD bars.

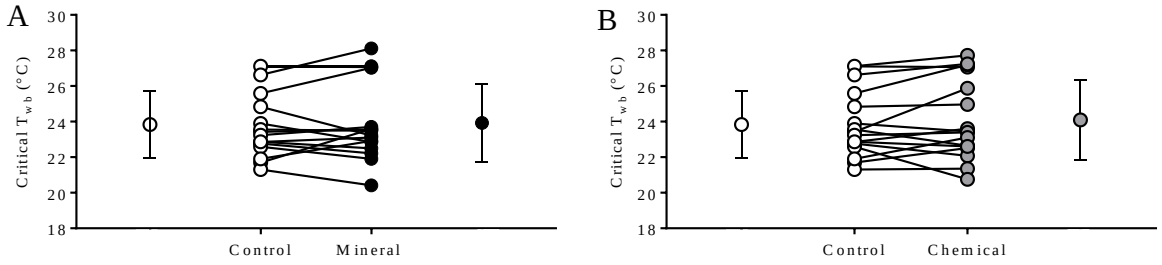

Figure 3-4. Comparisons of critical wet-bulb temperatures from both environments for mineral (panel A) and chemical (panel B) sunscreen compared to control (no sunscreen) conditions. Data are presented as connected, individual data points and the mean point with SD bars.

Table 3-3 presents the physiological responses to compensable heat stress at minute 5 of the experimental trial, minute 30, and the 5 minutes preceding the core temperature inflection. Between sunscreen and control conditions within the hot-dry and warm-humid environments, there were no differences in the T_c, $\overline{T}_{sk,}$ T_{mb}, or HR responses to compensable heat stress at each selected stage (all $P \geq 0.20$).

Table 3-3. Physiological responses during compensable heat stress.

Warm-Humid			
	No Sunscreen	**Mineral**	**Chemical**
Minute 5			
T_c, ºC	37.1 ± 0.4	37.2 ± 0.2	37.2 ± 0.2
$\overline{T}_{sk}$, ºC	33.7 ± 0.8	33.4 ± 1.1	33.9 ± 0.8
$\overline{T}_b$, ºC	35.9 ± 0.4	35.8 ± 0.3	36.0 ± 0.2
HR, bpm	111 ± 11	112 ± 13	115 ± 12
Minute 30			
T_c, ºC	37.9 ± 0.3	37.9 ± 0.4	37.8 ± 0.3
$\overline{T}_{sk}$, ºC	35.3 ± 0.4	34.9 ± 0.3	34.7 ± 0.8
$\overline{T}_b$, ºC	36.9 ± 0.3	36.8 ± 0.3	36.7 ± 0.5
HR, bpm	129 ± 10	129 ± 17	128 ± 15
Pre-Inflection			
T_c, ºC	37.9 ± 0.3	38.0 ± 0.3	37.8 ± 0.3
$\overline{T}_{sk}$, ºC	35.6 ± 0.3	35.3 ± 0.4	35.0 ± 0.7
$\overline{T}_b$, ºC	36.9 ± 0.4	36.9 ± 0.5	37.1 ± 0.2
HR, bpm	142 ± 24	136 ± 17	129 ± 19
Hot-Dry			
	No Sunscreen	**Mineral**	**Chemical**
Minute 5			
T_c, ºC	37.1 ± 0.5	37.3 ± 0.4	37.3 ± 0.4
$\overline{T}_{sk}$, ºC	33.3 ± 0.7	33.4 ± 0.6	33.2 ± 0.5
$\overline{T}_b$, ºC	35.8 ± 0.4	35.9 ± 0.2	35.8 ± 0.2
HR, bpm	118 ± 18	117 ± 16	117 ± 16
Minute 30			
T_c, ºC	37.9 ± 0.2	37.8 ± 0.4	37.8 ± 0.3
$\overline{T}_{sk}$, ºC	35.7 ± 0.6	35.3 ± 1.3	35.9 ± 0.6
$\overline{T}_b$, ºC	37.1 ± 0.4	36.9 ± 0.7	37.1 ± 0.4
HR, bpm	132 ± 18	134 ± 17	131 ± 17
Pre-Inflection			
T_c, ºC	38.0 ± 0.2	37.9 ± 0.3	37.9 ± 0.2
$\overline{T}_{sk}$, ºC	36.6 ± 1.1	36.2 ± 1.2	36.7 ± 0.8
$\overline{T}_b$, ºC	36.9 ± 0.2	36.9 ± 0.2	36.7 ± 0.3
HR, bpm	133 ± 20	138 ± 21	138 ± 19

Data are presented as means ± SD. T_c, core temperature; $\overline{T}_{sk}$, mean skin temperature; $\overline{T}_b$, mean body temperature; HR, heart rate.

As summarized in Table 3-4, rates of change in core temperature below (β_1) and above (β_2) T_c inflection points were not different between sunscreen conditions compared to control in either environment (all $P \geq 0.14$).

Table 3-4. Rates of change in core temperature below (compensable) and above (uncompensable) critical environmental limits (see Figure 3-1).

Environment	Condition	β_1	β_2
Hot-dry	No Sunscreen	0.11 ± 0.12	0.96 ± 0.66
	Mineral	0.16 ± 0.08	0.84 ± 0.51
	Chemical	0.28 ± 0.16	0.89 ± 0.55
Warm-humid	No Sunscreen	0.13 ± 0.11	0.65 ± 0.52
	Mineral	0.10 ± 0.10	0.70 ± 0.18
	Chemical	0.22 ± 0.15	0.56 ± 0.21

Data are presented as means ± SD. β_1; compensable heat stress. β_2; uncompensable heat stress.

Presented in Table 3-5 is the E, η_{sw}, ω and SR for sunscreen and control trials in each environment. E, η_{sw}, ω, and SR were not different between sunscreen and control conditions within a single environment. E and η_{sw} were significantly higher in the hot-dry compared to warm-humid environment in both sunscreen and the control conditions ($P \leq 0.01$).

Table 3-5. Characteristics of sweating and evaporation at the highest level of compensable heat stress.

Hot-Dry			
	No Sunscreen	**Mineral**	**Chemical**
E, W*m^{-2}	197 ± 39*	196 ± 32*	195 ± 35*
η_{sw}, W*m^{-2}	0.79 ± 0.24*	0.83 ± 0.28*	0.75 ± 0.28*
ω	0.35 ± 0.06	0.35 ± 0.05	0.34 ± 0.06
SR, g*m^{-2}*h^{-1}	396 ± 134	392 ± 154	422 ± 151
Warm-Humid			
	No Sunscreen	**Mineral**	**Chemical**
E, W*m^{-2}	147 ± 22	140 ± 19	146 ± 22
η_{sw}, W*m^{-2}	0.56 ± 0.15	0.60 ± 0.14	0.61 ± 0.15
ω	0.36 ± 0.08	0.36 ± 0.04	0.39 ± 0.09
SR, g*m^{-2}*h^{-1}	410 ± 126	370 ± 162	363 ± 169

Data are presented as means ± SD. E, evaporative heat loss; η_{sw}, sweating efficiency; ω skin wettedness; SR, sweat rate. $^{*}P < 0.05$ compared to warm-humid environment.

Figures 3-5 – 3-8 depict the perceptual ratings of thermal, humidity, and thermal comfort of the environments for sunscreen and control conditions. In the hot-dry environment, there was no differences in the thermal perception of the environment between sunscreen and control conditions at any of the three stages reported (P > 0.15). Likewise, in the warm-humid environment there was no difference in the humidity perception of the environment between sunscreen and control conditions at any of the three stages (P > 0.16). In both the warm-humid and hot-dry environments there were no differences in subject comfort between sunscreen and control conditions at any of the three stages (all P > 0.18).

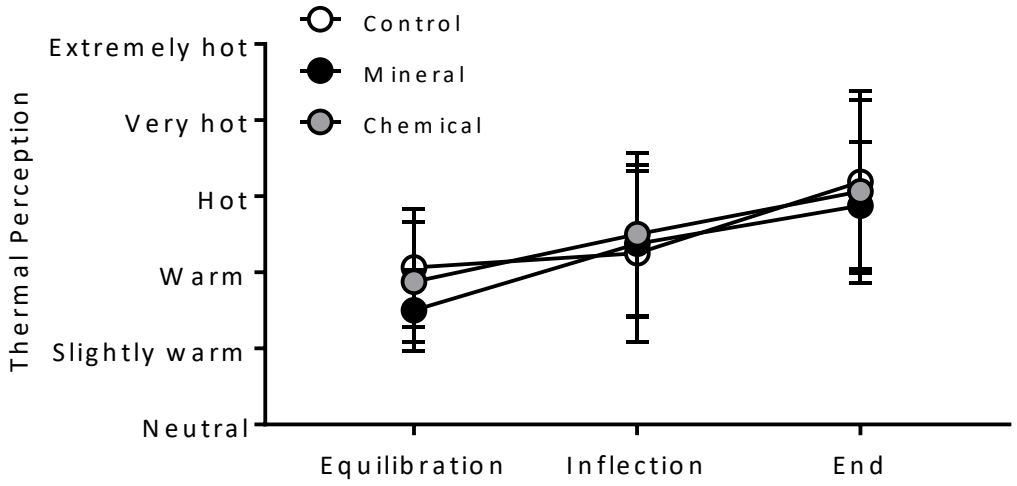

Figure 3-5. Comparisons of thermal perception between sunscreen and control conditions at equilibration, inflection, and at the end of the trials in the hot-dry environment. There were no differences in the rating of thermal perception at any stage in the hot-dry environment. Data are presented as overall means with SD bars.

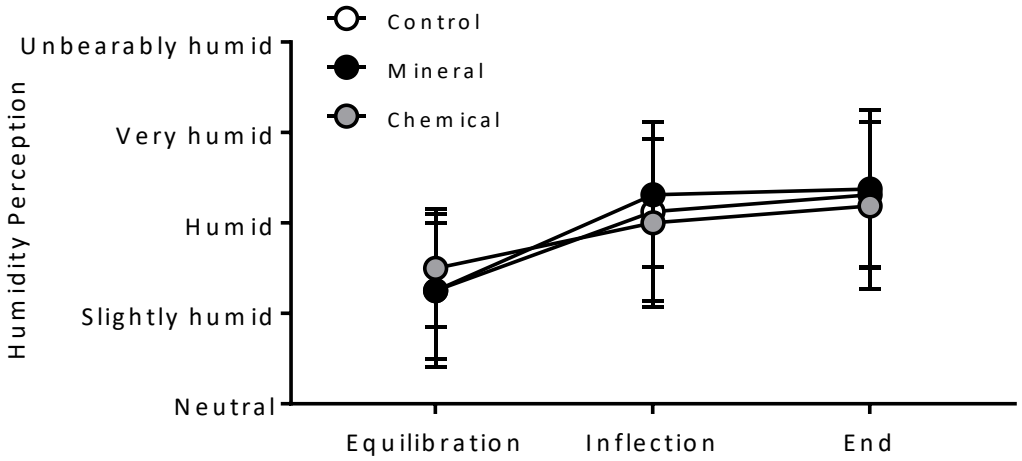

Figure 3-6. Comparisons of humidity perception between sunscreen and control conditions at equilibration, inflection, and at the end of the trials in the warm-humid environment. There were no differences in the rating of humidity perception at any stage in the warm-humid environment. Data are presented as overall means with SD bars.

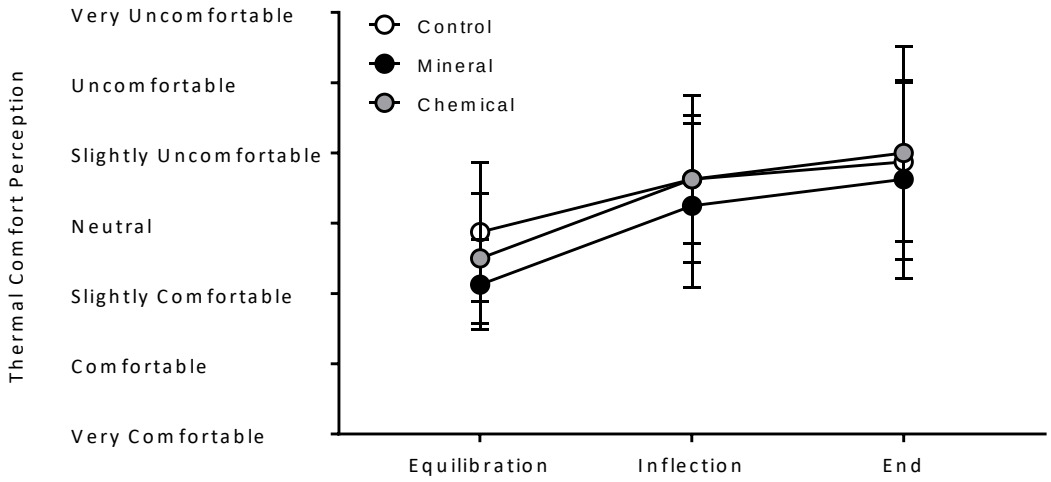

Figure 3-7. Comparisons of thermal comfort perception between sunscreen and control conditions at equilibration, inflection, and at the end of the trials in the hot-dry environment. There were no differences in the rating of thermal comfort perception at any stage in the hot-dry environment. Data are presented as overall means with SD bars.

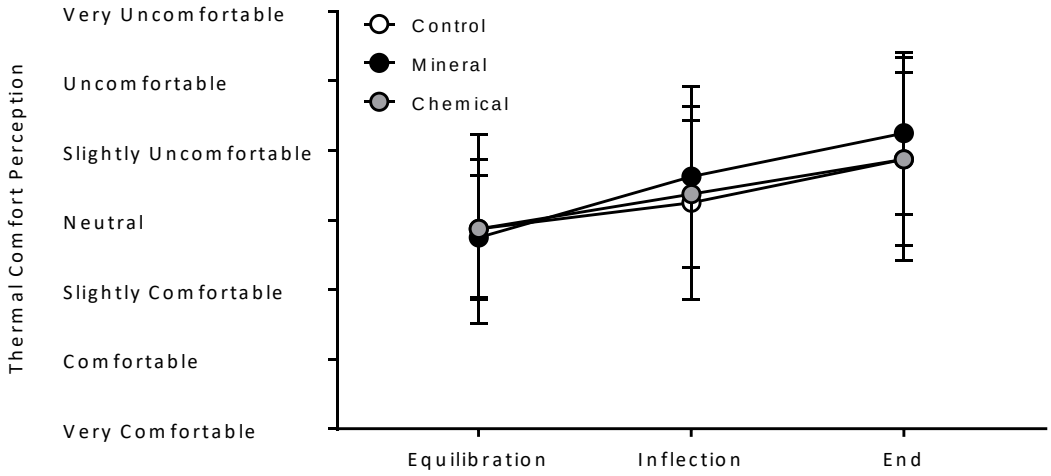

Figure 3-8. Comparisons of thermal comfort perception between sunscreen and control conditions at equilibration, inflection, and at the end of the trials in the warm-humid environment. There were no differences in the rating of thermal comfort perception at any stage in the warm-humid environment. Data are presented as overall means with SD bars.

DISCUSSION

Outdoor athletes are often exposed to large doses of UVR making them especially vulnerable to the deleterious effects of UVR such as sunburn [56]. Individuals who reported

being physically active outdoors experience significantly greater incidence of sunburn compared to inactive individuals [13]. A commonly reported barrier to using adequate sun protection is the belief that wearing sunscreen will impair athletic performance and thermoregulatory heat loss [18], although studies examining the effect of sunscreen on thermoregulation are equivocal [21-24]. Therefore, the purpose of the present study was to investigate the impact of both chemical and mineral sunscreen application on critical environmental limits and physiological responses to heat stress. Our findings demonstrate that whole body application of chemical or mineral-based sunscreen does not alter critical environmental limits in young adults. Additionally, application of either type of sunscreen does not alter the rates of change in core temperature or physiological responses of core and skin temperature below and above the environmental limits. Finally, sweating rate, evaporative heat loss, sweating efficiency and rate, and skin wettedness were unaffected by sunscreen. Therefore, our data suggest that perceived adverse thermoregulatory effects should not dissuade outdoor athletes from utilizing sunscreen lotion when exercising in the heat.

Heat Stress Protocol

Over a wide range of environments, core temperature will remain stable at a level proportional to the rate of metabolic heat production, independent of ambient conditions. Ambient conditions that exceed this threshold for equilibration result in a continuous rise in core temperature. Progressive heat stress protocols have been used to determine these safe upper limits of heat exposure in a variety of clothing and at various metabolic rates [26-29]. This protocol was originally developed by Belding and Kamon [57]and has been refined by our laboratory to identify critical environmental limits for a wide range of ambient temperatures and water vapor pressures [25, 50]. The protocol was used presently to investigate the impact of wearing sunscreen on these safe upper limits of heat exposure in warm-humid and hot-dry environments.

The PSU HEAT progressive heat stress protocol can be used to examine integrative thermoregulatory responses in environments associated with heat balance and imbalance. The protocol has been used recently to determine critical environmental limits in young adults during minimal activity and activities of daily living [25]. In the present study, the protocol was used to determine the integrative physiological effects of sunscreen application on critical environmental limits in young adults. Thus providing a comprehensive analysis of the impact of two common sunscreens on thermoregulation in warm-humid and hot-dry environments.

Limits of Compensability

Thermal imbalance results in heat storage that drives the change in T_c during progressive heat stress. As such, we calculated the rates of change in core temperature during compensable and uncompensable heat stress and heat storage in the sunscreen and control conditions in both environments. There were no differences in heat storage (Table 3-2) or the rates of change in core temperature above and below environmental limits (Table 3-4) between sunscreen conditions in either environment. These findings suggest that wearing sunscreen does not increase dry heat gain and therefore does not alter the change in mean body temperature during progressive heat stress.

Environmental conditions at which core temperature inflections occurred are summarized in Table 3-2 and depicted in Figures 3-2, 3-3, and 3-4. As expected, in the hot-dry environment T_{db} was significantly higher compared to the warm-humid environment and P_a, and T_{wb} were significantly higher in the warm-humid compared to the hot-dry environment. However, and important to our purpose and hypothesis, there were no differences T_{db}, P_a, or T_{wb} between sunscreen and control conditions in each environment. Therefore, the ambient conditions at which core temperature begins to continuously rise are not impacted by the application of sunscreen.

This suggests that wearing sunscreen does not change environmental limits associated with the onset of uncompensable heat stress in warm-humid and hot-dry environments.

Physiological Response to Compensable Heat Stress

To better understand the impact of wearing sunscreen on physiological responses to compensable heat stress we examined T_c, $\overline{T}_{sk}$, T_{mb}, and HR during the first five minutes of the heat stress protocol, 30 minutes into the protocol, and for the 5 minutes preceding the core temperature inflection (Table 3-3). At each selected time stage in both environments, there were no differences in the measured physiological variables between sunscreen and control conditions. During the compensable heat stress portion of the progressive heat stress protocol, wearing sunscreen did not change T_c, $\overline{T}_{sk}$, T_{mb}, or HR responses in either the warm-humid or hot-dry environment. Our findings are in corroboration with prior reports that during exercise in the heat skin temperatures, and core temperature were not different between sunscreen-treated and control conditions, suggesting sunscreen does not alter thermoregulatory responses, including sweating and evaporation, during exercise in the heat. The lack of differences in T_c and $\overline{T}_{sk}$ between sunscreen and control conditions additionally strengthens our calculations that heat storage is not impacted by the application of sunscreen as heat storage is a function of T_{mb}. These findings are important in the context of outdoor athletes and recreationists who are operating at levels of compensable heat stress as they should not eschew using sunscreen out of fear it may elevate core and skin temperature or strain cardiovascular responses.

Characteristics of Sweating and Evaporation

Perceived adverse effects of sunscreen on evaporative heat loss is a common barrier to using sunscreen as protection from UVR [56]. Therefore, in addition to setting environmental

limits and examining core and skin temperature responses to sunscreen, we investigated the impact of wearing sunscreen on sweating and evaporative heat loss characteristics. Table 3-5 summarizes the evaporative heat loss, sweating efficiency, skin wettedness, and sweat rates for each sunscreen condition in both environments. There was significantly greater evaporative heat loss and greater sweating efficiency in the hot-dry compared to warm-humid environments. This is expected as the lower ambient water vapor pressure in the hot-dry environment creates a larger vapor pressure gradient between wet skin and the air for the evaporation of sweat. However, within a given environment, evaporative heat loss, sweating efficiency, skin wettedness, and sweat rate were not altered by the application of sunscreen. In contraction to our findings, a previous study reported that mineral-based sunscreen may impair sweat rate or evaporation during exercise [23]. However, this discrepancy may be explained by sweat rate determination from total body mass loss in the present study, rather than local scapular sweat rate measured by sweat collection patches in the prior experiment [23]. In summary, our data suggest that wearing sunscreen does not change evaporative cooling nor the efficiency at which sweat is evaporated in hot-dry and warm-humid environments. Therefore, perceived adverse effects on evaporative heat loss should not dissuade outdoor athletes from utilizing sunscreen.

Limitations

We are limited in our calculation of SR as it is measured based on body mass loss across the entire trial, therefore, it is one measurement that may not be representative of sweating responses immediately prior to core temperature inflection. There was no attempt made in this study to account for heat acclimatization. However, subjects were tested throughout the year in a variety of seasons to mitigate the potential for acclimatization status to influence critical environmental limits. Likewise, no attempt was made to control for menstrual cycle or contraceptive use in female participants. While core temperature can vary across the menstrual

cycle the changes in core temperature during exercise in the heat are likely unaffected [58]. Therefore, it is unlikely that critical environmental limits are altered by menstrual cycle phase or contraceptive use.

Conclusion

In summary, the findings of the present study demonstrate that critical environmental limits are unaffected by mineral-based and chemical-based sunscreen application, suggesting that sunscreen does not alter integrative thermoregulatory responses during exercise in the heat. Additionally, the rates of change in core temperature in response to compensable and uncompensable heat stress are not changed by wearing sunscreen. Further, evaporative heat loss and efficiency of sweat evaporation are unaffected by sunscreen. Therefore, these data suggest that perceived adverse thermoregulatory effects should not dissuade outdoor athletes from utilizing sunscreen lotion as protection from UVR.

Chapter 4

CONCLUSIONS AND FUTURE DIRECTIONS

Outdoor athletes experience UVR exposure and as a result sunburn at a greater rate than less physically active individuals. This places outdoor athletes are greater risk of the negative effects of UVR exposure such as skin cancer and, in the case of acute UVR exposure, microvascular dysfunction. Despite the established adverse effect of UVR, many athletes choose not to apply sunscreen as a form of protection from the UVR. The notion that sunscreen may impair thermoregulation via limits sweating and/or evaporation is a common barrier to sunscreen use. Therefore, it is imperative to better understand both the UVR exposure impact on cutaneous vascular function and the impact of sunscreen application on thermoregulatory responses.

The studies comprising this thesis were aimed at investigating (1) the impact of within-limb variation in skin pigmentation secondary to UVR exposure on cutaneous microvascular function and (2) the impact of sunscreen application on integrative thermoregulatory responses during exercise in the heat. The findings of the first study concluded seasonal UVR exposure does not alter NO-dependent cutaneous vasodilation, suggesting microvascular function in unaffected by seasonal UVR exposure resulting in tanning. The findings of the second study suggest whole body sunscreen application does not alter critical environmental limits during exercise in the heat. Therefore, perceived adverse thermoregulatory effects should not dissuade outdoor athletes from utilizing sunscreen. Together these studies can provide outdoor athletes with more complete information regarding the impact of UVR exposure on cutaneous microvascular health and sunscreen on thermoregulatory function so that adequate sun protection may take place.

Future Directions

The study comprising Chapter 2 of this thesis was conducted that in lightly pigmented individuals and concluded that seasonal UVR exposure did not alter cutaneous microvascular function. However, this study was completed without controlling for the magnitude of exposure and in a region of the country that experiences moderate UVR exposure on average during the summer. Therefore, it cannot be ruled out that a higher magnitude of exposure in a region with high to very high UVR exposure would result in cutaneous microvascular dysfunction in lightly pigmented individuals.

The study that comprises Chapter 3 of this thesis tested the effect of a chemical-based sunscreen lotion and mineral-based sunscreen lotion. These were chosen based on their differing chemical makeup and common usage. However, whether varying SPF, spray sunscreen, or other chemical compositions alter thermoregulatory processes is unknown. Therefore, further research could investigate alternate sunscreens with differing mechanisms of action on thermoregulatory heat loss during exercise in the heat.

BIBLIOGRAPHY

1. Moehrle, M., *Outdoor sports and skin cancer.* Clin Dermatol, 2008. **26**(1): p. 12-5.
2. Gilchrest, B.A., et al., *The pathogenesis of melanoma induced by ultraviolet radiation.* N Engl J Med, 1999. **340**(17): p. 1341-8.
3. Bauer, A., T.L. Diepgen, and J. Schmitt, *Is occupational solar ultraviolet irradiation a relevant risk factor for basal cell carcinoma? A systematic review and meta-analysis of the epidemiological literature.* Br J Dermatol, 2011. **165**(3): p. 612-25.
4. Schmitt, J., et al., *Occupational ultraviolet light exposure increases the risk for the development of cutaneous squamous cell carcinoma: a systematic review and meta-analysis.* Br J Dermatol, 2011. **164**(2): p. 291-307.
5. Stanhewicz, A.E. and W.L. Kenney, *Role of folic acid in nitric oxide bioavailability and vascular endothelial function.* Nutr Rev, 2017. **75**(1): p. 61-70.
6. Crabtree, M.J. and K.M. Channon, *Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease.* Nitric Oxide, 2011. **25**(2): p. 81-8.
7. Wolf, S.T., et al., *Acute ultraviolet radiation exposure attenuates nitric oxide-mediated vasodilation in the cutaneous microvasculature of healthy humans.* J Appl Physiol (1985), 2018. **125**(4): p. 1232-7.
8. Wolf, S.T., et al., *Sunscreen or simulated sweat minimizes the impact of acute ultraviolet radiation on cutaneous microvascular function in healthy humans.* Exp Physiol, 2019. **104**(7): p. 1136-1146.
9. Bruning, R.S., et al., *Endothelial nitric oxide synthase mediates cutaneous vasodilation during local heating and is attenuated in middle-aged human skin.* J Appl Physiol (1985), 2012. **112**(12): p. 2019-26.
10. Minson, C.T., L.T. Berry, and M.J. Joyner, *Nitric oxide and neurally mediated regulation of skin blood flow during local heating.* J Appl Physiol (1985), 2001. **91**(4): p. 1619-26.
11. Holowatz, L.A., *Human cutaneous microvascular ageing: potential insights into underlying physiological mechanisms of endothelial function and dysfunction.* J Physiol, 2008. **586**(14): p. 3301.
12. Ziegler, A., et al., *Sunburn and p53 in the onset of skin cancer.* Nature, 1994. **372**(6508): p. 773-6.
13. Jardine, A., et al., *Does physical activity increase the risk of unsafe sun exposure?* Health Promot J Austr, 2012. **23**(1): p. 52-7.
14. Moehrle, M., et al., *Extreme UV Exposure of Professional Cyclists.* Dermatology, 2000. **201**(1): p. 44-45.
15. Moehrle, M., *Ultraviolet exposure in the Ironman triathlon.* Med Sci Sports Exerc, 2001. **33**(8): p. 1385-6.
16. Rigel, E.G., et al., *Ultraviolet Radiation in Alpine Skiing: Magnitude of Exposure and Importance of Regular Protection.* Archives of Dermatology, 2003. **139**(1): p. 60-62.
17. Protection, I.C.o.N.-I.R., *ICNIRP STATEMENT—PROTECTION OF WORKERS AGAINST ULTRAVIOLET RADIATION.* Health Physics, 2010. **99**(1): p. 66-87.
18. Wysong, A., et al., *Sunscreen use in NCAA collegiate athletes: identifying targets for intervention and barriers to use.* Prev Med, 2012. **55**(5): p. 493-6.
19. Hamant, E.S. and B.B. Adams, *Sunscreen use among collegiate athletes.* J Am Acad Dermatol, 2005. **53**(2): p. 237-41.
20. Ellis, R.M., et al., *Sunscreen use in student athletes: a survey study.* J Am Acad Dermatol, 2012. **67**(1): p. 159-60.

21. Wells, T.D., G.T. Jessup, and K.S. Langlotz, *Effects of Sunscreen Use During Exercise in the Heat.* The Physician and Sportsmedicine, 1984. **12**(6): p. 132-142.
22. Connolly, D.A. and A.R. Wilcox, *The effects of an application of suncream on selected physiological variables during exercise in the heat.* J Sports Med Phys Fitness, 2000. **40**(1): p. 35-40.
23. Aburto-Corona, J. and L. Aragón-Vargas, *Sunscreen Use and Sweat Production in Men and Women.* J Athl Train, 2016. **51**(9): p. 696-700.
24. Ou-Yang, H., et al., *Sunscreen formulations do not interfere with sweat cooling during exercise.* Int J Cosmet Sci, 2018. **40**(1): p. 87-92.
25. Wolf, S.T., et al., *Critical environmental limits for young, healthy adults (PSU HEAT Project).* Journal of Applied Physiology, 2022. **132**(2): p. 327-333.
26. Kenney, W.L., et al., *Physiologically derived critical evaporative coefficients for protective clothing ensembles.* Journal of Applied Physiology, 1987. **63**(3): p. 1095-1099.
27. Kenney, W.L. and M.J. Zeman, *Psychrometric limits and critical evaporative coefficients for unacclimated men and women.* Journal of Applied Physiology, 2002. **92**(6): p. 2256-2263.
28. Bernard, T.E., et al., *WBGT clothing adjustment factors for four clothing ensembles and the effects of metabolic demands.* J Occup Environ Hyg, 2008. **5**(1): p. 1-5; quiz d21-3.
29. Kenney, W.L., D.E. Hyde, and T.E. Bernard, *Physiological evaluation of liquid-barrier, vapor-permeable protective clothing ensembles for work in hot environments.* Am Ind Hyg Assoc J, 1993. **54**(7): p. 397-402.
30. Alexander, L.M., J.L. Kutz, and W.L. Kenney, *Tetrahydrobiopterin increases NO-dependent vasodilation in hypercholesterolemic human skin through eNOS-coupling mechanisms.* Am J Physiol Regul Integr Comp Physiol, 2013. **304**(2): p. R164-9.
31. Moan, J., K.P. Nielsen, and A. Juzeniene, *Immediate pigment darkening: its evolutionary roles may include protection against folate photosensitization.* FASEB J, 2012. **26**(3): p. 971-5.
32. Off, M.K., et al., *Ultraviolet photodegradation of folic acid.* J Photochem Photobiol B, 2005. **80**(1): p. 47-55.
33. Steindal, A.H., et al., *Photodegradation of 5-methyltetrahydrofolate: biophysical aspects.* Photochem Photobiol, 2006. **82**(6): p. 1651-5.
34. Wolf, S.T., et al., *Acute ultraviolet radiation exposure attenuates nitric oxide-mediated vasodilation in the cutaneous microvasculature of healthy humans.* J Appl Physiol (1985), 2018.
35. Parra, E.J., R.A. Kittles, and M.D. Shriver, *Implications of correlations between skin color and genetic ancestry for biomedical research.* Nat Genet, 2004. **36**(11 Suppl): p. S54-60.
36. Holick, M.F., *Vitamin D Deficiency.* 2007. **357**(3): p. 266-281.
37. Wolf, S.T., et al., *Skin pigmentation is negatively associated with circulating vitamin D concentration and cutaneous microvascular endothelial function.* Am J Physiol Heart Circ Physiol, 2022. **323**(3): p. 490-498.
38. Young, A.R., et al., *Melanin has a Small Inhibitory Effect on Cutaneous Vitamin D Synthesis: A Comparison of Extreme Phenotypes.* J Invest Dermatol, 2020. **140**(7): p. 1418-1426 e1.
39. Wolf, S.T. and W.L. Kenney, *The vitamin D-folate hypothesis in human vascular health.* Am J Physiol Regul Integr Comp Physiol, 2019. **317**(3): p. R491-R501.
40. Kim, D.-H., et al., *Vitamin D and Endothelial Function.* 2020. **12**(2): p. 575.

41. Wolf, S.T., et al., *Four weeks of vitamin D supplementation improves nitric oxide-mediated microvascular function in college-aged African Americans.* Am J Physiol Heart Circ Physiol, 2020.
42. Sachdeva, S., *Fitzpatrick skin typing: applications in dermatology.* Indian J Dermatol Venereol Leprol, 2009. **75**(1): p. 93-6.
43. Johnson, J.M., et al., *Effect of local warming on forearm reactive hyperaemia.* Clin Physiol, 1986. **6**(4): p. 337-46.
44. Minson, C.T., *Thermal provocation to evaluate microvascular reactivity in human skin.* J Appl Physiol (1985), 2010. **109**(4): p. 1239-46.
45. Nair, R. and A. Maseeh, *Vitamin D: The "sunshine" vitamin.* J Pharmacol Pharmacother, 2012. **3**(2): p. 118-26.
46. Libon, F., E. Cavalier, and A.F. Nikkels, *Skin color is relevant to vitamin D synthesis.* Dermatology, 2013. **227**(3): p. 250-4.
47. Ames, B.N., W.B. Grant, and W.C. Willett, *Does the High Prevalence of Vitamin D Deficiency in African Americans Contribute to Health Disparities?* 2021. **13**(2): p. 499.
48. Wolf, S.T., et al., *Skin Pigmentation is Negatively Associated with Circulating Vitamin D Concentration and Cutaneous Microvascular Endothelial Function.* Am J Physiol Heart Circ Physiol, 2022.
49. Cottle, R.M., Z.S. Lichter, and D.J. Vecellio, *Core temperature responses to compensable versus uncompensable heat stress in young adults (PSU HEAT Project).* 2022. **133**(4): p. 1011-1018.
50. Cottle, R.M. and S.T. Wolf, *Validity and reliability of a protocol to establish human critical environmental limits (PSU HEAT Project).* 2022. **132**(2): p. 334-339.
51. Kenefick, R.W. and S.N. Cheuvront, *Hydration for recreational sport and physical activity.* Nutr Rev, 2012. **70 Suppl 2**: p. S137-42.
52. Notley, S.R., R.D. Meade, and G.P. Kenny, *Time following ingestion does not influence the validity of telemetry pill measurements of core temperature during exercise-heat stress: The journal Temperature toolbox.* 2021. **8**(1): p. 12-20.
53. Ramanathan, N.L., *A NEW WEIGHTING SYSTEM FOR MEAN SURFACE TEMPERATURE OF THE HUMAN BODY.* J Appl Physiol, 1964. **19**: p. 531-3.
54. Cramer, M.N. and O. Jay, *Partitional calorimetry.* 2019. **126**(2): p. 267-277.
55. Jetté, M., K. Sidney, and G. Blümchen, *Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity.* 1990. **13**(8): p. 555-565.
56. Wolf, S.T., L.E. Kenney, and W.L. Kenney, *Ultraviolet Radiation Exposure, Risk, and Protection in Military and Outdoor Athletes.* Curr Sports Med Rep, 2020. **19**(4): p. 137-141.
57. Belding, H.S. and T.F. Hatch, *Relation of skin temperature to acclimation and tolerance to heat.* Fed Proc, 1963. **22**: p. 881-3.
58. Kolka, M.A. and L.A. Stephenson, *Interaction of menstrual cycle phase, clothing resistance and exercise on thermoregulation in women.* Journal of Thermal Biology, 1997. **22**(2): p. 137-141.

Appendix A

Informed Consent for Chapter 2

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:
    a. Height;
    b. Weight;
    c. Waist circumference;
    d. Blood pressure;
    e. Heart rate;
    f. 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 your socioeconomic status.

_____ initial **B. Microdialysis Experiment**

1. Preparation for the MD experiment:
   a. Do not eat or drink caffeine (ex. Coffee, tea, Coca Cola, chocolate) or alcohol for 12 hours before the MD study.
   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.
   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. 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.

4. 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.
   a. We collect data for 15 minutes.

b. We increase the temperature of the skin to 42◦C (107.6◦F). You may end the local heating phase at any time.

c. We hold the skin temperature at 42^{o}C until skin blood flow becomes stable (~40 minutes).

d. 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 43◦C (109.4◦F). 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 43◦C (109.4◦F) 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.

6. 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.

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, 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.

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)

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

Appendix B

Informed Consent for Chapter 3

CONSENT FOR RESEARCH
The Pennsylvania State University

Title of Project: Identification of Critical Thermal Environments for Aged Adults

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 – 30 and 65 – 85-year-old adults. We think that you may be a good fit for this study.

What is the purpose of this research study?

This study is being done to learn about limits of body temperature control in the heat. We also want to learn how taking aspirin affects these limits in older adults.

How long will the research study last?

The study will take about 3 months to complete. You will be asked to return to the research site for the following visits:
Screening (1 visit): About 1.5 hours
Maximal exercise testing (1 visit): About 1 hour
Experimental Visits (4-12 visits): About 3 hours each

Total: About 14.5-38.5 hours

You may be asked to repeat each visit after taking Aspirin for a week. You will be asked to return to the research site for the following visits:

Screening (1 visit): About 1.5 hours
VO_2max testing (1 visit): About 1 hour
Experimental Visits (8-24 visits): About 3 hours each
Total: About 26.5-74.5 hours

What will you need to do?

You will be asked to exercise in conditions of heat and humidity. You will be asked to come into the lab for testing on at least 4 days, up to 12 days. Each day will have different heat, humidity, and exercise conditions.

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

For this study, the main risks to know about are: discomfort with exercising in hot and humid conditions; discomfort with needles during blood draws; allergies to tape or latex; infection from blood draws. More information about 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 learn about how your body works. The study may benefit people in the future by helping us learn more about the limits of body heat control.

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?

The earth's climate is warming, and the number of heat waves has increased in recent years. At the same time, the number of adults over the age of 65 is growing. Humans sweat and increase blood flow to the skin to cool their body when they get hot. Older adults do not do this as well

as young adults. This makes it harder to safely exercise in warm and/or humid conditions. It is important to learn about safe limits of heat and humidity for older adults to exercise. Also, nearly 40% of adults over age 50 take aspirin to lower their risk for heart disease. Our lab has shown that aspirin lowers the control of body heat.

In this study, we will look at body temperature control during exercise in the heat and humidity and how this changes with age. We will also look at how aspirin may change the control of body heat in older adults. About 32 people will take part in this study.

2. What will happen in this research study?

_____ initial **A. Screening**

You come to the lab for a screening visit to see if you qualify 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:
    a. Height;
    b. Weight;
    c. Waist circumference;
    d. Blood pressure;
    e. Heart rate;
3. You complete a health history questionnaire.
4. You complete the 2017 Physical Activity Readiness Questionnaire for Everyone (PAR-Q+)
5. Women of childbearing age have a urine pregnancy test.
6. The research staff will draw 30 mL (2 Tbsp) of blood from a vein in your arm. Some of the blood is 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 the blood tests are common tests to determine your health status. If you are eligible, we invite you back to the lab for the study visits.
7. Adults aged 65+ only: The research staff performs a resting ECG to ensure you have no heart conditions that preclude you from exercise. The results are sent to a study physician to review.

_____ initial **B. Maximal Exercise Test**

You will complete a maximal exercise test on a treadmill before the experimental visits. This will allow us to see how hard you should exercise during the visits. Trained laboratory members and/or a physician will oversee your test.

1. Do not eat or drink caffeine or alcohol for 3 hours before the test.
2. Do not exercise hard for 24 hours before the test.
3. Wear clothing and shoes that you will be comfortable exercising in.
4. We place a blood pressure cuff around your upper arm to measure your blood pressure. We place ECG stickers on your torso to measure your heart rate and rhythm during the test.

5. We take resting blood pressure and heart rate and rhythm measurements. We then take your blood pressure at the end of each stage of the test.
6. We explain how to rate your effort during the test.
7. We show you hand signals to communicate with us during the test.
8. You wear a snorkel-like mouthpiece and nose clip. This allows us to look at how hard you are working during the test.
9. You choose a comfortable walking or jogging pace on the treadmill while we monitor you.
10. After you choose a pace on the treadmill, the grade and/or speed is increased every 2 minutes. This continues until: (1) you reach your maximal capacity; (2) you ask to stop; or (3) study personnel see something that causes them to stop the test.
11. Once the test is over, you walk on the treadmill at a slow speed while we monitor you.
12. You then sit until your heart rate and blood pressure start to return to pre-exercise levels.

_____ initial **C. Measurements of Body Core Temperature**

During each experimental visit, body core temperature will be measured in two ways:

Esophageal probe insertion:

1. A temperature probe of ~3 mm in diameter is placed through your nostril into the esophagus.
2. You sip water through a straw while the probe is placed through the nostril.
3. A numbing jelly can be put on the probe to lower discomfort.
4. The probe is placed to a depth of 40 cm past the nostril, or at the same level as your heart.
5. This temperature is measured throughout the study.

Temperature-sensing pill:

1. Intestinal temperature is measured with a temperature-sensing pill.
2. You swallow the pill with water.
3. You return the pill wrapping to the research team.
4. This temperature is measured every 5 minutes using a remote detector.
5.

_____ initial **D. Measurements of Skin Temperature**

1. Four sites are used to measure skin temperature:
    a. Leg
    b. Thigh
    c. Arm
    d. Chest
2. Each site is cleaned with alcohol wipes.
3. A temperature probe is applied to each site using double-sided stickers.
4. Skin temperature is recorded at all four sites throughout the test.

_____ initial **E. Environmental Chamber Experiments**

You complete two protocols, three times each, in an environmental chamber. The protocols are: (1) temperature increases while humidity stays the same, and (2) humidity increases while

temperature stays the same. You complete each condition twice exercising at different intensities.

Critical Temperature Experiments:

1. You complete 1-3 experiments, each with a constant water vapor pressure (humidity):
    a. 12 Torr
    b. 16 Torr
    c. 20 Torr
2. You enter the chamber and rest for 30 minutes.
3. A blood pressure cuff is placed around the upper arm.
4. ECG electrodes are placed on your chest to measure heart rate/rhythm during the test.
5. Resting blood pressure and heart rate are measured.
6. Blood pressure and heart rate are measured every 5 minutes during the test.
7. Core temperature is measured throughout the study.
8. For each humidity condition, you perform two trials of light exercise:
    a. You pedal a bicycle with low force.
    b. You walk on a treadmill at a speed of about 2 mph.
9. After 30 minutes of rest, you start exercising.
10. Chamber temperature is increased by 1°C every 5 minutes.
11. Your core temperature will rise, and then become stable.
12. Twice during exercise you put on the snorkel-like mouth piece and nose clip for 5 minutes to see how hard you are working. This happens at the 30 and 60 minute time points. The mouthpiece is removed at the end of the 5 minutes.
13. You exercise until your core temperature starts to rise again.
14. After this rise happens, the test ends.
15. The chamber is returned to normal conditions.
16. The esophageal probe is removed.

Critical Vapor Pressure Experiments:

1. You complete 1-3 experiments, each with a constant temperature:
    a. 36°C
    b. 38°C
    c. 40°C
2. You enter the chamber and rest for 30 minutes.
3. A blood pressure cuff is placed around the upper arm.
4. ECG electrodes are placed on your chest to measure heart rate/rhythm during the test.
5. Resting blood pressure and heart rate are measured.
6. Blood pressure and heart rate are measured every 5 minutes during the test.
7. Core temperature is measured throughout the study.
8. For each temperature condition, you perform two trials of light exercise:
    a. You pedal a bicycle with low force.
    b. You walk on a treadmill at a speed of about 2 mph.
17. After 30 minutes of rest, you start exercising.
18. Chamber water vapor pressure is increased by 1 Torr every 5 minutes.
19. Your core temperature will rise, and then become stable.

20. Twice during exercise you put on the snorkel-like mouth piece and nose clip for 5 minutes to see how hard you are working. This happens at the 30 and 60 minute time points. The mouthpiece is removed at the end of the 5 minutes.
9. You exercise until your core temperature starts to rise again.
10. After this rise happens, the test ends.
11. The chamber is returned to normal conditions.
12. The esophageal probe is removed.

_____ initial **F. Aspirin Supplementation (Older adults only)**

1. You may be asked to repeat all trials after taking 81 mg/dL aspirin for at least 7 days before testing.
2. After 7 days of taking aspirin, you repeat each of the 12 trials.
3. You continue taking aspirin until you finish all study visits.
4. You may choose not to complete this part of the study.

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

Esophageal probe: The probe is inserted through the nose into the esophagus. The probe rests at the level of the heart. There can be mild discomfort and/or a mild gagging reflex from swallowing the probe. However, this feeling should pass soon (2-3 min). Lidocaine jelly can be put on the probe to reduce discomfort. While very rare, the probe can puncture the esophagus. To lower this risk, trained personnel will insert the probe. This procedure will not be performed if there are any contraindications based on their medical history. Our lab has used this technique for many years with no problems.

Temperature-sensing pill: Although very rare, there are some risks to taking this pill. The pill could be inhaled into the lungs. The pill could also cause a puncture, blockage or infection of the intestines. This may require endoscopy or surgery to remove it. This pill should not be taken by anybody weighing less than 80 pounds, or by anybody who has or has had any gastrointestinal disease or surgery.

Maximal exercise test: Risks of this test include fatigue, irregular heart rate, changes in blood pressure, and fainting. Cardiac events are very rare. The risk of a cardiac event during the test, not resulting in death, is less than 4/10,000. The risk of a fatal cardiac event is less than 1/10,000. The research staff will watch closely to minimize the chance of injury. General Indications for Stopping an Exercise Test will be followed, per the American College of Sport Medicine (see 19.7 Stopping Rules). You may request to stop the test at any time.

Exercise: It is possible that you may experience faintness, fatigue, muscle pain, or chest pain during the exercise bouts. These possible side effects are not uncommon in activities that require physical exertion. Your heart rate, blood pressure, and core temperature will be monitored for the duration of the protocol. You may communicate any difficulty you experience. You may end the experiment at any time. All lab personnel are certified and have current

CPR/AED and First Aid qualifications. There is an AED in the laboratory. In any emergency, 911 will be called immediately.

Environmental Chambers: The chamber uses a three-mode controller to optimize stability and response. Air enters through the ceiling and returns through base molding on three sides. Because air returns behind the walls, wall temperature increases linearly with air temperature. You will complete 4-12 trials for 2-6 environmental conditions (2 trials in each condition at differing intensities). These environments are as follows: temperatures of 36, 38, and 40°C while ambient vapor pressure remains constant; ambient vapor pressures of 12, 16, and 20 Torr while temperature remains constant. In these environments, your core temperature will rise before becoming steady by ~min 40 and remains at an elevated steady state. You may feel some discomfort associated with increased body temperature and sweat on the skin. This is normal in hot and humid conditions. These chambers have been used for 25 years in our laboratory without any incidence of adverse events.

Blood Pressure (manual, CardioCap 5): The manual method and CardioCap5 use a cuff that inflates on the upper arm. The cuff slowly deflates while the researchers listen to pulse-sounds at the inside of the elbow with a stethoscope, or the CardioCap 5 takes a measurement. The inflated cuff may make the arm feel tingly and numb. The cuff may temporarily bruise the arm. Efficient and competent measurement technique minimizes the duration of cuff inflation. These techniques are unlikely to produce lasting ill effects.

Blood draw: Blood draws can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection. A nurse performs blood draws using standard procedures and techniques that minimize the chance of infection. Participants may recline for the procedure.

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 occurs, we call for medical help right away.

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.

ECG: The researchers attach three to twelve electrodes to your chest and then attach the electrode wires to an ECG machine. The machine records the electrical activity of the heart. There are no adverse effects from this measure. A subject may be shy about electrodes applied to the chest. The staff carefully remove the tape afterward. They conduct the test professionally and privately.

Thermoregulation and Microvascular Lab Websites: You may enter data into the screening form or questionnaires via the REDCap website. REDCap is a secure website and survey application designed to support data capture for research studies. You may be concerned about data security. All web traffic to and from the REDCap application website is done via a Secure Socket Layer (SSL) that encrypts the data in transmission. The questionnaire contains statements advising of the limitations of technology and that there is no confidentiality guarantee. Subjects may choose a personal interview instead.

Latex: Some gloves and medical materials are made of latex rubber. Some people may be sensitive to latex. We exclude those with a known latex allergy.

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?

This research may help scientists better understand age-related changes in the limits of body temperature control during exercise in the heat. It may also help to better understand how taking aspirin may alter control of body temperature in older adults. 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 3 months to complete the study. Subjects will be asked to return to the research site for the following sessions and times:

Screening (1 visit): About 1.5 hours
VO_2max testing (1 visit): About 1 hour
Experimental Visits (4-12 visits): About 3 hours each
Total: About 14.5 – 38.5 hours, depending on how many trials are completed

A sub-group of older adults will be asked to take Aspirin for a week and then repeat each of the experimental visits. Should you agree to this, you will be asked to return to the research site for the following sessions and times:

Screening (1 visit): About 1.5 hours
VO_2max testing (1 visit): About 1 hour
Experimental Visits (8-24 visits): About 3 hours each
Total: About 26.5 – 74.5 hours, depending on how many trials are completed

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 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 to 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.

Environmental chamber experiments: $75 per experiment
Total: Up to $900 (12 experiments)

Aspirin subset total: Up to $1800 (24 experiments)

The researchers pay an amount of money equal to the part completed. For instance, if a subject completes 6 experiments, they will receive a total payment of $450. Researchers may ask subjects to repeat a trial. If subjects agree to repeat a trial, they receive payment

for the repeated trial as stated above. They are reimbursed for gasoline if they 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 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.

- Rachel Cottle (W: 814-863-8556, C: 949-728-8870)
- 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