SEASONAL VARIATIONS IN ENDOTHELIAL FUNCTION AND BLOOD PRESSURE CONTROL IN YOUNG HEALTHY ADULTS

by

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(Under the Direction of S. Tony Wolf)

ABSTRACT

Cardiovascular risk is higher in the winter compared to other seasons. Endothelial dysfunction is a non-traditional risk factor for cardiovascular disease (CVD) that precedes the development of hypertension and increases cardiovascular risk. Exaggerated blood pressure (BP) responses could potentiate this risk. Endothelial function and BP control were assessed in 15 young adults across seasons. Intradermal microdialysis coupled with local heating and flow-mediated dilation (FMD) measured endothelial function. A cold pressor test (CPT) measured BP reactivity. Meteorological conditions and [25(OH)D] were collected. Winter/early-spring FMD was not different from summer/early-fall, but %NO contribution to local heating was attenuated (P = 0.0003). Wet bulb globe temperature (WBGT) was associated with the %NO contribution to local heating (r = 0.52), but UV and [25(OH)D] were not related. There were no variations in BP responses to the CPT. Young adults exhibit attenuated NO-mediated cutaneous vasodilation in the winter/early-spring, which may be mediated by decreased WBGT.

INDEX WORDS: Seasonal variations, endothelial function, nitric oxide, blood pressure control, temperature, ultraviolet radiation, vitamin D

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DEDICATION

To my parents — for inspiring me to do hard things and bring joy to the world.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cardiovascular disease (CVD) is the leading cause of death in the United States and globally (1, 2). Seasonal trends in CVD morbidity and mortality are evident in both the northern and southern hemispheres with higher incidences of CVD in the winter months (3). Hypertension is a major risk factor for the development of CVD and future cardiovascular events and elevated arterial blood pressures during the winter months have been well documented globally across a variety of populations (4, 5). Endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is a non-traditional risk factor for CVD and typically precedes the development of hypertension (6, 7). NO, a critical cardioprotective molecule, is produced by the vascular endothelium and contributes to relaxation of smooth muscle. Attenuated endothelial function in the winter may contribute to increased blood pressure and cardiovascular risk; however, current research in this area is limited in its scope and methods. Another component that is associated with cardiovascular risk, independent of resting blood pressure, is blood pressure reactivity (8–11). Blood pressure reactivity characterizes the cardiovascular system's response to psychophysiological stressors (12). Exaggerated increases in blood pressure to particular stimuli may contribute to an increased risk to developing CVD or having a cardiovascular-related event. Despite strong evidence of increased cardiovascular risk in the winter, there is a lack of research investigating seasonal variations in blood pressure reactivity as a potential contributor to this phenomenon.

Temperature and ultraviolet radiation (UVR) are two major environmental factors that demonstrate significant seasonal variation in many parts of the world. Wintertime is typically associated with decreased temperatures and decreased UVR. Additionally, there is robust evidence of reductions in physical activity during the winter and increased sedentary behavior is strongly associated with increased risk of cardiovascular disease and all-cause mortality (13, 14). These variables have been hypothesized to play a role in to the increases in blood pressure and cardiovascular risk during the winter, but their physiological mechanisms are not entirely understood. The purpose of this section is to review the current evidence on seasonal variations in cardiovascular function and examine the role of specific factors that may influence this phenomenon.

Seasonal Variations in Cardiovascular Outcomes and Arterial Blood Pressure

Wintertime increases in the incidence of numerous cardiovascular events (e.g. pulmonary embolism, stroke, heart failure, atrial fibrillation, myocardial infarction, etc.) have been well documented (3). A meta-analysis of 47 studies, primarily in temperate regions of the globe, found that there was a 20% increase in hospitalizations and 23% increase in CVD-related deaths during the peak season compared trough season (typically winter vs. summer) (15). Extreme increases in heat during the summer and extreme decreases in cold during the winter both contribute to excess deaths; however, higher relative risks are observed in temperatures below the 2.5 percentile (extreme cold) compared to those above the 97.5th percentile (extreme heat) (16).

One of the potential contributing factors to increased cardiovascular risk in the winter is elevated arterial blood pressure (4). Seasonal changes in blood pressure have been well-recorded. Rose et al. (1961) first reported increased clinical blood pressures in the United Kingdom during

the winter in 56 middle-aged men with ischemic heart disease (18). Sega et al. (1998) demonstrated that this pattern extended to home and 24-hour ambulatory blood pressure measurements in over 2000 normotensive and hypertensive adults in Italy (5). In recent decades, the aggregation of numerous population-based studies has allowed researchers to show this variation extends across the globe. Marti-Soler et al. (2014) took cross-sectional data from 24 population-studies in 15 countries (19) and found mean seasonal differences (winter – summer) in clinical systolic blood pressure (SBP) were 2.9 mmHg and diastolic blood pressure (DBP) were 1.4 mmHg in the Northern Hemisphere. Similar values of 3.4 mmHg SBP and 0.9 mmHg DBP were found in the Southern Hemisphere. Blood pressure peaks occurred in December in the Northern Hemisphere with troughs in June. In the Southern Hemisphere, the peaks were in July and May while the troughs were observed in January and November (19). Notably, these populations were primarily from high SES European countries and only 3 studies from Australia and New Zealand represented the Southern Hemisphere. However, a more extensive meta-analysis of 47 studies that included countries like the United States, Argentina, South Africa, Iran, and China presented similar patterns (20). Furthermore, these findings looked at various measurement methods and found significant seasonal differences in office BP, daytime ambulatory BP, and home BP. Night-time ambulatory BP was not significantly different. They conclude that summer SBP and DBP are around 5 and 3 mmHg lower, respectively, than in winter.

Blood pressure variation in geographical areas that experience little variation in their climate has not been as widely studied. When examining hypertensive patients in Singapore, a country that experiences minimal seasonal variation (minimal changes in temperature, humidity, and daylight hours), Wong et al. (2015) did not report any significant differences in a cross-

sectional analysis of ambulatory blood pressure in a cohort from May to July compared to October to December. A significant increase ($p = 0.022$) in night systolic dip was reported in the winter; however, there were no other differences in type of dipper, diastolic dip or duration of nighttime dip (21). This is the only study to our knowledge examining blood pressure variations in this type of climate. Evidently, more research needs to be conducted in these equatorial regions.

It does appear that seasonal variations in blood pressure may affect particular populations more than others. Age seems to play a factor, as studies have shown greater variation in older individuals (22–25). Evidence for the effect of BMI on seasonal variation is inconclusive. Some have reported that individuals with lower BMIs have greater seasonal variation, while others have reported no significant correlation (20, 22, 24, 26). There are conflicting results regarding variation by sex. Tu et al. (2013) observed greater variation in men, while others reported no differences in sexes (20, 22, 24). Socioeconomic status appears to be negatively correlated with seasonal variation. A South African study observed greater seasonal variation in subjects with low socioeconomic status and living in unplanned settlements (27). This may be partially mediated by increased exposure to environmental conditions and reduced access to central heating (25).

Determinants of BP

Regulation of blood pressure involves the complex interaction of neural, hormonal, and endothelial mechanisms. Neural control of blood pressure is primarily regulated by the sympathetic nervous system (SNS) (28). Acute changes in blood pressure are primarily modulated by the arterial baroreflex. Stretch receptors located in the aorta and carotid arteries sense perturbations in blood pressure and send signals to the sympathetic nervous system to

modulate vascular resistance and cardiac output via negative feedback (29). Baroreflex sensitivity was found to be negatively associated with healthy aging, hypertension, and myocardial infarction (30). In addition to acute control of blood pressure, the SNS also plays an important role in more long-term term control of blood pressure by innervating important endocrine tissues. There are numerous hormonal mechanisms responsible for regulating blood pressure. Norepinephrine, a circulating catecholamine, is released by the adrenal medulla in response to acute stressors. It binds to α-adrenergic receptors in blood vessels, resulting in vasoconstriction (31). The kidneys release renin, which converts angiotensinogen into angiotensin and subsequently is synthesized into angiotensin II (Ang II) by angiotensin-converting enzyme. Ang II not only directly causes vasoconstriction by binding to Ang II receptors on smooth muscle, but it also stimulates the adrenal glands to secrete a hormone called aldosterone, which increases sodium reabsorption and, subsequently, increases plasma volume, resulting in increased blood pressure. Ang II receptor blockers (ARBs) and ACE inhibitors are common blood pressure lowering medications and considerable research has demonstrated their efficacy in preventing cardiovascular events (32, 33). Another important player in plasma volume regulation is arginine vasopressin, which is secreted by the posterior pituitary gland and stimulates water reabsorption (34). Diuretics are another commonly prescribed medication to help counteract the effects of increased plasma volume due to Ang II and vasopressin (32). Additionally, Ang II interacts with the endothelium by stimulating the release of endothelin-1 (ET-1), which binds to ET-1 receptors on smooth muscle, amplifying vasoconstrictive effects (35). Increased ET-1 signaling is also implicated with direct and indirect reductions in NO bioavailability. ET-1 can directly inhibit NO production through the uncoupling of endothelial nitric oxide synthase (eNOS) (36). Additionally, ET-1 is associated with increased reactive

oxygen species (ROS) production, which can scavenge bioavailable NO (37). Impaired NO bioavailability and impaired endothelial nitric oxide-dependent endothelial function leads to pathophysiological states that contribute to the progression of atherosclerosis, hypertension, and cardiovascular disease (6, 38).

Seasonal Variations in Determinants of Blood Pressure

Central and hormonal mechanisms

Seasonal variation in central mechanisms of blood pressure control have not received much attention. The most direct quantification of sympathetic outflow utilizes microneurography to measure muscle sympathetic nerve activity (MSNA). The level of sympathetic activity to the smooth muscle is significantly correlated with total peripheral resistance, an important determinant of arterial blood pressure (39). Only one study has examined the relation of season with resting MSNA, demonstrating higher burst rates in the winter compared to the spring, summer, and fall (40). There were not any differences in blood pressure between seasons, however, which could potentially be due to the relatively young age of the cohort or lower cardiac output counteracting increases in TPR. Seasonal variations in cardiac output, another determinant of blood pressure, have also received little attention. There is one example of a study reporting significantly lower CO in the winter compared to the summer, however it is likely that these reductions in CO are due to increased afterload caused by increased TPR (41). Another technique that has been used in conjunction with measurement of MSNA is the cold pressor test (CPT) (42–44). Typically, the test involves placing a limb (i.e. hand or foot) in ice water while monitoring the participants hemodynamic responses to a sympathoexcitatory stimulus. The relative increase in blood pressure during the CPT has been utilized as a predictor for the development of hypertension and as a measure of sympathetic reactivity (44). To our knowledge,

no studies have investigated this potential relation between season and hemodynamic responses to the CPT. This could be important given the extensive evidence supporting increased blood pressure in the winter coupled with exaggerated blood pressure responses to physiological stressors (e.g. exercise), which have been found to be independent predictors for the future development of hypertension, heart failure, stroke, atherosclerosis, and cardiovascular-related deaths (8, 45–49).

Some data have suggested that variations in circulating hormones may have an impact on blood pressure and blood pressure control, however the current evidence is inconclusive. One study reported peak concentrations of plasma epinephrine and norepinephrine in the winter while others have reported no seasonal variations (50, 51). The same study that reported peak catecholamine levels in the winter surprisingly found that endothelin-1 and angiotensin II, potent vasoconstrictors, peaked in the summer (50). In contrast, others have reported peak endothelin-1 levels in January/February and no seasonal variations in angiotensin II (56,57). Plasma aldosterone and vasopressin (using copeptin as a surrogate marker) have also shown conflicting evidence. Some have reported increased aldosterone levels in the winter while others have reported no seasonal differences (51, 54, 55). Likewise, vasopressin concentrations were found to be significantly higher in the winter, but others found higher values in the summer (53, 56, 57). Given these results, it does not appear that these measures are currently useful in identifying potential mechanisms that could cause seasonal variations in blood pressure and blood pressure control.

Endothelial mechanisms

Endothelial function is primarily focused on endothelial production of NO. One technique often considered the gold standard for assessing endothelial function is FMD. Briefly,

FMD measures the change in diameter of a major conduit artery in response to reactive hyperemia (typically induced by the inflation and deflation of a sphygmomanometer cuff). The increase in blood flow and subsequent increase in shear stress results in the release of vasodilatory substances (like NO) from the endothelium, leading to an increase in diameter of the conduit artery (58). The magnitude of conduit artery vasodilation is strongly correlated with the development of cardiovascular disease (59). Currently, six studies have utilized FMD to assess seasonal variations in endothelial function. Four studies (including cross-sectional and longitudinal designs) found a significant decrease in FMD% in the winter compared to the summer (60–63). Two studies (one cross-sectional and one longitudinal) did not observe any significant winter/summer differences in FMD% (64, 65). Although FMD is primarily mediated by NO, other mechanisms including the release of prostaglandins and endothelium-derived hyperpolarizing factors may explain a substantial portion of the response (66). Intradermal microdialysis coupled with local heating of the skin allows for direct quantification of NO-mediated cutaneous vasodilation, a model that reflects vascular endothelial function in other circulatory beds (e.g., the renal circulation, coronary arteries, etc.) (67, 68). To our knowledge, no studies have mechanistically examined seasonal variations in NO-mediated vasodilation. Given that microvascular dysfunction may occur before the development of more overt macrovascular dysfunction, early identification of reduced NO bioavailability may be beneficial in managing potential seasonal variations in vascular function in at-risk populations (69).

Potential Contributors to Seasonal Variations in Determinants of Blood Pressure

Temperature

One of the proposed factors contributing to seasonal differences in blood pressure, blood pressure control, and endothelial function is ambient temperature. Quantitatively, a meta-analysis

of 14 studies found that each 1°C decrease in outdoor temperature was associated with a 0.26/0.13 mmHg increase in SBP/DBP. Conversely, a 1°C decrease in indoor temperature was associated with a 0.38 mmHg increase in SBP. There was not enough data on DBP to estimate the effects of colder temperatures in that study (70). Cardiovascular disease significantly modified this relation, as individuals with CVD experienced greater changes in blood pressure with changes in temperature.

It appears that temperature may have disparate effects on blood pressure and blood pressure control depending on the time of day. This is particularly important because it has been demonstrated that morning hours (6 AM – 12PM) are associated with a 40% increased risk of myocardial infarction, 49% with stroke, and 29% with cardiac events (71, 72). Exaggerated morning surges in blood pressure have been implicated in this phenomenon. Morning surges in blood pressure are a normal physiological event that are partially influenced by the a-adrenergic component of the sympathetic nervous system (73). Cold stimulates a-adrenergic vasoconstriction of the smooth muscle, and it has been demonstrated that elderly hypertensives have a significantly increased morning surge in blood pressure in cold temperatures (74, 75). Conversely, temperature has been positively associated with nighttime SBP and decreased nighttime dipping of blood pressure. It has been hypothesized that heat-related disruptions of sleep may be a major contributor to this outcome.

Along with seasonal variations in temperature, there is also considerable variation in individual experienced temperature. An analysis of the National Health and Examination Survey (NHANES) from 2009-2012 found that 44% of adults spent 30 min or less outdoors on workdays and 20% reported 30 min or less outdoor on non-work days (61). Therefore, outdoor ambient air temperature likely does not accurately reflect personal exposure. Heating,

ventilation, and air-conditioning infrastructure throughout a small geographical area may have significant variation, so one individual may be exposed to quite a different thermal environment than another in close proximity (76). Given the diurnal variation in blood pressure and potential individual variability in temperature exposure, it is suggested that 24-hr ABP monitoring with integrated with personal exposure temperature devices would best allow investigators to examine potential relations between the two variables, including at specific timepoints throughout the day (77).

The potential mechanisms responsible for temperature's impact on endothelial function have not been entirely elucidated; however, research investigating passive heating has highlighted the importance of increased blood flow and increased shear stress for inducing endothelial adaptations. Green et al. (2010) performed 8 weeks of bilateral forearm immersion with a cuffed and uncuffed arm in 42°C water. The cuff prevented increased flow and shear stress. Cutaneous vascular conductance (CVC) significantly improved in response to a gradual local heating protocol in the uncuffed arm, whereas no changes were observed in the cuffed arm. This suggests that hyperemia and shear stress are likely necessary factors to stimulate endothelial adaptations (78). During 8 weeks of lower limb heating at 40°C with subsequent increases in core temperature, Carter et al. (2014) observed improvements in forearm blood flow responses to local heating. These responses were attenuated when forearm temperature was clamped to 30°C or a pneumatic cuff was inflated to reduce skin blood flow, suggesting the importance of increased skin temperature and blood flow in peripheral adaptations (79). Unfortunately, the investigators did not utilize a maximal vasodilation phase in the local heating protocol, limiting conclusions regarding whether the improvements were due to endothelial function or simply the smooth muscle's response to endothelial vasodilators.

More recent studies have utilized intradermal microdialysis to directly quantify NO contribution to a local heating protocol. 8 weeks of hot water immersion (the arm used in microdialysis was kept out of the water to isolate the effects of core temperature) in sedentary young adults showed improved NO-dependent vasodilation to local heating while the sham therapy showed no change (80). Conversely ten days of forearm heating – core temperature remained unchanged – was insufficient to induce improvements in NO-dependent vasodilation (81). This might simply be due to the short duration of the protocol, and improvements may have been observed if it was extended, or it may suggest that elevations in core temperature are an important contributor to endothelial adaptations in response to heat.

In contrast, cold exposure generally appears to have a negative effect on endothelial function. Exposure to cold water via the CPT acutely diminishes FMD% in healthy young adults, which is likely to due to sympathetic-induced vasoconstriction reducing peripheral blood flow and shear stress, resulting in reduced NO bioavailability. Controlled studies examining long-term cold exposure have not been done in humans; however, 8 weeks of cold exposure (4 hours at 5°C/day) in Wistar rats significantly increased blood pressure, reduced acetylcholine-induced vasorelaxation, and eNOS expression (82).

UV

Along with temperature, UVR levels exhibits significant seasonal and geographical variation. The sun produces three main types of UV radiation: ultraviolet A (UVA), ultraviolet B (UVB), and ultraviolet C (UVC). 95% of UVR that reaches the earther's surface is UVA and the other 5% is UVB. UVC is blocked by the ozone layer, and therefore does not have a physiological impact (83). UVR levels are higher in the summer than the winter and it decreases at greater distances from the equator, with the poles experiencing the lowest amount of UVR

(84). Observational studies have shown relatively strong correlations between UVR and blood pressure. Data from the INTERSALT study shows a significant positive relation with mean BP, hypertension prevalence, and latitude (85). In a study of over 300,000 hemodialysis patients in the United States, Weller et al. (2020) found that UVA and UVB irradiation were inversely correlated with pre-dialysis SBP. The relation remained after accounting for temperature (86). The physiological mechanisms for UVR's effect on blood pressure have not been fully elucidated; however several hypotheses have been proposed. UVA and UVB both appear to influence vascular function by modulating NO bioavailability. On one hand, UVA and UVB can potentially negatively impact NO bioavailability through the acute production of reactive oxygen species (ROS). ROS are highly unstable and reactive molecules that can damage DNA, RNA, and proteins. Excessive levels can result in the uncoupling of endothelial nitric oxide synthase (eNOS), formation of reactive nitrogen species like peroxynitrite (ONOO-), or the scavenging folate, an important cofactor for eNOS (87, 88) (89). Conversely, UVA and UVB may also contribute to increases in NO bioavailability. Liu et al. (2014) demonstrated that 22 minutes of whole-body UVA irradiation in 24 normotensive young adults causes an acute decrease in DBP and MAP, but not SBP, for up to 30 minutes post-exposure. They also observed increases in relative forearm blood flow independent of nitric-oxide synthase (NOS) (90). Similarly, Oplander et al. (2009) demonstrated acute decreases in SBP and DBP for up to 60 minutes after 15 minutes of whole-body UVA exposure in 7 healthy adults (91). A subset (n = 4) also demonstrated acute increases in FMD and forearm blood flow post-irradiation. The mechanism for the acute improvements in endothelial function and blood pressure is not entirely clear, but it has been proposed that UVA irradiation leads to the liberation of "skin-bound" stores of NO from NO metabolites like nitrite and nitrosothiols (90–94). At this moment, there are very few

studies examining the effect of more long-term intervention strategies. 12 weeks of whole-body UVA irradiation (2x/week) was ineffective in reducing 24hr blood pressure in healthy older adults (95). Additionally, 6 weeks (3x/week) and 14 days of whole-body UVA irradiation was ineffective in reducing 24-hr ambulatory BP in patients with mild hypertension (96, 97).

The evidence for UVB's indirect effect on endothelial and cardiovascular function, via UVB-induced vitamin D synthesis, appears to be more substantial. UVB photolyzes 7-dehyrdrocholesterol (7-DHC) into cholecalciferol, or vitamin D3 (98). Vitamin D3 binds to Vitamin D receptors (VDR) that upregulate eNOS and superoxide dismutase expression (SOD), inhibits NADPH oxidase (NOX) production, and inhibits the proinflammatory transcription factor nF-kB (34). Observational studies have demonstrated that increased 25(OH)D concentrations strongly associated with reduced cardiovascular disease incidence and risk factors (99). Some investigators have argued that there are no interventional studies demonstrating the benefit of vitamin D supplementation on cardiovascular disease (99, 100). However, Wolf et al. (2024) astutely commented that the majority of these interventional studies are not effective because they focus on middle-aged and older adults, many of whom already have cardiovascular morbidities and may have been vitamin D deficient for decades prior (101). More darkly pigmented individuals are at a greater risk for vitamin D deficiency because their increased melanin content absorbs more UVB, preventing it from eliciting vitamin D synthesis (102). Additionally, it has been demonstrated that young, otherwise healthy, African-Americans have reduced NO-mediated cutaneous vasodilation compared to more lightly pigmented individuals (103). However, four-weeks of vitamin D supplementation alleviated any of the significant differences between the groups, which suggests that vitamin D supplementation early in life may

be an effective measure in preventing cardiovascular disease to those who are at greatest risk of deficiency (103).

There are few interventional studies utilizing whole-body UVB irradiation as a treatment to improve vascular function. Krause et al. (1998) performed 6 weeks (3x/week) of UVB irradiation on 9 patients with mild hypertension and observed a 6 mmHg reduction in SBP (-14,-1 mmHg) and DBP (-12,-2 mmHg), whereas Scragg et al. (2011) saw no changes in BP in 58 mostly healthy participants with vitamin D insufficiency (96). Evidently, more work needs to be done in this area, particularly in populations with hypovitaminosis D, but it appears that vitamin D supplementation may be a potential treatment avenue.

There is no research examining the effect of UVR on blood pressure control or reactivity; however, a few studies have examined the effects of vitamin D on SNS activity. Young, otherwise healthy adults who were vitamin D insufficient had increased circulating norepinephrine compared to vitamin D sufficient controls. After 90 days of vitamin D supplementation, this difference was attenuated (104). A study in which rats were fed a vitamin D deficient diet and then supplemented with vitamin D showed similar responses (105).

Physical Activity

An inverse dose response association has been found with the level of recreational physical (PA) and the incidence of hypertension and cardiovascular disease, such that higher levels are of PA are associated with a decreased incidence of hypertension (106, 107). Additionally, PA is one of the primary recommended treatment options to reduce elevated blood pressure (108). There is evidence that exercise can cause acute and chronic reductions in blood pressure reactivity and sympathetic activity. Ebbesen et al. (1992) demonstrated that one hour of

cycling at 55% VO2max resulted in transient reductions in the DBP response to a CPT 1 and 3 hours post-exercise (109). Likewise, Milatz et al. (2015) reported reductions in SBP and DBP one hour after cycling for 60 min at 45% VO2max (110). They postulate that exercise may cause a transient desensitization of the alpha-adrenergic stress response. Grassi et al. (1994) showed that 10 weeks of endurance training in young, sedentary, adults resulted in reductions in resting MSNA. They suggest that exercise training may improve baroreceptor control of the SNS (111). Other studies, however, have shown no differences between trained and untrained individuals and resting MSNA levels and MSNA responses to the cold pressor test (112, 113).

Black et al. (2008) demonstrated that fit older adults have improved NO-mediated endothelial function compared to age-matched sedentary adults and 12 weeks of exercise training improves endothelial function in the latter group (114). Additionally, DeSouza et al. (2000) showed that forearm blood flow (FBF) responses to acetylcholine were significantly lower in sedentary middle-aged and older men compared to young endurance-trained men, whereas older endurance-trained men showed no difference in FBF compared to their younger counterparts (115). 3 months of aerobic exercise (primarily walking) in a subset of the sedentary but healthy middle-aged and older men eliminated the difference in endothelial function between that group and the endurance-trained middle-aged and older-men (115). The evidence is less clear regarding whether regular exercise training improves endothelial function in healthy young adults without evidence of impaired vascular function. The previous study showed no differences in endothelial function between the young sedentary and endurance-trained men (115). Additionally, there were no differences in highly endurance-trained and sedentary, young men and women (116, 117).

Specific Aims and Hypotheses

Despite significant evidence of increased blood pressures in the winter accompanied with increased risk for cardiovascular morbidity and mortality, there is a dearth of research examining seasonal variations in endothelial function and blood pressure control. Additionally, numerous factors have been hypothesized to contribute to seasonal variations in blood pressure and cardiovascular risk; however, nobody has quantified the relative contributions of those factors to endothelial function and blood pressure control. For this purpose, this thesis tested the following specific aims and hypotheses:

Aim 1: Determine whether there are seasonal differences in endothelial function and blood pressure control in young healthy adults.

> Hypothesis 1a: Endothelial function will be reduced in winter and early-spring months compared to summer and early-fall.
>
> Hypothesis 1b: Blood pressure control will be reduced (i.e. greater blood pressure reactivity) in winter and early-spring months compared to summer and early-fall.

Exploratory Aim 2: Determine the relative contributions of temperature, UVR, and serum vitamin D concentrations on endothelial function and blood pressure control in young healthy adults.

CHAPTER 3

SEASONAL VARIATIONS IN ENDOTHELIAL FUNCTION AND BLOOD PRESSURE CONTROL IN YOUNG HEALTHY ADULTS[1]

[1] James F. Bangle Jr. and S. Tony Wolf. To be submitted to the Journal of Applied Physiology

Abstract

Cardiovascular events and death rates are highest during the winter months. Endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is a non-traditional risk factor for cardiovascular disease (CVD) that typically precedes the development of hypertension and increases cardiovascular risk. Exaggerated blood pressure (BP) responses to physiological stressors could potentiate this risk. Therefore, we assessed 15 young, healthy (23 ± 2; 7 men, 8 women) adults in the summer/early-fall and winter/early-spring to determine if there were seasonal variations in endothelial function and BP control. Intradermal microdialysis coupled with a standard local heating (42ºC) protocol and flow-mediated dilation (FMD) measured endothelial function. Continuous blood pressure measurement (finger photoplethysmography) measured BP responses to a 3-min hand cold pressor test (CPT). Mean daytime (sunrise to sunset) ambient temperature, wet bulb globe temperature (WBGT), and UV index were recorded the 30 days prior to experimental visits. Serum vitamin D concentrations [25(OH)D] were measured at each visit. Winter/early-spring FMD was not different ($P = 0.54$), but %NO contribution to local heating was attenuated (41.89 ± 15.11 vs. 67.41 ± 10.32 %, $P = 0.0003$) compared to summer/early-fall. WBGT was positively associated with the %NO contribution to local heating ($P = 0.01$, r = 0.52), but UV and [25(OH)D] were not related. There were no seasonal variations in peak BP responses to the CPT [SBP ($P = 0.33$), DBP ($P = 0.47$), and MAP ($P = 0.39$)]. Young, healthy, adults exhibit attenuated NO-mediated cutaneous vasodilation in the winter/early-spring, which may be partially mediated by decreased WBGT.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States and the world (1, 118). Seasonal trends in CVD mortality are evident across the globe with higher incidences of cardiovascular-related events in the winter months (3). Hypertension is a major risk factor for the future development of CVD and cardiovascular events, and elevated arterial blood pressure (BP) during the winter months has been well documented globally with home, office, and ambulatory methods (4, 20). Endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is a non-traditional risk factor for CVD that typically precedes the development of hypertension and increases cardiovascular risk (6, 38, 119, 120). Therefore, reduced endothelial function may be an important contributor to elevated winter BP.

Reduced flow-mediated dilation (FMD) has been observed across a variety of populations in the winter compared to the summer (60–63). Although FMD is primarily mediated by NO, other mechanisms including the release of prostaglandins and endothelium-derived hyperpolarizing factors may explain a substantial portion of the response (66). Intradermal microdialysis coupled with local heating of the skin allows for direct quantification of NO-mediated cutaneous vasodilation, a model that reflects vascular endothelial function in other circulatory beds (e.g., the renal circulation, coronary arteries, etc.) (67, 68). To our knowledge, no studies have mechanistically examined seasonal variations in NO-mediated vasodilation.

In addition to potential reductions in endothelial function contributing to seasonal variations in CV-related events, exaggerated BP responses to physiological stimuli could potentiate the winter increase in cardiovascular mortality. Exaggerated BP responses to physiological stressors have been found to be independent predictors for the future development

of hypertension, heart failure, stroke, atherosclerosis, and cardiovascular-related deaths (8, 45–49). However, to our knowledge, no study has yet examined seasonal variation in BP reactivity.

Despite the preliminary evidence that endothelial dysfunction and BP control may be impaired in the winter months, the specific factors contributing to these impairments is debated. Two environmental factors that demonstrate significant annual variation in temperate zones are ambient temperature and ultraviolet radiation (UVR). Ambient temperature is negatively associated with BP and BP variability (86, 121–126). Similarly, there is a negative relation between UVR and BP, and it has been hypothesized that UV light has direct (via liberation of NO stores independent of eNOS) and indirect (via synthesis of vitamin D) effects that may contribute to seasonal variations in BP (86, 127, 128). However, there have yet to be any studies that specifically examine the relative influence of seasonal variation in temperature, UVR intensity, and circulating vitamin D concentrations on endothelial function and blood pressure control.

Thus, the purpose of this study was to determine whether there are seasonal differences in endothelial function and BP control in healthy young adults. We hypothesized that 1) endothelial function would be reduced in the winter and early-spring months compared to summer and early-fall, and 2) BP control (i.e. greater BP reactivity to a sympathoexcitatory stimulus) would be reduced in the winter and early-spring months compared to summer and early-fall. An additional, exploratory aim was to determine the relative contributions of temperature, UVR, and circulating vitamin D concentrations on endothelial function and BP control in young healthy adults.

METHODS

Participants

All experimental protocols were approved by the Institutional Review Board at the University of Georgia. Written and verbal consents were obtained from all subjects prior to participation, according to the Declaration of Helsinki. All participants underwent an initial screening that included a health history questionnaire, measures of height, weight, and BP. Fifteen healthy individuals (7 men, 8 women) aged 19 - 27 yrs were enrolled. Participants were normotensive [systolic BP (SBP) < 130 and diastolic BP (DBP) < 85 mmHg], non-obese (BMI < 30 kg/m^2), nonsmokers who were free of cardiovascular disease, kidney disease, skin disease, pigmentation disorders or skin allergies, and were not taking any medications that have known vascular effects. Women had regular menstrual cycles (n = 4), were taking oral contraceptives (n = 3), or using other alternative birth control methods (n = 1). A urine pregnancy test confirmed the absence of pregnancy before experimental visits. To increase generalizability of our findings, and based on data demonstrating negligible impacts (129, 130), women were tested without regard to menstrual cycle or oral contraceptive phase.

Experimental Procedures

Data were collected from July 2024 to June 2025 in a thermoneutral laboratory. Summer/early-fall was defined as June-October and winter/early-spring as January-April. These timepoints were selected because there are significant differences in temperature in the US and adults have lower serum vitamin D levels in the winter compared to the summer (131). Furthermore, serum Vitamin D [25(OH)D] has a relatively long half-life of 15 days, therefore the time between October – January allowed sufficient time for [25(OH)D] to significantly decline

(132). A subset of participants (4 men, 3 women) performed repeated-measures visits separated by ~6 months. Participants were fasted and had not consumed caffeine for at least 8 hours prior to their visit. Additionally, they refrained from vigorous exercise for 24 hours prior to testing.

Intradermal Microdialysis

With the participant in a semi supine position, one intradermal microdialysis fiber (10 mm, 55-kDa cutoff membrane; CMA, Kista, Sweden) was placed in the dermal layer of the ventral aspect of the left forearm for local delivery of pharmacological agents. Pharmacological agents were mixed before use, dissolved in lactated Ringer's solution, sterilized using syringe microfilters (Acrodisc; Pall, Port Washington, NY), and wrapped in foil to prevent degradation due to light exposure. Solutions were perfused through the microdialysis fiber at a rate of 2 μL/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems) (133). Local red blood cell flux was measured directly over the microdialysis site throughout local heating via an integrated laser-Doppler flowmetry probe placed in a local heating unit (Moor Instruments SHO2, Moor Instruments, Inc., Wilmington, DE). After fiber placement, a ~60-min period of hyperemia resolution due to needle trauma (during which FMD tests were performed). After the resolution of hyperemia, baseline data were collected (~20 min) before starting a standard local heating (42°C) protocol, during which lactated Ringer's solution was perfused through the microdialysis fiber (134). The local heating response is characterized by an initial axon-mediated rise and brief nadir in skin blood flow, followed by a gradual rise and eventual plateau (45-60 min) in blood flow. After observing a local heating plateau, 15 mM of NG-nitro-l-arginine methyl ester (L-NAME; NO synthase inhibitor) was perfused, allowing for quantification of NO-dependent vasodilation (%NO) (67). Following the observation of a stable L-NAME plateau, 28mM sodium nitroprusside (SNP; Sigma-Aldrich, St. Louis, MO) was

perfused and local temperature was increased to 43°C to elicit maximal vasodilation (CVC_{max}) (134).

Flow-mediated dilation (FMD)

Brachial artery FMD was assessed as described by Thijssen et al. (2019) using high-resolution ultrasonography (GE Logiq E, GE Medical, Milwaukee, WI) (135). Briefly, a rapid inflator BP cuff (Hokanson, Bellevue, WA) was attached to the right forearm below the antecubital fossa. Brachial artery diameter and shear rate were continuously captured using an automated edge-detection software (Quipu, Pasa, Italy). After a 1-min baseline measurement period, the cuff was inflated to a suprasystolic pressure (220 mmHg) for 5 min. The cuff was deflated and the vascular response to reactive hyperemia was recorded for 3 min post-occlusion. FMD was calculated as the percentage change in baseline arterial diameter to peak arterial diameter during post-occlusion. Additionally, to account for potential differences in shear rate (the stimulus for brachial artery vasodilation), FMD was normalized to shear rate (SR) area under the curve (AUC) to peak dilation. All FMDs were performed by the same trained sonographer and analyzed by two trained technicians. Inter-rater reliability was excellent with an intraclass correlation coefficient of 0.99.

Cold Pressor Test (CPT)

With participants in a semi-supine position, a small cuff was placed on the left middle finger for continuous beat-to-beat BP measurement (Human NIBP Nano, ADInstruments, Bella Vista, NSW, Australia). After 5 min of a stable baseline measurement, the participant's hand was placed up to the wrist in ice water (0-3°C) for 3 min. After 3-min CPT, the participant's hand was removed from the ice water and measurements continued for 5 min post-CPT.

Meteorological Data

Meteorological data were extracted in the 30 days prior to each participant's experimental visit. Mean daytime (sunrise to sunset) ambient temperature, wet bulb globe temperature (WBGT), and UV index were recorded using the closest WeatherSTEM station to the participant's location.

Serum Vitamin D Analysis

Blood samples were collected in serum separator tubes during each visit. Serum was isolated via centrifugation and stored at -80°C for future analysis. Serum [25(OH)D], the primary circulating metabolite of vitamin D, were quantified in triplicate using an ELISA kit according to the manufacturer's instructions (CrystalChem, Elk Grove Village, IL).

Data Acquisition and Statistical Analysis

Skin blood flow data were continuously recorded and stored for off-line analysis (PowerLab/LabChart, ADInstruments, Bella Vista, NSW, Australia). Mean arterial pressure (MAP) was calculated for each phase of the local heating protocol using BP from an automated BP monitor (CONNEX Spot Monitor, Hill-Rom, Chicago, IL). Cutaneous vascular conductance was calculated as red blood cell flux divided by MAP and expressed as a percentage of cutaneous vascular conductance (CVC_{max}; $\%CVC_{max}$) for each phase of the local heating protocol (134). The NO contribution to cutaneous vasodilation was calculated as the difference between the local heating and L-NAME plateau responses (67). A two-way ANOVA was used to assess the effects of season (winter/early-spring vs. summer/early-fall) and phase (baseline, peak, local heating plateau, L-NAME plateau, and %NO contribution) for the local heating protocol. Post hoc comparisons with Tukey's corrections were performed for specific planned

comparisons. Student's unpaired *t* tests were used to compare CVC_{max}. Forward entry, stepwise multiple regression analyses were used to determine the relative influences of WBGT, UV index, and serum [25(OH)D] on local heating responses. WBGT was used instead of ambient temperature because it accounts for multiple environmental factors (e.g. radiation, wind speed, and humidity) in addition to dry-bulb temperature that contribute to the imposed thermal load (42). The threshold for inclusion was $P < 0.05$. Absence of multicollinearity was confirmed for all analyses with variance inflation factor (VIF) < 5.0 for all variables.

A two-way ANOVA was used to evaluate the effect of season (winter/early-spring vs. summer/early-fall) and phase (baseline diameter, peak diameter, Δ diameter, %FMD, and %FMD normalized to SR). Post hoc comparisons with Tukey's corrections were performed for specific planned comparisons.

A two-way ANOVA was used to evaluate the effect of season (winter/early-spring vs. summer/early-fall) on resting blood pressure (SBP, DBP, and MAP). Baseline hemodynamic data were determined as the average of 5 min of quiet rest before the CPT. During the 3 min CPT and 5 min recovery, data were analyzed in 30 sec bins to determine relative changes from baseline. An additional two-way ANOVA was used to evaluate the effects of season (winter/early-spring vs. summer/early-fall) and time (every 30 sec) on CPT responses. Post hoc comparisons with Tukey's corrections were performed for specific planned comparisons. Peak relative hemodynamic responses were recorded as the highest 5 sec average during the cold pressor response. Student's unpaired *t* tests were used to compare the peak change in responses.

Additionally, student's unpaired *t* tests were used to compare temperature, WBGT, UV index, and serum [25(OH)D]. All statistical measures were also separately performed in the

repeated-measures cohort. Data were analyzed using GraphPad Prism 10.5.0 (GraphPad Software, San Diego, CA) and SPSS 29.0.1.0 (IBM, Armonk, NY).

RESULTS

Subject characteristics are presented in Table 1. All anthropometric characteristics and blood pressures were within normal limits for this age group.

Table 1. *Subject Characteristics*

	All Participants	**Repeated Measures**
n (men/women)	15 (7/8)	7 (4/3)
Age, yr	23 ± 2	24 ± 2
BMI, kg/m^2	23.3 ± 2.3	22.9 ± 2.6
Systolic BP, mmHg	112 ± 9	112 ± 10
Diastolic BP, mmHg	68 ± 7	68 ± 7

Values are means ± SD, n = number of participants. BMI, body mass index; BP, blood pressure

Meteorological data for each season is presented in Table 2. Daytime temperature and WBGT (both $P < 0.0001$) and daytime UV index ($P = 0.04$) were significantly lower in the winter/early-spring compared with summer/early-fall. Temperature and WBGT were still different in the repeated-measures cohort, however UV was no longer significant between seasons ($P = 0.18$). There were no seasonal differences in serum [25(OH)D] ($P = 0.36$), however, in the repeated-measures cohort, serum [25(OH)D] was slightly reduced in the winter/early-spring compared to summer/early-fall (36.23 ± 15.42 vs. 40.20 ± 14.12 ng/mL, $P = 0.04$).

Table 2. *Meteorological Data*

	Summer/early-fall	Winter/early-spring
Daytime Temperature, °C	25.12 ± 1.94	14.15 ± 4.72*
Daytime WBGT, °C	24.08 ± 2.43	12.53 ± 4.06*
Daytime UV Index (0-11+)	1.82 ± 0.46	1.32 ± 0.70*

*Values are means ± SD. WBGT, wet bulb globe temperature; UV, ultraviolet. *P < 0.05 compared with summer/early-fall.*

Endothelial function

Figure 1 depicts endothelium-dependent vasodilation assessed by FMD. The connecting lines represent the repeated-measures participants who were tested in both timepoints. There were no differences in baseline ($P = 0.34$), peak ($P = 0.38$), or absolute change in artery diameter ($P = 0.65$). Likewise, there was no change in FMD (%) ($P = 0.54$; Figure 1A) or FMD normalized to SR_{AUC} ($P = 0.52$; Figure 1B). The results did not change in the repeated-measures group ($P \geq 0.30$).

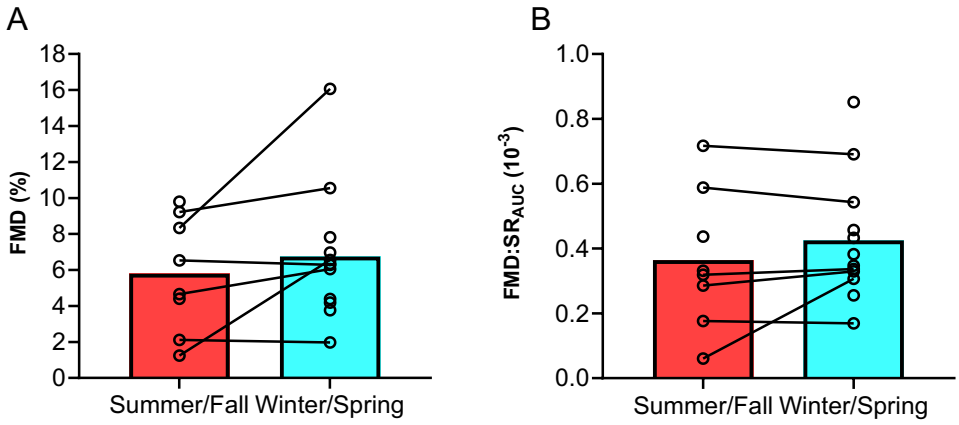

Figure 1. No difference in endothelium-dependent vasodilation of the brachial artery assessed via flow-mediated dilation in the summer/early-fall (n = 8) compared to winter/early-spring (n = 12). Values are represented as the peak change in brachial artery diameter (FMD%) (A) and the FMD% normalized to shear rate area under the curve to peak (SR_{AUC}) (B). The connecting lines represent the participants who were tested in both seasons (n = 6).

Figure 2 depicts the $\%CVC_{max}$ for each phase of the local heating protocol in both timepoints and the %NO contribution to the local heating response. There were no differences in baseline ($P = 0.95$), the initial axon reflex-mediated peak ($P = 0.06$), or the L-NAME plateau ($P = 0.13$). The plateau phase was significantly diminished in the winter/early-spring compared to summer/early-fall (58.52 ± 16.17 vs. 79.36 ± 13.72 $\%CVC_{max}$, $P = 0.004$; Figure 2A). Likewise, the %NO contribution to local heating was attenuated in winter/early-spring compared to summer/early-fall (41.89 ± 15.11 vs. 67.41 ± 10.32 %, $P = 0.0003$; Figure 2B). There were no differences in maximal CVC values between seasons ($P = 0.08$). When only looking at the repeated-measures cohort, the initial axon reflex-mediated peak was attenuated in the winter/early-spring compared to summer/early-fall (33.28 ± 14.15 vs. 55.48 ± 13.63, $P = 0.02$). There were no changes in the other phases.

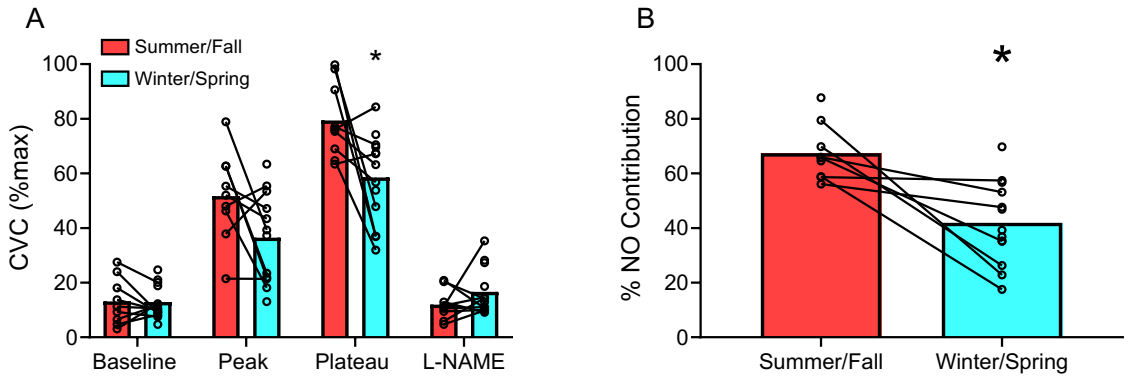

Figure 2. Seasonal variations (summer/early-fall (n = 9) vs. winter/early-spring (n = 13)) in cutaneous vascular conductance ($\%CVC_{max}$) throughout each phase of the local heating protocol (A) and percent contribution of nitric oxide (%NO Contribution) to the local heating plateau (B). The connecting lines represent the participants who were tested in both seasons (n = 7). *P < 0.05 compared with summer/early-fall.

There was a positive relation between WBGT and %NO contribution to the local heating response (Fig. 3; $P = 0.01$, r = 0.52). Neither daytime UV index ($P = 0.45$) nor serum [25(OH)D] ($P = 0.52$) were independently related to the %NO contribution to the local heating response. Consequently, only WBGT entered the forward stepwise regression model (Table 3).

Table 3. *Results of forward stepwise linear regression analysis*

	Standardized Coefficient	***t***	***P***	***R²***	**Adjusted *R²***
WBGT	0.53	2.68	0.02	0.29	0.25

WBGT, wet bulb globe temperature (°C)

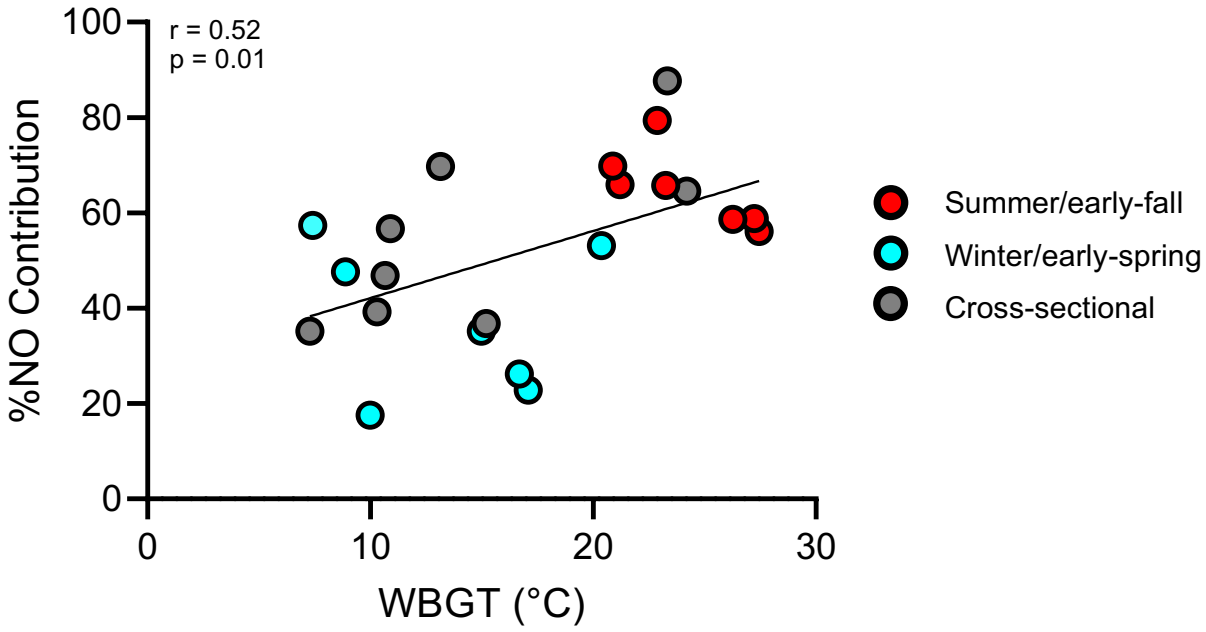

Figure 3. The relation between WBGT and the %NO contribution to the cutaneous vasodilation response to local heating.

Blood pressure and blood pressure control

There were no differences in resting SBP ($P = 0.52$), DBP ($P = 0.36$), and MAP ($P = 0.37$). The results were the same in the repeated-measures cohort ($P \geq 0.18$). Figure 3displays the

relative blood pressure responses to the CPT between seasons. As expected, SBP, DBP, and MAP significantly increased during the CPT independent of season ($P < 0.0001$). However, there were no significant differences in the SBP, DBP, and MAP responses between seasons (A, C, E). There were also no differences in 5-sec peak SBP ($P = 0.33$), DBP ($P = 0.47$), or MAP ($P = 0.39$) responses during the CPT (B, D, F). These results remained the same in the repeated-measures group ($P \geq 0.25$).

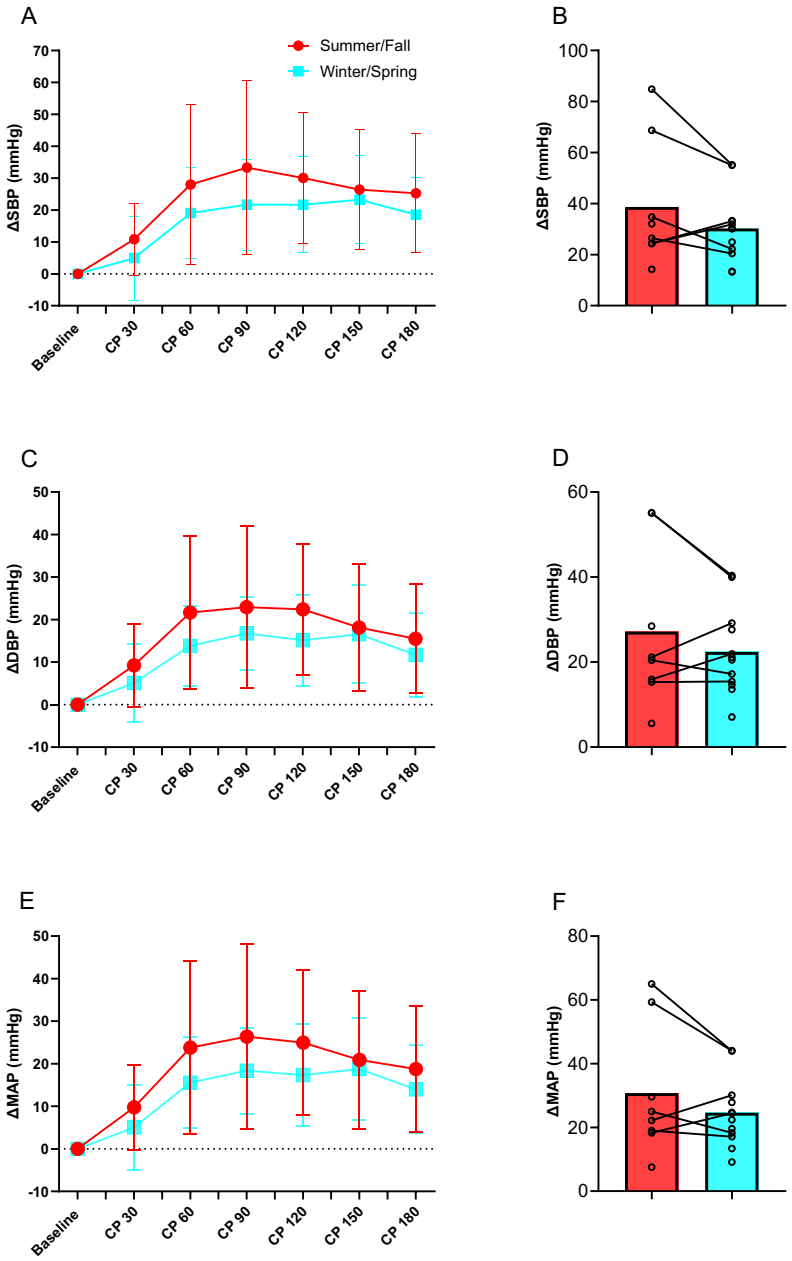

Figure 4. Blood pressure responses during a 3-min cold pressor test (CPT) in summer/early-fall compared to winter/early-spring. There were no differences in systolic (ΔSBP), diastolic (ΔDBP), and mean arterial blood pressure (ΔMAP) responses during the CPT. Likewise, there were no differences in peak ΔSBP, ΔDBP, or ΔMAP.

DISCUSSION

The aims of this study were to determine if young healthy adults exhibit seasonal variations in endothelial function and blood pressure control, and to quantify the relative contributions off temperature, UVR, and vitamin D to these outcomes. The primary findings were that NO-mediated cutaneous vasodilation was attenuated in the winter/early-spring compared to summer/early-fall and that WBGT was positively associated with the NO component of the response to local heating, whereas UVR and circulating [25(OH)D] were not. Additionally, there were no seasonal variations in FMD or blood pressure responses to the CPT.

Previous work has primarily focused on seasonal variation in endothelial function in older populations and/or those with cardiometabolic diseases. In an older cohort without evidence of CVD, Widlansky et al. (2007) found lower winter FMD% compared to the summer (62). Maruhashi et al. (2023) did not find any seasonal difference in FMD% in an older population, many of whom had CVD or risk factors for CVD (64). Of note, both of these studies were cross-sectional in nature, so inter-subject variability over time could not be assessed. Iwata et al. (2012) and Honda et al. (2020) both used longitudinal designs and found decreased wintertime FMDs in populations of hypertensive and type II diabetes patients, respectively (60, 63).

Research on younger populations is also conflicting. A longitudinal study of 21 young women with primary Raynaud's phenomenon and 22 controls found no seasonal differences in FMD%, while another longitudinal study in healthcare workers found significantly lower winter FMD% compared to the summer (61, 65). We did not observe any seasonal differences in FMD in our young healthy cross-sectional or repeated-measures participants. NO mediates ~72% of dilation in FMD healthy individuals; therefore, it is likely that the redundancy of vasodilatory

mechanisms (e.g. endothelium-derived hyperpolarizing factors (EDHFs) and prostacyclin) in young healthy individuals may have accounted for the lack of seasonal variation in this endothelial measure (66). Interestingly, we did find reductions in NO-mediated cutaneous vasodilation in the winter/early-spring compared to summer/early-fall. Endothelial dysfunction is antecedent to the development of hypertension and more overt cardiovascular dysfunction, and apparently healthy young adults can exhibit endothelial dysfunction without any clinical cardiovascular risk factors (136–138). Although young healthy individuals may have alternative mechanisms to alleviate decreased NO bioavailability in the winter/early spring, it is likely that the reduced baseline NO bioavailability and declines in redundant mechanisms in older populations and individuals with cardiovascular disease is a contributing factor to increased winter cardiovascular morbidity and mortality (139).

Our study demonstrated that WBGT, but not UV index or circulating [25(OH)D], was associated with NO-mediated cutaneous vasodilation. No other studies have evaluated the relation of ambient temperature (or WGBT) and NO-mediated cutaneous vasodilation; however, 8 weeks of passive heat therapy improved NO-mediated cutaneous vasodilation in sedentary, but otherwise healthy, young adults (80). In addition, passive heat therapy has also demonstrated improvements in FMD responses (51–53). Mechanistically, increased shear stress associated with heat-induced increases in peripheral blood flow elicits an upregulation in eNOS expression, thus improving NO production and bioavailability (140, 141). Indeed, Green et al. (2010) demonstrated that the increases in blood flow and shear stress are obligatory in inducing endothelial adaptations (78). Conversely, reduced peripheral blood flow at cold temperatures may be partially responsible for the reductions we observed in NO-mediated cutaneous vasodilation.

Contrary to our hypothesis, neither UVR nor circulating 25(OH)D were associated with NO-mediated cutaneous vasodilation. UVB, in particular, plays an important role in the synthesis of vitamin D by photolyzing 7-dehyrdrocholesterol (7-DHC) into cholecalciferol. Vitamin D3 binds to Vitamin D receptors (VDR) that upregulate eNOS, the protein that synthesizes NO, and superoxide dismutase (SOD), an antioxidant that neutralizes superoxide (34). eNOS activity and antioxidant balance are both critical to NO bioavailability. In a diverse cohort of healthy young adults with a wide range of skin pigmentation, serum vitamin D levels were directly related to the NO-mediated cutaneous vasodilation during a 39ºC local heating protocol (102). The contrasting findings may be explained, at least in part, by the observed range of serum [25(OH)D]. Most of the participants in the study by Wolf et al. (2022) were vitamin D deficient (<20 ng/mL; n = 10) or insufficient (21–30 ng/mL; n = 17), with only 6 of the participants being sufficient (>30 ng/mL) (49). In contrast, 76% of our values were sufficient and there were no participants who were vitamin D deficient. Therefore, the difference in the proportion of subjects who were sufficient, insufficient, or deficient between the two studies is likely explained by the lack of more darkly-pigmented subjects in the current study, which may have limited the potential for discovering a significant relation between vitamin D and NO-mediated cutaneous vasodilation responses. Future studies should incorporate a more diverse cohort with more darkly-pigmented individuals who are at higher risk for vitamin D insufficiency and deficiency (88).

Cardiovascular events and death rates are highest during the winter months (3). One of the primary risk factors for the development of cardiovascular disease is hypertension, and seasonal elevations in winter blood pressure are prevalent across the globe, particularly in older and diseased populations (4, 20). In the present study, there were no differences in blood

pressure between seasons, but the magnitude of changes were similar to those previously reported in young healthy adults (20, 27, 143–146). Another factor that may contribute to increased cardiovascular risk in the winter is increased blood pressure reactivity, particularly when combined with a higher baseline winter blood pressure. The sympathetic nervous system is an important regulator of blood pressure and vascular function and it has been demonstrated that higher resting MSNA activity is associated with greater daytime blood pressure variability (28, 147). In a cohort of older participants with risk factors for CVD, Narita et al. (2022) observed increased day-by-day home SBP in the winter and found that winter variability was significantly related to future CVD events (148). Increases in MSNA during the CPT are well correlated with increases in blood pressure (149, 150). To our knowledge, we are the first to evaluate seasonal variations in blood pressure control using the CPT. We did not observe any seasonal variation in blood pressure responses to the CPT in our healthy young cohort. Increased sympathetic activity is associated with healthy aging and may be related to stiffening of the baroreflexes, which could result in a poorer blood pressure control in response to stressors (28). Our young healthy participants likely have well-maintained autonomic control of their blood pressure; therefore, seasonal fluctuations may have relatively little effect on blood pressure regulation. Future work should consider examining CPT responses in older adults and/or other cohorts with increased CVD risk.

An important implication of our findings relates to study design. The attenuation of NO-mediated cutaneous vasodilation in the winter/early-spring suggests that future studies should consider testing individuals and groups at the same time of year to eliminate the potential effects of seasonal variation. One limitation of this study is the use of weather stations to assess the contributions of environmental exposures to seasonal variation in endothelial function. Although

we used nearby weather stations to assess ambient environmental temperatures in the weeks leading up to each experimental visit, these conditions only represent the potential exposures at the individual level. An analysis of the National Health and Examination Survey (NHANES) from 2009-2012 found that 44% of adults spent 30 min or less outdoors on workdays and 20% reported 30 min or less outdoor on non-work days (151). Therefore, the significant amount of time spent indoors may reduce the potential effects of outdoor temperature and UVR, and we have no way in the current study to assess actual exposures at the individual level. Future work should look to use personal ambient temperature and UVR monitoring to better understand the relation between these environmental factors and vascular function.

Another potential limitation is that neither physical activity (PA) levels nor cardiovascular fitness were tested in this study. PA levels are typically higher in the summer compared to other seasons and moderate-to-vigorous PA is greater in the summer compared to the winter (13, 152). Similar to heat stress, PA elicits elevations in core temperature, resulting in increased blood flow to the skin to promote heat loss and maintain thermoregulation (153). Older fit adults have improved NO-mediated endothelial function compared to age-matched sedentary adults, and 12 weeks of exercise training improves endothelial function in the latter group (114). The evidence is less clear regarding whether regular exercise training improves endothelial function in younger healthy adults without evidence of impaired vascular function; therefore, it seems unlikely that variations in PA or fitness would significantly modulate vascular outcomes in this population (115–117, 154, 155). However, given that chronic exercise-independent heat exposure has been shown to improve endothelial function in a variety of populations, future work should consider accounting for both of these factors when analyzing seasonal variations in

vascular outcomes, particularly in older populations or in individuals with greater cardiometabolic disease risk (80, 156–158).

In summary, this is the first study to our knowledge to demonstrate seasonal variations in NO-mediated cutaneous vasodilation and its relation with WBGT. Season did not mediate FMD or blood pressure reactivity to the CPT; however, these outcomes should continue to be examined with larger cohorts including older and special populations, as these populations are at greater risk for cardiovascular-related events and deaths in the winter. These findings reinforce the importance of considering seasonality when examining vascular function and suggest the need for further research in this area to find solutions to mitigate excess cardiovascular morbidity and mortality in the winter months.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

The primary aim of this study was to better understand the effects of season on endothelial function and blood pressure control in young adults. These findings suggest that there are seasonal variations in NO-mediated endothelial function in young, healthy adulmay be partially mediated by WBGT. However, blood pressure and blood pressure control were not different between seasons. This chapter summarizes this study's findings and discusses the future directions for this area of research.

Nitric Oxide-Mediated Vasodilation in the Cutaneous Microvasculature Is Attenuated in the Winter/Early-Spring vs. Summer/Early-Fall in Young, Healthy Adults

The primary finding was that NO-mediated cutaneous vasodilation was attenuated in the winter/early-spring compared to summer/early-fall in young healthy adults, whereas there were no seasonal differences in FMD. Furthermore, this study demonstrated that WBGT is independently related to the NO component of the response to local heating, while UV and serum [25(OH)D] were not.

Implications

This is the first study to demonstrate seasonal variations in NO-mediated cutaneous vasodilation in young healthy adults, substantiating previous research reporting seasonal variations in endothelial function via other measures. It also suggests that WBGT may partially contribute to the seasonal variation in NO-mediated cutaneous vasodilation. Together, this provides evidence

that seasonal variations in ambient temperature may influence seasonal variations in endothelial function.

No Seasonal Differences in Blood Pressure or Blood Pressure Control in Young Healthy Adults

The primary finding was that resting blood pressure did not differ in the winter/early-spring vs. summer/early-fall in young healthy adults. Moreover, there were no seasonal differences in blood pressure responses to the CPT.

Implications

The lack of seasonal differences in blood pressure supports previous research suggesting relatively minor seasonal changes in blood pressure in young healthy adults. Moreover, season did not affect blood pressure responses to the CPT. Together, these data suggest that season does not affect resting blood pressure or blood pressure reactivity to a sympathoexcitatory stimulus in young healthy adults.

Future Directions

1) Previous work has highlighted the important role of NO as a cardioprotective molecule and shown that reductions in NO bioavailability precede the development of more traditional cardiovascular risk factors and cardiovascular disease (6, 38, 119, 120). The attenuation of NO-mediated cutaneous vasodilation in young healthy adults during the winter may not result in an increased cardiovascular risk in the short term, as young adults have redundant mechanisms that compensate for reduced NO bioavailability (66). This may have been reflected by the lack of seasonal differences in FMD responses. However, this mechanism

likely still contributes to the increased rate of cardiovascular morbidity and mortality during the winter.

Older individuals and those with CVD already demonstrate reduced endothelial function; therefore, further reductions could magnify the risk of cardiovascular events (133). Previous research has already demonstrated reduced FMD% during the winter in these populations; thus, future studies should consider investigating the effect of season on NO-mediated cutaneous vasodilation and blood pressure reactivity, as they may further elucidate the mechanisms contributing to increased cardiovascular risk (60–63). Additionally, given the heterogeneity in vascular health among older adults, longitudinal designs following the same participants may help eliminate any potential confounding factors.

2) The relation between WBGT and the NO-component of the response to local heating highlights the important role temperature may play in augmenting endothelial function. Passive heating (e.g., saunas, hot water immersion) has been demonstrated to improve endothelial function in a variety of populations. Therefore, future work should consider studying the effects of passive heating during the winter to potentially mitigate the increased risk of cardiovascular-related events and deaths (80, 157–160). Nevertheless, WBGT only accounted for 19% of the variance in our study, so considerably more work needs to be done to elucidate the underlying mechanisms causing seasonal variation in endothelial function.
3) The small sample size and lack of diversity in our participants likely limited our ability to elucidate the potential roles of UVR and vitamin D on endothelial function. Previous research has demonstrated the importance of vitamin D in cardiovascular function, and these factors should still be considered when looking at potential contributors to seasonal variations (103, 161). Moreover, given the heterogeneity in individuals' environments and

behaviors, future studies should consider using personal environmental monitors and activity tracking to more accurately understand the potential contributors to seasonal variations in vascular function.

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