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Relation of Season and Temperature to Endothelium-Dependent Flow-Mediated Vasodilation in Subjects Without Clinical Evidence of Cardiovascular Disease (From The Framingham Heart Study)

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Abstract

Multiple studies have documented an increased incidence of cardiovascular events in the winter, but the pathophysiological mechanisms remain incompletely understood. We hypothesized that brachial flow and flow-mediated dilation (FMD) would vary by season and temperature. We related season and temperature to ultrasonic brachial artery endothelium-dependent FMD% (n=2587), baseline flow velocity and maximal reactive hyperemia (n=1973) in the Framingham Offspring cohort (mean age 61±10 years; 53% women). We obtained outdoor temperature from National Climate Data Center records for Bedford, Massachusetts (about 14 miles from testing site) and we measured the examination room temperature. In multivariable models, FMD% was highest in summer and lowest in winter (3.01±0.09 vs. 2.56±0.10%, respectively; P=0.02 for differences across all 4 seasons). FMD % was highest in the warmest and lowest in the coldest outdoor temperature quartiles. In stepwise models adjusting for risk factors, and selecting among season, outdoor temperature, and room temperature, FMD% was associated with season (P=0.02); temperature did not enter the model. In contrast, hyperemic flow velocity was significantly lower for cooler, and higher for warmer room temperatures (P=0.02 overall); season did not enter the model. Season, outdoor and room temperature were each retained in a stepwise model of baseline flow velocity (P<0.0001, P=0.02, P<0.0001, respectively). In conclusion, we observed a significant association between season and FMD. Microvascular vasodilator function, as reflected by hyperemic flow, was more strongly related to temperature than season. In conclusion, we speculate that endothelial dysfunction may be 1 of the mechanisms influencing seasonal variation in cardiovascular events.

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Keywords

endothelium; epidemiology; temperature; season

Large epidemiological studies have consistently demonstrated an increased incidence of myocardial infarction in the winter months.[1-3] The pathophysiological mechanisms that link winter and cardiovascular events are remain incompletely understood, but in part relate to cold temperatures.[4] Prior studies have reported that colder months are associated with deleterious alterations of many traditional[5-8] and novel[9-11] cardiovascular risk factors. Further, it is well recognized that endothelial dysfunction contributes to the pathogenesis of cardiovascular events,[12] and is related to many of the risk factors that vary by season. Seasonal variation in endothelial function based on the effect of temperature on endothelium derived nitric oxide synthase[13;14] might also contribute to increased cardiovascular risk in colder time periods.[12-14] We hypothesized that winter would be associated with impaired endothelial function, in part due to colder outdoor and room temperature and seasonal variation of risk factors. To test this hypothesis, we investigated seasonal variation in flow-mediated dilation (FMD%) of the brachial artery and reactive hyperemia in the community-based Framingham Heart Study.

METHODS

Participants

The design of the Framingham Offspring Study has been described elsewhere.[15] Participants receiving the 7th examination (1998-2001) were eligible for the present investigation (n=3539; 86% of those alive at examination 7). A total of 2587 participants (73% of eligible) were available for FMD analyses after exclusions for the following indications: room temperature not recorded at the time of examination [n=237], missing data for outdoor temperature on examination day [n=37]; nursing home examination [n=205]; radical mastectomy [n=34]; significant Raynaud's disease [n=9]; subject refusal [n=83]; equipment malfunction/ miscellaneous [n=15]; examination prior to digital image capture [n=177]; technically inadequate study [n=133]; or missing covariate data [n=22]. A subset of 1973 participants (56% of eligible, 68% of those with data for FMD%) provided information on brachial artery Doppler flow; digital acquisition of Doppler flow was phased in later in the examination cycle. The Boston University Medical Center Institutional Review Board approved the study protocol; participants provided written informed consent.

All participants underwent routine medical history, physical examination, and laboratory assessment. Participants were instructed not to eat or drink after 8 PM the previous evening, except water, or decaffeinated black coffee or tea. Cigarette smoking (within 6 hours) was ascertained by self-report. Heart rate and blood pressure (BP) were measured by automatic device (Dinamap, Model#1846SX, Critikon, Inc.) at the time of brachial testing. Diabetes mellitus was defined as fasting glucose ≥126 mg/dl or use of diabetes medication. A panel of 3 investigators blinded to risk factor data determined cardiovascular disease events (coronary heart disease, heart failure, stroke, intermittent claudication) from medical records.[16] The examination included a 6 minute walk test (Bruce protocol Stages I and II), in participants without contraindications (known coronary heart disease, chest pain on test day, or inability to perform test).

Determination of Season, Outdoor and Room Temperature

Participants were classified with respect to the season based on date of their examination at the Heart Study: winter (December 21st to March 20th), spring (March 21st to June 20th), summer (June 21st to September 20th), and fall (September 21st to December 20th). Outdoor

temperature at 8 AM on the day of participants' tests was obtained from data recorded in Bedford, Massachusetts (about 14 miles from testing site) by the National Climate Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html). Participants generally entered the Framingham Study clinic between 8 and 9 AM. Approximate outdoor temperature quartile was classified as follows: quartile 1: -18.9 to 0.6 C°, quartile 2: 1.1 to 6.7 C°, quartile 3: 7.2 to 13.3 C°, quartile 4: 13.9-23.9 C°. Examination room temperatures were determined to the nearest degree by digital thermometer (Fisher Scientific), and were classified into 3 categories: <25°C, 25°C (median), and >25°C. Whereas the indoor temperature minimum and maximum were 20°C and 30°C, the 25th and 75th percentile values of indoor temperature categories were 24°C and 26°C, respectively.

Brachial Artery Vasodilator Function Assessment

Brachial artery diameters and flow velocities were examined by high-resolution vascular ultrasound (Toshiba #SSH-140A with 7.5 MHz linear array transducer) pre- and post-inflation of a forearm occlusive cuff (HokansonAG101, Bellevue, Washington), as previously described.[17;18] Images were analyzed off-line using commercially available software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa, version 3.2.3.sp2) by sonographers blinded to participant status. FMD% and hyperemic flow velocity were chosen a priori as our primary measures of conduit and microvascular function, respectively. Hyperemic flow velocity was selected as the primary measure of microvascular function because it can be directly measured and therefore limits error that may be introduced by using calculated variables such as hyperemic flow or shear stress. Baseline brachial artery diameter and flow velocity were considered secondary measures. FMD% and flow velocity measurements had good reproducibility (correlation coefficients ranging between 0.78 to 0.92 for FMD% and 0.99 for baseline and hyperemic flow velocity measurements).[17]

Statistical Analysis

We analyzed Spearman correlations between season, outdoor temperature quartile and room temperature category. Separate multivariable general linear regression models were created using analysis of covariance to examine the least square means (±s.e.) for each vascular function measure (baseline diameter, FMD%, baseline flow velocity, and hyperemic flow velocity) by 1) season; 2) outdoor temperature quartile; and 3) room temperature category. These models adjusted for published correlates of vascular function including age, sex, body mass index, smoking 6 hours prior to brachial test, diabetes, systolic blood pressure, total to high density lipoprotein cholesterol ratio, heart rate, walk test (before test, after test, not done), lipid lowering therapy, hypertension treatment, hormone replacement therapy, and prevalent cardiovascular disease.[17;18]

To assess relative importance of season versus temperature in association with the brachial measures, we conducted stepwise linear regression with covariates noted above forced into the models to determine whether season, outdoor, and room temperature would be retained. Variance inflation factors were calculated[19] for season, outdoor temperature quartiles, and room temperature categories to evaluate the degree of collinearity of these variables. All variance inflation factors were <5, so it is reasonable to include season and temperature variables in the same model. Model R^2 and partial R^2 for season and temperatures were reported for the different models. To evaluate the possibility that either baseline flow or brachial artery diameter could be confounding conduit vessel and microvascular findings, we also performed stepwise regression with baseline flow forced into models for FMD% and hyperemic flow and baseline diameter forced into the model for FMD%. Secondary models were constructed to examine interactions between the season and temperatures with sex, age (≥60 vs. <60 years), or cardiovascular disease status (present vs. absent). Two-sided $p<0.05$ were considered

statistically significant. Analyses were conducted with procedures REG and GLM in SAS version 8.1.[20] The authors had full access to the data and take responsibility for its integrity.

RESULTS

Participant Characteristics

Characteristics of the 2587 eligible participants (1973 with flow velocity data) from the Framingham Offspring cohort (53% women, mean age 61±10 years old), categorized by season, are presented in Table 1. The vascular measurements were made between 8:00 AM and 1 PM and the mean time of study did not vary according to season (P=0.55). In winter, participants had higher systolic and diastolic blood pressures (unadjusted). Participants examined during winter and spring were younger than those examined in summer and fall; clinic schedulers report that elderly participants were more likely to avoid winter appointments because of inclement weather. As expected, season correlated with outdoor (r=0.75; p<0.0001) and room (r=0.56; p<0.0001) temperatures, and outdoor and room temperatures correlated with each other (r=0.54; p<0.0001).

Association of Season, Temperature, and Measures of Brachial Reactivity

The brachial measures varied by season and temperature (Table 2). The overall model R^2 values for each brachial artery measurement were nearly identical whether adjusted for season, outdoor temperature quartile, or room temperature category [R^2 0.57 (season) to 0.58 (room and outdoor temperature), 0.18, 0.22, 0.16, and 0.31 for brachial artery diameter, FMD%, FMD % adjusted for brachial artery diameter, baseline flow velocity, and hyperemic flow velocity, respectively, for models adjusted by season, outdoor temperature, and room temperature]. The large R^2 for brachial artery diameter was largely attributable to expected sex differences in brachial artery diameter (partial R^2=0.32). As shown in Table 2A, except for hyperemic flow, the brachial variables varied by season; FMD% and baseline flow velocity were lowest during winter (18% and 17% lower than during summer, respectively). Outdoor temperature also was associated with FMD% and baseline flow velocity (Table 2B). FMD% was lowest in the coldest (15% lower in the coldest compared to the warmest quartile) and baseline flow velocity was highest in the warmest quartile of outdoor temperature. Table 2C displays that baseline and hyperemic flow velocity were related to room temperature category; baseline and hyperemic flow velocity were lowest at the coolest room temperature (20-24°C).

Stepwise Model and Model R^2 for Associations between Season, Temperatures and Brachial Reactivity Measures

To investigate the relative importance of season and temperature as correlates of vascular function, we forced in clinical covariates and conducted stepwise models allowing selection between the season and the 2 temperatures (Table 3). Baseline diameter and FMD% were both associated with season (changes in R^2 of 0.002 and 0.003, respectively, between the covariate adjusted and the covariate + season model). All 3 components (season and both temperatures) were retained in the model as correlates of baseline flow velocity (change in R^2 of 0.033 between the covariate-adjusted, and season and temperatures adjusted). Room temperature entered the baseline flow velocity model first with a partial R^2 of 0.02 followed by season and outdoor temperature (partial R^2 of 0.01 and 0.004, respectively). Both outdoor and room temperature were retained in the model for hyperemic flow velocity (change in R^2 of 0.006; partial R^2 of 0.003 for both outdoor and room temperature). The amount of variability in FMD % explained by season (0.003), outdoor temperature (0.003), and indoor temperature (0.001) was smaller than some of the predictors of FMD% including age (partial R^2 0.025 to 0.026), systolic blood pressure (partial R^2 0.035 to 0.036), female sex (partial R^2 0.007) for the separate multivariable models for season, outdoor temperature quartile, and indoor temperature category.

Given the statistically significant relations between baseline flow and season, outdoor temperature, and room temperature, we considered the possibility that variation in baseline flow might account for our FMD% and reactive hyperemia results. However, if baseline flow was forced into the stepwise models with the other clinical covariates, season remained a correlate of FMD% (P=0.01) and outdoor temperature remained a correlate of reactive hyperemia (P=0.01).

Secondary Models

Additionally adjusting the models for FMD% in Table 2 for baseline diameter did not substantively alter the association between season and outdoor temperature with FMD%, and the lack of association between FMD% and indoor temperature persisted. The observed relations between season, outdoor or room temperature and the brachial measures did not vary by sex (male vs. female), age (≥60 vs. <60 years) and cardiovascular disease (present, absent). The minimum p-value was 0.07 for the interaction of room temperature by age for hyperemic flow velocity.

DISCUSSION

In the Framingham Offspring Cohort, we observed that brachial artery FMD was lowest in winter and that the relation between season and this measure of conduit artery endothelial function remained significant after adjusting for clinical covariates and baseline flow velocity. Whereas conduit artery dilator function related most strongly to season, we observed that microvascular vasodilator function, as assessed by hyperemic flow, was more strongly related to ambient temperature. Thus, reactive hyperemia was associated with outdoor and room temperatures. Interestingly, baseline flow was lowest in winter and also related positively to both outdoor and room temperature in the stepwise model. The portions of the variation in measures of conduit and microvascular function accounted for by season and temperature were small; although statistically significant, the clinical significance of these associations remains uncertain. Overall, our findings support the hypothesis that endothelial function is associated with season and ambient temperature.

Several prior studies have examined the association between endothelial function and season or warmer temperatures. A small study by Klein-Weigel and colleagues failed to demonstrate differences in FMD% measured serially in summer and winter in 22 young adults with Raynaud's phenomenon and in 21 healthy referents.[21] However, the results of other studies are consistent with our findings. For example, Imamura and colleagues observed improved FMD% in humans with at least 1 cardiac risk factor,[14] and in humans with chronic heart failure,[22] following 2 weeks of daily "sauna therapy" (15 minutes in a 60°C, dry sauna bath followed by 30 minutes in bed covered with blankets).[14] In contrast to the current study, a recent study by Nawrot and colleagues reported an inverse association between FMD% and outdoor temperature in 274 subjects recruited from participants in the Flemish Study on Environment, Genes and Health Outcomes.[23] The etiology of these discrepant findings remains a matter of conjecture, but it may relate to several factors including a smaller sample size in Nawrot et al, dissimilarities in study sample characteristics, analytical issues, and differences in temperatures ranges examined.

Prior human studies also have examined the vascular effects of cold temperature. It has long been recognized that cooling the body is associated with activation of the sympathetic nervous system leading to peripheral vasoconstriction and decreased resting flow, a response that contributes importantly to the regulation of body temperature.[24] The cold pressor test (immersing the hand in ice water) also produces constriction of the conduit brachial and coronary artery and decreased blood flow, particularly if there is evidence of endothelial dysfunction.[25] Of historical interest, in 1958 Coffman and colleagues reported a decrease in

reactive hyperemia in the foot as room temperature was decreased from 28 to 20°C in healthy subjects.[26] Lind and colleagues reported that the cold pressor test was associated with an acute reduction in FMD%.[27] Thus, the findings of our large community-based observational study are consistent with several relatively small human studies and show a direct correlation between colder temperature and lower resting blood flow, reactive hyperemia, and endothelium-dependent FMD.

Recent experimental studies suggest that alterations in temperature might also influence vascular function through an effect on endothelial nitric oxide synthase and the bioavailability of nitric oxide. Acute and short-term exposure of rats to elevated environmental or core body temperatures has been shown to increase endothelial nitric oxide synthase expression. Conversely, repeated cold exposure of rats (4°C for 4 hours per day for 1 week) led to the development of hypertension and impaired endothelial vasodilator function in isolated arterial tissue.[28] Cold exposure also produces other relevant changes in the endothelial phenotype, including activation of the pro-inflammatory transcription factor nuclear factor-κB.[29] Thus, experimental studies suggest that cold temperature may alter endothelial biology in a manner consistent with our findings, particularly in regard to microvascular function.

Our study has a number of limitations. The Framingham participants were predominantly white and middle-aged to senior; the applicability of our findings to individuals who are younger or other ethnicities/races is unknown. In addition, we cannot exclude the possibility that seasonal variation in clinic attendance may be a proxy for participants' lifestyle and risk factor burden, which may have accentuated the observed seasonal differences in endothelial function. Given the location of the Framingham Study in a temperate climate, our highest temperature quartile temperatures were from 13.9 C° to 23.9 C°. Thus, we cannot comment on the influence of hot temperatures on endothelial function. Given the cross-sectional nature of this study, the same participants were not measured during each of the 4 seasons, making it possible that some of the differences measured may be due to residual inter-individual variation after multivariable adjustment. Although FMD is recognized as an endothelium-dependent response, we were not able to administer nitroglycerin in our observational study. Thus, we cannot exclude the possibility that we are detecting variation in general vasodilator capacity in part governed by nervous system activity or the function of vascular smooth muscle. The study design also does not allow us to withhold vasoactive medications prior to study of vascular function. However, recent data suggest there are no significant differences in brachial reactivity measures after acutely withholding non-nitrate vasoactive medications in individuals chronically on these medications.[30] Balanced against these limitations are the large single-site community-based cohort with routine ascertainment of clinical covariates and brachial function measures, facilitating multivariable models and minimizing referral biases.

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Table 1

Temperature and Participant Characteristics by Season

Variable	Winter (N=599)	Spring (N=755)	Summer (N=733)	Fall (N=500)
Room temperature, Celsius	24±1	25±1	26±1	25±1
Outdoor temperature, Celsius	-2.7±6.9	7.1±5.8	15.5±4.1	4.9±6.0
Age (years)*	60±10	60±10	61±9	62±9
Women*	48%	54%	57%	53%
Systolic blood pressure (mm Hg)*	128±18	124±18	122±17	123±17
Diastolic blood pressure (mm Hg)*	72±12	71±11	69±11	70±11
Heart rate, (beats/min*)	65±11	65±10	63±11	63±11
Body mass index, (kg/m^2)	28.1±5.1	28.2±5.5	28.0±5.2	27.9±5.3
Total/high density lipoprotein cholesterol	4.2±1.3	4.0±1.3	4.0±1.4	4.0±1.4
Diabetes mellitus	13%	14%	13%	13%
Blood glucose (mg/dL)	105±27	106±30	103±26	104±26
Smoked 6 hours prior to test	9%	9%	8%	10%
Hormone replacement therapy	30%	31%	36%	32%
Lipid lowering medication	20%	23%	20%	21%
Walk test before brachial testing	39%	39%	40%	35%
Walk test after brachial testing	38%	33%	37%	37%
Prevalent cardiovascular disease	13%	14%	13%	13%

Unadjusted data reported as mean±SD for continuous and % for dichotomous variables;

* $p<0.05$

Table 2

Association of Brachial Artery Reactivity Measures by Season and Temperature

A: Comparison by Season	Winter (N=599)	Spring (N=755)	Summer (N=733)	Fall (N=500)	Change Winter to Summer	P value for Season
Baseline diameter (mm)	4.29±0.02	4.29±0.02	4.26±0.02	4.18±0.03	-1%	**0.002**
Flow Mediated Dilation (%)	2.56±0.10	2.79±0.09	3.01±0.09	2.87±0.11	+18%	**0.02**
Flow Mediated Dilation (%) adjusting for baseline diameter	2.59±0.10	2.82±0.09	3.01±0.09	2.79±0.11	+16%	**0.03**
Baseline flow velocity (cm/sec)	7.2±0.3	8.5±0.2	8.4±0.2	7.5±0.2	+17%	**<0.0001**
Hyperemic flow velocity (cm/sec)	50.4±1.0	51.9±0.7	50.7±0.7	52.1±0.9	+1%	0.39

2B: Comparison by Outdoor Temperature Quartile	Quartile 1 -18.9 to 0.6 C° (N=659)	Quartile 2 1.1 to 6.7 C° (N=616)	Quartile 3 7.2 to 13.3 C° (N=652)	Quartile 4 13.9-23.9 C° (N=660)	Change from Quartile 1 to 4	P value for Outdoor Temperature Quartile
Baseline diameter (mm)	4.26±0.02	4.23±0.02	4.27±0.02	4.28±0.02	0.5%	0.54
Flow Mediated Dilation%	2.59±0.10	2.80±0.10	2.88±0.10	2.98±0.10	+15%	**0.048**
Flow Mediated Dilation% adjusting for baseline diameter	2.59±0.10	2.77±0.10	2.89±0.10	2.99±0.10	+15%	**0.03**
Baseline flow velocity (cm/sec)	7.6±0.2	7.4±0.2	8.2±0.2	8.8±0.2	+16%	<**0.0001**
Hyperemic flow velocity (cm/sec)	51.5±0.9	50.2±0.8	53.0±0.8	50.5±0.7	-2%	0.054

2C: Comparison by Room Temperature Category	<25 C° (N=746)	Room Temperature 25 C° (N=1036)	>25 C° (N=805)	Change from lowest to highest temperature	P value for Room Temperature Category
Baseline diameter (mm)	4.27±0.02	4.24±0.02	4.28±0.02	0.2%	0.32
Flow Mediated Dilation%	2.67±0.09	2.84±0.08	2.91±0.09	+9%	0.15
Flow Mediated Dilation% adjusting for baseline diameter	2.67±0.09	2.82±0.08	2.93±0.09	+10%	0.13
Baseline flow velocity (cm/sec)	7.1±0.2	7.9±0.2	8.9±0.2	+25%	<**0.0001**
Hyperemic flow velocity (cm/sec)	49.4±0.8	52.2±0.6	51.5±0.7	4%	**0.02**

Data reported as least squares mean±SE, adjusted for age, sex, BMI, smoking within 6 hours, diabetes, systolic blood pressure, total/HDL cholesterol, heart rate, walk test, medication (hypertension, lipid lowering, hormone replacement), prevalent cardiovascular disease and season or temperature. P values are for the overall differences between season, outdoor temperature quartile and room temperature category, respectively.

Table 3

Stepwise Models of Brachial Reactivity Measures Selecting Between Season, Outdoor and Room Temperature, Adjusted for Clinical Confounders

	Basic Multivariable*	Multivariable Model with Stepwise Selection of Season and Temperatures			
Variable	**R^2**	**Final Model R^2**	**Season**	**Outdoor P value**	**Room**
Baseline diameter (mm)	0.573	0.575	**0.002**	—	—
Flow Mediated Dilation (%)	0.178	0.181	**0.02**	—	—
Baseline flow velocity (cm/sec)	0.144	0.177	**<0.0001**	**0.02**	**<0.0001**
Hyperemic flow velocity (cm/sec)	0.311	0.317	—	**0.03**	**0.01**

See Table 2 for covariates included in all multivariable models.

*Without season or temperatures. —Signifies not entering stepwise model. P value indicates if season, outdoor or room temperature entered the stepwise models for brachial reactivity measures.