Sympathetic Neural and Hemodynamic Responses During Cold Pressor Test in Elderly Blacks and Whites

Yoshiyuki Okada, Sara S. Jarvis, Stuart A. Best, Jeffrey G. Edwards, Joseph M. Hendrix, Beverley Adams-Huet, Wanpen Vongpatanasin, Benjamin D. Levine, Qi Fu

Abstract—The sympathetic response during the cold pressor test (CPT) has been reported to be greater in young blacks than whites, especially in those with a family history of hypertension. Because blood pressure (BP) increases with age, we evaluated whether elderly blacks have greater sympathetic activation during CPT than age-matched whites. BP, heart rate, cardiac output, and muscle sympathetic nerve activity were measured during supine baseline, 2-minute CPT, and 3-minute recovery in 47 elderly (68±7 [SD] years) volunteers (12 blacks and 35 whites). Baseline BP, heart rate, cardiac output, or muscle sympathetic nerve activity did not differ between races. Systolic and diastolic BP and heart rate increased during CPT (all P<0.001) with no racial differences (all P>0.05). Cardiac output increased during CPT and ≤30 s of recovery in both groups, but was lower in blacks than whites. Muscle sympathetic nerve activity increased during CPT in both groups (both P<0.001); the increase in burst frequency was similar between groups, whereas the increase in total activity was smaller in blacks (P=0.030 for interaction). Peak change (Δ) in diastolic BP was correlated with Δ total activity at 1 minute into CPT in both blacks (r=0.78, P=0.003) and whites (r=0.43, P=0.009), whereas the slope was significantly greater in blacks (P=0.007). Thus, elderly blacks have smaller sympathetic and central hemodynamic (eg, cardiac output) responses, but a greater pressor response for a given sympathetic activation during CPT than elderly whites. This response may stem from augmented sympathetic vascular transduction, greater sympathetic activation to other vascular beds(s), or enhanced nonadrenergically mediated vasoconstriction in elderly blacks. (Hypertension. 2016;67:951-958. DOI: 10.1161/HYPERTENSIONAHA.115.06700.) • Online Data Supplement

Key Words: aging ■ blood pressure ■ cardiac output ■ heart rate ■ hypertension

Hypertension is more common in blacks than whites, especially among older individuals. However, the number of hypertensive patients with controlled blood pressure (BP) is lesser in blacks even with antihypertensive treatment, which may predispose them to cardiovascular and renal complications. Despite these problems, the mechanism(s) underlying the racial differences in BP control in the elderly remain unknown.

The cold pressor test (CPT) evokes an increase in muscle sympathetic nerve activity (MSNA), resulting in a pressor response with or without an increase in heart rate (HR). Wood et al reported that an exaggerated increase in BP during CPT was related to the future development of hypertension and its severity. Indeed, previous studies showed that young blacks had a greater pressor response to CPT than young whites, which was accompanied by a greater increase in MSNA. This was particularly evident in blacks who had a family history of hypertension. These results suggest that greater peripheral sympathetic activation to the vasculature during CPT may be related to the higher prevalence of hypertension in blacks when compared with whites. However, Stein et al found that forearm vascular resistance (FVR) was higher during CPT in young blacks than age-matched whites, but the sympathetic neural response was similar between groups. We recently found that a rise in BP during orthostasis was greater in elderly blacks than whites, which was accompanied by a smaller increase of MSNA in blacks. These results suggest that the enhanced stress-induced pressor response observed in blacks may arise from augmented sympathetic vascular transduction, greater sympathetic activation to other vascular beds(s), or enhanced nonadrenergically mediated vasoconstriction in elderly blacks.

The literature lacks information about racial differences in pressor and sympathetic neural responses to CPT in the elderly. Given that the incidence of hypertension is high and the rate to reduce BP is low among elderly blacks, it is important to understand how BP is regulated in this challenging population. Because both MSNA and BP increase with age, and some previous reports have demonstrated a greater MSNA response to CPT in hypertensive compared with normotensive individuals, we speculated that the augmented sympathetic neural response to CPT would be more predominant in elderly individuals.
blacks compared with similarly aged whites. Therefore, the purpose of this study was to evaluate the differences of MSNA and hemodynamic responses to CPT between elderly blacks and whites. We hypothesized that elderly blacks would have a greater pressor response to CPT, which is accompanied by a greater increase in MSNA (neurovascular component) during CPT (defined as an efficacy of MSNA) is greater in elderly blacks than whites. Evaluating the contribution of each component to BP control may provide insight into the selection of an appropriate strategy against hypertension in elderly blacks.

Methods

Subjects
Forty-seven sedentary elderly volunteers (age range, 60–83 years; 12 blacks and 35 whites) participated in this study. All subjects were screened with a careful medical history, physical examination, 12-lead ECG, echocardiogram, and 24-hour ambulatory BP monitoring. They were nonsmokers and had no history of chronic diseases except for mild hypertension. They were excluded if they exercised at moderate-to-high intensity levels as defined as ≥30 minutes/d for ≥3×/wk. Women taking hormone replacement therapy were excluded. Subjects gave their written informed consent to participate in the study that was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas.

Measurements

Muscle Sympathetic Nerve Activity
MSNA signals were obtained with microneurography. Briefly, a recording electrode was placed in the peroneal nerve at the popliteal fossa, and a reference electrode was placed subcutaneously 2 to 3 cm apart from the recording electrode. The nerve signals were amplified (70000- to 160000-fold), band-pass filtered (700 to 2000 Hz), full-wave rectified, and integrated with a resistance–capacitance circuit (time constant 0.1 s). Adequate MSNA recording was assessed by (1) pulse synchrony; (2) facilitation during hypotension phase of the Valsalva maneuver, and suppression during the hypertensive overshoot phase after release; (3) increase in response to breath holding; and (4) insensitivity to a gentle skin touch or a loud shout.

Hemodynamics
HR was determined from lead II of the ECG (Hewlett-Packard). Arm cuff BP was measured by electrophysmomanometry (SunTech) with a microphone placed over the brachial artery to detect Korotkoff sounds. Resting steady-state cardiac output (Qc) was measured via the modified acetylene rebreathing technique. Stroke volume was calculated from Qc divided by HR, and total peripheral resistance (TPR) was calculated as the quotient of mean BP (diastolic BP [DBP]+systolic BP–DBP)/3 and Qc, multiplied by 80.

Before, during, and after CPT, beat-by-beat BP was measured by finger photoplethysmography (Nexfin). Beat-by-beat Qc was derived from the Modelflow method and calibrated by the acetylene rebreathing technique, so that baseline Modelflow Qc was made equal to the resting steady-state acetylene Qc for each subject. Beat-by-beat TPR was calculated as the quotient of mean BP and the Modelflow Qc multiplied by 80, whereas beat-by-beat mean BP was derived from finger photoplethysmography. All beat-by-beat variables were averaged for every 30 s. Respiratory excursions were detected by a nasal cannula.

Study Design and Protocol
After screening, subjects who had been taking antihypertensive medications spent 1 to 2 weeks to wean themselves from these drugs to avoid any effects of the drugs on MSNA and hemodynamic responses. Subjects then underwent a 3-week run-in period in which they were instructed to maintain a healthy lifestyle according to the Joint National Committee standard guidelines to avoid lifestyle effects on MSNA. A 24-hour ambulatory BP monitoring was measured at the end of the run-in period, by which we assigned the subjects to hypertensives or normotensives. Three days before testing, all subjects began an isocaloric constant diet consisting of: 100-mEq sodium, 100-mEq potassium, and 1000-mg calcium daily. Fluid intake was ad libitum and assessed by 24-hour urine output the day before testing to verify dietary compliance.

The experiment was performed in the morning ≥2 hours after a light breakfast, ≥72 hours after the last caffeinated or alcoholic beverage, and ≥24 hours after strenuous physical activity. Experiments were performed in a quiet, environmentally controlled laboratory with an ambient temperature of ≥25°C. The subject was placed in the supine position, and an intravenous catheter was inserted into the antecubital vein of the left arm for withdrawal of blood sampling. After at least 20 minutes in the supine position, resting steady-state Qc was measured via the acetylene rebreathing technique. A blood sample was taken for assessment of plasma catecholamine, aldosterone, vasopressin, and direct renin concentrations. At least 15 minutes after a satisfactory nerve recording site had been found, supine resting data were collected for 6 minutes. After that, forearm blood flow was measured by venous occlusion plethysmography and was expressed as (mL/100 mL of tissue/min). After 1 minute of baseline with spontaneous breathing, the dominant hand was immersed up to the wrist in an ice water bath (4°C) for 2 minutes, which was followed by 3 minutes of recovery. Subjects were instructed to avoid breath holding and to stay as relaxed as possible. Throughout the entire CPT, beat-by-beat BP, HR, Qc, respiratory waveforms, and MSNA were recorded continuously. The microneurographic needles were removed after the recovery period.

After a sufficient break (i.e., ≥200 minutes), blood volume was measured using a modified carbon monoxide rebreathing method in the sitting position.

Data Analysis
Data were sampled at 625 Hz and stored on personal computer with a commercial data acquisition system (Biopac). Off-line data analyses were performed using signal-processing software (LabView). Beat-by-beat HR was calculated from the R–R interval measured by ECG. Beat-by-beat systolic BP and DBP were obtained from the arterial blood pressure waveform. FVR was calculated as mean BP divided by forearm blood flow. Sympathetic bursts were identified by a computer program, and then confirmed by an experienced microneurographer. The integrated neurogram was normalized by assigning a value of 100 to the largest amplitude of a sympathetic burst during the 1-minute baseline. Burst area was measured as the area under the curve of each sympathetic burst of the normalized integrated neurogram on a beat-by-beat basis. The number of bursts per minute (burst frequency) and the burst area per minute (total activity) were used as quantitative indices. Changes (Δ) in these variables from baseline were calculated to compare responses between races. We also estimated baseline sympathetic vascular transduction using FVR divided by MSNA burst frequency and the efficacy of MSNA from both intraindividual ratio and interindividual slope derived from peak ∆DBP and Δtotal activity at 1 minute into CPT.

Statistical Analysis
Values are expressed as means±SEM. Subject characteristics, ambulatory BP monitoring results, baseline hemodynamic and MSNA variables, and the ratio of ∆DBP to ∆total activity between groups were compared by using unpaired t tests if normality and equality tests passed. If normality and equality tests failed, we used a Mann–Whitney Rank-sum test (eg, body mass index, plasma norepinephrine, plasma epinephrine, aldosterone, vasopressin, direct renin, stroke volume, FVR, and FVR/burst frequency). Changes during CPT in hemodynamics and MSNA indices all passed normality tests and were examined using 2-way repeated measures ANOVA with
factors for race, time, and the interaction (race*time). The Holm–Sidak method was used as post hoc for multiple comparisons if a significant interaction between race and time was observed. Linear regression analysis was used to evaluate the interindividual correlations between ∆DBP and ∆total activity within elderly blacks and whites. The slope of the relationship was compared between blacks and whites by using the permutation test. A P value of <0.05 was considered statistically significant.

Power and sample size calculations were based on the study of Calhoun et al., showing that the differences in group means of increases in MSNA burst frequency and total activity were 10 bursts/min and 173%, and the within group SDs were 9 bursts/min and 84%. If the ratio of blacks to whites was 1:3, we need to study at least 9 elderly blacks and 27 elderly whites to reject the null hypothesis that the population means of blacks and whites are equal with power 0.80, and the type I error probability is 0.05.26

Results

Subject Characteristics

As shown in Table 1, there were no differences in physical characteristics between elderly blacks and whites. The ratios of men/women and normotensives/hypertensives were similar between groups. Twenty-four-hour ambulatory systolic BP was not different, whereas 24-hour ambulatory DBP was higher in blacks than whites (P=0.012). Blood volume, resting plasma catecholamine, aldosterone, vasopressin, and direct renin concentrations were similar between races.

Hemodynamic and Neural Responses

Representative hemodynamic and MSNA responses to CPT from 1 black and 1 white subject were demonstrated in Figure 1. There were no racial differences in resting steady-state hemodynamics, or MSNA burst frequency during supine baseline (Table 2), whereas FVR tended to be greater (P=0.059) and the ratio of FVR/MSNA burst frequency was significantly greater in elderly blacks than whites. Systolic BP, DBP, and HR significantly increased during CPT, whereas there were no differences in changes of these variables between elderly blacks and whites (Figure 2). Qc increased during CPT and ≤30 s of recovery in both groups, whereas it was lower in elderly blacks than whites. TPR increased during CPT without a significant racial difference. Both MSNA burst frequency and total activity also increased during CPT. However, the increase in burst frequency was similar between groups, whereas the increase in total activity was smaller in elderly blacks than whites (P=0.030 for interaction). The change of total activity at 1 to 1.5 minutes into CPT was greater in whites (both P<0.05).

Relationship of Neural Response and BP

Intraindividual relationship between ∆DBP and ∆total activity (the ratio of ∆DBP to ∆total activity) was significantly higher in elderly blacks than whites (Figure 3A). Moreover, ∆DBP was interindividually correlated with ∆total activity within both elderly blacks and whites (Figure 3B), and the slope of the relationship was greater in blacks than whites (P<0.007).

Discussion

The main findings of this study are that: (1) resting steady-state hemodynamics and baseline sympathetic nerve activity were not different between elderly blacks and whites; (2) BP similarly increased during CPT, whereas the increases in total activity of MSNA and Qc were smaller in elderly blacks than whites; and (3) both inter- and intraindividual efficacies of the change in MSNA on BP rise during CPT were significantly greater in elderly blacks than whites. These results suggest that elderly blacks may have a blunted sympathetic neural and central hemodynamic responsiveness during CPT but a greater pressor response for a given change of MSNA compared with elderly whites, which may be attributable to augmented sympathovascular transduction, greater sympathetic activation to other vascular bed(s), or enhanced nonadrenergic vasoconstrictor mechanisms.

Central Hemodynamic and Sympathetic Neural Responses

The smaller increase of Qc demonstrated in elderly blacks during CPT in this study was also observed in younger subjects during behavioral challenges, including CPT.27,28 It was reported that greater increases in Qc and stroke volume in whites than blacks were eliminated with β-blockade,28 suggesting that β-receptor sensitivity may be blunted in blacks. Because β-receptor sensitivity attenuates with age,29 the degree of attenuation may be greater in elderly blacks than whites. Alternatively, circulating catecholamines affecting myocardial responses may increase more in elderly whites than blacks. This speculation seems to be supported by the greater increase in total activity of MSNA in whites. Despite a smaller Qc in blacks during CPT, the pressor response was similar between groups. Thus, BP control via the peripheral vasculature may be predominant compared with central hemodynamics (eg, Qc) in elderly blacks.
Contrary to young blacks,7–9 MSNA response to CPT was smaller in elderly blacks than elderly whites. In this study, total activity, but not burst frequency, was blunted. This discrepancy between MSNA indices could be explained by displacement of the recording electrodes during CPT; however, we cannot think of any reasons that such a displacement only occurred in 1 group but not the other group. Kienbaum et al30 have proposed that there are 2 sites for modulation of sympathetic activity; one determines whether a burst will occur and another determines the strength of the discharge (eg, burst area). It is possible that elderly blacks have a smaller response in the strength of central sympathetic outflow for a given stress, such as afferent signals from c-fiber during CPT. Indeed, previous studies showed attenuated total activity responses to orthostasis in young25 and elderly blacks,10 when the response in burst frequency was similar between groups and only burst strength was the factor of difference in total activity. It is also possible that the attenuated increase of total activity in elderly blacks may be the result of buffering via the sympathetic baroreflex. Specifically, the operating point of BP during CPT increased similarly between elderly blacks and whites; however, elderly blacks had a greater degree of vasoconstriction for a given increase in MSNA, which may require less sympathetic nerve activation. In other words, an augmented response in the peripheral arc (neurovascular component) of the whole baroreflex loop induces an attenuated response in the neural arc between baroreceptors and MSNA efferent signals (neural component).31 When the baroreflex loop is then considered as a closed-loop feedback system,
BP reaches a similar operating point of the baroreflex curve, which is shifted rightward during CPT.

Impact of Sympathetic Activation on BP

The sympathetic neural response was smaller in elderly blacks, while their BP rose similarly to whites during CPT, resulting in a higher ratio of ΔDBP to Δtotal activity (namely, a greater efficacy of MSNA). Because Qc response was smaller in elderly blacks, this greater efficacy of MSNA on BP increase should be attributable to augmented mechanism(s) concurrently responsible for vasoconstriction between sympathetic nerve terminal and the vasoactive receptors. One possible mechanism may be a greater sympathetic vascular transduction, which was found during orthostatic stress using lower body negative pressure in young blacks.²¹ To our knowledge, there is no information available on the racial difference of adrenergic receptor sensitivity in elderly individuals; however, young blacks were reported to have a greater vasoconstriction by intrabrachial artery administration of phenylephrine, an α-adrenergic agonist.²²⁻²³ Moreover, Fairfax et al.²⁴ recently reported that forearm vascular conductance, the reciprocal of FVR, decreased in association with preceding total activity, and the reduction was eliminated by α-adrenergic blockade.

Therefore, the greater FVR for a given MSNA burst frequency in elderly blacks (Table 2) may indicate a greater α-adrenergic sensitivity in this group. Conversely, others²⁵ reported similar α-adrenergic sensitivity between blacks and whites by systemic infusion of norepinephrine. This discrepancy may be because of systemic reflexes, such as the baroreflex. Another possibility is that elderly blacks may have augmented noradrenaline release, such as what has been observed in young blacks,²⁶ or impaired noradrenaline clearance, leading to a greater vasoconstriction. Indeed, the peak increase in TPR was observed in the later stage of CPT, and TPR was still above the baseline level during recovery in blacks compared with whites when MSNA had already returned to baseline. β-adrenergic receptor sensitivity (vasodilation) has been assessed by local artery infusion of isoproterenol and was found to be attenuated in young blacks than whites.²⁷⁻²⁸ Thus, the findings on α- or β-adrenergic sensitivities could explain the augmented sympathetic vascular transduction in elderly blacks. Indeed, Stein et al.²⁹ showed a greater vascular resistance during CPT in blacks even with similar norepinephrine spillover compared with whites. Given the fact that adrenergic sensitivities decrease with age,³⁰⁻³¹ the racial difference of sympathetic vascular transduction may be augmented by a smaller reduction of α-adrenergic sensitivity or a greater reduction of β-adrenergic sensitivity with age in blacks. However, future studies are needed to evaluate the contributions of α-adrenergic vasoconstriction and β-adrenergic vasodilation to racial differences of vasoreactivity in the elderly. Finally, it is possible that sympathetic activation to other vascular bed(s), such as the renal or splanchnic vascular beds, rather than the skeletal muscle, may be greater in elderly blacks than whites.

A strong intraindividual relationship between changes in MSNA and BP was found; however, the slope of the relationship varies interindividually,³² probably because of interindividual differences in nonadrenergic vasoconstriction or nonvasoactive BP control systems, as well as those of adrenergic vasoconstrictor sensitivity. We observed significant interindividual correlations between Δtotal activity and ΔDBP within both groups, but the interindividual slope was significantly steeper in elderly blacks. These results indicate that the interindividual differences within the groups may be smaller than the group difference of the relationship, and impact of MSNA on BP during CPT may be greater in elderly blacks compared with whites. The racial difference in the relationship between ΔMSNA and ΔDBP disappeared when it was assessed with burst frequency (Figure S2 in the online-only Data Supplement), indicating that the racial difference of the
efficacy of MSNA on BP depended on burst strength but not burst frequency. Although we did not assess nonadrenergic vasoconstrictor mechanisms, nonadrenergically mediated vasoconstriction could have occurred with a concomitant increase in MSNA, which may have contributed to the steeper increase in BP for a given rise in MSNA in elderly blacks. The renin–angiotensin–aldosterone system is one possible mechanism for nonadrenergically mediated vasoconstriction; however, renal–adrenal hormones were similar between races in this study. In addition, it was previously reported that plasma renin and aldosterone did not change from baseline during a 3-minute CPT, indicating little impact of this system on MSNA and hemodynamic responses during CPT. A previous study suggested that endothelium-dependent, nitric oxide (NO)–mediated vasodilation decreased during CPT and contributed to the BP rise independently of the increase in MSNA. Because NO-mediated vasodilation was found to be impaired in blacks compared with whites, it is possible that the attenuation of NO-mediated vasodilation by CPT may influence the relationship between BP and MSNA in elderly whites only. The r value of the interindividual relationship was higher in elderly blacks than whites, suggesting that the non-MSNA contribution of BP control may have less impact on the pressor response to CPT compared with the sympathetic transduction (MSNA contribution) in elderly blacks. Taken together, the smaller increase in total activity with greater vasoconstricotor effects in elderly blacks may elicit a pressor response during CPT comparable with elderly whites. Thus, peripheral vascular function, rather than central MSNA control, may be responsible for BP rise in elderly blacks.

Limitations

First, as we did not measure FVR during CPT, we cannot provide direct evidence about vasoconstrictor responsiveness to the increase in MSNA elicited by CPT. Therefore, we discussed the possibility of not only α- and β-adrenergic sensitivity but also nonadrenergically mechanisms during CPT, such as NO-mediated vasodilation. These speculations need to be verified in future research. Second, 1 minute baseline before CPT may be too short for MSNA data analysis. However, MSNA burst frequency and total activity in blacks and whites calculated from 1 minute baseline were similar to those obtained during 6-minute resting period before the measurement of forearm blood flow. Third, despite elderly blacks and whites being well matched for physical characteristics, the men/women ratio and the normotensives/hypertensives ratio, we did not evaluate socioeconomic status and the well-matched ratios between groups in this study may not match the ratios in real society. Furthermore, we did not separate normotensives and mild hypertensives within each racial group. It was extremely difficult to find individuals (especially in blacks) who met inclusion criteria (eg, normotension or mild hypertension otherwise healthy, sedentary, but not overweight) in the Dallas–Fort Worth area, we were unable to enroll similar numbers of elderly blacks and whites as subjects for this study. However, the sample size calculations showed that we had sufficient power to detect differences between groups. In addition, we performed supplementary data analysis to compare hemodynamic and sympathetic neural responses to CPT in normotensive and hypertensive blacks and whites (see online-only Data Supplement for detail). These preliminary results need to be confirmed in more subjects in future studies. Fourth, we studied healthy normotensive and mild hypertensive individuals. It is possible that sympathetic neural control may be different in patients with moderate and severe hypertension.

Perspectives

MSNA increases with age, which is thought to be one of the main risk factors for hypertension in the elderly. In this study, elderly blacks exhibit augmented BP for a given increase in MSNA. Therefore, the age-related increase of basal MSNA may have a larger influence on the age-related increase of BP via vasoconstrictor effects in blacks than whites. Furthermore, enhanced vasoconstrictor sensitivity (neurovascular component) may cause an excessive BP rise even with a lower sympathetic neural activation (neural component) during physiological stresses, which may be a crucial element of the higher prevalence of hypertension-related target-organ damages (eg, cardiovascular and renal complications) in elderly blacks. This study may provide insight into not only the pathophysiology but also antihypertensive treatment in elderly blacks. Certainly, to verify which vasoconstrictor mechanism is most responsible for hypertension in elderly blacks is important in future research for selection of the appropriate strategy to reduce the prevalence of hypertension in elderly blacks.
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Disclosures
None.

References


**Novelty and Significance**

**What Is New?**
- The pressor response during the cold pressor test was similar between elderly blacks and whites. However, we found: a blunted sympathetic neural response but greater vasoconstrictor responsiveness in elderly blacks and an attenuated increase in cardiac output in elderly blacks.

**What Is Relevant?**
- Enhanced vasoconstrictor responsiveness for a given change of sympathetic nervous activity in elderly blacks.
- This may be one of the causes of the high prevalence of hypertension in blacks.

**Summary**
Vasoconstrictor responsiveness to sympathetic stimuli rather than sympathetic nerve activation may be amplified in elderly blacks than whites during cold pressor test.

**These results may provide important information on the treatment of hypertension in blacks.**
Sympathetic Neural and Hemodynamic Responses During Cold Pressor Test in Elderly Blacks and Whites
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Correction to: Sympathetic Neural and Hemodynamic Responses During Cold Pressor Test in Elderly Blacks and Whites

In the article by Okada et al, “Sympathetic Neural and Hemodynamic Responses During Cold Pressor Test in Elderly Blacks and Whites,” which published online on March 28, 2016, and appeared in the May 2016 issue of the journal (Hypertension. 2016;67:951–958. DOI: 10.1161/HYPERTENSIONAHA.115.06700), a correction is needed.

Figures 2 and 3 that had missing text due to a publication error have been replaced.

We apologize for this error.

This correction has been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/67/5/951.
ONLINE SUPPLEMENT

SYMPATHETIC NEURAL AND HEMODYNAMIC RESPONSES DURING COLD PRESSOR TEST IN ELDERLY BLACKS AND WHITES

Yoshiyuki Okada\(^1,2,3\), Sara S. Jarvis\(^1,2,4\), Stuart A. Best\(^1,2\), Jeffrey G. Edwards\(^1,2\), Joseph M. Hendrix\(^2\), Beverley Adams-Huet\(^2\), Wanpen Vongpatanasin\(^2\), Benjamin D. Levine\(^1,2\) and Qi Fu\(^1,2\)

\(^1\) Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas

\(^2\) UT Southwestern Medical Center, Texas, USA

\(^3\) Department of Special Care Dentistry, Matsumoto Dental University, Nagano, Japan

\(^4\) Department of Biological Sciences, Northern Arizona University, Arizona, USA

Running head: Race and sympathetic response to cold pressor test

Corresponding author: Qi Fu, M.D., Ph.D.,

UT Southwestern Medical Center
Institute for Exercise and Environmental Medicine
Texas Health Presbyterian Hospital Dallas
7232 Greenville Avenue, Suite 435, Dallas, Texas 75231
Phone: (214) 345-8125; Fax: (214) 345-4618
Email: QiFu@TexasHealth.org
Supplementary Data Analysis

Analysis in subgroups
Elderly blacks and whites were divided into normotensive and hypertensive subgroups. Then, we re-analyzed numerical data for the subgroups and compared racial differences within normotensives and hypertensives, separately.

Efficacy of MSNA burst frequency on blood pressure
The efficacy of muscle sympathetic nerve activity (MSNA) from both intra-individual ratio and inter-individual slope was derived from peak change (Δ) of diastolic blood pressure (DBP) and Δburst frequency at 1 min into cold pressure test (CPT).

Statistical analysis
Values are expressed as means ± SEM. Subject characteristics, baseline hemodynamic and MSNA variables were examined using 2-way repeated measures ANOVA with factors for race, group (hypertensives and normotensives), and the interaction (race×group). Changes during CPT in hemodynamics and MSNA indices (burst frequency and total activity) were examined using 2-way repeated measures ANOVA with factors for race, time, and the interaction (race×time) within normotensives and hypertensives, separately. In addition, these variables were also assessed with factors for group, time, and the interaction (group×time) within elderly blacks. The Holm-Sidak method was used as post-hoc test for multiple comparisons if a significant interaction was observed. The ratio of ΔDBP to Δburst frequency between races was compared by using a Mann-Whitney Rank Sum test because normality and equality tests failed. Linear regression analysis was used to evaluate the inter-individual correlations between ΔDBP and Δburst frequency within elderly blacks and whites. The slope of the relationship was compared between blacks and whites by using the permutation test. A P value of <0.05 was considered statistically significant.

Supplementary Results

Subject characteristics
Table S1 depicts similar physical characteristics between races, as well as between hypertensives and normotensives. 24-h ambulatory blood pressure monitoring (ABPM) was higher in hypertensives than normotensives in both races, while blacks had a higher ABPM than whites within hypertensives. Blood volume was greater in elderly blacks than whites within normotensives and it was greater in hypertensives than normotensives within whites, but blood volume was similar between races in hypertensives.

Supine resting plasma norepinephrine was similar between races and between hypertensives and normotensives, while plasma epinephrine was greater in hypertensives than normotensives only in whites. Aldosterone and vasopressin were similar between races and between hypertensives and normotensives. Direct renin was greater in blacks than whites within normotensives and it was smaller in hypertensives than normotensives in blacks, but direct renin was similar between races in hypertensives.

Hemodynamic and neural responses
There were no racial differences in resting steady-state hemodynamics, or MSNA burst frequency during supine baseline, while forearm vascular resistance (FVR) and the ratio of FVR/MSNA burst frequency were significantly greater in elderly blacks than whites within hypertensives (Table S2). Systolic BP (SBP), DBP, and total peripheral resistance (TPR) were higher in hypertensives than normotensives both in elderly blacks and whites.
SBP, DBP, and TPR increased during CPT and these responses did not differ between races within normotensives and hypertensives (Figure S1). ΔSBP, ΔDBP, and ΔTPR were also similar between hypertensives and normotensives within elderly blacks. Δheart rate (HR) was greater in hypertensive blacks than hypertensive whites and normotensive blacks. Δcardiac output (Qc) was smaller in elderly blacks than whites within normotensives, while it tended to be higher in hypertensives than normotensives within blacks, and ΔQc was similar between races in hypertensives.

MSNA burst frequency also increased during CPT. The increase in burst frequency was similar between races both in normotensives and hypertensives, while the increase was greater in hypertensives than normotensives within elderly blacks. Total activity increased during CPT in both normotensives and hypertensives and the racial difference within each subgroup seemed to be similar to that evaluated with all subjects (Figure 2 in the main manuscript), while the racial difference did not reach statistical significance in either group.

Relationship of neural response and blood pressure

Contrary to the intra-individual relationship between ΔDBP and Δtotal activity (Figure 3A in the main manuscript), the ratio of ΔDBP to Δburst frequency was similar between elderly blacks and whites (Figure S2A). Inter-individual correlation coefficients between ΔDBP and Δburst frequency in elderly blacks and whites were 0.41 and 0.42, respectively, and the slopes of the relationship were similar between races (blacks, 0.443 and whites, 0.337 mmHg/bursts/min; P=0.753) (Figure S2B).

Supplementary Discussion

Central hemodynamic and sympathetic neural responses

Preliminary data from the subgroups suggest that racial differences in hemodynamic and neural responses during CPT reported in the main manuscript were mainly driven by the differences in normotensive blacks and whites. Hypertension appeared to diminish these race-specific differences in the elderly. Given that Qc is determined by HR and stroke volume, greater increases in HR during CPT in hypertensive blacks compared to normotensive blacks and hypertensive whites may be a primary cause of similar increases of Qc between races in hypertensives. These preliminary observations need to be confirmed in more subjects in future studies.

Impact of MSNA burst frequency on blood pressure

Because burst frequency similarly increased during CPT between blacks and whites (Figure 2 in the main manuscript), the correlation between changes in burst frequency and DBP should be similar between races. Indeed, both slopes of the inter- and intra-individual correlations between burst frequency and DBP were similar between elderly blacks and whites, which were different from those evaluated with total activity and DBP. Therefore, the greater efficacy of MSNA calculated with total activity must be attributable to burst strength, but not burst frequency. These results further support that elderly blacks have a smaller response in the strength of central sympathetic outflow for a given stress, such as CPT (see line 14 on page 10 and line 2 on page 13 in the main manuscript).

Supplementary Limitations

In addition to the limitations discussed in the main manuscript, the sample size was too small (especially in blacks) to evaluate racial differences in hemodynamic and neural responses during CPT within each subgroup (e.g., hypertensives and normotensives). However, we can use these preliminary data to calculate the sample size and power needed for a large study in the future.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Blacks</th>
<th>Whites</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensives</td>
<td>Hypertensives</td>
<td>Normotensives</td>
<td>Hypertensives</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Age, years</td>
<td>65 ± 2</td>
<td>65 ± 2</td>
<td>68 ± 2</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 3*</td>
<td>168 ± 4</td>
<td>167 ± 2</td>
<td>172 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84 ± 6</td>
<td>79 ± 5</td>
<td>75 ± 3</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.0</td>
<td>1.9 ± 0.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.7 ± 2.0</td>
<td>28.1 ± 1.5</td>
<td>26.9 ± 0.6</td>
<td>26.2 ± 0.7</td>
</tr>
<tr>
<td>24h ABPM SBP, mmHg</td>
<td>124 ± 3</td>
<td>150 ± 5*</td>
<td>122 ± 2</td>
<td>141 ± 2*</td>
</tr>
<tr>
<td>24h ABPM DBP, mmHg</td>
<td>74 ± 2</td>
<td>88 ± 1*</td>
<td>69 ± 1</td>
<td>78 ± 2*</td>
</tr>
<tr>
<td>Blood volume, mL/kg</td>
<td>69.7 ± 3.6*</td>
<td>61.1 ± 3.1</td>
<td>60.5 ± 1.7</td>
<td>66.6 ± 1.7*</td>
</tr>
<tr>
<td>Plasma Norepinephrine, pg/mL</td>
<td>373 ± 54</td>
<td>341 ± 60</td>
<td>374 ± 46</td>
<td>332 ± 33</td>
</tr>
<tr>
<td>Plasma Epinephrine, pg/mL</td>
<td>23.6 ± 6.6</td>
<td>14.6 ± 1.9</td>
<td>17.5 ± 2.2</td>
<td>24.1 ± 3.6*</td>
</tr>
<tr>
<td>Aldosterone, ng/dL</td>
<td>4.8 ± 0.9</td>
<td>7.3 ± 3.1</td>
<td>4.7 ± 0.6</td>
<td>4.6 ± 0.6</td>
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<tr>
<td>Vasopressin, pg/mL</td>
<td>0.50 ± 0.00</td>
<td>0.50 ± 0.00</td>
<td>0.50 ± 0.00</td>
<td>0.58 ± 0.06</td>
</tr>
<tr>
<td>Direct Renin, pg/mL</td>
<td>19.9 ± 1.3 (n=3)*</td>
<td>6.7 ± 1.4 (n=4)†</td>
<td>10.2 ± 1.4 (n=16)</td>
<td>9.9 ± 1.1 (n=15)</td>
</tr>
</tbody>
</table>

ABPM indicates ambulatory blood pressure monitoring; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are means ± SEM. * vs. whites and † vs. normotensives within the same race at P<0.05.
**Table S2. Resting steady-state hemodynamics and sympathetic nerve activity**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Blacks</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensives</td>
<td>Hypertensives</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>120 ± 2</td>
<td>147 ± 8†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>65 ± 4</td>
<td>79 ± 4†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>63 ± 3</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>SV, ml</td>
<td>68 ± 6</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>Qc, l/min</td>
<td>4.7 ± 0.4</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>TPR, dyn·s/cm²</td>
<td>1376 ± 99</td>
<td>1826 ± 109†</td>
</tr>
<tr>
<td>FVR, mmHg/ml/100ml/min</td>
<td>51 ± 7</td>
<td>73 ± 13*</td>
</tr>
<tr>
<td>MSNA burst frequency, bursts/min</td>
<td>47 ± 7</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>FVR/burst frequency, mmHg/ml/100ml/burst</td>
<td>1.31 ± 0.22</td>
<td>1.52 ± 0.32*</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SV, stroke volume; Qc, cardiac output; TPR, total peripheral resistance; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity. Values are means ± SEM. *, vs. whites and †, vs. normotensives within the same race at P<0.05.
Figure S1: Changes (Δ) from baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), cardiac output (Qc), total peripheral resistance (TPR), and muscle sympathetic nerve activity (MSNA) burst frequency and total activity during the cold pressor test (CPT) and recovery in elderly normotensive and hypertensive blacks and whites. Values are means±SEM.
**Figure S2**: Means±SEM intra-individual ratio of a change (Δ) in diastolic blood pressure (DBP) to Δ MSNA burst frequency (A) and inter-individual relationship between Δ DBP and Δ MSNA burst frequency (B) during the cold pressor test in elderly blacks and whites.