Aging augments renal vasoconstrictor response to orthostatic stress in humans

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Running Title: Aging effects on renal vascular responses to LBNP in humans

AUTHOR CONTRIBUTIONS

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The ability of the human body to maintain arterial blood pressure (BP) during orthostatic stress is determined by several reflex neural mechanisms. Renal vasoconstriction progressively increases during graded elevations in lower body negative pressure (LBNP). This sympathetically mediated response redistributes blood flow to the systemic circulation to maintain BP. However, how healthy aging affects the renal vasoconstrictor response to LBNP is unknown. Therefore, ten young (25 ± 1 years; mean ± SE) and ten older (66 ± 2 years) subjects underwent graded LBNP (-15 and -30 mmHg) while beat-to-beat renal blood flow velocity (RBFV; Doppler ultrasound), arterial blood pressures (BP; Finometer), and heart rate (HR; electrocardiogram) were recorded. Renal vascular resistance (RVR), an index of renal vasoconstriction, was calculated as mean BP/RBFV. All baseline cardiovascular variables were similar between groups, except diastolic BP was higher in older subjects ($P < 0.05$). Increases in RVR during LBNP were greater in the older group compared to the young group (older: -15 mmHg Δ10 ± 3%, -30 mmHg Δ20 ± 5%; young: -15 mmHg Δ2 ± 2%, -30 mmHg Δ6 ± 2%; $P < 0.05$). RBFV tended to decrease more ($P = 0.10$) and mean BP tended to decrease less ($P = 0.09$) during LBNP in the older group compared to the young group. Systolic and diastolic BP, pulse pressure, and HR responses to LBNP were similar between groups. These findings suggest that aging augments the renal vasoconstrictor response to orthostatic stress in humans.

**Key Words:** Aging, renal vasoconstriction, orthostasis, sympathetic nervous system, baroreflex
INTRODUCTION

The ability of the human body to maintain arterial blood pressure (BP) and cerebral perfusion during orthostatic stress, such as standing up or remaining upright, is determined by several reflex neural mechanisms (14). The baroreflex regulates second-to-second BP and can activate the sympathetic nervous system in order to increase or decrease BP during a drop or rise in BP, respectively (13). Sympathetic outflow from the medulla oblongata in the brainstem directed towards the kidneys causes renal vasoconstriction and consequently reduces renal blood flow (10). This shunting of blood away from the kidneys helps to redistribute blood flow to areas of the body where it is more critically needed at that time, such as the systemic circulation to maintain BP. As the kidneys receive ~25% of the body’s cardiac output at rest (30), they play an integral role in systemic BP regulation.

The incidence of orthostatic hypotension increases with advancing age (24) and is linked with greater mortality (15). Therefore, investigating the mechanisms involved in the physiological response to a drop in BP is of clinical relevance. Reflex responses to changes in BP can be investigated using stressors that unload the baroreflexes, as occurs during application of lower body negative pressure (LBNP). LBNP causes blood pooling in the venous circulation of the lower limbs, which unloads cardiopulmonary baroreceptors due to the decrease in central venous pressure. The resultant increase in sympathetic outflow serves to augment peripheral resistance in the vasculature to restore the diminished cardiac output due to the reduction in venous return, with heart rate (HR) also increasing in an attempt to restore cardiac output (2, 9, 26). The effect of healthy aging on cardiovascular
responses to LBNP has been examined in some studies, with various findings. Mean BP in response to LBNP in older subjects has been shown to be similar (8, 25), to decrease more (25), or increase (4, 27) compared to young subjects, while similar (8, 22) or smaller (4, 6, 25, 27) HR increases to LBNP in older compared to young subjects have been found. Other studies have reported that older subjects demonstrate augmented increases in sympathetic outflow with central hypovolemic stress, but an attenuated forearm vasoconstrictor responsiveness (4), an augmented increase in peripheral resistance (6), yet similar muscle sympathetic nerve activity increases in response to LBNP (22) compared to young subjects. The lack of consistent findings warrants further investigation in this area, particularly due to the clinical relevance of this issue.

Few studies have assessed the effect of aging on renal responses to orthostatic stress in humans. Similar decreases in renal blood flow in response to head-up tilt have been reported in young and older subjects (12, 16). Conversely, attenuated increases in renal vascular resistance (RVR) during standing (1) and lower plasma renin activity in response to LBNP (3) have been detected in older compared to young subjects. In healthy young subjects, renal vasoconstriction progressively increases during graded LBNP (19). The physiological purpose of this renal vasoconstriction would be to attempt to restore blood volume to maintain BP. However, how healthy aging affects the renal vasoconstrictor response to LBNP is unknown. Unlike muscle sympathetic nerve activity, it is not feasible to directly measure renal sympathetic nerve activity in humans. Renal blood flow velocity can be measured using Doppler ultrasound in humans (18, 29) and RVR, calculated as mean BP divided by renal blood flow velocity measured by Doppler ultrasound, can be
used as an index of renal vasoconstriction (18, 29). Therefore, we examined the effect of healthy aging on the renal vasoconstrictor response to LBNP in humans. As advancing age is associated with an increased incidence of orthostatic hypotension (24), we hypothesized that the renal vasoconstrictor response to LBNP would be attenuated in healthy older people.

**METHODS**

*Ethical approval.* The experimental protocol was approved by the Institutional Review Board at the Pennsylvania State University College of Medicine and complied with Declaration of Helsinki standards. The purpose of the study and risks of involvement were explained to the subjects, and signed informed consent was obtained.

*Subjects.* Ten young (5 men and 5 women; age 25 ± 1 year; height 1.78 ± 0.02 m; weight 82.2 ± 4.3 kg) and ten older (3 men and 7 women; age 66 ± 2 years; height 1.68 ± 0.03 m; weight 68.6 ± 3.8 kg) healthy subjects participated in this study. All subjects met the following inclusion criteria: good health, normotensive, non-smokers, recreationally active with at least 2-3 hours of aerobic exercise per week, no history of cardiovascular disease or autonomic disorders, and not taking any medications that could impact autonomic or cardiovascular function. Subjects were instructed to refrain from performing strenuous exercise, ingesting caffeine and alcohol for 24 hours, and ingesting food for 8 hours prior to their study visits. Blood samples were obtained from all subjects for measurement of baseline plasma creatinine and blood urea nitrogen (BUN) to assess renal function (Clinical Laboratories, Penn State Milton S. Hershey Medical Center).
Experimental protocol. Supine subjects were encased up to the level of the iliac crest in an LBNP chamber and then instrumented. After at least 30 minutes, a 3-minute baseline period occurred before LBNP was sequentially applied in two 10-minute phases (-15 mmHg followed by -30 mmHg). Graded levels of LBNP were applied to assess ‘dose-dependent’ responses in both groups.

Cardiovascular measurements. Renal blood flow velocity (RBFV) was measured using Doppler ultrasound (HDI 5000; ATL Ultrasound, Bothell, WA) via the anterior abdominal approach (18, 29). The renal artery was imaged using a 2-5 MHz curved-array transducer with a 2.5-MHz pulsed Doppler frequency. The probe was held in the same location for each trial, with an insonation angle of less than or equal to 60° to the renal artery, and the focal zone set to the artery’s depth. Beat-to-beat systolic BP, diastolic BP, and mean BP were measured with a photoplethysmographic finger cuff (Finometer; FMS, Arnhem, Netherlands). Three BP measurements were taken at baseline with a semi-automated upper arm cuff (Dinamap; GE Medical System; Milwaukee, WI) and were used to calibrate the baseline finger cuff signal in offline analysis. HR was measured using a three-lead electrocardiogram (ECG, Cardiocap/5; GE Healthcare, Waukesha, WI). RBFV measurements were taken at baseline and during LBNP. BP and HR were measured continuously throughout baseline and during LBNP.

Data and statistical analyses. Doppler images were analyzed using HDI 5000 software to produce beat-to-beat values for RBFV. RVR, an index of renal vasoconstriction (18, 29), was calculated as mean BP/RBFV. An analog-to-digital converter sampled data at 400 Hz, and data were displayed and recorded for offline analysis (MacLab 8e; AD
Instruments; Castle Hill, NSW). Raw data files were analyzed to produce beat-to-beat values for systolic, diastolic, and mean BPs, pulse pressure (PP), and HR. Absolute and relative (change from baseline) RVR, RBFV, mean BP, HR, systolic and diastolic BP, and PP values were calculated for the 3-minute baseline, the last 3 minutes of -15 mmHg LBNP, and the last 3 minutes of -30 mmHg LBNP for both the young and older groups. The data are shown as mean ± SEM.

Baseline differences in young and older subject characteristics were assessed using independent samples t-tests. Differences in cardiovascular responses to LBNP were assessed using a one within- (phase: -15 mmHg LBNP and -30 mmHg LBNP) and one between- (age: young vs. old) factor, repeated measures mixed ANOVA. Statistical significance was set at $P < 0.05$, and all statistical analysis was performed using SPSS (IBM, Armonk, NY).

RESULTS

**Subject characteristics at rest.** RVR, RBFV, HR, systolic BP, and PP were similar in young and older adults at rest (Table 1). Diastolic BP was higher ($P < 0.05$) and mean BP tended to be higher ($P = 0.07$) in the older group than in the young group at rest. The older group weighed less than the young group ($P < 0.05$). Plasma creatinine and BUN were similar in both groups (creatinine: young 0.81±0.03 vs. older 0.85±0.03 mg/dl; BUN: young 12.8±1.1 vs. older 15.2±0.9 mg/dl).

**Effects of aging on renal responses to LBNP.** RVR increased (Fig. 1) and RBFV decreased (Fig. 2) in an intensity-dependent fashion during LBNP in both young and older
subjects ($P < 0.05$). Additionally, RVR increases were greater in the older group compared to the young group ($P < 0.05$). Decreases in RBFV during graded LBNP tended to be greater in the older than in the young group ($P = 0.10$).

Effects of aging on BP and HR responses to LBNP. Mean BP (Fig. 3A), systolic BP (Fig. 4A), and PP (Fig. 4C) decreased and HR increased (Fig. 3B) in an intensity-dependent fashion during LBNP in both young and older subjects ($P < 0.05$). Diastolic BP did not change significantly in either group with increasing LBNP intensity (Fig. 4B). Mean BP during graded LBNP tended decrease less in the older than in the young group ($P = 0.09$). Systolic and diastolic BP, PP, and HR responses to LBNP did not differ significantly between groups.

DISCUSSION

The main finding from this study is that older subjects exhibited an augmented RVR increase during LBNP compared to young subjects. BP and HR responses to LBNP were similar in young and older subjects, suggesting that the older subjects relied on enhanced renal vasoconstriction in order to support their BP. Therefore, aging appears to augment the renal vasoconstrictor response to orthostatic stress in humans.

The aging-induced augmentation in the RVR increase during LBNP was counter to our hypothesis. We hypothesized that older subjects would exhibit an attenuated RVR, as older people are more prone to experiencing orthostatic hypotension (24). However, we observed a greater increase in RVR during LBNP in the older compared to young subjects, suggesting that renal vasoconstriction is enhanced in older people when experiencing a
hypovolemic stimulus. It would appear that older people might rely on a greater renal vasoconstrictor response in order to support their BP when its maintenance is challenged. Augmented renal vasoconstrictor responses to other sympathetic stimuli in older people have been observed in other studies. A greater RVR increase during fatiguing handgrip exercise (17), and an augmented decrease in renal vascular conductance (analogous to an increase in RVR) during fatiguing handgrip with additional forearm heating (11), have been reported. Additionally, this enhanced renal vasoconstriction during handgrip and local heating was associated with augmented muscle sympathetic nerve activity in the older subjects.

BP and HR responses to LBNP were similar in young and older subjects in this study. This is in agreement with some studies that have investigated age-related cardiovascular responses to LBNP (8, 22, 25) but different to others (4, 6, 25, 27). Several factors could explain the differing BP and HR responses to LBNP that have been observed in young and older subjects. Venous compliance could affect the level of venous pooling caused by LBNP, and this can be affected by age, gender, and physical fitness. Venous compliance is reduced with increasing age (7, 20, 23), although this may not affect responses to LBNP (7). Young women have lower venous compliance at rest, yet venous compliance decreases in young men but not young women during LBNP (21). Physical fitness increases venous compliance (7, 20), and may partially offset the age-related reduction in venous compliance (20), although again this may not affect responses to LBNP (7). Variations in application of LBNP may also contribute to different BP and HR responses observed in young and older subjects. Differences in LBNP intensities, durations,
and whether it is applied to one leg or the whole lower body could affect the magnitude of responses seen in these groups. It is possible that if we had applied LBNP at a higher intensity and/or for a longer duration, age-related differences in BP and HR might have been unmasked, in addition to the already-enhanced renal vasoconstriction observed in the older subjects. This may warrant further investigation.

Both mean BP and RBFV decreased during LBNP across the young and older groups. As RVR is calculated as mean BP divided by RBFV, an increase in mean BP and/or a decrease in RBFV would result in a higher RVR. As RBFV decreased and mean BP did not increase (it decreased) during LBNP, this therefore suggests that the decrease in RBFV had a greater relative contribution to the increase in RVR in response to LBNP in both the young and older groups than the decrease in mean BP. Although RBFV only tended to decrease more ($P = 0.10$) and mean BP only tended to decrease less ($P = 0.09$) during LBNP in the older compared to the young group, the direction of these changes in RBFV and mean BP indicate that RBFV was likely contributing more to the significantly greater increase in RVR during LBNP in the older compared to the young group than mean BP. Also, mean BP, systolic BP and PP, but not diastolic BP, decreased with LBNP across the young and older groups. It is likely that as LBNP reduced central blood volume and consequently venous return, this decreased systolic BP. This lower systolic BP with an unchanged diastolic BP resulted in a reduced PP, as well as a decreased mean BP.

It is intriguing that RVR increased to a greater extent during LBNP in the older compared to young subjects, yet BP and HR responses were similar. This suggests that the older subjects’ greater renal vasoconstriction, in addition to an apparently adequate HR
increase, was sufficient to support their BP to a similar extent compared to the young subjects. This may seem like an effective physiological response, as the older group could maintain their BP during LBNP similarly to the young group. However, it appears that the older subjects had to rely on an augmented renal vasoconstriction to maintain an adequate BP. The potential cumulative effect of this greater renal response occurring repeatedly when older people experience a drop in BP may have a negative impact on their renal function. Renal function is known to decline with advancing age (28), and it may be the case that when a fall in BP is detected, augmented renal vasoconstriction occurs in older people to restore BP, but the repeated enhanced reduction in renal blood flow may negatively affect renal function. This could be due to exaggerated activation of the renin-angiotensin system, with greater subsequent sodium and fluid reabsorption, and/or detrimental effects on the renal glomeruli (10).

BP did not drop to a large extent in this study (mean BP decreased ~4 mmHg from baseline during -30 mmHg LBNP in the older group) in comparison to the larger falls associated with orthostatic hypotension, but small drops in BP are likely to occur more often than larger drops in BP. Therefore, the augmented renal vasoconstriction observed during this small drop in BP may occur quite frequently in older people. As older people are more prone to falls due to a large drop in BP, it may be the case that they rely on an enhanced renal vasoconstrictor response to even a small drop in BP. As BP declines further, the level of renal vasoconstriction would likely increase but could eventually reach a ceiling effect where older people cannot constrict their kidneys further. This mechanism to maintain BP would therefore become ineffective at this time, potentially resulting in the
continued decline in BP and subsequent cerebral hypoperfusion that could cause a fall in an older person.

*Perspectives and Significance.* Our findings suggest that aging augments the renal vasoconstrictor response to orthostatic stress in humans. This appears to be a physiological compensatory adaptation that occurs with older age that serves to support BP in the face of orthostatic stress when BP would not be maintained otherwise. The clinical significance of this finding is that it provides potential insight into the physiological mechanisms involved during an episode of orthostatic hypotension. Orthostatic hypotension is associated with conditions such as Parkinson’s disease, pure autonomic failure, and multiple system atrophy (5). It may be that in these conditions, the renal vasoconstrictor response that occurs in response to orthostatic stress is not augmented, like in healthy older people, but is unchanged or even attenuated. This lack of an appropriate level of renal vasoconstriction would provide insufficient support of BP, leading to a large drop in BP during orthostatic stress. As the incidence of orthostatic hypotension is linked with greater mortality (15), investigating renal vasoconstrictor responses to orthostatic stress in these populations will be of clinical importance.
ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

There are no conflicts of interest.
REFERENCES


FIGURES AND LEGENDS

Figure 1 – Changes from baseline in renal vascular resistance for young and older groups during -15 mmHg and -30 mmHg lower body negative pressure (LBNP). * = different from -15 mmHg LBNP (P < 0.05). † = different from young (P < 0.05).

Figure 2 – Changes from baseline in renal blood flow velocity for young and older groups during -15 mmHg and -30 mmHg lower body negative pressure (LBNP). * = different from -15 mmHg LBNP (P < 0.05).

Figure 3 – Changes from baseline in A) mean arterial pressure and B) heart rate for young and older groups during -15 mmHg and -30 mmHg lower body negative pressure (LBNP). * = different from -15 mmHg LBNP (P < 0.05).

Figure 4 – Changes from baseline in A) systolic blood pressure, B) diastolic blood pressure, and C) pulse pressure for young and older groups during -15 mmHg and -30 mmHg lower body negative pressure (LBNP). * = different from -15 mmHg LBNP (P < 0.05).
Table 1. Baseline cardiovascular values of the young and older groups. Diastolic BP, diastolic blood pressure; HR, heart rate; mean BP, mean arterial blood pressure; PP, pulse pressure; RBFV, renal blood flow velocity; RVR, renal vascular resistance; systolic BP, systolic blood pressure. Data are shown as means ± SEM. * = different from young ($P < 0.05$).

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<td>RBFV (cm.sec$^{-1}$)</td>
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<td>Diastolic BP (mmHg)</td>
<td>66 ± 2</td>
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<td>PP (mmHg)</td>
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<td>53 ± 3</td>
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<tr>
<td>HR (b.min$^{-1}$)</td>
<td>58 ± 2</td>
<td>63 ± 3</td>
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Change in renal vascular resistance (%)

-15 mmHg

-30 mmHg

LBNP

Young

Older

*.

†.
Change in renal blood flow velocity (cm.sec\(^{-1}\))

-15 mmHg
-30 mmHg

Young
Older

*
A

LBNP

-15 mmHg

-30 mmHg

Change in mean arterial pressure (mmHg)

Young
Older

*
Change in heart rate (b.min\(^{-1}\))

-15 mmHg

-30 mmHg

LBNP

Young

Older

*
Change in diastolic blood pressure (mmHg)

LBNP

-15 mmHg
-30 mmHg

Young
Older
Change in pulse pressure
(mmHg)

LBNP

-15 mmHg

-30 mmHg

Young
Older

*