

Age-Related Changes in Vasomotor Reflex Control of Calf Venous Capacitance Response to Lower Body Negative Pressure in Humans

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Abstract: The present study was performed to test the hypothesis that calf venous capacitance would be reduced by mild gravitational stress through a vasomotor reflex in humans, and this response could be diminished with advancing age. Nine young (31 ± 1 years, mean \pm SE) and 9 elderly (69 ± 1 years) healthy males were exposed to a lower body negative pressure (LBNP) of 15 mmHg. Venous occlusion plethysmography was used to measure calf venous capacitance and calf blood flow. Muscle sympathetic nerve activity (MSNA) was recorded microneurographically from the tibial nerve along with cardiovascular variables. It was found that baseline MSNA was higher [21 ± 4 (mean \pm SE) vs. 37 ± 5 bursts \cdot min $^{-1}$, young vs. elderly; $p < 0.05$] and calf venous capacitance was lower (1.71 ± 0.12 vs. 1.44 ± 0.10 , ml \cdot 100 ml $^{-1}$, young vs. elderly; $p < 0.05$) in the elderly group. At 15 mmHg-LBNP,

heart rate and mean arterial pressure both remained unchanged, MSNA was enhanced, and calf blood flow was reduced in all subjects. Calf venous capacitance during LBNP decreased in the young, but did not change in the elderly. A significant negative correlation between percent changes in MSNA and percent changes in calf venous capacitance existed in the young group ($y = -0.171x - 11.863$, $r = -0.682$; $p = 0.0432$), but disappeared in the elderly group. The ratio of percent changes in calf venous capacitance to percent changes in MSNA was markedly lower in the elderly ($p < 0.01$). In conclusion, these results substantiate our hypothesis that calf venous capacitance is reduced by mild LBNP through the vasomotor reflex, and this response is diminished in the elderly. [Japanese Journal of Physiology, 52, 69–76, 2002]

Key words: muscle sympathetic nerve activity, cardiopulmonary baroreflex, arterial baroreflex, calf blood flow, gravitational stress.

The venous system plays an important role in fluid shifts and blood pressure regulation. In humans, it has been supposed that the capacity of the leg veins may be reduced by a vasomotor reflex when standing [1]. If this is so, this is a portion of the compensatory response to gravitational stress. However, how the vasomotor reflex controls calf venous capacitance is unclear.

Currently, little information is available concerning a relationship between vasomotor sympathetic activ-

ity, which can be recorded as muscle sympathetic nerve activity (MSNA) [2, 3], and calf venous capacitance, especially under the effect of aging. Advancing age produces several changes in cardiovascular structure and function in humans [4, 5], among which an impairment in cardiopulmonary, integrative baroreflex sympatho-circulatory control [6, 7], and a stiffness of the peripheral venous wall [8, 9] have mainly been proposed to have influences on cardiovascular stability in the elderly.

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Peripheral veins are well known to be under thermoregulatory reflex control [10, 11], but it is uncertain how MSNA responses exert an influence on venous capacitance. It has been proposed that changes in venous capacitance may represent venoconstriction, and they cannot be adequately described by another parameter—venous compliance [12]. Halliwill *et al.* [13] reported that the whole-limb venous compliance was under negligible sympathetic control in humans; however, venous capacitance is closely related to the arterial inflow and venous outflow, and therefore it can be under the vasomotor control. Venous capacitance can change without a measurable change in compliance, and this stems from the distributed nature of venous compliance within the organs [14].

The present study was conducted to test the hypothesis that calf venous capacitance was under the vasomotor reflex control. Specifically, we investigated the relationship between MSNA and calf venous capacitance and how advancing age influences this relationship. Some of these results have been presented in preliminary form [15].

METHODS

Subjects. Nine young and nine elderly healthy, nonobese men participated in the study. Their averaged ages were 31 ± 1 in the young and 69 ± 1 in the elderly. The weight, body fat percentage, and height were 66 ± 2 kg in the young vs. 55 ± 2 kg in the elderly, $11 \pm 1\%$ in the young vs. $13 \pm 2\%$ in the elderly ($p > 0.05$, N.S.), and 174 ± 2 cm in the young vs. 160 ± 1 cm in the elderly, respectively. None had a history of cardiovascular disease, kidney disease, diabetes, venous insufficiency, or other diseases and were not on any medication at the time of the study. All had abstained from alcohol and caffeine use for 24 h before the examination, and all reported no recent use of tobacco or other pharmacological agents. The subjects were informed of the purpose and the procedures used in the study and gave their consent to participate in the experiment. The present study was conducted under the guidelines proposed by the Japan Microneurography Society and was approved by the Human Research Committee of the Research Institute of Environmental Medicine, Nagoya University.

Experimental protocol and procedure. The experimental protocols were performed in the morning or at noon 1 h after a light meal and normal hydration. All experiments were carried out with the subject supine, dressed in shorts without shirt, and in a room with an ambient temperature of 24–26°C. MSNA was recorded microneurographically from the left tibial

nerve at the popliteal fossa. The heart rate (HR, beats \cdot min⁻¹) obtained from electrocardiogram (ECG) and blood pressure (BP, mmHg) waves obtained by tonometry (model BP-508S, Nippon Colin, Komaki, Japan) were simultaneously recorded. Respiration waves were recorded by a nasal airflow with a thermistor. Peripheral venous pressure (PVP, mmHg) was measured from a 20-gauge catheter placed in a large antecubital vein of the left arm through a transducer kit (Baxter, Tokyo) and connected to a pressure amplifier (model AP-641G, Nihon Kohden, Tokyo). Calf blood flow ($\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$) and calf venous capacitance ($\text{ml} \cdot 100 \text{ ml}^{-1}$) were determined by mercury strain gauge venous occlusion plethysmography (Hokanson EC5R plethysmograph, Hokanson, Bellevue, WA). This method was proved to be comparable with the measurement by ultrasonic Doppler flowmetry even in animal study [16].

Having rested for >30 min, the data of MSNA, HR, BP, respiration, PVP, calf blood flow, and calf venous capacitance were recorded during 12 min of baseline control, followed by 12 min of 15 mmHg-LBNP, and then returned to 0 mmHg for recovery. All variables were monitored throughout the procedures and stored on a digital audio tape recorder (PC 216Ax, Sony Precision Technology, Tokyo) for later analysis.

Lower body negative pressure (LBNP). The LBNP facility affiliated with the Space Medicine Center, Research Institute of Environmental Medicine, Nagoya University, was employed for the study. LBNP was applied distally to the subject's iliac crest by sealing the subject within a customized pressure box at the level of the iliac crest. A freely movable saddle could be fixed at 20–60 cm inside from the orifice of the chamber. This saddle was capable of supporting the inguinal region of the subject to prevent it from being sucked into the LBNP chamber without muscle contraction. Pressure was regulated within the LBNP chamber by controlling valves that adjusted vacuum into the chamber with the help of a computer using a closed-loop servomechanism. The pressure applied was read via a pressure transducer connected to the inside of the chamber. Since this LBNP device has the suction pump outside the room with a large reservoir, there would be no suction noise during the change in chamber pressure.

Recording of MSNA. MSNA was recorded from the left tibial nerve at the popliteal fossa by a microneurographic technique using a tungsten microelectrode with a tip diameter of $\sim 1 \mu\text{m}$ and an electrode impedance of 2–5 M Ω (model 26-05-1, Frederic Haer, Bowdoinham, ME). Nerve signals were fed through a high-input impedance preamplifier with a

500–5,000 Hz band-pass filter. MSNA was then full-wave rectified and integrated with a time constant of 0.1 s. The identification of MSNA was based on the presence of the following discharge characteristics described elsewhere [2, 3, 17]: briefly, (1) pulse-synchronous and rhythmic efferent burst discharges; (2) afferent activity evoked by a tapping of the appropriate muscle but not in response to a gentle touch; (3) modulation by respiration; and (4) enhancement by maneuvers increasing intrathoracic pressure, such as the Valsalva maneuver.

Venous occlusion plethysmography measurements and calculations. For all measurements, the subject relaxed in the supine position with his right leg elevated 3–5 cm above the heart level. A mercury-in-silastic strain gauge was placed at the maximal circumference of the right calf. A contoured 22 cm wide thigh cuff was positioned around the proximal right thigh. The subject was informed of the importance of muscle relaxation during the testing period. In this investigation, the foot blood circulation was not excluded. The occlusion cuff pressure used in this study was relative to the outside of the negative pressure chamber.

For measuring calf blood flow, the occlusion cuff was inflated quickly to 50 mmHg to stop blood from leaving the measurement site, but not hindering the arterial inflow. After a 5 s inflation, the cuff was deflated for a 25 s interval. The calf swelling as a result of the arterial inflow and the rate of blood flow was determined by measuring the rate of increase in volume. The inflow rate was determined by drawing a line on the recorded output tangent to the first few pulses following cuff inflation. The slope of this line was defined as the rate of volume change caused by arterial inflow. The flow rate was expressed as volume change per unit time such as [ml of blood flow] · [100 ml tissue]⁻¹ · min⁻¹. The measurement was repeated 8 times in each stage, and the mean value was obtained.

Figure 1 illustrates how calf venous capacitance is detected. In the supine position, the vein in an elevated leg is flat, so the early phase of expansion of the vein involves no actual stretching of the elastic material in its wall, and a small change in distending (transmural) pressure merely changes the geometry of the vein [18]. Once the vein has assumed a circular cross section, subsequent increases in its transmural pressure are opposed by the development of increased tension in the wall, and stiff collagen fibers must be stretched to increase the volume [19]. It was observed that when the transmural pressure was increased over 18 mmHg, the vein had a circular cross section either

in young or in elderly healthy subjects [20]. Moreover, the transmission of externally applied pressure to the underlying tissue was found to be almost identical in the young and elderly [20]. Therefore, in the present study we applied a low counterpressure of 20 mmHg to both groups to equalize the circular cross section of the vein before measurement. After the control baseline was obtained, the occlusion cuff was quickly inflated to 20 mmHg for 1 min, and a small curve with a steeply ascending beginning followed by a plateau was observed. The cuff pressure was then rapidly increased to 50 mmHg, and a similar curve appeared with a larger range of a steeply ascending beginning followed by another plateau because of arterial inflow without venous outflow. In the resting supine position, especially for an elevated leg, the venous pressure in the leg vein does not exceed the atrium level (5 mmHg) [21], and thus 50 mmHg of counterpressure can completely block the venous return. After a venous stasis period, the cuff pressure was abruptly released to 0 mmHg. The criteria for deflating the cuff here were (1) the calf circumference increment had reached its maximum, and (2) the curve had reached a plateau. In our measurement, 50 mmHg cuff inflation for venous occlusion was maintained for 2.5 min. Because the capacitance response is usually terminated within about 3 min [22], transcapillary fluid filtration was ignored in the present study. After cuff deflation, the curve first dropped quickly, then more slowly until it reached the previously noted baseline.

Calf venous capacitance was calculated by measuring the distance in mm from the maximum height of the first small curve to the maximum height of the second large curve and dividing this distance by the height of the calibration marker (CAL=1%Δ). This marker represents a 1% change in the electrical resistance of the conductor inside the gauge, which is equal to a 1% change in limb volume or a 0.5% change in gauge length. The resulting number was referred to as percent increase, or volume in [ml blood] · [100 ml tissue]⁻¹.

Statistics and data analysis. An unpaired *t*-test was used to test the differences of physical constitution of the subjects in the young and elderly groups. We employed a two-way repeated-measure ANOVA to determine the effects of aging on calf venous capacitance, MSNA, and cardiovascular responses to LBNP. Session (young vs. elderly) and period (control vs. 15 mmHg-LBNP) were set as the main factors. Post hoc comparisons were performed by Fisher's test. Simple linear regression and correlational analyses were generated to determine the relationship between percent changes in MSNA and percent changes in calf

Table 1. Cardiovascular and MSNA variables at resting control and 15 mmHg-LBNP.

	Young group (n=9)		Elderly group (n=9)	
	Control	15 mmHg-LBNP	Control	15 mmHg-LBNP
HR (beats · min ⁻¹)	69.9±2.3	70.6±2.6	67.8±1.7	68.9±1.8
MAP (mmHg)	87.1±2.2	80.0±2.0	94.6±3.2	88.5±2.9
PVP (mmHg)	1.3±0.7	-1.2±0.8*	1.2±0.4	-0.3±0.4 ^{#,*}
CBF (ml · 100 ml ⁻¹ · min ⁻¹)	3.7±0.5	3.0±0.4*	3.4±0.3	2.9±0.2*
MSNA (bursts · min ⁻¹)	20.6±4.2	29.6±4.6*	36.8±3.5 [#]	43.7±3.1 ^{#,*}

Values are means±SE. MSNA, muscle sympathetic nerve activity; LBNP, lower body negative pressure; HR, heart rate; MAP, mean arterial pressure; PVP, peripheral venous pressure; CBF, calf blood flow. * $p<0.05$ vs. the control; [#] $p<0.05$ vs. the young group.

venous capacitance. An unpaired *t*-test was also employed to compare the ratio of percent changes in calf venous capacitance to percent changes in MSNA in the young and elderly groups. $p<0.05$ was considered statistically significant. All analyses were conducted with a computerized statistical analysis system (StatView J-4.5, Power PC Version, 1992-98, Abacus Concepts) on a Power Macintosh computer (6300/120).

RESULTS

Complete data were obtained in all subjects. There were no untoward effects from the study and none of the subjects complained of any discomfort during the experiment. The results are shown in Table 1 and Figs. 2-5. During the procedure, no breath holding was observed from the recording of respiratory curve.

Cardiovascular and MSNA variables at rest and 15 mmHg-LBNP

Resting HR was not different between the young and elderly subjects, but baseline MAP tended to be elevated in the elderly ($p=0.067$, Table 1). Resting MSNA level was significantly higher in the elderly group ($p<0.05$), but baseline CBF was not different between the two groups (Table 1, Fig. 2).

At 15 mmHg-LBNP, neither HR nor MAP changed markedly in any subject (Table 1). PVP was reduced, but the reduction (Δ PVP) was smaller in the elderly subjects (-2.5 ± 0.4 vs. -1.5 ± 0.6 mmHg, young vs. elderly; $p<0.05$). MSNA was enhanced in all subjects ($p<0.05$, Table 1); however, the enhancement (Δ MSNA) was not different between the two groups (9.0 ± 3.2 vs. 6.9 ± 3.8 bursts · min⁻¹, young vs. elderly; $p>0.05$). CBF was reduced during LBNP, and the reduction was not different between the two groups (Table 1).

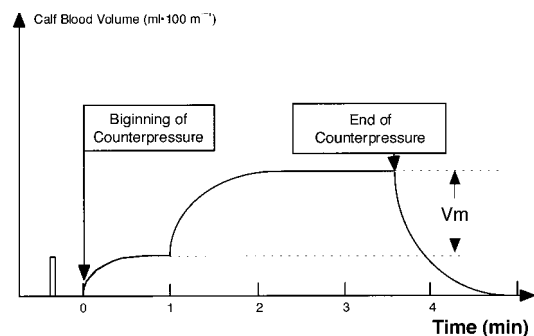


Fig. 1. Illustration of plethysmographic curve. Calf volume changes following applied counterpressures. V_m , the distance in mm from the maximum height of the first curve to the maximum height of the second curve. Calf venous capacitance was calculated from V_m divided by the height of the calibration marker (CAL=1% Δ). This marker represents a 1% change in the electrical resistance of the conductor inside the gauge, which is equal to a 1% change in limb volume or a 0.5% change in gauge length.

Calf venous capacitance at rest and 15 mmHg-LBNP

Baseline calf venous capacitance was significantly lower in the elderly ($p<0.05$, Fig. 3). During 15 mmHg-LBNP, calf venous capacitance was decreased in the young group ($p<0.01$), but it did not change in the elderly group (Fig. 3). A significant negative correlation between percent changes in calf venous capacitance and percent changes in MSNA was observed in the young group ($y=-0.171x-11.863$, $r=-0.682$; $p=0.0432$), but the relationship disappeared in the elderly group (Fig. 4). The ratio of percent changes in calf venous capacitance to percent changes in MSNA was significantly lower in the elderly ($p<0.01$, Fig. 5).

DISCUSSION

The major findings from the present study were (1) the changes from the baseline MSNA were not signifi-

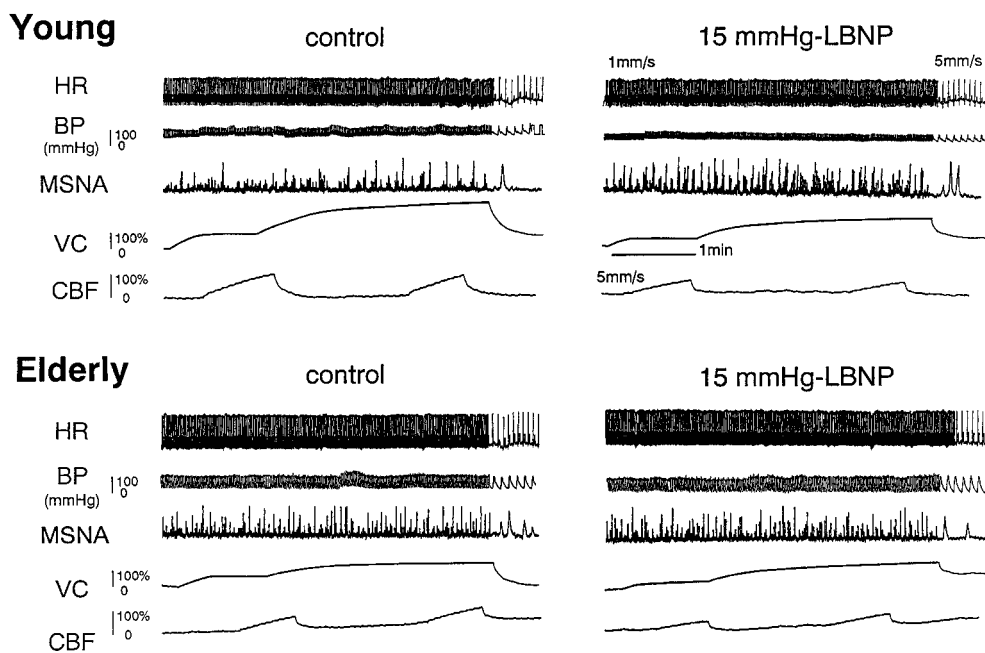


Fig. 2. Original tracings of electrocardiogram (HR), blood pressure (BP), integrated muscle sympathetic nerve activity (MSNA), calf venous capacitance response (VC), and calf blood flow (CBF) in one young and one elderly subject at control and during 15 mmHg of lower body negative pressure (15 mmHg-LBNP).

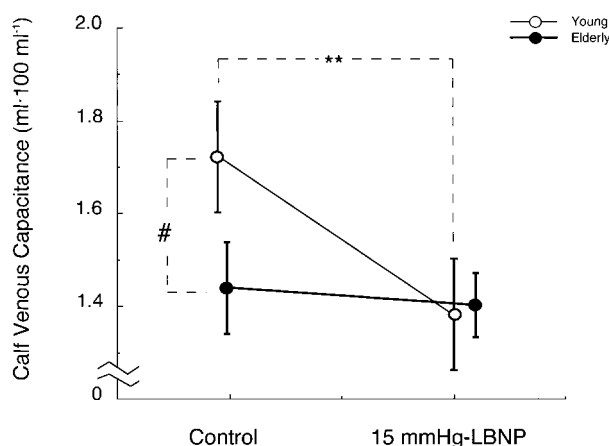


Fig. 3. Calf venous capacitance in response to 15 mmHg-LBNP in the young (○) and elderly (●) groups. Each curve represents averaged group data (means±SE; n=9 in the young group and n=9 in the elderly group). ** p<0.01 vs. the control; # p<0.05 vs. the young group.

cantly different between the young and the elderly; (2) the enhanced MSNA response reduced calf venous capacitance in the young group; and (3) the sensitivity of calf venous capacitance in response to MSNA was markedly decreased in the elderly.

In the present study, we found that baseline MSNA was higher in the elderly group, but MSNA response to 15 mmHg-LBNP was nearly the same as that of the young group. This was consistent with the results of Iwase *et al.* [23], who observed that aging could increase resting MSNA, and of Davy *et al.* [24], who reported that MSNA responses were the same or even

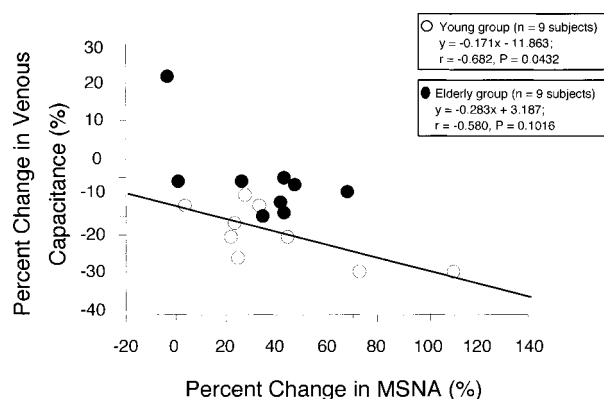


Fig. 4. Correlation of percent changes in MSNA and percent changes in calf venous capacitance at 15 mmHg-LBNP in the young (○) and elderly (●) subjects.

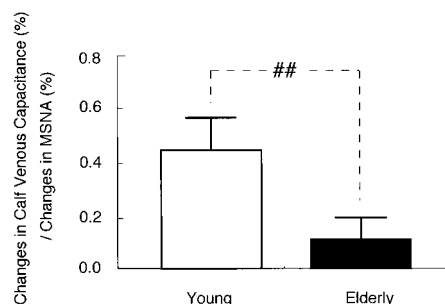


Fig. 5. A comparison of the ratio of percent changes in calf venous capacitance to percent changes in MSNA in the young (□) and elderly subjects (■). Each bar represents averaged group data (means±SE; n=9 in the young group and n=9 in the elderly group). ## p<0.01 vs. the young group.

greater in the older than in the young subjects during LBNP.

Contributions of enhanced MSNA to calf venous capacitance. It was observed that percent changes in calf venous capacitance was negatively correlated with percent changes in MSNA in the young subjects. This result substantiates the hypothesis that the capacity of the leg veins in humans was under vasomotor reflex control. This vasomotor reflex was induced by the unloading of cardiopulmonary receptors, which was transmitted via the vagus nerve. The afferent signal entered the nucleus tractus solitarius, was input to the rostral ventrolateral medulla, the vasomotor center, then it activated the MSNA [25].

The exact mechanisms could not be well elucidated in the present investigation, but we would suppose that the contribution of the enhanced MSNA to the reduction in calf venous capacitance was through the limitation of calf blood flow. It is well known that veins are highly compliant, and small changes in intravenous pressure because of the changes in blood flow may cause great changes in venous volume [19].

Thus the distribution of blood volume depends on the distribution of resistance and blood flow. In the present study, it was unlikely that the reduction in calf venous capacitance was direct via active venoconstriction because muscular venules are without sympathetic innervation or are sparsely innervated [26]; moreover, human studies have indicated that venous changes may follow passively from changes in blood flow, which in turn are influenced by the baroreceptors [27, 28].

Cutaneous veins are accepted to be influenced directly by sympathetic vasomotor impulses in skin nerves, and this could in theory also be an important contributing mechanism. However, it was reported that skin sympathetic nerve activity did not change during mild LBNP [29]; thus the effects of skin sympathetic nerve activity on calf venous capacitance could be ignored in the present study.

Reduced baseline calf venous capacitance and diminished response to MSNA in the elderly. We found that baseline calf venous capacitance was smaller in the elderly subjects, for which there might be two possibilities. First, there should be age-related structural changes in the peripheral venous walls. It is well known that the visco-elasticity of the peripheral venous wall is decreased with advancing age [9] because the collagen/elastin ratio has been reported to be increased and the venous wall has been observed to be thickened in aged people [30]. Since elastic tissue is decreased and collagen content in the smooth muscle is increased in the elderly, these two

factors can stiffen the venous wall and reduce the venous distensibility. Second, there was a higher baseline MSNA level in the elderly subjects. It has been suggested that the lower venous capacitance in the elderly might be due to a higher baseline sympathetic tone [20]. Therefore the reduced baseline calf venous capacitance might be derived not only from the structural changes, but also from the functional changes with advancing age. The diminished calf venous capacitance response to MSNA in the elderly might also be attributable to the structural changes in the peripheral venous walls; the venous distensibility is decreased in aged people, resulting in an attenuated reaction of venous capacitance to MSNA.

A significant negative correlation between percent changes in MSNA and percent changes in calf venous capacitance was observed in the young group, but it disappeared in the elderly group. We noticed that one elderly subject nearly had no change in MSNA at 15 mmHg-LBNP; it is interesting that his calf venous capacitance was increased during LBNP (Fig. 4, left upper part, filled circle). The exact reason for his different response was unclear, but a lesser fluid shift by mild LBNP might be one explanation. When his data was excluded, the significant correlation between percent changes in MSNA and percent changes in calf venous capacitance still did not exist in the elderly group ($y = -0.0614x - 5.308$, $r = -0.235$, $p = 0.575$, $n = 8$), indicating a diminished venous capacitance response to MSNA with advancing age.

Limitations. A low level of LBNP was used in the present study to simulate mild gravitational stress. However, LBNP may not actually be identical to true orthostasis. During LBNP, a uniform distending pressure is applied to the lower body (including the abdominopelvic regions and legs) within the chamber, whereas in orthostasis, a hydrostatic pressure gradient exists from the heart level to the feet. Therefore the first limitation of our study might be that the abdominopelvic blood pooling may be larger, and the leg venous pooling may be smaller during LBNP than those in true orthostasis.

The second limitation is that we did not exclude the foot blood circulation before measuring calf venous capacitance and calf blood flow in the present study. Our LBNP facilities did not allow us to put two cuff inflation tubes into the chamber; therefore we selected only the thigh cuff inflation tube. It is possible that the foot blood circulation influences the responses of calf venous capacitance and calf blood flow to LBNP.

In summary, during 15 mmHg-LBNP, MSNA was enhanced in both groups, but calf venous capacitance was decreased significantly only in the young group.

A negative correlation between the percent changes in calf venous capacitance and percent changes in MSNA existed in the young subjects, but it disappeared in the elderly subjects. With these results we conclude that the vasomotor reflex control of calf venous capacitance during gravitational stress is diminished with advancing age.

Perspectives. This study demonstrates the first description of calf venous capacitance in response to the enhancement of MSNA with advancing age. Our findings suggest that the vasomotor reflex control of the capacity of the leg veins during gravitational stress may serve as one of the protective mechanisms against orthostatic hypotension. It is conceivable that the impairment of this response in elderly people may sometimes at least partially play a role in the pathophysiology of orthostatic intolerance. The present results may be relevant to an understanding of orthostatic intolerance in the general population, in astronauts after spaceflights, and/or in subjects after simulated microgravity exposure.

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