Cardiovascular response to acute hypovolemia in relation to age. Implications for orthostasis and hemorrhage

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Olsen, Henrik, Einar Vernersson, and Toste Länne. Cardiovascular response to acute hypovolemia in relation to age. Implications for orthostasis and hemorrhage. Am. J. Physiol. Heart Circ. Physiol. 278: H222–H232, 2000.—Venous compliance in the legs of aging man has been found to be reduced with decreased blood pooling (capacitance response) in dependent regions, and this might lead to misinterpretations of age-related changes in baroreceptor function during orthostasis. The hemodynamic response to hypovolemic circulatory stress was studied with the aid of lower-body negative pressure (LBNP) of 60 cmH2O in 33 healthy men [18 young (mean age 22 yr) and 15 old (mean age 65 yr)]. Volumetric technique was used in the study of capacitance responses in the calf and arm as well as transcapillary fluid absorption in the arm. LBNP led to smaller increase in heart rate (P < 0.001) and peripheral resistance (P < 0.01) and reduced transcapillary fluid absorption in the arm (P < 0.05) in old subjects. However, blood pooling in the calf was reduced in old subjects (1.66 ± 0.10 vs. 2.17 ± 0.13 ml/100 ml tissue; P < 0.01). Accordingly, during similar blood pooling in the calf (LBNP 80 cmH2O in old subjects), no changes in cardiovascular reflex responses with age were found. The capacitance response in the arm (mobilization of peripheral blood to the central circulation) was still reduced, however (0.67 ± 0.10 vs. 1.37 ± 0.11 ml/100 ml tissue; P < 0.01). Thus the reduced cardiovascular reflex response found in the elderly during orthostatic stress seems to be caused by a reduced capacitance response in the legs with age and a concomitant smaller central hypovolemic stimulus rather than a reduced efficiency of the reflex response. With similar hypovolemic circulatory stress, no changes in cardiovascular reflex responses are seen with age. The capacitance response in the arm (mobilization of peripheral blood toward the central circulation) is reduced, however, by ~50% in the elderly. This might seriously impede the possibility of survival of an acute blood loss.

CARDIOVASCULAR RESPONSE TO HYPOVOLEMIC CIRCULATORY STRESS: HUMAN DATA

Several groups have found reduced baroreceptor efficiency with aging, which might reduce the capacity to preserve homeostasis during hypovolemic circulatory stress induced by orthostasis or hemorrhage (13, 22). Also, the possibility of compensating for reduced circulating blood volume by means of transcapillary fluid absorption from skeletal muscle and skin to the intravascular space might be reduced, because this is controlled by sympathetic α- and β-receptors in the microcirculation (37, 38). When experimental approaches such as lower-body negative pressure (LBNP) and tilting are used, age-related differences in unloading of central baroreceptors might lead to misinterpretations in changes of baroreceptor function, and techniques that do not unload baroreceptors, such as the coldpressor test, have shown unchanged sympathetic reflex responses (8, 15). One confounding factor might be a reduced compliance of the cardiopulmonary walls, where volume (stretch) receptors are situated, because an attenuated reduction in left ventricular diastolic diameter is seen with age during hypovolemic stress caused by LBNP (8). An alternative explanation might be a decline of the venous capacitance response in the lower limbs with age, thereby reducing the decrease in central blood volume during orthostatic stress and thus the deactivation of baroreceptors. Ebert et al. (14) found a smaller decrease in thoracic blood volume during similar levels of lower body suction in old compared with young individuals, suggesting a smaller shift in thoracic blood volume to the lower extremities.

MATERIALS AND METHODS

A total of 33 healthy male volunteers were divided into two separate age groups: 18 young (mean age 22 yr) and 15 old (mean age 65 yr) volunteers. Physical examination showed the absence of varicose veins, hypertension, diabetes, or other serious systemic diseases. All subjects were nonsmokers and were not taking any medication. Each subject gave informed consent to the experiments approved by the Ethics Committee of Lund University, Sweden. The experiments were started 1 h after a regular meal in the morning or at noon and were performed at a room temperature of 22–24°C. The subjects were instructed to abstain from coffee or tea on the day of the investigation. Throughout the experiments, which lasted ~3 h, continuous efforts were made to maintain a relaxed, quiet atmosphere.

Cardiovascular response to hypovolemic circulatory stress. The first part of the study was conducted on 24 of the 33 subjects: 12 young (mean age 22 yr; range 20–25 yr) and 12 old (mean age 65 yr; range 61–70 yr) subjects.

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The subjects were placed in a supine position with the legs enclosed in an airtight box up to the level of the iliac crest with a rubber seal fitted hermetically around the waist. The box was connected to a vacuum source, permitting stable negative pressure to be rapidly produced (within 5 s) (LBNP). LBNP is a well-established technique in the study of orthostatic stress, is noninvasive and relatively comfortable to the subjects, and can also be discontinued quickly, promoting safety. The advantage of LBNP compared with passive tilt is that the subject remains at rest in the supine position, which facilitates physiological measurements and minimizes the likelihood of confounding activity in skeletal muscle. Furthermore, the transmural pressure change over the vascular walls is easier to define. LBNP of −40 to −50 mmHg and passive tilt of 90° cause similar shifts in blood volume, although the distribution of the pooled blood is probably not the same because of the differences in transmural pressure gradients (3, 49, 65). The negative pressure in the LBNP chamber was measured continuously by a manometer (DT-XX disposable transducer, Viggo Spectramed, Helsingborg, Sweden) and held constant by a rheostat.

After at least 45 min of supine rest LBNP was rapidly instituted within 5 s and maintained for 8 min at 60 or 80 cmH2O. Two experiments were performed with at least 30 min between each investigation to ensure that the basal state was restored. To define the hypovolemic stimulus caused by LBNP, the blood pooling in the legs was measured by means of strain-gauge plethysmography (64). This method is designed for measuring volume changes (ml/100 ml) of a limb by measurement of the circumference. Comparison with air-filled plethysmography showed that reliable results may be obtained (5). The strain gauge was applied at the maximal circumference of the calf ~15 cm distal to the knee. Care was taken to place the midpoint of the calf 5 cm below heart level in all subjects. To avoid any confounding external pressure, the lowest part of the calf was at least 2 cm above the floor of the vacuum chamber. At onset, LBNP evoked an initial rapid increase of calf volume (capacitance response) followed by a slower but continuous rise caused by net transcapillary fluid filtration from blood to tissue. At cessation, there was a rapid decrease of the volume corresponding well with the increase at onset of LBNP (33, 46). The capacitive response was calculated from the volume increase at the onset of LBNP to the line defined from the filtration slope between 3 and 8 min, because the capacitance response is terminated within ~3 min (50).

Transcapillary fluid absorption from the upper arm was measured by plethysmography. The air plethysmographs were cylindrical, 8 cm long, and made of 3.5-mm-thick transparent plastic. They had openings of different sizes to fit the upper arms of the subjects. When the subjects were in the supine position, the right arm was placed at the level of the heart. To avoid venous stasis caused by the plethysmograph, the size of the proximal and distal openings was chosen to be slightly larger than the circumference of the arm. The air slits between the openings and the skin of the arm were then sealed with a soft latex compound (Nordsjö, Malmö, Sweden) that did not cause any additional pressure or irritation to the skin. The proximal, middle, and distal circumferences of the enclosed segment were measured, and the volume was calculated. Changes in tissue volume were measured with a piston recorder connected to the plethysmograph. LBNP was applied when upper arm volume was stable. In the analysis of the tissue volume responses, abrupt changes are considered to reflect alterations of regional blood volume, whereas gradual volume changes reflect net transcapillary fluid transfer (see Fig. 2). This interpretation of tissue volume changes during acute hypovolemic stress has been validated with the aid of simultaneously measured blood and tissue volume changes in both in animals (1, 42) and in humans (38). Thus, the capacitance response in the upper arm was measured from the rapid volume change between baseline volume and the line defined from the transcapillary fluid absorption both at onset and at cessation of LBNP, and the mean value was taken as the prevailing capacitance response. At the time of the capacitance response at onset of LBNP the paper speed was also increased to make it possible to calculate the time(s) for 50% of the total capacitance response to be developed (C50). Mean transcapillary fluid absorption during the LBNP stimulus was calculated from the volume decrease after cessation of LBNP compared with baseline volume before LBNP divided by the duration (8 min; see Fig. 2). To avoid any influence of the hyperemic reaction, present in some of the recordings immediately after LBNP, and to be sure that the blood volume had returned to baseline level, the measurement of the change in volume in relation to baseline was performed 1 min after discontinuation of LBNP. Two experiments were performed at each LBNP step, and the mean value was taken as the prevailing capacitance response as well as transcapillary fluid absorption.

Arterial blood pressure was measured noninvasively in the left upper arm with a semiautomatic blood pressure device (model HEM-700C, Omron, Tokyo, Japan). Mean arterial pressure (MAP) was taken as the diastolic pressure plus one-third of the pulse pressure.

The electrocardiogram signal, the plethysmograph recording, the pressure in the LBNP chamber, and the calf volume were amplified (PC polygraph, Synetics Medical, Stockholm, Sweden) and collected with a modified computer program for medical examination (Gastrosoft polygram, Synetics Medical) on a personal computer (SPC 386, SPC Trading, Uppsala, Sweden).

In separate experiments on the subjects, blood flow was measured on the right forearm by standard venous occlusion (50 mmHg) mercury-in-silicone elastomer (Silastic) strain-gauge plethysmography (Hokanson EC-4 D.E, Hokanson). The forearm was placed ~5 cm above the level of the right atrium, and the strain gauge was placed 5 cm distal to the elbow. Occlusion of hand blood flow was accomplished by a wrist cuff inflated to 100 mmHg above systolic arterial pressure 1 min before measurements (32). A computerized R wave-triggered system was used for measurement of forearm blood flow using the first three to six heart beats after institution of occlusion plethysmography (7). The blood flow was measured six times at baseline and twice at 30 s and 1, 3, 6, and 8 min after institution of LBNP and 1, 2, and 4 min after LBNP was discontinued. Peripheral resistance was calculated as MAP divided by blood flow. Data are given with reference to soft tissue weight excluding bone. Bone is taken as 10% in the upper arm and calf (29) and 13% in the forearm (9).

In other experiments on 6 of the young and 10 of the old subjects, a polyethylene catheter was inserted into an antecubital vein for blood sampling to measure plasma norepinephrine, which has been shown to be a good marker for the general sympathetic activation (16). LBNP at 60 and 80 cmH2O was applied as described above. Blood samples (3 ml) were taken from the vein before and at the end of each LBNP level. The blood samples were collected in prechilled tubes containing 5.7 mg of EGTA and 3.6 mg of reduced glutathione and kept on ice until centrifuged at 4°C within 30 min. The plasma was stored at −80°C. Venous plasma (VP) norepinephrine was analyzed with HPLC (18). The duration of the LBNP stimulus was 4 min because, during this period, the increase
in venous norepinephrine is almost completely developed (17).

Venous compliance in the calf. The second part of the study was performed on 11 of 33 subjects [6 young (median age 22 yr, range 20–24 yr) and 5 old (median age 64 yr, range 60–66 yr) subjects], in whom venous compliance was studied in the right calf. With the subjects placed in a supine position, the skin and muscle fascia of the posterolateral muscle tissue compartment at the level of maximal circumference of the lower leg, ~15 cm distal to the knee, was anesthetized by 1–2 ml of lignocaine (10 mg/ml; Astra, Södertälje, Sweden). A needle surrounded by a polyethylene catheter with an outer diameter of 1.7 mm was inserted perpendicularly to the skin into the lateral gastrocnemius muscle (Venflon, Viggo Spectramed). The needle was withdrawn, and a 0.7-mm (ID) Teflon catheter with four side holes (Myopress, Athos Medical, Höby, Sweden) was inserted via the Venflon catheter, after which the Venflon was withdrawn. The pressure catheter was fixed with adhesive tape to the skin and connected to a pressure transducer (DT-XX disposable transducer, Viggo Spectramed), which was placed at the height that corresponded to the midpoint of the studied tissue segment of the calf ~5 cm below heart level. A three-way stopcock close to the pressure transducer was used to connect the pressure catheter to a pump (Perfusor Secura FT, B. Braun, Kronberg, Germany) delivering saline at 0.5 ml/h during the experiment to preserve catheter patency. In each subject, tests on the dynamic function of the pressure-recording system were performed at the beginning of the experiment by applying external compression to the tissue and asking the subject to perform active muscle contractions. These procedures normally resulted in rapid changes in the recorded pressure. When occasionally such tests produced unusually slow and small pressure deflections, or if there was retrograde filling of blood into the catheter, this was taken as a sign of inadequate catheter patency. In these cases, the catheter was removed and a new one was inserted at an adjacent site. The test with active muscle contractions was also performed at intervals between the experiments, and in a few cases there were signs of deterioration in catheter patency. Data from such recordings were omitted from the results presented.

After the catheter was inserted at a 4-cm depth, the legs were encased in the LBNP chamber as described in Cardiovascular response to hypovolemic circulatory stress. Applied negative external pressure has been shown to be transmitted into the tissue, causing an increase in transmural vascular pressure with only transient effects on intravascular pressure (2, 36). Because compliance of the arterial bed is only ~3% of that of the venous bed, almost exclusively venous blood is pooled in the lower part of the body, with the degree of pooling proportional to the negative pressure. The subsequent volume increase was calculated by means of strain-gauge plethysmography with the strain gauge placed around the maximal calf circumference ~15 cm below the knee and adjacent to the insertion of the pressure catheter. The subject was lying supine in the box with the left foot resting on a wooden plate to counter the suction force created by the negative box pressure. The right foot had no contact with the plate, to avoid muscle tension in the calf because this may affect muscle pressure and induce mechanical compression of the vascular tree (51, 54). Care was taken to place the midpoint of the right calf 5 cm below heart level in all subjects. To avoid any confounding external pressure the lowest part of the calf was at least 2 cm above the floor of the vacuum chamber.

Between each period of reduced external pressure, tissue pressure and calf volume were allowed to return to control levels. Repetitive analyses gave no indication that pressure transmission deteriorated with time or that control pressure increased with time because of possible edema formation. Tissue pressure was not affected by the discrete saline infusion, because no pressure changes were seen during arrested infusion. The applied transmural vascular pressure gradient was calculated from the measured tissue pressure change during LBNP, with the prevailing control level as baseline.

The basic data from these experiments have already been reported (46), and this part of the study only addressed the possibility of mobilizing capacitance blood from skeletal muscle and skin to the central circulation during a standardized reduction of venous transmural pressure. Thus, initially, LBNP of 42 mmHg (increase in venous transmural pressure) was applied for 4 min to allow complete filling of the capacitance vessels (50). The applied external negative pressure was then reduced in a standardized linear fashion (0.35 mmHg/s) from 42 to 0 mmHg (120 s), during which the changes in tissue pressure and calf volume, i.e., capacitance response, were continuously collected. Because the veins account for 97% of the vascular compliance, this is denoted venous compliance (Cv, ml·100 ml⁻¹·mmHg⁻¹). Cv was then calculated

\[
C_v = \frac{\Delta V}{\Delta P}
\]

where \(\Delta V\) denotes a change in calf volume (ml/100 ml) and \(\Delta P\) denotes a change in muscle pressure (mmHg). The calculations were performed for every 5-cmH2O (3.7 mmHg) decrease of tissue pressure and were related to the induced level of transmural pressure. At least two experiments were performed in all individuals, and the mean values were calculated.

Statistical evaluation. Values are expressed as means ± SE. Area under the curve for the changes in heart rate, blood pressure, forearm blood flow, and peripheral resistance was calculated. The significance of difference between the two groups was tested by unpaired Student’s t-test. To study Cv in the calf, a nonlinear mixed model was used that included Cv as a dependent variable and transmural pressure change as a covariate variable. The two groups of subjects (young and old) were included as fixed parameters, and the individual subjects as random-effect parameters. P < 0.05 was considered statistically significant.

RESULTS

Table 1 shows resting hemodynamic values in young and old subjects. It is seen that the old subjects had higher diastolic pressure and MAP (P < 0.001). Also, VP norepinephrine was higher in the old than in the young subjects (1.9 ± 0.2 vs. 1.1 ± 0.1 pmol/l; P < 0.05).

Both the young and old subjects showed good homogeneity of responses to hypovolemic circulatory stress and tolerated LBNP of 60 cmH2O (LBNPortho) well with no symptoms. One of the young men developed a more pronounced fall in systolic blood pressure and decrease in heart rate (vagal reaction) on one occasion. The results from this experiment are excluded.

Figure 1 shows the cardiovascular responses in young and old subjects during the hypovolemic stress caused by 8-min LBNPortho. This shows a progressive increase in heart rate and decrease in pulse pressure. The forearm blood flow decreased because of an increase in peripheral resistance. The increase in heart rate was
norepinephrine in young and old volunteers

<table>
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<th>Young</th>
<th>Old</th>
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<td>n</td>
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<tr>
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<td>180 ± 2</td>
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<td>Weight, kg</td>
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<td>MAP, mmHg</td>
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<td>93 ± 2</td>
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<td>PR, PRU</td>
<td>25 ± 2</td>
<td>33 ± 3</td>
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<tr>
<td>VP norepinephrine</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.2</td>
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Values are means ± SE. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FBF, forearm blood flow; PR, peripheral vascular resistance; PRU, PR units; VP, venous plasma; NS, not significant.

Table 1. Resting heart rate, systolic and diastolic blood pressure, mean arterial pressure, forearm blood flow, peripheral vascular resistance, and venous plasma norepinephrine in young and old volunteers

lower (P < 0.001) and the decrease in systolic blood pressure was less prominent (P < 0.05) in old compared with young subjects. Furthermore, the increase in peripheral vascular resistance was lower in old subjects (P < 0.01), with a less-pronounced reduction in forearm blood flow (P < 0.01).

Figure 2, left, shows a representative original recording in a 20-year-old subject, illustrating the changes of tissue volume in the upper arm caused by LBNP, orthostatic. There is an initial rapid decline of tissue volume, followed by a much slower but continuous decrease throughout the 8-min period of LBNP. At cessation of LBNP, tissue volume rapidly increased again and tended to stabilize for a few minutes on a new level lower than that before LBNP. Later, there was a slow and gradual increase toward the initial control level. Previous analyses (see MATERIALS AND METHODS) showed that this train of events reflects 1) an initial mobilization of regional blood toward the central circulation at onset of LBNP (capacitance response), followed by 2) a net transcapillary fluid absorption of fluid from the extra- to the intravascular space, and 3) on cessation of LBNP, by a rapid regain of regional blood content back to control level. The subsequent slow increase of tissue volume represents a transcapillary filtration of fluid that gradually restores the fluid volume prevailing in the tissue before LBNP. The capacitance response was 1.40 ml/100 ml, and the transcapillary fluid absorption was 0.097 ml·100 ml⁻¹·min⁻¹. Figure 2, right, shows a representative recording in a 61-year-old subject during 8-min LBNP, orthostatic. The capacitance response was reduced by 50% to 0.61 ml/100 ml and the transcapillary fluid absorption by 30% to 0.062 ml·100 ml⁻¹·min⁻¹ compared with the young subject.

Figure 3A shows that the capacitance response, i.e., the blood mobilization from the upper arm to the central circulation, during LBNP, orthostatic was decreased in old compared with young subjects (0.64 ± 0.07 vs. 1.37 ± 0.11 ml/100 ml; P < 0.001). Also, transcapillary fluid absorption (Fig. 3B) was lower in old compared with young subjects (0.068 ± 0.007 vs. 0.091 ± 0.008 ml·100 ml⁻¹·min⁻¹; P < 0.05).

During LBNP, orthostatic VP norepinephrine increased significantly (95 ± 28% in young and 61 ± 7% in old subjects; P < 0.001). The difference in increase between young and old was not significant (NS).

The blood pooling in the calf during LBNP, orthostatic was significantly lower in old compared with young subjects (1.66 ± 0.10 vs. 2.17 ± 0.13 ml/100 ml; P < 0.01). To accomplish similar hypovolemic circulatory stress (blood pooling in the lower part of the body), the LBNP stimulus was increased in a separate study to 80 cmH₂O in old subjects (LBNP, hypostatic). Of the 12 old indi-

![Fig. 1. Heart rate (HR), systolic blood pressure (SBP), mean arterial blood pressure (MAP), diastolic blood pressure (DBP), forearm blood flow (FBF), and peripheral vascular resistance (PR) in percentage of resting values in young (solid lines) and old (dashed lines) subjects during graded hypovolemic circulatory stress caused by lower-body negative pressure (LBNP) of 60 cmH₂O. SBP fall and HR increase were smaller in old subjects. Increase in PR was reduced with a concomitant reduction in FBF decrease in old subjects. Vertical bars denote SE. *P < 0.05; **P < 0.01; ***P < 0.001.](image-url)
Individuals investigated with LBNP ortho, 10 agreed to be included in the LBNP hypo study. One of these developed a more pronounced fall in systolic blood pressure and decrease in heart rate (vagal reaction) 1 min after onset of LBNP and was excluded. Thus data from nine old subjects were included when the hemodynamic circulatory responses to LBNP hypo were calculated. The hemodynamic responses to LBNP ortho in the three individuals who did not participate in the LBNP hypo study did not differ compared with the study group. Blood pooling in the calf during LBNP hypo was found to be similar ($2.23 \pm 0.24$ vs. $2.17 \pm 0.13$ ml/100 ml in old and young subjects; NS).

Figure 4 shows the hemodynamic responses to LBNP hypo in the two groups. There were no significant differences in the changes of heart rate or blood pressure between the young and old subjects. There was a slightly lower heart rate increase in the old subjects ($P = 0.06$). Furthermore, the increase in peripheral resistance was similar, with a concomitant equal reduction in forearm blood flow.

Figure 5A shows that the capacitance response, i.e., the blood mobilization from the upper arm to the central circulation, during LBNP hypo was still significantly decreased by $\sim 50\%$ in the old subjects ($0.67 \pm 0.10$ vs. $1.37 \pm 0.11$ ml/100 ml in old and young subjects; $P < 0.001$). Figure 5B shows that the transcapillary fluid absorption during LBNP hypo was similar ($0.091 \pm 0.008$ vs. $0.090 \pm 0.009$ ml·100 ml$^{-1}$·min$^{-1}$ in young and old subjects; $P = $ NS). No difference in venous norepinephrine increase ($\%$) during LBNP hypo was seen ($95 \pm 28$ and $87 \pm 15\%$ in young and old subjects, respectively; $P = $ NS).

Figure 6 shows the venous compliance in the calf in young and old subjects. Only the decrease in transmural pressure from 18 mmHg downward is shown. This reveals that the compliance increased significantly both in young ($P < 0.0001$) and old ($P < 0.005$) subjects.
during a decrease in transmural pressure gradient. In the young subjects the increase in compliance, especially at low transmural pressures, was nonlinear and much more apparent than in the old subjects (P < 0.0001). For comparison, earlier published values on Cv calculated at higher transmural pressure gradients (interval 18–51 mmHg) are depicted at right; this was reduced in old subjects and was not pressure dependent.

The time for 50% of the capacitance response in the upper arm to be developed (C50), i.e., the mobilization of the regional blood content from the upper arm to the central circulation, was calculated during LBNPhypox (similar hypovolemic circulatory stress in young and old subjects). This showed that C50 was significantly longer in the old than in the young subjects (16.4 ± 1.1 vs. 9.4 ± 0.9 s, respectively; P < 0.001).

Fig. 4. Hemodynamic response to hypovolemic circulatory stress caused by LBNP of 60 cmH2O in young (solid lines) compared with LBNP of 80 cmH2O in old (dashed lines) subjects, leading to similar hypovolemic circulatory stress in the 2 groups. HR, SBP, MAP, DBP, FBF, and PR in percentage of resting values in young and old subjects are shown. Vertical bars denote SE. No significant hemodynamic changes were found between young and old subjects.

Fig. 5. A: capacitance response (mobilization of regional blood from upper arm to central circulation) in upper arm during hypovolemic circulatory stress caused by LBNP of 60 cmH2O in young and 80 cmH2O in old subjects, leading to similar hypovolemic circulatory stress in the 2 groups. Vertical bars denote SE. Capacitance response was reduced by 50% in old subjects. B: transcapillary fluid absorption from tissue to blood in upper arm during hypovolemic circulatory stress caused by LBNP of 60 cmH2O in young and 80 cmH2O in old subjects, leading to similar hypovolemic circulatory stress in the groups. Transcapillary fluid absorption was similar in young and old subjects. NS, not significant. ***P < 0.001.

Fig. 6. Venous compliance in calf in young (○) and old (□) subjects. Compliance increased significantly both in young (P < 0.0001) and old (P < 0.005) subjects during a decrease in transmural pressure gradient (arrows). In young subjects, increase was nonlinear and much more apparent at low transmural pressures than in old subjects (P < 0.0001). Vertical bars denote SE. For comparison, earlier published values (44) on venous compliance calculated at higher transmural pressure gradients (18–51 mmHg) are depicted at right; this was reduced in old subjects and was not pressure dependent.
DISCUSSION

Venous compliance $C_v$ in the legs has been found to be reduced with age in humans, with a concomitant reduction in capacitance response (33, 46). This affects the blood pooling in dependent regions and might be a confounding factor in the study of cardiovascular responses during hypovolemic circulatory stress with experimental approaches such as LBNP and tilting, leading to misinterpretations in the changes of baroreceptor responses with age. The aim of this study was to reevaluate the changes found in baroreceptor function with age with confounding factors borne in mind. A reduced compensatory baroreceptor reflex response was found with age during a LBNP-induced orthostatic stimulus ($\text{LBNP}_{\text{ortho}}$ 60 cmH$_2$O in young and old subjects), with smaller increases in heart rate and peripheral resistance as well as reduced transcapillary fluid absorption from skeletal muscle and skin to blood. This was found to be caused by reduced blood pooling (capacitance response) in the legs in response to LBNP with a concomitant smaller central hypovolemic stimulus in the old subjects rather than reduced efficiency of the reflex response. With similar blood pooling in the lower part of the body [LBNP 60 cmH$_2$O vs. 80 cmH$_2$O ($\text{LBNP}_{\text{novo}}$) in young and old subjects], and thus presumably similar central hypovolemic circulatory stress, no differences in the compensatory baroreceptor reflex responses with age were found. Our study implies that the reduced efficiency in the baroreceptor reflex axis found in earlier studies (22, 13) is well compensated with age. However, the capacitance response in skeletal muscle and skin not exposed to an increased hydrostatic pressure load, i.e., the first line of defense during hypovolemic circulatory stress (leading to mobilization of peripheral blood toward the central circulation, increasing the effective circulating blood volume), was reduced in the old subjects (Fig. 5). This might seriously impede the possibility of survival of an acute blood loss.

LBNP was used in this study to pool blood in the capacitance vessels in the lower part of the body to create central hypovolemia to explore baroreceptor function changes in aging. During LBNP$_{\text{ortho}}$ in young and old healthy subjects we found a reduced increase in heart rate with age, which earlier was related at least partly to an attenuation of responses to $\beta$-adrenergic agonists (Fig. 1; Refs. 13, 22, 56, 60), although limitations in the withdrawal of parasympathetic tone affecting the initial phase of cardioacceleration may also play a role (59, 63).

Reduced $\alpha$-receptor-mediated responsiveness with attenuated vasoconstriction was found with increasing age in humans in both in vivo and in vitro studies (31, 44, 57). This may be the reason for the elevated muscle sympathetic nerve activity (MSNA) found in older humans, being compensatory sympathetic adjustments that offset declining effector responses (12, 15, 43, 57). Furthermore, the active transmitter reuptake mechanism seems to be attenuated, and these mechanisms are likely to explain the elevated resting plasma norepinephrine level found in the old subjects (Table 1; Ref. 20). MSNA responses to intravenous administration of vasodilatory drugs are not impaired, and baroreflex control of MSNA during orthostatic stress seems rather to be augmented with advancing age in healthy humans (12). Despite these facts, we found an attenuated forearm vasoconstrictor response to sympathetic stimulation induced by LBNP$_{\text{ortho}}$ in the old subjects (Fig. 1). This could have been caused by a smaller release from sympathetic nerve endings and consequent lower synaptic concentration of norepinephrine. This seems to be refuted, however, by the increase in norepinephrine concentration during orthostatic stress that was similar in the two groups and by the fact that norepinephrine clearance decreases with age (19, 20).

There was a compensatory increase in circulating effective blood volume in response to the hypovolemic circulatory stress caused by LBNP$_{\text{ortho}}$ accomplished both by mobilization of blood from capacitance vessels and by net transcapillary fluid absorption from skeletal muscle and skin not exposed to hydrostatic pressure load (Fig. 2; Ref. 38). The transcapillary fluid absorption is caused by deactivation of central baroreceptors with a concomitant sympathetic stimulation. This establishes a reflex decline in capillary pressure caused by $\alpha$- and $\beta$-adrenergic adjustment of the precapillary-to-postcapillary resistance ratio, initiating a transcapillary driving force (37, 41). Both capacitance response and fluid absorption were significantly reduced in the old subjects with a defective compensatory increase in effective circulating blood volume in response to the hypovolemic situation (Fig. 3). The reduced fluid flux could have been caused by decreased responses to $\alpha$- and $\beta$-receptor agonists (31, 44, 57, 60). Another possibility that would affect the fluid absorption is a reduction of capillary fluid permeability and area in skeletal muscle and skin available for fluid exchange with age. This possibility, however, seems incompatible with earlier studies that showed no such changes in capillary fluid permeability characteristics (25, 33).

Thus, in older humans, there seems to be a generalized reduction in baroreceptor reflexes in response to orthostasis (Figs. 1 and 3; Refs. 8, 14, 22, 61). These findings could result in more pronounced blood pressure alterations, and postural hypotension has been reported to be common in the elderly. Most studies on the capacity of the cardiovascular system to adapt to orthostatic stress in old age, however, have included more or less disabled persons or patients with physical inactivity, bed rest, obesity, or cardiovascular disease, which can independently influence autonomic circulatory control during orthostasis (24, 39, 40, 55). In contrast, Mader (40) reported that postural hypotension is a relatively uncommon finding in healthy elderly persons and that its prevalence is significantly related to risk factors. In fact, our data show a less-pronounced blood pressure fall during orthostatic stress in the old subjects despite a smaller increase in peripheral vascular resistance or reflex tachycardia (Fig. 1) and confirm earlier studies on healthy old subjects (12, 58). The lack
of decline in arterial systolic and pulse pressures in the old subjects during orthostasis suggests that the central baroreceptors may not have been unloaded to the same extent as that observed in the young subjects. Thus the absence of a significant increase in peripheral vascular resistance and heart rate should not necessarily be interpreted as evidence for impaired arterial baroreflex control with aging (Fig. 1). An alternative explanation for the maintained regulation of arterial pressure despite an attenuated peripheral vasoconstriction in the old could be a smaller challenge to arterial blood pressure maintenance caused by decreased venous capacitance response with age in the lower limbs. This would reduce the decrease in central blood volume and, thus, the deactivation of baroreceptors. A smaller decrease in thoracic blood volume as well as in cardiac output was found during orthostatic stress in old compared with young subjects, suggesting a smaller shift in central blood volume to the lower extremities (14, 21). This is in accordance with the data presented here as well as earlier findings in our laboratory (33) showing a reduced capacitance response of the lower limbs with age. Frey and Hoffler (23) did not find such a reduction, however, but the age range in their study was lower, making the putative differences more difficult to detect. The causative factor for the reduction in capacitance response seems to be a reduced venous compliance in the lower limbs that decreases ~45% between 20 and 60 yr of age in healthy subjects (46). A possible explanation for the changes in vein compliance with a concomitant decrease in capacitance function might be the increase in collagen-to-elastin ratio as well as wall thickening found in veins with age (4).

To reveal the true physiological changes with age resulting from the chain of baroreceptor deactivation–sympathetic discharge–effector response, it is of fundamental importance to equalize the hypovolemic stimulus between young and old subjects. This was accomplished in separate experiments by using LBNP at 80 cmH2O (LBNP<sub>hyp</sub>) instead of 60 cmH2O in the old subjects, which led to similar blood pooling in the lower part of the body and, thus, presumably similar central hypovolemic circulatory stress in the two groups (see RESULTS).

The reflex cardiovascular response in the old subjects now reveals systolic blood pressure fall and reduction in pulse pressure similar to that in the young subjects (Fig. 4), suggesting that the baroreceptors may have been unloaded to the same extent in young as in old subjects. The resulting increases in peripheral vascular resistance and heart rate as well as the transcapillary fluid absorption from skeletal muscle and skin to blood were similar in the two groups (Figs. 4 and 5). These results are consistent with the study of Cléroux et al. (8) that showed unchanged sympathetic reflex responses to cold-pressor test with age, and they might be interpreted as evidence for a sustained arterial baroreflex control during aging (Fig. 4).

Although the baroreflex control seems to be maintained in elderly subjects, it is of interest to note the association between substantial hypotension and gastrointestinal vasodilatation during digestion as well as fluid loss or sodium depletion that has been found in elderly subjects (35). Blood volume is lower in older than in young humans, which might be related to total cell mass as well as to the level of physical activity in the elderly (11). Furthermore, fluid intake was reported to be decreased and fluid excretion by the kidneys increased in older subjects (52). Thus dehydration from whatever cause was shown to have more profound effects in the elderly, and a limitation in blood pressure homeostasis may be unmasked after a modest physiological stress such as diuretic-induced loss of sodium and reduction of extracellular volume (53).

A possible explanation for the increased cardiovascular susceptibility to hypovolemic challenge might be the fact that the capacitance response to hypovolemic circulatory stress, i.e., the mobilization of venous blood to the effective circulating blood volume, was reduced by 50% in old subjects (Figs. 3A and 3A). The veins can be looked upon as a voluminous reservoir containing 85% of the total blood volume that is designed to preserve a proper inflow of blood into the heart (49). The pronounced capacity and low resistance of this vascular section imply that even small pressure reductions in the central veins are followed by substantial mobilization of blood from peripheral vascular beds toward the heart. Although in vivo experiments on subcutaneous veins provided evidence for sympathetic constrictor responses (34), no evidence exists that active vеноconstriction of capacitance vessels in skeletal muscle (40–45% of body wt) provides an important mechanism translocating blood into the central circulation (28). Thus the main part of the venous reservoir is adjusted simply by means of passive changes. The reduced capacitance response in the old subjects could be a result of several factors. The differences in body weight between the groups, with a larger weight in the old subjects, might introduce an error in the estimation of the soft tissue-to-bone ratio (Table 1). An increased amount of soft tissue in the arm would indicate an overestimation of the capacitance response and an underestimation of the differences between the groups. Furthermore, muscle atrophy that may occur with aging seems to increase C<sub>euv</sub> at least in the calf, and would thus also lead to an underestimated of the differences in capacitance response between young and old subjects (6). Another confounding factor might be differences in venous filling before LBNP. The capacitance of an anatomic area relates the total volume contained within the vasculature to the prevailing transmural pressure. In our experiments, no measurements were made of the amount of blood held in the upper arm before the application of suction (V<sub>0</sub>), and there is no reason to believe that this volume is constant. To avoid inappropriate differences in V<sub>0</sub> between individuals, care was taken to place the arms at the same level. Furthermore, the subjects rested at least 45 min before institution of LBNP, during which time arm volume and blood flow became stabilized (see MATERIALS AND METHODS). Our experiments were then concerned with the quantity of blood that was mobi-
lized from the upper arm in response to the hypovolemic circulatory stress caused by LBNP. Because of the high Cv, small changes in intravenous pressure, owing to changes in blood flow, will have marked effects on venous volume. During LBNP arteriolar resistance increases by sympathetic stimulation of the arterial smooth muscle, and the flow tends to decrease (Figs. 1 and 4). This in turn decreases the pressure gradient from capillaries to large veins and the average small vein pressure decreases (48). Because the decrease in blood flow was attenuated during LBNPortho in the old subjects (Fig. 1), this might have led to differences between the groups, with a relatively larger venous volume in the older group which would result in an overestimation of the differences in capacitance response (48). During LBNPortho, however, with a similar reduction in blood flow between the groups, the capacitance response was still 50% lower in the old subjects (Fig. 5). Thus the conclusion is reached that the hypothesis of venous capacitance decline with age is valid.

The decline in capacitance might be caused by the increase in collagen-to-elastin ratio as well as wall thickening found in the veins with age (4), with stiffening as a consequence. An increased venous stiffness was shown earlier in both arms and legs in humans, analogous to the known increase in arterial wall stiffness with age (26, 30, 46). The stiffness of a vein can be ascribed quantitatively in terms of a relationship between its volume and distending (transmural) pressure. This is nonlinear, and at low pressures a small change in pressure leads to a large change in volume, so that compliance is high. The early expansion phase of the veins involves no actual stretch of the elastic material in its wall, and a small change in distending pressure merely changes the geometry of the veins (45, 47). Once the veins have assumed a circular cross section, subsequent increases in their transmural pressure are opposed by the development of increased tension in the walls, and at higher pressure compliance is lower (62). In the experiments on Cv in the calf no data on baseline venous pressure were collected, which might be a confounding factor in the study of Cv. The precautions adopted in our protocol, however, make this unlikely (see MATERIALS AND METHODS). Another factor of concern might be differences in transmission of the applied external negative pressure around the calf in young and old subjects, with a concomitant difference in applied transmural pressure gradient. This seems refuted, however, by the fact that 80% of the pressure is found to be transmitted in both young and old subjects (46). Our recent study (46) showed reduced Cv in old subjects and was focused on the part of the volume-pressure curve that had assumed linearity (transmural pressure increase 18–51 mmHg), indicating that the venous section containing the majority of the blood volume, i.e., the venules, had assumed a circular cross-sectional area. In the present investigation, however, the attention was focused on the lower, more compliant level of the volume-pressure curve, i.e., where even small transmural pressure changes in the peripheral veins are followed by substantial differences in central blood volume. This part of the curve is the principal culprit for mobilization of venous blood to the effective circulating blood volume during hypovolemic circulatory stress. It is of interest to note that the venous compliance showed a much more rapid increase, especially in the lowest part of the curve, in the young rather than in the old subjects (Fig. 6).

The rate of the capacitance response (mobilization of blood from skeletal muscle and skin) in the arm during hypovolemic circulatory stress was also found to be slower in the old subjects. Thus it seems that not only the amount of blood that may be mobilized but also the rate of mobilization is reduced in the old. The volume-pressure curve of a limb at rest represents the distributed properties of all veins (microvessels to large veins). Factors other than venous properties may affect this curve, such as rigid fascia that restricts expansion, especially in the upper part of the curve. In the lower part of the volume-pressure curve, extensive tethering of veins may limit their emptying and changes in the tethering might perhaps be an explanation for the demonstrated differences with age; however, data are lacking in this context. The volume-pressure relationship will also be affected by the vascular anatomy, which determines how large a fraction of the total volume is distributed within the smallest veins as opposed to the largest ones. This means that total Cv of the limbs depends on the size, relative number, and wall structure of each venous segment. Another factor of importance is the resting state of the muscles, because muscle contraction increases muscle pressure and affects Cv (10, 51).

The pathophysiological significance of the changes in the venous system with age has clinical implications. In fact, this crucial first line of defense comes into play within seconds during an acute hypovolemic circulatory stress, where both the compensatory increase in effective circulating blood volume and the rate of compensation are important factors to preserve homeostasis (Fig. 2). Thus this might seriously impede the possibility of survival of acute blood loss in the aging.

In conclusion, a reduced compensatory baroreceptor reflex response was found with increasing age during a LBNP-induced orthostatic stimulus, with a smaller increase in heart rate and peripheral resistance as well as reduced transcapillary fluid absorption from skeletal muscle and skin into blood. This seems to be caused by reduced blood pooling (capacitance response) in the legs in response to LBNP with a concomitant, smaller central hypovolemic stimulus in the elderly rather than a reduced efficiency of the reflex response. With similar blood pooling in the lower part of the body as in the young, and thus presumably similar central hypovolemic circulatory stress, no differences in the compensatory baroreceptor reflex responses with age were found. This implies that the reduced efficiency in the baroreceptor deactivation–sympathetic discharge–effector response that was reported previously, is well compensated with age. However, the capacitance response in skeletal muscle and skin not exposed to an increased
hydrostatic pressure load, i.e., the first line of defense during hypovolemic circulatory stress (leading to mobilization of peripheral blood toward the central circulation increasing the effective circulating blood volume), showed a 50% reduction in the old subjects. This might seriously impede the possibility of survival of an acute blood loss.

The authors thank Dr. Britt-Marie Jacobson, Bioanalytical Chemistry, Astra Hässle AB, Mölndal, Sweden, for the analyses of norepinephrine.

This study was supported by grants from the Medical Faculty, Lund University, Medical Research Council Grant 12661, and the Funds of Malmö University Hospital.

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Received 5 April 1999; accepted in final form 11 August 1999.

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