Evidence for unloading arterial baroreceptors during low levels of lower body negative pressure in humans

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For decades, low levels (i.e., <20 mmHg) of lower body negative pressure (LBNP) have been firmly believed to be a useful tool for separating the contribution of cardiopulmonary and arterial baroreflexes to circulatory control in humans. Whether such transient reductions in blood pressure and heart rate have been found unchanged at such levels. However, transient reductions in blood pressure (BP), followed by reflex compensation, may occur without detection, which could unload arterial baroreceptors. The purposes of this study were to test the hypothesis that the arterial baroreflex is engaged even during low levels of LBNP and to determine the time course of changes in hemodynamics. Fourteen healthy individuals (age range 20–54 yr) were studied. BP (Portapres and Suntech), HR (ECG), pulmonary capillary wedge pressure (PCWP) or pulmonary artery diastolic pressure (PDP) and right atrial pressure (RAP) (Swan-Ganz catheter) and hemodynamics (Modelflow) were recorded continuously at baseline and −15- and −30-mmHg LBNP for 6 min each. Application of −15-mmHg LBNP resulted in rapid and sustained falls in RAP and PCWP or PDP, progressive decreases in cardiac output and stroke volume, followed subsequently by transient reductions in both systolic and diastolic BP, which were then restored through the arterial baroreflex feedback mechanism after ∼15 heartbeats. Additional studies were performed in five subjects using even lower levels of LBNP, and this transient reduction in BP was observed in three at −5 and in all at −10-mmHg LBNP. The delay for left ventricular stroke volume to fall at −15-mmHg LBNP was about 10 cardiac cycles. An increase in systemic vascular resistance was detectable after 20 heartbeats during −15-mmHg LBNP. Steady-state BP and HR remained unchanged during mild LBNP. However, BP decreased, while HR increased, at −30-mmHg LBNP. These results suggest that arterial baroreceptors are consistently unloaded during low levels (i.e., −10 and −15 mmHg) of LBNP in humans. Thus “selective” unloading of cardiopulmonary baroreceptors cannot be presumed to occur during these levels of mild LBNP.

baroreflexes; arterial pressure; hemodynamics

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With invasive pulmonary artery catheterization, we measured beat-by-beat right atrial pressure and pulmonary capillary wedge pressure or pulmonary artery diastolic pressure (the latter two were used as an index of left atrial and thereby left ventricular end-diastolic pressure) at the onset, as well as during mild LBNP in healthy individuals. We also recorded noninvasively beat-by-beat arterial pressure and heart rate during the entire procedure, while beat-by-beat hemodynamic variables were derived from the Modelflow method (29, 35, 39), calibrated by a modified acetylene rebreathing technique (47). The purposes of this study were 1) to test the hypothesis that the arterial baroreflex is engaged during mild LBNP in humans; and 2) to determine the time course of changes in cardiac filling and central hemodynamics during low levels of LBNP.

METHODS

Subjects

Nineteen healthy volunteers (16 men, 3 women) were studied. They were 35 ± 11 yr old (mean ± SD), 178 ± 10 cm in height, and 77 ± 13 kg body wt. No subject smoked, used recreational drugs, or had significant medical problems. None was an endurance-trained athlete, and subjects were excluded if they exercised for >30 min/day and >3 times/wk (either dynamic or static exercise). No woman was pregnant or in the early follicular phase (menstruation) of her menstrual cycle during the experiments. The subjects were screened with a careful history, physical examination, and 12-lead electrocardiogram. All subjects were informed of the purpose and procedures used in the study and gave their written, informed consent to a protocol approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas.

Measurements

Heart rate and blood pressure. Heart rate was determined from lead II of the electrocardiogram, and beat-by-beat arterial pressure was measured noninvasively using finger photoplethysmography (Portapres, TNO-BMI, Amsterdam, The Netherlands). Cuff blood pressure was measured by electrosphygmomanometry (model 4240, SunTech Medical Instruments, Raleigh, NC) with a microphone placed over the brachial artery to detect Korotkoff sounds, and arm...
cuff mean arterial pressure and diastolic blood pressure were used as references for the finger arterial pressure measurements. Respiratory excursions were monitored continuously via a piezoelectric transducer (Pneumotrace II, UFI, Morro Bay, CA).

**Cardiac filling pressure.** A 6F balloon-tipped, flow-directed pulmonary arterial catheter (Edwards Swan-Ganz, Edwards Lifesciences, Irvine, CA) was placed under fluoroscopic guidance through a right antecubital vein into the pulmonary artery. Beat-by-beat right atrial pressure was measured at the proximal port of the catheter. Left ventricular end-diastolic pressure was estimated from pulmonary capillary wedge pressure or pulmonary artery diastolic pressure with the balloon of the Swan-Ganz catheter inflated or deflated. All intracardiac pressures were referenced to atmospheric pressure, with the pressure transducer (Transpac IV, CIVCO Medical Instruments, Kalona, IA) zero reading set at 5 cm below the sternal angle in the supine position. Pressure waveforms were amplified (Agilent M1165, Agilent Technologies, Andover, MA) and displayed on a strip-chart recorder (Astromed MT 95000, Astro-Med, West Warwick, RI) with 0.5 mmHg resolution.

**Hemodynamic variables.** Beat-by-beat cardiac output and stroke volume were derived from the Modelflow method (29, 35, 39), calibrated by the acetylene rebreathing technique (47), so that the baseline Modelflow cardiac output was made equal to the baseline acetylene cardiac output for each subject. Beat-by-beat systemic vascular resistance was calculated as \( \frac{\text{mean arterial pressure}}{\text{cardiac output}} \times 80 \) (expressed as dyn·s·cm\(^{-5}\)), while beat-by-beat mean arterial pressure was derived from the Beatfast Modelflow program (BeatScope 1.1a, Finapres Medical System BV, Amsterdam, the Netherlands).

Steady-state cardiac output was measured intermittently with the modified acetylene rebreathing technique (model MGA1100, Marquette, Milwaukee, WI) (47). This method has been validated in our laboratory against standard invasive techniques, including thermodilution and direct Fick during orthostatic stress with a typical error (expressed as coefficient of variation) of 4–5% (18). Steady-state stroke volume was calculated from cardiac output and the heart rate measured during the rebreathing. Steady-state systemic vascular resistance was calculated as \( \frac{\text{mean arterial pressure} - \text{right atrial pressure}}{\text{cardiac output}} \times 80 \). Mean arterial pressure was calculated using arm cuff blood pressure, where right atrial pressure was averaged during steady state.

**Protocol**

All experiments were performed in the morning >2 h after a light breakfast and >12 h after the last caffeinated or alcoholic beverage, in a quiet, environmentally controlled laboratory with an ambient temperature of ~25°C. The subject was placed in a Plexiglas LBNP tank sealed at the level of the iliac crests in the supine position. Suction was provided by a vacuum pump controlled with a variable autotransformer and calibrated against a mercury manometer. After at least 30 min of quiet rest, baseline data were collected for 6 min. Then ~15-mmHg LBNP was started over ~5 s at the end of expiration with breath held for ~10–15 cardiac cycles in 14 subjects. LBNP ~15-mmHg stage lasted for 6 min, followed by ~30-mmHg LBNP for 6 min, and then finally by a 3-min recovery period. Heart rate, blood pressure, respiratory waveforms, right atrial pressure, and pulmonary artery diastolic pressure were recorded continuously. Steady-state cardiac

![Fig. 2. Beat-by-beat RAP (A), PCWP and pulmonary artery diastolic pressure (PDP; B), systolic blood pressure (SBP; C), and diastolic blood pressure (DBP, D) before and at the onset of ~15-mmHg LBNP. BL, baseline; B5–B40: the 5th through 40th heartbeat after initiation of LBNP. Values are expressed as median and 25th as well as 75th percentile. *P < 0.05 compared with baseline.](image-url)
output was measured at baseline before data collection, and at the 6th min of −15- and −30-mmHg LBNP. In 9 of the 14 subjects, the balloon of the Swan-Ganz catheter was inflated at baseline and during initiation of −15-mmHg LBNP for ~20 s each to make sure that pulmonary artery diastolic pressure tracked pulmonary capillary wedge pressure and, therefore, left ventricular end-diastolic pressure accurately.

In a separate study, after ≥30 min of supine rest, LBNP of −5 and −10 mmHg was applied for 3 min each in an additional five healthy young subjects with invasive pulmonary artery catheterization. The balloon of the catheter was inflated in all subjects before and during initiation of LBNP, and, therefore, right atrial pressure and pulmonary capillary wedge pressure were measured continuously. In addition, beat-by-beat finger blood pressure and heart rate were recorded during the entire experimental procedures.

Data Analysis and Statistics

Data were sampled at 200 Hz with a commercial data-acquisition system (Biopac System, Santa Barbara, CA). Baseline data for −5- and −10-mmHg LBNP from five additional subjects were analyzed on a beat-by-beat basis and averaged for every five beats over 40 cardiac cycles before the onset of LBNP. Baseline data for −15- and −30-mmHg LBNP were averaged for 6 min. During −5-, −10-, and −15-mmHg LBNP, data were analyzed on the beat-by-beat basis for the initial 40 heartbeats; however, pulmonary capillary wedge pressure was analyzed for the initial 12 heartbeats, since the balloon of the Swan-Ganz catheter was inflated for only 20 s. Beat-by-beat pulmonary capillary wedge pressure and pulmonary artery diastolic pressure data during −15-mmHg LBNP were combined. Steady-state data during −15- and −30-mmHg LBNP were averaged from the 2nd to the 5th min.

Data are expressed as means ± SD (if normality test passed) or median (25th, 75th percentile) (if normality test failed). Variables at baseline and the initial 40 heartbeats during −15-mmHg LBNP were analyzed using one-way repeated-measures analysis of variance (RM ANOVA), if normality test passed, and using Friedman RM ANOVA on ranks (nonparametric test), if normality test failed. The Holm-Sidak or Dunn’s method was used post hoc for multiple comparisons vs. baseline. The correlation between the mean values of combined pulmonary capillary wedge pressure and pulmonary artery diastolic pressure, and pulmonary capillary wedge pressure alone, at baseline and during initiation of −15-mmHg LBNP was analyzed using the least squares linear regression model. Steady-state hemodynamic variables at baseline and during −15- and −30-mmHg LBNP were also analyzed using one-way RM ANOVA or Friedman RM ANOVA on ranks. Both individual and mean data obtained during −5- and −10-mmHg LBNP in five subjects were presented, and no statistics were carried out for these additional data. All statistical analyses were performed with a personal computer-based analysis program (SigmaStat, SPSS). A P value of <0.05 was considered statistically significant.

RESULTS

Beat-by-Beat Hemodynamic Responses

Figure 1 depicts original tracings of beat-by-beat arterial pressure, heart rate, and cardiac filling pressure before and at the onset of −15-mmHg LBNP from one subject. Application
of −15-mmHg LBNP resulted in a rapid and sustained fall in right atrial pressure (Fig. 2A), followed subsequently by a fall in pulmonary capillary wedge pressure or pulmonary artery diastolic pressure (Fig. 2B). Systolic and diastolic blood pressures decreased transiently and then were restored through the arterial baroreflex feedback mechanism after −15 heartbeats (Fig. 2C and 2D). Heart rate remained stable during −15-mmHg LBNP (Fig. 3A). There was a delay (i.e., 10 cardiac cycles) for stroke volume to fall during application of −15-mmHg LBNP (Fig. 3B). Cardiac output decreased progressively during −15-mmHg LBNP (Fig. 3C). Systemic vascular resistance did not change significantly during initiation of −15-mmHg LBNP, but started to increase after 20 heartbeats (Fig. 3D). The combined pulmonary artery diastolic pressure and pulmonary capillary wedge pressure data tracked pulmonary capillary wedge pressure alone accurately at baseline and during initiation of −15-mmHg LBNP (Fig. 4A). They were highly correlated (Fig. 4B), and the typical error for these two data sets was 5.9%.

In the additional study, the transient reduction in arterial pressure was observed in three subjects during −5-mmHg LBNP, and in all five subjects during −10-mmHg LBNP (Fig. 5). During −5-mmHg LBNP, systemic vascular resistance increased more rapidly in one of the two subjects who did not have the transient reduction in arterial pressure than those who did; however, the other subject had a similar systemic vascular resistance response to those who had the transient reduction in arterial pressure. Both right atrial pressure and pulmonary capillary wedge pressure decreased at −5- and −10-mmHg LBNP (Fig. 6). Heart rate remained stable during −5- and −10-mmHg LBNP in these subjects.

Steady-state Hemodynamic Responses

Table 1 shows steady-state hemodynamic responses. Steady-state cuff systolic blood pressure decreased during −30-mmHg LBNP (P < 0.001), while diastolic pressure did not change significantly during −15- or −30-mmHg LBNP (ANOVA, P = 0.783). Steady-state heart rate remained unchanged during −15-mmHg LBNP (P = 0.226), but increased during −30-mmHg LBNP (P < 0.001). Both right atrial pressure and pulmonary artery diastolic pressure decreased progressively during −15- and −30-mmHg LBNP (both ANOVA, P < 0.001). Steady-state cardiac output and stroke volume decreased during −15-mmHg LBNP and further decreased during −30-mmHg LBNP (both ANOVA, P < 0.001). Systemic vascular resistance increased gradually during −15- and −30-mmHg LBNP in all subjects (ANOVA, P < 0.001).

DISCUSSION

The major findings of this study are that 1) application of −15-mmHg LBNP resulted in a rapid and sustained fall in right atrial pressure, a subsequent fall in left ventricular end-diastolic pressure, a progressive decrease in cardiac output, followed by transient reductions in systolic and diastolic blood pressure; 2) both systolic and diastolic pressures were restored, presumably through the arterial baroreflex feedback mechanism, after −15 heartbeats; and 3) the transient reduction in arterial pressure was also observed in most subjects during initiation of −5-mmHg LBNP and in all during initiation of −10-mmHg LBNP. These results support our hypothesis and suggest that arterial baroreceptors are unloaded and the arterial baroreflex is consistently engaged during −10- and −15-mmHg of LBNP.

Time Course of Changes in Hemodynamics

We showed the time course of changes in cardiac filling and central hemodynamics during application of mild LBNP in healthy humans. The rapid and sustained fall in right atrial pressure at the onset of LBNP was caused by a quick reduction in venous return due to blood pooling in lower body capacitance vessels (54). Thus right ventricular output to the pulmonary vascular bed must have decreased instantly upon application of LBNP. We did not measure right ventricular output in the present study. However, Wolthuis et al. (55) found a reduction in plasma-bound isotope activity, as detected over the right anterior chest during LBNP, indicating a decrease in pulmonary blood flow and thereby pulmonary blood volume. Followed subsequently, left ventricular end-diastolic pressure decreased, suggesting a reduction in left ventricular end-diastolic volume. Although heart rate remained stable at the onset and during −15-mmHg LBNP, cardiac output decreased progressively.

The transient fall in systolic and diastolic blood pressure during initiation of −15-mmHg LBNP appeared to be attrib-
utable to the fall in cardiac output. In the present study, LBNP was started over \(-5\) s. Blood pressure was found decreased transiently at either rapid (0.3 s) or slow (15 s) initiation of \(-20\)-mmHg LBNP in previous investigations (22, 23). Our data are in agreement with those of Hisdal et al. (22, 23), and together demonstrate convincingly that low levels of LBNP generally do indeed produce a detectable transient reduction in blood pressure. The restoration of arterial pressure later on during low levels of LBNP was due to an increase in systemic vascular resistance.

However, Zoller et al. (56) did not find any transient changes in arterial pressure at the onset of mild (i.e., \(-10\) mmHg) LBNP, which appears to conflict with our findings. One potential explanation for these discrepancies may be attributable to different methodologies utilized to measure blood pressure. Zoller et al. inserted a polyethylene cannula into the brachial artery and advanced it 10–20 cm proximal to the antecubital fossa; thus blood pressure measured was closer to aortic (i.e., central) pressure. In our study, blood pressure was measured noninvasively using finger (i.e., peripheral) photoplethysmography. As suggested by Pawelczyk and Raven (37), it is conceivable that the peripheral amplification of a small change in central/aortic pressure undetected by Zoller et al. (56) may account for the differences between our findings and those of Zoller et al. It is of note that Zoller et al. did not apply \(-15\)-mmHg LBNP to their subjects; although \(-20\)-mmHg LBNP was applied, they did not report any dynamic changes in arterial pressure during initiation of \(-20\)-mmHg LBNP.

Consistent with the results of Hisdal et al. (23), we also observed a delayed (i.e., after 10 heartbeats) reduction in stroke volume during \(-15\)-mmHg LBNP in our subjects by using a different (Modelflow vs. ultrasound) method. Similar observations were made by Wieling et al. (41, 53) in healthy individuals on moving from the supine position to upright tilt. A reservoir of blood between the right and left ventricles (24, 50) was likely to account for the delayed fall in left ventricular stroke volume. Some of this blood is undoubtedly stored in the pulmonary circulation (i.e., the upstream reservoir), since Dubois and Marshall (14) showed that, usually during normal or deep respiration, the pulmonary capillary blood flow did not vary. With positron emission tomography, Cai et al. (7) showed that the lung (pulmonary) blood volume decreased by \(-22\%\) (i.e., \(-80\) ml) during \(-20\)-mmHg LBNP in healthy young men, supporting the concept of pulmonary circulation being a reservoir for filling of left ventricle.

**Evidence of Arterial Baroreflex Engagements**

We found in this study that application of \(-10\)- or \(-15\)-mmHg LBNP resulted in universal transient decreases in both SBP and DBP.
systolic and diastolic blood pressures, which were then re-
stored after ~15 heartbeats. Undoubtedly, the restoration of
blood pressure was, at least in part, through the arterial barore-
flex feedback mechanism. However, as has been noted for
decades, steady-state arterial pressure and heart rate were not
altered significantly during mild LBNP, appearing to argue
against the notion that the arterial baroreceptors are engaged
during this period. Heart rate remained unchanged, despite the
fact that the arterial baroreceptors were clearly unloaded at the
onset of and during ~15-mmHg LBNP. It is possible that vagal
withdrawal and sympathetic activation to the heart during the
transient reductions in arterial pressure could have been offset
or obscured by the simultaneous unloading of atrial, ventricu-
lar, or aortic receptors that activate cardiac-specific sympatho-
excitatory reflexes. For example, Flores et al. (15) proposed
that, if a tonically active Bainbridge reflex was functionally
important in humans, nonhypertensive LBNP should exert the
opposite effect, i.e., decrease cardiac sympathetic and increase
efferent vagal firing. The increase in systemic vascular resis-
tance but not heart rate accounted for the restoration of blood
pressure in our subjects. This observation supports the notion
that peripheral vascular rather than cardiac mechanisms are
essential to maintaining arterial pressure during orthostatic
stress in humans (48). Certainly, we cannot exclude the pos-
sibility that the cardiopulmonary baroreflex was also involved
in the blood pressure restoration.

It has been proposed that baroreceptors sense the mechanical
deformation of the arterial wall rather than the level of blood
pressure exclusively (3, 9). We did not measure the deforma-
tion of aortic and carotid arterial wall in this study. However,
using echo-tracking methods, it was observed in humans that stroke changes in common carotid arterial diameter were significantly modified during mild LBNP, suggesting that alterations in carotid geometry might modify the stretch applied at the site of the carotid receptors (30, 31, 36). A deformation of the ascending thoracic aorta was also found during mild LBNP in healthy men with magnetic resonance imaging techniques (45).

In addition, animal studies have shown that aortic baroreceptors may respond not only to changes in aortic pressure and/or deformation, but also to changes in aortic flow (10, 19). The location of arterial baroreceptors in the aortic arch and carotid sinuses seems to be ideal for sensing changes in cardiac output or cerebral blood flow. We found in this study that both cardiac output and stroke volume decreased progressively upon application of mild LBNP and reached statistically significant levels during the steady-state period. It is well known that stroke volume is the key variable in the heart rate-stroke volume-total peripheral resistance ("triple-product") relation that is directly affected by hydrostatic gradients (33), and it is a major determinant of flow in baroreceptive arteries, which modulates baroreceptor activity (19). Stroke volume changes translate into pulse amplitude and pressure changes, and these clearly modulate arterial baroreceptor activity (3, 8). Taken together, the transient fall in arterial pressure, along with the decreases in cardiac output and stroke volume in our subjects, provide strong evidence for unloading of arterial baroreceptors at the onset and during −10- and −15-mmHg LBNP. However, our study does not preclude unloading of cardiopulmonary baroreceptors during low levels of LBNP, although it should be noted that very low levels (i.e., −3 or −6 mmHg) of LBNP do not even consistently result in increased sympathetic nerve activity (13). Moreover, we cannot determine the proportional influence of cardiopulmonary vs. arterial baroreceptors on the restoration of blood pressure during low levels of LBNP. We can say though that arterial baroreceptors are definitely engaged and unloaded at −10- and −15-mmHg LBNP.

Study Limitations

There are at least two limitations in this study. First, arterial pressure was measured noninvasively and indirectly with the Portapres. This method might have reflected waves that do not reflect true central arterial pressure that the arterial baroreceptors see. Further work with left heart catheterization is necessary to confirm our findings. Second, in the present study, beat-by-beat hemodynamic variables were derived from the Modelflow method. Although this method has been validated in different populations under different conditions (4, 6, 11, 32, 39, 52), the absolute values of cardiac output obtained have never been shown to be the same as those from “gold-standard” invasive methods. Nevertheless, it has been shown that the Modelflow method tracks fast changes in hemodynamics during various experimental protocols, including postural stress and exercise (21, 25, 42, 44, 49, 51). In this study, tracking of changes rather than getting the absolute values are more important. Furthermore, we used the modified acetylene rebreathing technique as a reference and calibrated the Modelflow values to this external standard. Thus the proportion and time course of changes in hemodynamics reported in this study are likely to be valid.

In summary, we found, in the present study, that application of −15-mmHg LBNP resulted in a rapid and sustained fall in right atrial pressure, a fall in pulmonary capillary wedge pressure or pulmonary artery diastolic pressure (indicating a decrease in left atrial pressure, and then left-ventricular end-diastolic pressure), progressive decreases in cardiac output and stroke volume, followed by transient reductions in both systolic and diastolic pressures, which were restored through the arterial baroreflex feedback mechanism after −15 heartbeats. This transient reduction in arterial pressure was even observed in most subjects at −5- and in all at −10-mmHg LBNP. These results suggest that arterial baroreceptors are unloaded during low levels (i.e., −10 and −15 mmHg) of LBNP. Thus “selective” unloading of cardiopulmonary baroreceptors cannot be presumed to occur during low levels of LBNP in healthy humans.

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