Am J Physiol Regulatory Integrative Comp Physiol
281: R213–R221, 2001.

Beat-to-beat cardiovascular responses to rapid, low-level LBNP in humans

JONNY HISDAL, KARIN TOSKA, AND LARS WALLØE
Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, N-0317 Oslo, Norway

Received 29 November 1999; accepted in final form 7 March 2001

Hisdal, Jonny, Karin Toska, and Lars Walløe. Beat-to-beat cardiovascular responses to rapid, low-level LBNP in humans. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R213–R221, 2001.—The hypothesis tested was that there are significant transient changes in the cardiovascular variables after rapid onset and release of mild lower body negative pressure (LBNP, −20 mmHg), even in experimental situations where there is no detectable change in steady-state values. Twelve subjects participated in the study. Heart rate, stroke volume (SV), cardiac output, mean arterial pressure (MAP), total peripheral resistance (TPR), acral and nonacral skin blood flow, and blood flow velocity in the brachial artery were continuously recorded during the pre-LBNP period (0–120 s), during LBNP (120–420 s), and during the post-LBNP period (420–600 s). The main finding was that MAP is transiently but strongly affected by rapid changes in LBNP as small as −20 mmHg. There was also a characteristic asymmetry in cardiovascular responses to the onset and release of LBNP, particularly in the responses in SV. The transient changes in MAP indicate that the neural responses that affect TPR are not fast enough to compensate for the rapid changes in LBNP. In this case, the arterial baroreceptors will be activated as well as the low-pressure baroreceptors that sense central venous pressure. This must be taken into consideration in future discussions of the results of LBNP protocols.

lower body negative pressure; cardiovascular control; mean arterial pressure; stroke volume

THE LOWER BODY NEGATIVE PRESSURE (LBNP) technique was introduced by Stevens and Lamb (20) in 1965 to study circulatory responses to simulated gravitational shifts of blood in humans. LBNP induces fluid shifts by pooling blood in the legs and abdomen and activates a number of cardiovascular adjustments that tend to maintain central blood volume and arterial pressure. The cardiovascular responses to different levels and durations of LBNP have been widely studied during the last 40 years (1, 2, 6, 7, 15, 20), and the steady-state responses to different levels of LBNP are well known. Although different techniques have been used to measure the cardiovascular variables and to apply LBNP, there is relatively good agreement between the steady-state results in comparable studies. It is generally agreed that there is a decrease in central venous pressure (CVP), stroke volume (SV), cardiac output (CO), and forearm and leg blood flow, and an increase in total peripheral resistance (TPR) and heart rate (HR) on the introduction of LBNP. It is also generally accepted that mean arterial pressure (MAP) is not affected by mild LBNP (0 to −20 mmHg) (15). During lower levels of LBNP (0 to −20 mmHg), most authors report no change in MAP (6, 13, 14, 16); however, others have reported a small increase (12) or decrease in MAP (9, 17).

Because mild LBNP has traditionally been expected not to affect MAP, it has frequently been used to study the influence of cardiopulmonary baroreceptors on the human circulatory system. Some studies have questioned the concept that arterial pressure is maintained perfectly during mild hypovolemia by reflexes triggered by cardiopulmonary receptors (17, 21). Taylor et al. (21) used nuclear magnetic resonance imaging to measure the dimensions of a major barosensory area, the thoracic aorta, during different levels of LBNP. They were able to detect significant decreases in the ascending aortic pulse area during LBNP as mild as −10 mmHg. These authors found no significant changes in arterial pressure during −10 mmHg LBNP with uncontrolled breathing, but they showed that during controlled breathing, −10 mmHg LBNP increased systolic and pulse pressure. Pannier et al. (17) studied the pulsatile changes in blood pressure and arterial diameter with applanation tonometry and echo-tracking techniques at the sites of the common carotid artery and the carotid arterial bulb during −10 and −40 mmHg LBNP. They reported cyclic changes in tension for even −10 mmHg LBNP. Those studies show that the arterial baroreceptors are probably affected during the "steady-state" period of mild LBNP as well, even though changes in MAP are not normally detected.

A common feature of all studies using the LBNP technique to study circulatory responses to simulated gravitational shifts of blood in humans is that cardiovascular responses have not been continuously measured but only sampled once or a few times at different levels of LBNP.

Address for reprint requests and other correspondence: J. Hisdal, Dept. of Physiology, Inst. of Basic Medical Sciences, Univ. of Oslo, PO Box 1103 Blindern, N-0317 Oslo, Norway.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Thus, despite of the large numbers of studies where the LBNP technique has been used to study cardiovascular responses to gravitational shifts of blood, the time course of these cardiovascular responses has not yet been studied systematically. This is probably because of a lack of methods to measure beat-to-beat SV noninvasively or because it has not been possible to control the LBNP chamber with sufficient accuracy at the onset and release of LBNP. Regardless of the reason, knowledge of the transient cardiovascular responses caused by gravitational shifts of blood is still poor.

We expected that there might be significant transient changes in the cardiovascular variables that are different from those found during the steady-state periods of mild LBNP. We assumed such changes have not been detected in previous studies, because the time resolution of recordings of the cardiovascular variables has been too low or because they have been smoothed out because data have been averaged over specific time periods. If such transient changes occur during the onset and release of LBNP, they may contribute to the precise regulation of MAP reported during mild LBNP.

Our group previously developed and improved an ultrasound Doppler technique for continuous recording of beat-to-beat SV for long periods of time (5). To be able to study the transient cardiovascular responses caused by rapid shifts of blood, we developed and constructed an LBNP chamber that allows us to regulate precisely both the rate of pressure change and the set LBNP level. We can thus record the cardiovascular variables with a high sample frequency and regulate the onset, release, and extent of LBNP very precisely.

Our hypothesis is that there are significant transient changes in the cardiovascular variables after rapid onset and release of mild LBNP (−20 mmHg) even in experimental situations where there is no detectable change in steady-state values.

METHODS

Subjects. Twelve volunteers, six female and six male {age 23.3 (2.9) yr [mean (SD)], height 175.5 (6.0) cm, weight 67.7 (9.2) kg}, were studied. All were nonsmokers and in good physical shape. None was taking any medication. The subjects were not allowed to drink coffee or tea on the experimental day or to exercise or eat for at least 2 h before the start of the experiment. Written informed consent was obtained from all participants, and the study was approved by the local ethics committee.

Experimental design. The experiments were carried out at the University of Oslo. Each test subject was lightly clothed and lay comfortably on a bench, with the lower body inside the LBNP chamber, which was sealed at the level of the iliac crest. The ambient temperature was between 25 and 28°C and was adjusted to obtain large fluctuations in the brachial arterial blood flow velocity, indicating that the subjects were in their thermoneutral zone (22). The subjects were acclimatized for 30 min before the experiment started. The experiment started with a 2-min period at room pressure in the LBNP chamber. This period is defined as the pre-LBNP period. Two minutes after recording started, the pressure in the LBNP chamber was rapidly lowered to 20 mmHg below room pressure. This period lasted 5 min and is defined as the LBNP period. Five minutes after the onset of LBNP, the pressure in the LBNP chamber was released and returned to room pressure. This period lasted 3 min and is defined as the post-LBNP period. Five identical experiments were run for each test subject, two on the first visit to the lab and three on the second visit. All experiments were carried out between 9 AM and 4 PM, and each test subject was asked to arrive at the lab to start the experiment at the same time for both visits to reduce any effects of his or her circadian rhythm.

LBNP. LBNP was applied using a custom-built chamber and pressure-control system designed to introduce rapid changes in LBNP. The chamber is a transparent polymethylmethacrylate (PMMA) tube that encloses the patient's lower body. This tube is connected to a vacuum pump and tank via hoses and motor-actuated valves. Pressure inside the chamber is monitored by a pressure sensor (Lucas Novasensor NPC-1210). A microcontroller compares the measured pressure with the pressure set point at a frequency of 50 Hz. If there is a difference, the controller sends signals to the valves to modulate chamber pressure and reduce the difference. In this study, the pressure was reduced to 20 mmHg below room pressure in <0.3 s and then returned to room pressure in <0.3 s. Figure 1 shows the pressure in the LBNP chamber during one experiment.

Measurements. Beat-to-beat SV was recorded by an ultrasound Doppler method (5). A bidirectional ultrasound Doppler velocimeter (SD-100, GE Vingmed Ultrasound, Horten, Norway) was operated in pulsed mode at 2 MHz with a hand-held transducer. The ultrasound beam was directed

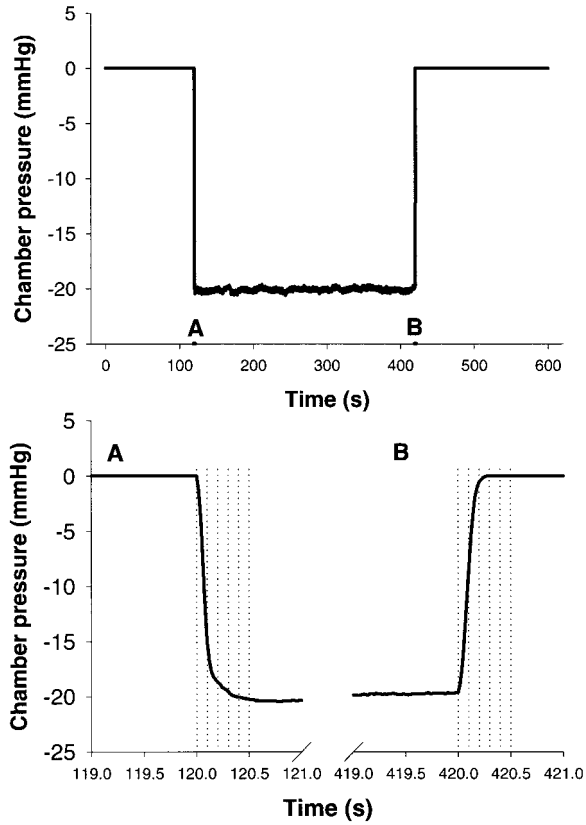

Fig. 1. *Top*: chamber pressure (mmHg) during 1 experiment. The pressure is sampled at a frequency of 50 Hz. 0–120 s, Pre-lower body negative pressure (LBNP) period; 120–420 s, LBNP period (−20 mmHg); and 420–600 s, post-LBNP period. *Bottom*: chamber pressure during the same experiment from 1 s before to 1 s after the onset (A) and release (B) of LBNP.

from the suprasternal notch toward the aortic root, and the sample volume range was adjusted so that measurements were made 1–2 cm above the aortic valve. We positioned the sample volume range centrally in the aorta by searching for the highest obtainable velocity signal. An angle of 20° between the direction of the sound beam and the bloodstream was assumed in the calculations. To remove vessel wall and valve motion artifacts, together with any recorded diastolic movement of blood, the built-in high-pass filter in the SD-100 was set to remove signals originating from velocities of <0.275 m/s. The output of the SD-100 maximum velocity estimator and a three-lead surface electrocardiogram (ECG) were interfaced online to a personal computer. In a separate session, the diameter of the rigid aortic ring was determined by parasternal sector scanner imaging (CFM-750, GE Vingmed Ultrasound). We assumed that the orifice was circular and used this diameter to calculate the area of the aortic valvular orifice. SV was calculated by multiplying the value obtained by numerical integration of the recorded instantaneous maximum velocity during each R–R interval by the area of the orifice. This calculation is based on the assumption that the velocity profile is rectangular at the level of the valves and that this velocity is conserved as the central maximum velocity of a jet 3–4 cm upward in the aortic root (4, 5). Instantaneous HR was obtained from the duration of each R–R interval of the ECG signal. Beat-to-beat CO was calculated from the corresponding HR and SV values. Blood flow velocity in the brachial artery was measured using the ultrasound Doppler technique (SD-50, GE Vingmed Ultrasound). The operating frequency was 10 MHz. The circular transducer had a fixed angle of 45° between the sound beam and the underlying skin surface. The transducer was fastened to the skin of the cubital fossa with adhesive tape, and the ultrasound beam was directed toward the brachial artery. The instantaneous cross-sectional mean velocity was calculated by the SD-50 and fed online to the computer for beat-by-beat time averaging, gated by the ECG R waves. Laser-Doppler technique (MBF3D, Moore Instruments, Devon, UK) was used to measure skin blood flow in the pulp of the left second finger (acral skin area) and in the skin of the forearm (nonacral skin area). The laser-Doppler probes were fastened to the skin with narrow double-sided tape (Kontron Instruments). The noise-limiting filter of the instrument was set at its highest level (21 kHz), and the emitted wavelength was 820 nm. The flux output signal was filtered with a time constant of 0.1 s and sent to the computer. The sampling frequency was 2 Hz. Finger arterial pressure was recorded continuously (2300 Finapress BP, monitor, Ohmeda, Madison, WI). Care was taken to adjust the arm so the finger was at heart level. The instantaneous pressure output was transferred online to the recording computer, and beat-to-beat MAP was calculated by numerical integration. Arterial pressure obtained by this method has been shown to be in good accordance with central intra-arterial pressure in various situations (11, 18). TPR was calculated as MAP divided by CO (mmHg min/l). MAP was used as an approximation to the perfusion pressure across the systemic circulation, assuming CVP to be zero and unchanged by LBNP. CO was used as an estimate for averaged flow through the resistance vessels. We did not measure CVP in this study and are aware that we have probably overestimated TPR during LBNP, because venous pressure is lower in this period than before and after LBNP.

Data analysis. SV and blood flow velocity in the brachial artery were sampled beat by beat, gated by the ECG R waves. CO, MAP, and TPR were calculated for every heart beat. Acral and nonacral skin blood flow were sampled at a frequency of 2 Hz. Before the data were analyzed, all recorded variables were converted into a 2-Hz sampled signal by interpolation. Throughout the registration period, there is considerable beat-to-beat variation in the recorded variables. This variation has been reported by other authors (5, 8) and is partly due to the influence of respiration (8, 23). Variations in the recorded variables not related to the onset and release of LBNP were partly eliminated by calculating the average response from five identical experiments run in each subject. This was done using the coherent averaging technique (19, 24) synchronized by the onset of LBNP. Finally, we pooled the individual average curves for the twelve subjects for calculation of the interindividual averaged responses by finding the mean value in each set of synchronous samples for each 2-Hz time step (24). Frey et al. (6) studied women's responses to different levels of LBNP in the follicular and luteal phases of the menstrual cycle. They concluded that there were no significant differences between these two phases in the responses to LBNP. Frey et al. (6) also concluded that the responses of the women in their study to LBNP were qualitatively similar to those reported for male subjects (24). We have therefore considered the results from the females and males together in the present study. The mean value for the steady-state part of the pre-LBNP period (0–120 s) for each cardiovascular variable was used as a reference value. In a first statistical analysis, this value was compared with the value for the same variable in the steady-state part of the LBNP period (180–420 s) and the value in the steady-state part of the post-LBNP period (480–600 s). ANOVA for repeated measures was used to test for significant differences. If there was a significant difference, the values were tested against each other. Bonferroni adjustment of the P value for multiple comparisons was used. In a second analysis, transient values of the variables, observed a specified time after the onset and release of LBNP, were added to the model and were tested in the same way as previously described. All the analyses were performed using the statistical program SPSS. Differences were considered significant at $P < 0.05$.

RESULTS

Mean SV, HR, CO, MAP, TPR, acral skin blood flow, and blood flow velocity in the brachial artery from the whole registration period (600 s) are shown at *left* of Fig. 2. Changes in the same variables in the 10 s before to 60 s after the onset (A) and the release (B) of LBNP are shown at *right*.

Steady-state levels. All the physiological variables showed relatively stable resting values in the pre-LBNP period (0–120 s). All the measured variables also showed stable values after the first 60 s of the LBNP period (180–420 s) and the post-LBNP period (480–600 s). Table 1 shows the mean values for SV, HR, CO, MAP, TPR, acral and nonacral skin blood flow, and blood flow velocity in the brachial artery in the pre-LBNP period (0–120 s), the steady-state part of the LBNP period (180–420 s), and the steady-state part of the post-LBNP period (480–600 s).

Onset of LBNP. There were dramatic changes in the measured variables shortly after the onset of LBNP. Approximately 8 s after the onset of LBNP, SV started to decrease. During the next 50 s, it gradually dropped about 18% (slope −0.3 ml/s) and then stabilized at this level during LBNP. There was a small transient in-

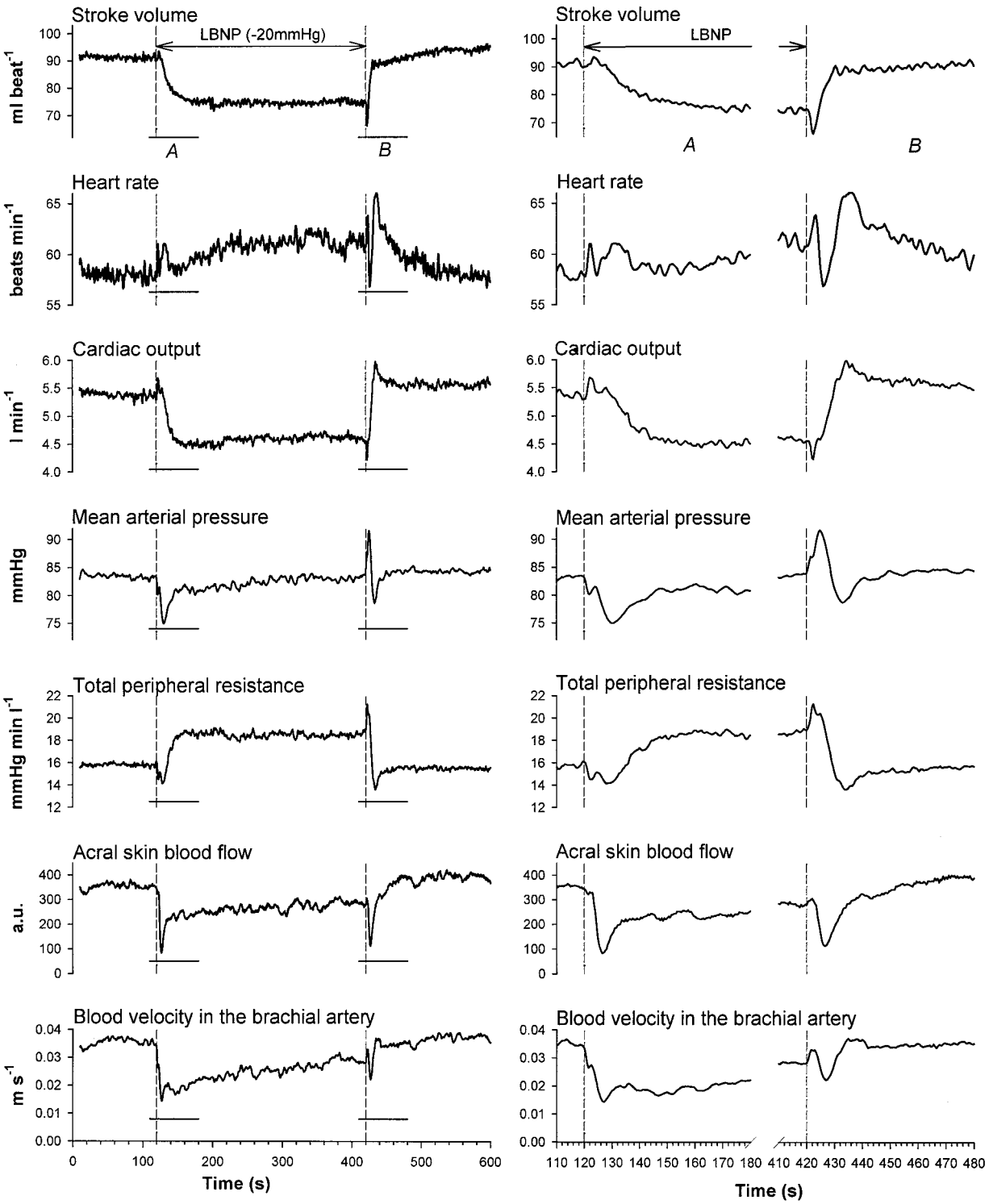

Fig. 2. *Left* shows mean stroke volume (SV), heart rate (HR), cardiac output, mean arterial pressure (MAP), total peripheral resistance, acral skin blood flow, and blood flow velocity in the brachial artery for the whole registration period (0–600 s). *Right* shows the corresponding values for the periods 110–180 s (A) and 410–480 s (B) or 10 s before to 60 s after the onset and release of LBNP.

crease in HR in the first seconds after the onset of LBNP, followed by a slow rise in the next 60 s, before HR stabilized. Because the changes in HR were relatively small, CO showed a very similar pattern to SV the first part of the LBNP period. CO stabilized after about 60 s at a level about 15% lower than in the control period (slope −8.7 ml/s).

MAP showed a transient decrease. First there was a small drop followed by a further decrease some seconds later, giving a 10% total reduction in MAP. This was a

Table 1. *Stroke volume, heart rate, cardiac output, mean arterial pressure, total peripheral resistance, acral skin blood flow, nonacral skin blood flow, and blood flow velocity in the brachial artery in the pre-LBNP period, steady-state part of the LBNP period, and steady-state part of the post-LBNP period*

	Pre-LBNP Period (0–120 s)	Last Part of LBNP Period (180–420 s)	Last Part of Post-LBNP Period (480–600 s)
Stroke volume, ml/beat	91.7 ± 13.8	74.8 ± 16.2*	93.9 ± 14.0
Heart rate, beat/min	57.9 ± 7.5	61.8 ± 8.4	58.1 ± 7.4
Cardiac output, l/min	5.4 ± 0.8	4.6 ± 0.8*	5.6 ± 0.8
Mean arterial pressure, mmHg	83.2 ± 7.3	82.2 ± 7.3	84.0 ± 7.6
Total peripheral resistance, mmHg·min/l	15.8 ± 3.3	18.5 ± 4.1*	15.8 ± 3.3
Nonacral skin blood flow, AU	18.8 ± 18.2	16.3 ± 18.5	18.9 ± 23.3
Acral skin blood flow, AU	364 ± 208	278 ± 196*	403 ± 207
Blood flow velocity in brachial artery, m/s	0.037 ± 0.023	0.028 ± 0.019*	0.040 ± 0.028

Values are means ± SD. LBNP, lower body negative pressure; AU, arbitrary units. *Significant difference ($P < 0.05$) from the pre-LBNP period.

significant reduction from MAP in the pre-LBNP period ($P < 0.0005$). After about 10 s, MAP started to rise and stabilized at approximately the resting value 30 s after the onset of LBNP. TPR also showed a transient decrease to a value about 12.5% lower than the pre-LBNP level. This was a significant drop from the value in the control period ($P = 0.001$). After this, TPR increased rapidly and stabilized at a level about 16% higher than the pre-LBNP level. There were no significant changes in nonacral skin blood flow after the onset of LBNP. Acral skin blood flow was not affected for the first 3 s after onset of LBNP but then started to decrease. During the next 4 s, it dropped significantly ($P = 0.004$) to the lowest values recorded during the test, ~77% below the pre-LBNP level. After this transient drop, it increased again, and about 14 s after the onset of LBNP it stabilized at ~25–30% below the pre-LBNP level. Blood flow velocity in the brachial artery also decreased in two steps after the onset of LBNP. The first step started immediately after the onset of LBNP, and blood velocity dropped by about 27% during the first 2 s. About 4 s after the onset of LBNP, velocity began to drop again and, during the next 4 s, fell significantly ($P = 0.003$) to about 50% of the mean velocity in the pre-LBNP period. It then started to rise again and had reached about 0.028 m/s at the end of the LBNP period, still 25% lower than before LBNP.

Release of LBNP. There were also dramatic changes in the physiological variables after the release of LBNP. After ~1 s we observed a significant transient drop in SV ($P = 0.008$). In the following 8 s, SV rose rapidly to about the same level as before LBNP (slope 2.62 ml/s). SV returned to pre-LBNP level during about 10–12 s after release of LBNP, whereas it took ~60 s before SV stabilized after onset of LBNP. There was a further slight increase in SV in the next 2 min. HR increased immediately after the release of LBNP and then dropped in the next 3 s before increasing again to a maximum level about 17 s after the release of LBNP. Figure 3 clearly shows the negative correlation between HR and SV in the first 6 s after the release of LBNP. We observed a drop in CO (9%) corresponding to the drop in SV ~1 s after the release of LBNP. Because the changes in SV were larger than those in HR, CO was still closely correlated with SV immediately after the release of LBNP. MAP rose significantly ~16% immediately after the release of LBNP ($P < 0.0005$). After ~5 s, it started to fall, reaching a minimum level significantly (~22%) under the mean level in the steady-state part of the LBNP period ($P = 0.002$). It then rose again and after ~21 s stabilized at the same level as before LBNP and in the steady-state part of the LBNP period. During the first 2 s after the release of LBNP, TPR rose significantly ($P < 0.0005$), to ~15% above the mean level in the steady-state part of the LBNP period. It then dropped gradually in the next 12 s to significantly (~26%) below the mean level in the steady-state part of the LBNP period ($P = 0.002$). After this it increased again and stabilized at the same level as before LBNP, ~25–30 s after the release of LBNP. We did not observe any significant changes in nonacral skin blood flow after the release of LBNP. Acral skin blood flow was also unchanged for the first 3 s after the release of LBNP, but after this we observed a significant decrease ($P = 0.049$). The time course and magnitude of this reduction in acral skin blood flow were very similar to what we observed immediately after the onset of LBNP. In the subse-

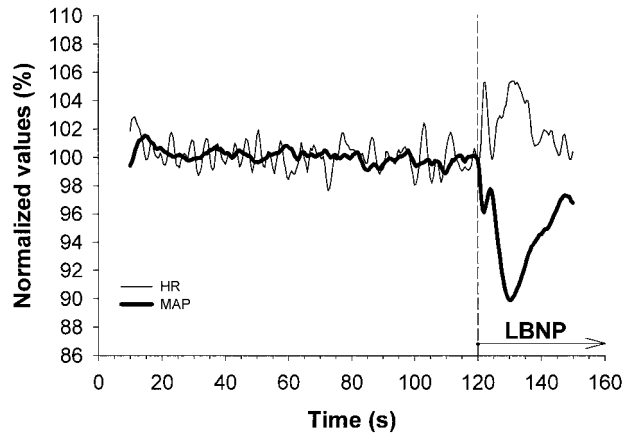

Fig. 3. HR and MAP in the pre-LBNP period and first 30 s of the LBNP period. The values are normalized to 100% for the average values of HR and MAP in the pre-LBNP period.

quent 10 s, acral skin blood flow increased again to about the same level as before LBNP. Blood flow velocity in the brachial artery increased in the first 3 s after the release of LBNP. A drop corresponding to the drop in acral skin blood flow followed this. Blood flow velocity in the brachial artery then increased steadily and stabilized at about the same level as before LBNP.

DISCUSSION

Cardiovascular changes at the onset and release of LBNP. Using our improved LBNP chamber, we observed cardiovascular changes at the onset and release of LBNP that to the best of our knowledge have not previously been described in the literature.

The most important finding in this study was that MAP is transiently but strongly affected by rapid changes in LBNP, even those as small as −20 mmHg. Because mild LBNP has traditionally been expected not to affect MAP, mild LBNP has frequently been used to study the influence of cardiopulmonary baroreceptors on the human circulatory system. Our findings, however, suggest that the neural responses that affect TPR are not fast enough to compensate for rapid changes in LBNP. In this case, the arterial baroreceptors will be activated in addition to the low-pressure baroreceptors that sense CVP, even for mild LBNP. Figure 3 shows the relationship between MAP and HR in the pre-LBNP period and during the onset of LBNP and the first part of the LBNP period. The transient changes in HR corresponding to the transient changes in MAP during the onset of LBNP clearly indicate a baroreflex involvement.

Taylor et al. (21) and Pannier et al. (17) have also both challenged the concept, and they provided results that indicated that the arterial baroreceptors are probably affected during steady state of mild LBNP, even though changes in MAP are not normally detected. These findings must be taken into consideration in future discussions of the results of LBNP protocols where the aim is to elicit reflex responses mediated by cardiopulmonary baroreceptors without activating the arterial baroreceptors.

After the rapid onset of LBNP we also observed a delay of ~8 s before SV started to fall. During this period, SV in the left ventricle is probably maintained by depleting the blood "stored" in the pulmonary vessels (26). Hoffman et al. (10) showed that when a dog was suddenly held upright for 20–30 s, right ventricular SV fell immediately, but the fall in left ventricular SV was delayed by 6–8 heartbeats. We did not measure CVP or right ventricle SV, but we believe that it is reasonable to expect the same pattern in our subjects after the onset of LBNP.

Eight seconds after the rapid onset of −20 mmHg LBNP, a steady decrease in SV started. SV stabilized at a level ~19% lower than the pre-LBNP level about 50 s after the onset of LBNP. After the release of LBNP, however, SV rose to its pre-LBNP value in ~10 s. Toska and Walløe (25) also described this asymmetry in the SV response in a study in which they recorded beat-to-beat SV in healthy humans during passive head-up tilt to 30°. They found that SV took 30 s to adjust on head-up tilt but stabilized at its pretilt value during the first 10 s after tilt back to a supine position. We believe that the asymmetry in the SV response after rapid changes in body position or LBNP can be explained mainly mechanically. After the onset of LBNP, the venous valves will restrict backward filling of the veins in the lower body. These veins must therefore be filled exclusively from the arterial side, in spite of the rapid reduction in LBNP. After the end of LBNP, the pressure around the veins suddenly increases, and the blood stored in the expanded veins during LBNP will rapidly be returned to the central circulation. This will cause an immediate increase in CVP and may result in a rapid adjustment of SV back to pre-LBNP values. Even if the CVP starts to decrease immediately after onset of LBNP, it appears to take 50 s before SV reaches a stable level.

Immediately after the release of LBNP, we also observed a transient fall in SV, the lowest values being reached ~2–2.5 s after the release of LBNP. SV was then ~12% below the stable level at the end of LBNP and 28% lower than before LBNP. In the following 8 s, SV returned to about the same level as before LBNP. The large drop in SV immediately after the release of LBNP is probably explained by many factors, one of which may be interventricular interaction. The sudden increase in filling of the right side of the heart may cause a reduction in the volume of the left side and thus a lower left ventricular SV. In addition, there may be an "afterload effect," i.e., a rise in end systolic volume resulting from the sudden increase in MAP. We observed a considerable increase in MAP immediately after the release of LBNP.

Figure 4 shows the relationship among MAP, SV, and HR after the release of LBNP. HR increased the first few seconds, but reached its peak level later than

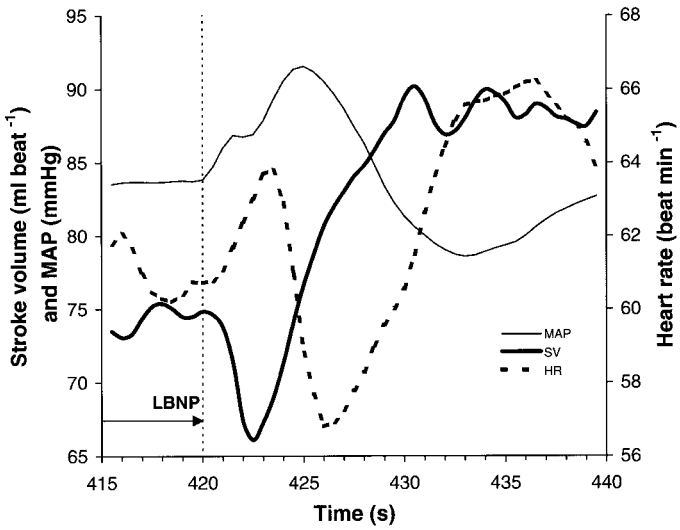

Fig. 4. SV, HR, and MAP in the period 415–440 s or 5 s before to 20 s after the release of LBNP. The dotted line at 420 s shows the release of LBNP.

SV reached its lowest value. The increase in HR is caused by reduced vagal activity. It is well known that the vagal response time is shorter than the sympathetic response time. During the period of stable LBNP, we expect the myocardial contractile force to be set at a stable level. The sympathetic response affecting myocardial contractile force is not fast enough to compensate for the sudden increase in MAP, and, as a result, the end systolic volume increases and SV decreases.

After the onset of LBNP, we observed an immediate fall in TPR. TPR fell in two steps before increasing again and stabilizing at a higher level during than before LBNP. We observed peripheral vasoconstriction in the period after the onset of LBNP. This will contribute to the rise in TPR. Because CO is maintained for the first 8 s after the onset of LBNP, other factors may also contribute to the decrease in TPR. One of these is probably the mechanical suction, which also affects the artery system in the legs. The final stable level of TPR was ~16% higher than before LBNP. These findings are in accordance with the observed vasomotor tone in acral skin. After the release of LBNP, we observed a transient increase in TPR before it fell again and stabilized at the same level as before LBNP.

Nonacral skin blood flow was found not to be affected by LBNP, indicating that nonacral skin probably does not participate in blood pressure regulation in the circumstances simulated in the present study. There were large differences between the subjects in nonacral skin blood flow. It is possible that despite careful probe positioning, veins draining remote skin areas pass nearby the optical probe, interfering with the measurements. Thus we cannot exclude the possibility that there were small changes in nonacral skin blood flow that we were unable to observe. Given the large area of nonacral skin, it is clear that even a small change in nonacral skin blood flow may affect TPR and MAP.

The changes in acral skin blood flow were larger. Circulation is relatively high in acral skin in thermoneutral humans, and we observed relatively high acral skin blood flow before LBNP (Fig. 2). Three seconds after the onset of LBNP, we observed a marked decrease in acral skin blood flow, probably caused by strong vasoconstriction. When we looked at the results of all the tests on each test subject, we noted that acral skin blood flow dropped to about the same level regardless of the level before LBNP (Fig. 5). This finding indicates that there was maximal vasoconstriction in acral skin areas in all subjects at this stage of the test. We also observed a similar drop in acral skin blood flow ~3 s after the release of LBNP. The time course and magnitude of the reduction in acral skin blood were very similar to what we observed immediately after the onset of LBNP. MAP, on the other hand, dropped immediately after the onset of LBNP and rose immediately after its release. This shows that the transient drop in acral skin blood flow starting ~3 s after the onset and release of LBNP is probably caused by psychological rather than physiological factors and may be described as a startle response. After the transient drop observed after the onset of LBNP, acral skin blood flow stabilized at a level ~25–30% lower than before LBNP. This indicates that vasomotor tone is higher during LBNP than before LBNP. This will result in higher TPR and is probably an important factor in maintaining MAP during LBNP.

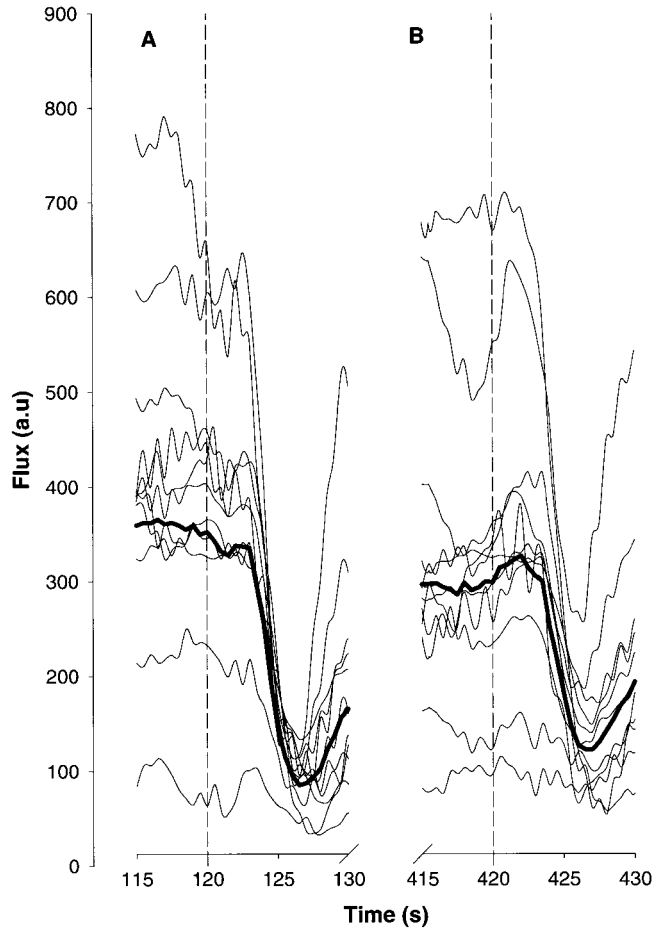

Fig. 5. Average acral skin blood flow for each of the 12 subjects in the periods 5 s before to 10 s after the onset (A) and release (B) of LBNP. The figures are found by coherent averaging for 5 identical tests in each subject. The bold line indicates the average response for all test subjects. The vertical dotted lines at 120 and 420 s indicate the onset and release of LBNP.

Blood flow velocity in the brachial artery dropped immediately after the onset of LBNP. In this study, we did not measure the brachial artery diameter, but a previous study in our group shows that the pulsatile diameter in small arteries is very stable (3). We therefore believe that the changes we observed in blood flow velocity during the LBNP period and after the release of LBNP reflect changes in blood flow in the brachial artery and not only changes in the artery diameter. Figure 2 clearly shows the strong correlation between acral skin blood flow, measured by the laser-Doppler technique, and the blood flow velocity in the brachial artery. The relationship between acral skin blood flow and brachial blood flow velocity supports our assumption that the method we used to measure brachial

blood flow velocity probably also reflects changes in blood flow in the brachial artery. The drop in blood flow velocity in the brachial artery occurs before vasoconstriction reduced blood flow in acral skin. This may be explained by increased resistance in the underarm muscles due to immediate vasoconstriction of the arterioles in the muscles. However, the method we used may also provide a partial explanation. The laser-Doppler probe was placed on the skin of the finger pulpa. We measured capillary flow in the skin, and the immediate fall in brachial blood flow velocity may be caused by vasoconstriction in the afferent arterioles and/or closure of the arteriovenous anastomoses (AVAs) in acral skin areas. These factors may raise TPR immediately after the onset and release of LBNP, leading to an immediate decrease in blood flow velocity in the brachial artery. Because we measured acral skin blood flow downstream of the arterioles and AVAs and because blood flow velocity in the capillary is rather low, it may take a few seconds before the vasoconstriction is observed by the laser-Doppler technique. During LBNP, blood flow in the brachial artery steadily increases despite the stable acral skin blood flow. This may indicate a drop in resistance in the underarm muscle. We also observed generally higher velocity in the brachial artery shortly after the release of LBNP than shortly after its onset, even though vasoconstriction in the acral skin was apparently of the same magnitude. This finding is probably explained by reduced resistance in the underarm muscles in this period.

Steady-state values during LBNP. In the present study, we obtained steady-state values during LBNP corresponding to a 19% reduction in SV, a 7% rise in HR, a 15% reduction in CO, no change in MAP or nonacral skin blood flow, a 24% reduction in acral skin blood flow, and a 25% reduction in blood flow velocity in the brachial artery. At the end of the post-LBNP period, the variables started to stabilize at around the same values as before. These findings are in accordance with those previously described for the steady-state periods before, during, and after exposure to −20 mmHg LBNP.

In conclusion, the main findings in this study were that MAP was transiently but strongly affected by the rapid onset and release of mild LBNP. This shows that the arterial baroreceptors will be activated as well as the low-pressure baroreceptors that sense CVP during rapid onset and release of mild LBNP. There was also a characteristic asymmetry in cardiovascular responses to rapid changes in LBNP, particularly in the responses in SV. The onset of LBNP resulted in a slow decrease in SV lasting some 50 s, whereas the release of LBNP caused a rapid increase of SV, back to the pre-LBNP level in <10 s.

Perspectives

Despite the large numbers of studies where the LBNP technique has been used to study cardiovascular responses to gravitational shifts of blood, the continuous time course of the cardiovascular variables has not yet been studied systematically. Therefore, knowledge of the transient cardiovascular responses caused by gravitational shifts of blood is still poor. In this study, we have revealed that there are significant transient changes in SV, HR, CO, TPR, acral skin blood flow, blood flow velocity in the brachial artery, and, most importantly, in MAP. Mild LBNP has traditionally been considered to elicit reflex responses mediated by cardiopulmonary baroreceptors only, without any arterial baroreflex involvement. However, because there are clear transient effects on MAP, at least during rapid onset and release of mild LBNP, it is possible that the arterial baroreceptors are in fact activated. If this is the case, they will contribute to the precise regulation of MAP reported during mild LBNP. This may be a potential artifact that must be taken into consideration in drawing conclusions from LBNP studies. In the future, care should be taken to observe transient changes in MAP during the onset and release of LBNP.

The authors thank the 12 volunteers for participation in this study. We also thank Professor Emeritus B. A. Waaler for valuable discussions and A. Sira, Ø. Løkeberg, and E. Salberg for technical assistance with the design and construction of the LBNP chamber.

This work was supported by the Norwegian Council on Cardiovascular Diseases.

Preliminary results from this study were presented as a poster at Experimental Biology, Washington, DC, 1999.

REFERENCES

1. **Brown E, Goei JS, Greenfield AD, and Plassaras GC.** Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to sub-atmospheric pressure. *J Physiol (Lond)* 183: 607–627, 1966.
2. **Chang CM, Cassuto Y, Pendergast DR, and Farhi LE.** Cardiorespiratory response to lower body negative pressure. *Aviat Space Environ Med* 65: 615–620, 1994.
3. **Eriksen M.** Effect of pulsatile arterial diameter variations on blood flow estimated by Doppler ultrasound. *Med Biol Eng Comput* 30: 46–50, 1992.
4. **Eriksen M, Waaler BA, Walloe L, and Wesche J.** Dynamics and dimensions of cardiac output changes in humans at the onset and at the end of moderate rhythmic exercise. *J Physiol (Lond)* 426: 423–437, 1990.
5. **Eriksen M and Walloe L.** Improved method for cardiac output determination in man using ultrasound Doppler technique. *Med Biol Eng* 28: 555–560, 1990.
6. **Frey MA and Hoffler GW.** Association of sex and age with responses to lower-body negative pressure. *J Appl Physiol* 65: 1752–1756, 1988.
7. **Frey MA, Mathes KL, and Hoffler GW.** Cardiovascular responses of women to lower body negative pressure. *Aviat Space Environ Med* 57: 531–538, 1986.
8. **Guz A, Innes JA, and Murphy K.** Respiratory modulation of left ventricular stroke volume in man measured using pulsed Doppler ultrasound. *J Physiol (Lond)* 393: 499–512, 1987.
9. **Halliwill JR, Lawler LA, Eickhoff TJ, Joyner MJ, and Mulvagh SL.** Reflex responses to regional venous pooling during lower body negative pressure in humans. *J Appl Physiol* 84: 454–458, 1998.
10. **Hoffman JI, Guz A, Charlier AA, and Wilcken DE.** Stroke volume in conscious dogs: effect of respiration, posture, and vascular occlusion. *J Appl Physiol* 20: 865–877, 1965.
11. **Imholz BP, Settels JJ, van der Meiracker AH, Wesseling KH, and Wieling W.** Non-invasive continuous finger blood pressure measurement during orthostatic stress compared with intra-arterial pressure. *Cardiovasc Res* 24: 214–221, 1990.

12. **Laszlo Z, Rossler A, and Hinghofer-Szalkay HG.** Cardiovascular changes during and after different LBNP levels in men. *Aviat Space Environ Med* 69: 32–39, 1998.
13. **Levenhagen DK, Evans JM, Wang M, and Knapp CF.** Cardiovascular regulation in humans in response to oscillatory lower body negative pressure. *Am J Physiol Heart Circ Physiol* 267: H593–H604, 1994.
14. **Lundvall J and Edfeldt H.** LBNP–a safe method in man capable of disclosing the wide range of baroreflex limb vascular resistance control exercised by the sympathetic vasomotor fibres. *Acta Physiol Scand* 146: 401–402, 1992.
15. **Mancia G and Mark AL.** Cardiopulmonary baroreflexes in humans. In: *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow*. Bethesda, MD: Am. Physiol. Soc., 1983, sect. 2, vol. III, pt. 2, chapt. 20, p. 795–813.
16. **Melchior FM, Thullier PH, Lejeune D, Kerguelen M, and Srinivasan RS.** Increase of LBNP tolerance using elastic compression stockings. *Aviat Space Environ Med* 67: 1161–1165, 1996.
17. **Pannier B, Slama MA, London GM, Safar ME, and Cuche JL.** Carotid arterial hemodynamics in response to LBNP in normal subjects: methodological aspects. *J Appl Physiol* 79: 1546–1555, 1995.
18. **Parati G, Casadei R, Groppelli A, Di Rienzo M, and Mancia G.** Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension* 13: 647–655, 1989.
19. **Rompelman O and Ros HH.** Coherent averaging technique: a tutorial review. Part 2: trigger jitter, overlapping responses and non-periodic stimulation. *J Biomed Eng* 8: 30–35, 1986.
20. **Stevens PM and Lamb LE.** Effects of lower body negative pressure on the cardiovascular system. *Am J Cardiol* 16: 506–515, 1965.
21. **Taylor JA, Halliwill JR, Brown TE, Hayano J, and Eckberg DL.** 'Non-hypotensive' hypovolaemia reduces ascending aortic dimensions in humans. *J Physiol (Lond)* 483: 289–298, 1995.
22. **Thoresen M and Walloe L.** Skin blood flow in humans as a function of environmental temperature measured by ultrasound. *Acta Physiol Scand* 109: 333–341, 1980.
23. **Toska K and Eriksen M.** Respiration-synchronous fluctuations in stroke volume, heart rate and arterial pressure in humans. *J Physiol (Lond)* 472: 501–512, 1993.
24. **Toska K, Eriksen M, and Walløe L.** Short-term cardiovascular responses to a step decrease in peripheral conductance in humans. *Am J Physiol Heart Circ Physiol* 266: H199–H211, 1994.
25. **Toska K and Walløe L.** The dynamic time course of left cardiac stroke volume after passive head-up tilt and tilt-back to supine in healthy humans (Abstract). *J Physiol (Lond)* 479: 82P, 1994.
26. **Waaler BA.** Physiology of the pulmonary circulation. *Angiologica* 266–284, 1971.