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J Appl Physiol 133: 20–26, 2022.
First published May 12, 2022; doi:10.1152/japplphysiol.00110.2022

american physiological society | JOURNAL OF APPLIED PHYSIOLOGY

RAPID REPORT

Graded lower body negative pressure induces intraventricular negative pressures and incremental diastolic suction: a pressure-volume study in a porcine model

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Abstract

Lower body negative pressure (LBNP) is a tool to study compensatory mechanisms to central hypovolemia for decades. However, the underlying hemodynamic mechanisms were mostly assessed noninvasively and remain unclear. We hypothesized that incremental LBNP reduces diastolic filling and thereby affects left ventricular (LV) diastolic suction (DS). Here, we investigated the impact of graded LBNP at three different levels of seal as well as during β-adrenergic stimulation by invasive pressure-volume (PV) analysis. Eight Landrace pigs were instrumented closed-chest for PV assessment. LBNP was applied at three consecutive locations: *I*) cranial, 10 cm below xiphoid process; *II*) medial, half-way between cranial and caudal; *III*) caudal, at the iliac spine. *Level III* was repeated under dobutamine infusion. At each level, baseline measurements were followed by application of incremental LBNP of −15, −30, and −45 mmHg. LBNP induced varying degrees of preload-dependent hemodynamic changes, with cranial LBNP inducing more pronounced effects than caudal. According to the Frank–Starling mechanism, graded LBNP progressively reduced LV stroke volume (LV SV) following a decrease in LV end-diastolic volume. Negative intraventricular minimal pressures were observed during dobutamine-infusion as well as higher levels of LBNP. Of note, incremental LV negative pressures were accompanied by increasing DS volumes, derived by extrapolating the volume at zero transmural pressure, the so-called equilibrium volume (V_0), related to LV SV. In conclusion, graded preload reduction via LBNP shifts the PV loop to smaller volumes and end-systolic volume below V_0, which induces negative LV pressures and increases LV suction. Accordingly, LBNP-induced central hypovolemia is associated with increased DS.

NEW & NOTEWORTHY This study examined the effects of incremental lower body negative pressure (LBNP) from −15 to −45 mmHg on hemodynamic regulation using invasive pressure-volume assessment in closed-chest pigs. Graded preload reduction via LBNP induces negative left ventricular (LV) pressures while increasing LV suction and thus allowing the ventricle to eject below the equilibrium volume at the end of systole. Accordingly, LBNP-induced central hypovolemia is associated with increased diastolic suction.

diastolic suction; invasive hemodynamics; lower body negative pressure; pressure-volume loops

INTRODUCTION

Lower body negative pressure (LBNP) has proved to be a useful tool to evaluate compensatory mechanisms for central hypovolemia for decades. As it induces pooling of venous blood in the lower part of the body, it reduces left ventricular (LV) end-diastolic volume (V_{ed}), stroke volume (SV), and cardiac output (CO) (1). Resulting compensatory mechanisms include early intrinsic neural reflexes such as vagal withdrawal and sympathetic activation as well as intrinsic and systemic neurohormonal activation of (nor-) adrenaline or volume regulating hormones. Therefore, LBNP can be used as a model to affect blood volume distribution, and thus stimulate hypovolemia from slight central blood

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Submitted 22 February 2022 / Revised 10 May 2022 / Accepted 10 May 2022

volume depletion progressing to simulated hemorrhagic shock (2, 3) or simulate effects of gravitational stress like an orthostatic challenge (4, 5).

Accordingly, there are numerous studies investigating the diverse effects of LBNP. Most studies are performed with human subjects and as very high levels of LBNP are associated with risk of cardiovascular decompensation this cannot be tested on human subjects. Mostly, effects are assessed by noninvasive measurements like Doppler probes or flow traces (6, 7), and therefore have limitations. As LBNP reduces preload it should have distinct effects on diastolic phase and ventricular relaxation. An important mechanism of diastolic filling is diastolic suction (DS), especially under conditions of stress (8, 9). In short, left ventricular (LV) suction describes the ability of the ventricle to generate an active suction force to pull in blood volume during diastole rather than just being passively expanded by the inflowing blood. There has been and still is controversy regarding the exact definition of DS (10). Although the debate on the exact physiological mechanisms behind remains open, it has been proposed to be linked to the rate of ventricular untwisting (11, 12) and end-systolic volume (13). As the ventricle contracts, it stores potential energy as elastic energy that is released in early diastole. DS is thought to play a role in valve disorders like mitral stenosis (14) and is reduced in patients with heart failure (15, 16). But to our knowledge, DS has not been evaluated in states of central hypovolemia.

Therefore, we set out to investigate the diastolic cardiovascular response mechanisms to incremental levels of LBNP including levels commonly considered potentially life-threatening by extensive invasive characterization of LV function using high-fidelity pressure-volume analysis in pigs. We hypothesized that incremental LBNP reduces diastolic filling and thereby affects LV DS.

MATERIAL AND METHODS

The experimental protocols were approved by the local bioethics committee of Vienna, Austria (Austrian Committee for Animal Trials, Registration Number 2020-0.272.246, in German "Tierversuchskommission") and conform to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No. 123, Strasbourg 1985).

Experimental Setup

The experimental setup has been described before (17). Briefly, eight human-sized female Landrace pigs (68 ± 9 kg) were intubated, and anesthesia was continued with 1.0–2.0 vol% sevoflurane (Sevorane, Abbott GmbH, Vienna, Austria), 30–35 μg/kg fentanyl (Fentanyl-Janssen, 0.1 mg ampoules, Janssen-Cilag GmbH, Vienna, Austria), 1–1.25 mg/kg midazolam, and 0.2 mg/kg pancuronium (Pancouroniumbromid 2 mg/mL ampoules, Ratiopharm GmbH, Ulm, Germany). Pigs were ventilated (Julian, Draeger, Vienna, Austria) with an FI_{O_2} (fraction of inspired oxygen) of 0.5 and *I*:*E*-ratio of 1:1.5, the positive end-expiratory pressure was set at 5 mmHg and tidal volume at 10 mL/kg. The respiratory rate was adjusted constantly to maintain an end-expiratory CO_2 partial pressure between 35 and 45 mmHg. Under fluoroscopic guidance, animals were instrumented with a Swan–Ganz catheter (Edwards Lifesciences CCO connected to Vigilance I, Edwards Lifesciences, Irvine, CA), an LV conductance catheter (5F, 12 electrodes, 7 mm spacing, MPVS Ultra, Millar Instruments, Houston, TX) and a PTBV-balloon (Osypka VACS II 20 mm, Rheinfelden, Germany), which was placed in the distal descending aorta at the level of diaphragm. Noninvasive oximetry and body core temperature were measured continuously. After instrumentation, pigs were allowed to stabilize until steady-state hemodynamics were reached. Animals remained supine throughout the trial and lower body was placed in a sealed plastic container. LBNP was generated inside the box by a vacuum pump. The pressure was continuously measured by a manometer and adjusted by regulating the air outflow through an adjustable leak.

Experimental Protocol

The protocol consisted of four measurement stages. The LBNP chamber was sealed at three different levels along the body: *I*) cranial, 10 cm below the xiphoid process, *II*) medial, half-way between cranial and caudal, *III*) caudal, at the anterior iliac spine and *IIId*) caudal level was repeated under dobutamine infusion (Fig. 1). Dobutamine was titrated to approximately double the maximum rate of LV pressure increase (dP/dT_{max}), as previously published (17). At each level, increasing LBNP was applied, i.e., −15, −30, and −45 mmHg. Due to experimental setup, the protocol was always started with LBNP at *level I*, followed by *level II* and *III* and ended with *level IIId*.

At each step, aortic occlusions were performed three times to acutely increase afterload and obtain pressure-volume relationships. Baseline was recorded over three respiratory cycles and end-expiratory PV-loops were averaged. Measurements taken after steady-state hemodynamics were attained. The steady state was defined as stable mean aortic pressure (mAOP), heart rate, and maximum pressure increase over at least 30 s. An average of 10–15 cardiac cycles per measurement step were studied, corresponding to the end-expiratory beats during three respiratory cycles. Premature ventricular contractions were excluded from the analysis. The protocol was discontinued if mAOP declined below 30 mmHg.

Data Processing and Statistical Analysis

Details on data analysis have been described previously (18). Conductance data were calibrated at each level by CO and injection of 3 boli of hypertonic saline (19). Pressure-volume data and time intervals were analyzed offline by CircLab software (custom made by P. Steendijk). End diastole was defined as the time point of zero crossing of dP/dT before its rapid upstroke. End systole was defined as the time point of maximum pressure/volume ratio. The end-diastolic pressure-volume relationship (EDPVR) was derived from an exponential fit of end-diastolic pressure and volume data points during aortic occlusion using the equation $P_{ed} = \alpha \times e^{\beta \times Ved}$.

The end-systolic pressure-volume relationship (ESPVR) was derived using a linear fit of end-systolic pressure and volume data points, characterized by the slope (end-systolic elastance) and volume axis intercept. To obtain single-point measurements of PV relationships, representative LV volumes at a fixed pressure were calculated at an end-diastolic pressure

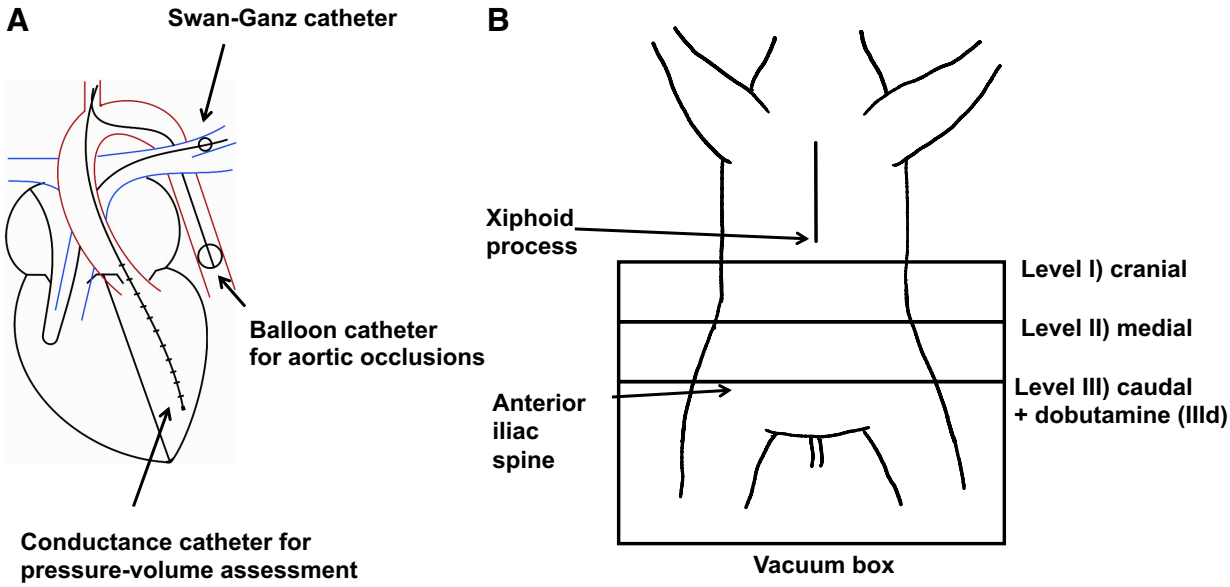

Figure 1. Sketch to visualize the trial arrangement. Eight female Landrace pigs were instrumented with a left ventricular (LV) conductance, Swan-Ganz, and a PTBV balloon catheter to invasively assess LV pressure-volume loops (*A*). The negative body pressure was generated in a vacuum box, which was sealed at three different body levels (*levels I–III*) (*B*). *Level I* lied 10 cm below xiphoid process, *level III* was at the anterior iliac spine, and *level II* was half-way in between *position I* and *III*. *Level III* was repeated under dobutamine infusion (*level IIId*). At every level a baseline was measured, after which pressure of −15, −30, and −45 mmHg was applied.

of 10 mmHg (VP_{ed10}) and end-systolic pressure of 100 mmHg (VP_{es100}). VP_{ed10} poses an index of capacitance, whereas VP_{es100} reflects contractility relatively independent from preload (20). The isovolumic relaxation constant τ (ms) was calculated based on the method described by Raff and Glantz (21). Time of diastole (t-dia) was calculated as time between end systole and end diastole. Equilibrium volume (V_0, volume of the resting ventricle at zero transmural pressure) was estimated as the latter intercept of the diastolic PV-loop with the volume line at $P = 0$ as the point where LV pressure returns to positive values. Suction volume (V_0^*) was calculated as difference of estimated V_0 and end-systolic volume ($V_0^* = V_0 - V_{es}$) (9, 22). The Frank–Starling curve in Fig. 2 was obtained by plotting SV and V_{ed} data points from aortic occlusions and LBNP at *level I*, and consequently applying a polynomial nonlinear regression (Fig. 2*A*).

All data are presented as means ± standard deviation. Steady-state data of *levels I*, *II*, and *III* at different steps were compared by two-way analysis of variance for repeated measurements. In the same way, dobutamine step (*level IIId*) was compared with *level III* only. If we stated a value to be not significantly influenced, there were no significant main or interaction effects. Correlation was assessed by linear regression and Spearman correlation. Post hoc testing was performed by Tukey test. A P value <0.05 was considered significant. All values are compared with baseline if not stated otherwise. Data analysis and graphical presentation were conducted with SigmaPlot Version 11.0 (Systat Software, Inc.).

RESULTS

Eight animals underwent catheterization. One animal was excluded as it suffered from dissection during positioning of the aortic balloon. Only two animals reached LBNP of −45 mmHg at *level I* without sign of hemodynamic instability. Aortic occlusions at −30 and −45 mmHg at *level I* could only be performed in one animal, so that corresponding aortic occlusion-derived data were excluded from analysis. Due to technical issues, a dobutamine step could not be performed in two of the animals.

Systemic Hemodynamics and LV Systolic Function

In general, LBNP was well tolerated up to −45 mmHg at *position II* to *IIId* whereas at cranial *level I* a maximal LBNP of −30 mmHg could be applied without inducing hemodynamic instability (mAOP < 30 mmHg).

Heart rate did not increase during any measurement steps (*level I*: 93 ± 13 to 88 ± 11; *level II*: 95 ± 21 to 98 ± 38; *level III*: 93 ± 21 to 88 ± 18; *level IIId*: 113 ± 43 to 114 ± 39 beats/min; interaction: $P = 0.309$), meaning reflex tachycardia, which is usually seen in human studies (1), could not be observed throughout our trial.

To visualize the main hemodynamic impact of LBNP, we plotted an exemplary Frank–Starling curve composed of data points from *level I* (Fig. 2*A*), as well as exemplary original tracings of PV loops of one animal at different levels and steps (Fig. 2*B*).

Graded LBNP led to a decrease of mAOP at all levels (*level I*: from 91 ± 18 to 34 ± 14, $P < 0.001$; *level II*: 97 ± 24 to 77 ± 35, $P = 0.020$; *level III*: 104 ± 16 to 76 ± 20, $P < 0.001$; *level IIId*: 118 ± 18 to 68 ± 40 mmHg, $P = 0.022$). LV end-diastolic pressure (P_{ed}) and LV end-diastolic volume (V_{ed}) were reduced at all levels, but the reduction was more pronounced at *level I* and *level IIId* (Fig. 3, *A* and *B*). This was paralleled by a CO decrease at all levels (*level I*: 6.3 ± 1.2 to 2.8 ± 1.9, $P < 0.001$; *level II*: 6.4 ± 1.4 to 3.8 ± 2.1, $P < 0.001$; *level III*: 6.1 ± 1.3 to 4.3 ± 0.9, $P < 0.001$; *level IIId*: 7.3 ± 2.1 to 3.2 ± 2.4 L/min, $P = 0.013$).

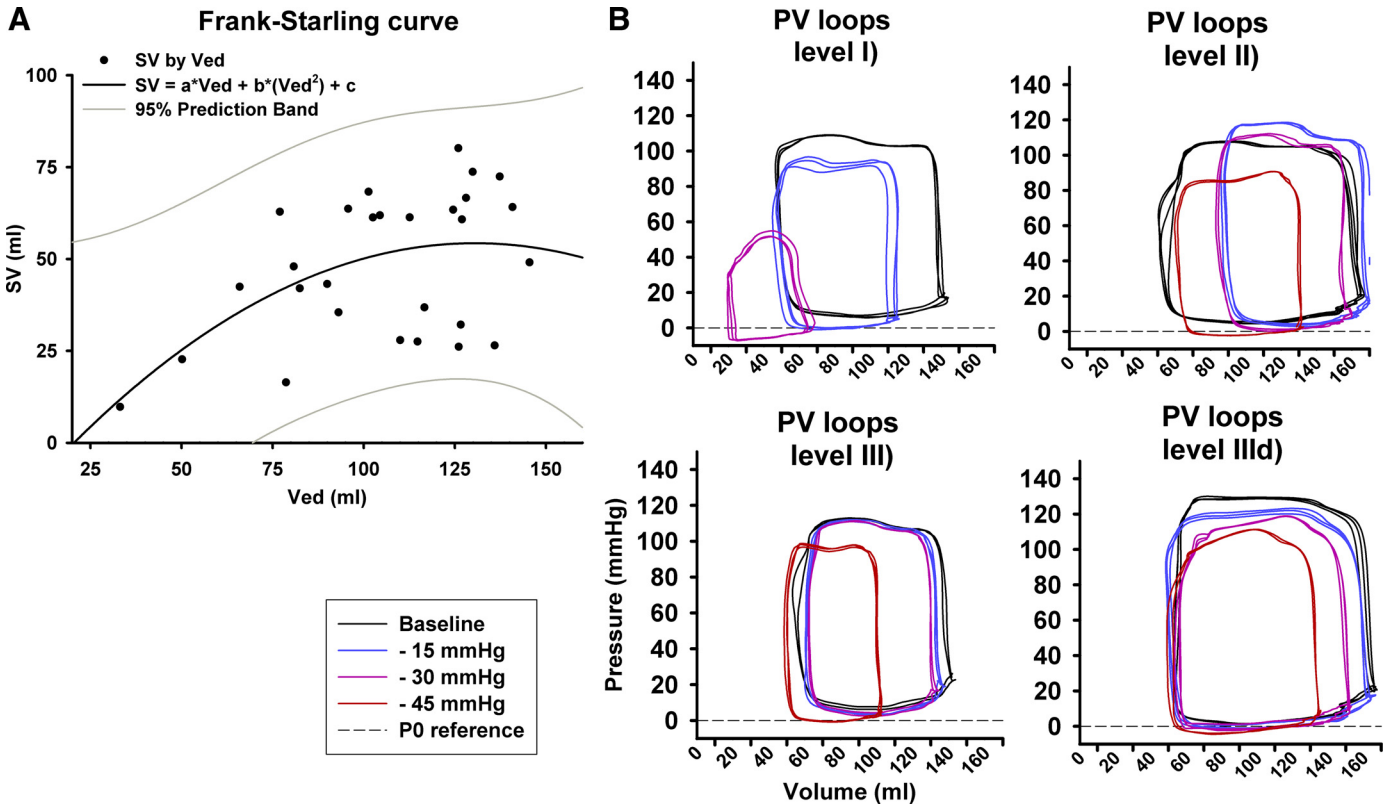

Figure 2. Frank–Starling plot and exemplary original tracings of pressure-volume loops (PV-loops). The Frank–Starling curve shows the dependency of stroke volume (SV) on ventricular pre-load, here represented by end-diastolic volume (V_{ed}). Regression line is applied as a polynomial fit and accompanied by 95% prediction band (*A*, *n* = 25 from *level I*). Exemplary PV-loops obtained at the different locations of lower body negative pressure (LBNP) show the pressure- and volume lowering effects of LBNP. Dashed line shows a reference at zero pressure. Note the more pronounced effects at cranial body location and the pressure reaching negative levels at higher amounts of LBNP (*B*).

Ejection fraction did not change significantly (*level I*: 50 ± 8 to 44 ± 27; *level II*: 48 ± 6 to 40 ± 10; *level III*: 49 ± 8 to 53 ± 20; *level IIId*: 55 ± 11 to 40 ± 16%; interaction: $P = 0.648$). LV dP/dT_{max} doubled under dobutamine infusion compared with *level III* and fell at *level I*, *level II*, and *level IIId* (*level I*: 1,832 ± 557 to 769 ± 470, $P < 0.001$; *level II*: 1,982 ± 563 to 1,664 ± 1,205, $P = 0.043$; *level III*: 2,060 ± 400 to 1,896 ± 609, $P = 0.806$; *level IIId*: 4,373 ± 743 to 2,557 ± 1,864 mmHg/s, $P = 0.025$). VP_{es100} showed no significant change during graded LBNP (*level I*: 70 ± 27 to 57 ± 29; *level II*: 72 ± 17 to 69 ± 25; *level III*: 61 ± 32 to 49 ± 35; *level IIId*: 49 ± 10 to 56 ± 6 mL; interaction: $P = 0.401$).

Diastolic Properties

Minimal rate of LV pressure decrease (dP/dT_{min}) was more negative in dobutamine at baseline, hinting to the lusitropic effects of dobutamine. dP/dT_{min} increased at all levels, again the effect was more pronounced at *level I* and *level IIId* (Fig. 3*C*) while peak flow rate fell in all levels (Fig. 3*D*).

The isovolumic relaxation coefficient τ was only prolonged at *level I* (*level I*: 43 ± 7 to 77 ± 41, $P = 0.010$; *level II*: 45 ± 6 to 61 ± 22, $P = 0.090$; *level III*: 49 ± 5 to 54 ± 10, $P = 0.691$; *level IIId* 39 ± 7 to 51 ± 18, interaction $P = 0.429$). The ratio of t-dia and τ (t-dia/τ) was relatively stable (Fig. 3*E*) and stayed well above the proposed cut-off value of 3.5, which would indicate incomplete relaxation (23). The LV EDPVR and VP_{ed10} showed no significant shifts on the *xy* plane (Fig. 3*G*).

LV minimal pressure (P_{min}) decreased significantly at all levels if LBNP of −15 mmHg or more was applied (Fig. 3*F*). The pressure fell below zero in all levels but the effect was most distinct during dobutamine infusion. When negative pressure was observed, we estimated V_0 and V_0^* (Fig. 4*A*). As applied LBNP increased, V_{es} and V_{ed} decreased and therefore V_0 and V_0^* were lowered as well, but the relation of suction volume to stroke volume (V_0^*/SV) seemed to increase. P_{min} showed a negative correlation with V_0^*/SV ($r^2 = 0.583$; $P < 0.001$, Fig. 4*B*).

DISCUSSION

This mechanistic study examined the effects of incremental lower body negative pressure from −15 to −45 mmHg on hemodynamic regulation using invasive pressure-volume assessment in closed-chest pigs. We hypothesized that incremental LBNP reduces diastolic filling and thereby affects LV DS. We were able to show, that preload reduction by graded increases in LBNP up to −45 mmHg is accompanied by increased DS, with β-adrenergic stimulation further enhancing this phenomenon.

Hemodynamic Effects of LBNP

LBNP is described in literature as a unique tool to investigate the physiology of integrated systemic compensatory responses to altered hemodynamic patterns during

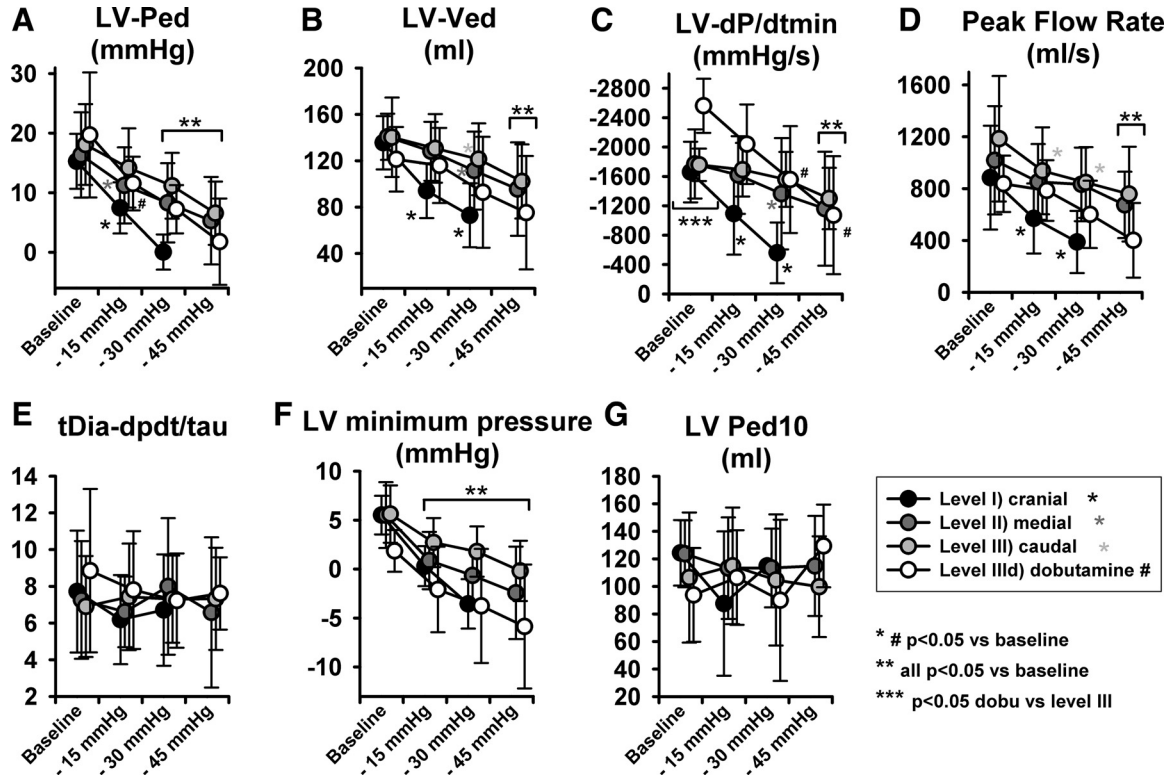

Figure 3. Hemodynamic effects of Lower body negative pressure (LBNP) (*levels I–III*: $n = 7$; *level IIId*: $n = 5$). Data were analyzed by two-way repeated measure ANOVA with the covariables level and LBNP pressure. *Levels I*, *II*, and *III* were compared with each other, *level IIId* was compared with *level III* only. *A*: LV-Ped, left-ventricular end-diastolic pressure (change at highest applied LBNP in all levels: $P < 0.001$); *B*: LV-Ved, end-diastolic volume (*level I–III*: $P < 0.001$; *level IIId*: $P = 0.001$); *C*: dP/dT_{min}, minimal rate of LV pressure decrease (*levels I*, *II*, and *IIId*: $P < 0.001$; *level III*: $P = 0.012$); *D*: Peak diastolic flow rate; *E*: t-dia-dpdt/τ, relation of time of diastole to relaxation coefficient τ (interaction: $P = 0.717$); *F*: P_{min}, minimal pressure (all levels: $P < 0.001$); *G*: V_{Ped10}, representative end-diastolic volume at a fixed pressure of 10 mmHg (interaction: $P = 0.262$).

conditions of central hypovolemia in humans (1). However, most studies draw conclusions from noninvasive measurements and low levels of negative pressures, because of safety issues in human subjects (6, 7). We here report data on the pathophysiological effect of high amounts of preload reduction and, in particular, its consequences on diastolic suction.

The effect of preload on cardiac function has been brought to attention by O. Frank and E.H. Starling more than 100 years ago (24, 25). To visualize this central physiologic mechanism, we plotted a Frank–Starling curve (Fig. 2*A*) as well as exemplary PV loops of one animal at different levels (Fig. 2*B*). A significant reduction in preload led to severe impairment of cardiac function indicated by significant decreases in CO and mAOP. Of interest, the induced reduction of preload was also mirrored in a decline of load-dependent dP/dT_{max} but load-independent contractility was not influenced as demonstrated by constant VP_{es100}. Furthermore, LV compliance was not affected by LBNP, while active relaxation was prolonged. However, we did not observe incomplete relaxation, as t-dia and τ increased proportionally, resulting in

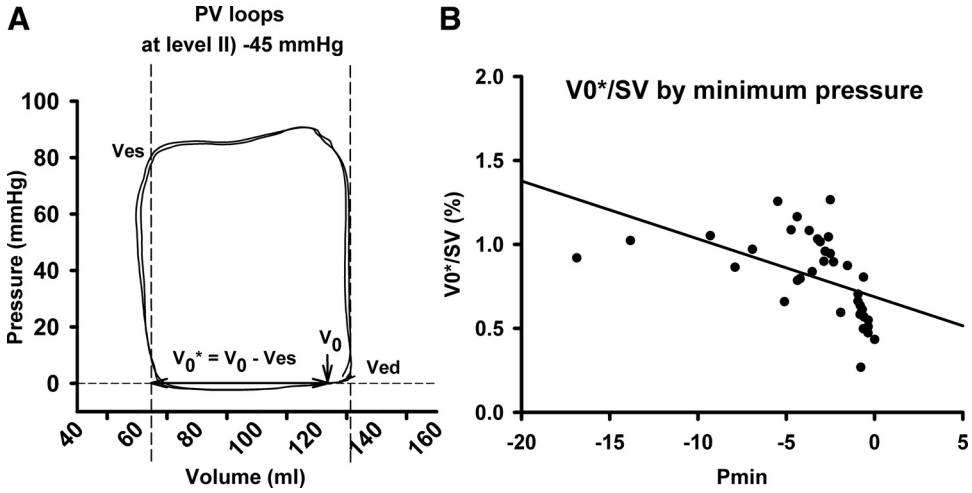

Figure 4. Exemplary pressure-volume loops to visualize diastolic suction and linear regression analysis. Solid lines show original tracing of one animal with lower body negative pressure (LBNP) applied at *position II* with −45 mmHg (*A*). The pressure falls below zero during early diastole and becomes positive at late diastole. The equilibrium volume V_0 is estimated as the latter interception with the volume-axis. Suction volume $V_0^* = V_0 - V_{es}$. Dashed lines indicate end-systolic volume (V_{es}) and end-diastolic volume (V_{ed}). With decreasing minimal pressure, the amount of suction volume accounting for stroke volume increased (*B*, $r^2 = 0.583$, $P < 0.001$, $n = 40$). Correlation was assessed by linear regression and Spearman correlation.

a stable t-dia/τ ratio above 3.5, a threshold previously reported in literature and validated in vivo by our group (23).

Dobutamine seemed to partly counter the impaired preload by increasing the contractility as well as lusitropy (26). An intrinsic countermeasure seemed to be increasing LV DS, as P_{min} became increasingly negative during intensifying LBNP (Fig. 3*F*), as previously reported in human subjects (9).

LBNP and Diastolic Suction

We were able to show that LBNP acutely reduces preload that leads to decreasing and even negative P_{min}. This P_{min} correlates with the suction volume related to SV (or V_0^*/SV). Interestingly, negative P_{min} was measured at baseline only under infusion of dobutamine that matches the effect of β-adrenergic stimulation or exercise on DS in humans (9, 27). The definition of DS has been controversial since its experimental demonstration (28) and still is (10). One of the first to define DS was Katz (28), who showed that in early diastole the LV can lower its pressure while volume increases, therefore $dP/dV < 0$, and defined this as an active filling process, opposed to the ventricle being filled from positive pressure where both P_{LV} and V_{LV} increase ($dP/dV > 0$). Later, Brecher (29) was able to measure negative intraventricular pressure in the static, isovolumic ventricle at small volumes. He further described the passive PV-relationship by increasing the volume of an almost empty ventricle step by step and obtained an S-shaped PV curve (30), where the intercept of this curve with the volume axis represents the equilibrium volume V_0 at zero transmural pressure. If the ventricle contracts below V_0, so $V_{es} < V_0$, in diastole the ventricle actively expands to V_0 by restoring elastic recoil. Nikolić et al. (22) further confirmed this approach. They closed the mitral valve remote-controlled at different points during diastole to regulate LV filling, defining $V_{es} < V_0$ as prerequisite for suction. On the contrary, Shmuylovich et al. (31) proposed a different approach. They took up Katz's definition of $dP/dV < 0$ and defined diastasis as a functional equilibrium volume where forces are balanced, and no net force or wall motion is present. Therefore, suction occurs independent of contraction below V_0 if the ventricle expands faster than it fills, as indicated by $dP/dV < 0$. Finally, this view has been opposed by Remme et al. (32). They computed a two-dimensional LV model and showed that it can lower its pressure while volume is expanding even when there are no restoring forces, so $V_{es} > V_0$.

These models have in common that they were conducted in an open-chest preparation, opposing to our closed-chest experimental setup, chosen for a relatively little manipulation of baseline hemodynamics. As we did not measure intrathoracic and pericardial pressure, we could not calculate exact transmural pressure, so analogous to Udelson et al. (9) we estimated V_0 as the intercept of the PV-loops at $P = 0$ for each animal and measurement step.

Translational Outlook

In the past decades, LBNP has been primarily adopted as a tool to study acute central hypovolemia and as a model of hemorrhagic shock. Furthermore, it has given insights into the physiology of blood pressure regulation, autonomic dysfunction, and adaptations to exercise training (1). Of note, it has recently received renewed attention as a tool to counter the impact on microgravity encountered over months during spaceflight, i.e., lack of blood pooling in the lower limbs in a zero gravity environment, leading to the so called "space-associated neuro-ocular syndrome" (33). Given the broad spectrum of applications of LBNP, we therefore believe to provide novel and relevant mechanistic insights for future and ongoing investigations in the aforementioned fields.

Limitations

The physiologic effects of high negative pressures of LBNP were assessed in healthy pigs, which might possess higher venous volume capacity in the gluteus region compared with humans, although the general effects might be comparable. Also, their carotid baroreceptors reside approximately at the same arterial hydrostatic indifference level as their cardio-pulmonary ones, unlike in humans where carotid receptors sense orthostatic challenges (34). This could be mirrored in the missing reflex tachycardia that is usually seen in humans undergoing LBNP. Due to easier housing and milder behavior, all the animals were females. We therefore cannot draw any relevant conclusions on the role of gender on the hemodynamic response to LBNP. Also, the sample size was relatively small, but still in line with the large animal literature and in accordance with the ethical principle of reducing *n* numbers in animal trials. In addition, pigs were deeply anesthetized to lower interference of autonomous nerve activity to allow stable measurement conditions. Possible effects of diaphragm position changes during LBNP on respiratory parameters were beyond the scope of this study. Finally, the investigation of an amount of LBNP as high as −45 mmHg induced a high degree of hemodynamic instability, especially at *level I*, leading to a certain amount of missing data. This phenomenon is a relevant readout of the study, hinting to the maximal amount of hemodynamically tolerated LBNP and of importance for future studies in the field. Furthermore, the missing data are not affecting the overall concept of the study.

Conclusions

The current study investigates the hemodynamic impact of graded LBNP. The significant reduction in preload, induced via LBNP, leads to a decrease in end-diastolic volume and pressure, shifting the PV loop to the left and downward. This shift is accompanied by incremental intraventricular negative pressure as well as increasing amounts of DS. Positive lusitropy via pharmacological stimulation of the β-adrenergic pathway further promotes DS already at a minimal vacuum pressure.

GRANTS

This study was supported by K.H. Tscheliessnigg-Foundation by paying for catheters, animals, their transport and accommodation as well as veterinarians. A. Alogna is a participant in the BIH-Charité Clinician Scientist Program funded by Charité—Universitätsmedizin Berlin and the Berlin Institute of Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.Z., H.M., and A.A. conceived and designed research; B.Z., M.M., and H.M. performed experiments; L.B. and A.A. analyzed data; L.B., H.M., and A.A. interpreted results of experiments; L.B. prepared figures; L.B. and A.A. drafted manuscript; L.B., B.Z., M.M., P.S., C.T., D.S., H.G.H.-S, N.G., L.G.P., H.M., and A.A. edited and revised manuscript; L.B., B.Z., M.M., P.S., C.T., D.S., H.G.H.-S., N.G., L.G.P., H.M., and A.A. approved final version of manuscript.

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