Lower-body negative pressure restores leg bone microvascular flow to supine levels during head-down tilt

Jamila H. Siamwala, Paul C. Lee, Brandon R. Macias, and Alan R. Hargens

Department of Orthopedic Surgery, University of California, San Diego, California

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Siamwala JH, Lee PC, Macias BR, Hargens AR. Lower-body negative pressure restores leg bone microvascular flow to supine levels during head-down tilt. J Appl Physiol 119: 101–109, 2015. First published April 30, 2015; doi:10.1152/japplphysiol.00028.2015.—Skeletal unloading and cephalic fluid shifts in microgravity may alter the bone microvascular flow and may be associated with the 1-2% bone loss per month during spaceflight. The purpose of this study was to determine if lower-body negative pressure (LBNP) can prevent microgravity-induced alterations of tibial microvascular flow. Head-down tilt (HDT) simulates the cephalad fluid shift and microvascular flow responses that may occur in microgravity. We hypothesized that LBNP prevents HDT-induced increases in tibial microvascular flow. Tibial bone microvascular flow, oxygenation, and calf circumference were measured during 5 min sitting, 5 min supine, 5 min 15° HDT, and 10 min 15° HDT with 25 mmHg LBNP using photoplethysmography (PPG), near-infrared spectroscopy (NIRS), and strain-gauge plethysmography (SGP). Measurements were made simultaneously. Tibial microvascular flow increased by 36% with 5 min 15° HDT [2.2 ± 1.1 V; repeated-measures ANOVA (RMANOVA) P < 0.0001] from supine (1.4 ± 0.8 V). After 10 min of LBNP in the 15° HDT position, tibial microvascular flow returned to supine levels (1.1 ± 0.5 V; RMANOVA P < 0.001). Tibial oxygenation did not change significantly during sitting, supine, HDT, or HDT with LBNP. However, calf circumference decreased with 5 min 15° HDT (−0.7 ± 0.4 V; RMANOVA P < 0.0001) from supine (−0.5 ± 0.4 V). However, with LBNP calf circumference returned to supine levels (−0.4 ± 0.1 V; RMANOVA P = 0.002). These data establish that simulated microgravity increases tibial microvascular flow and LBNP prevents these increases. The results suggest that LBNP may provide a suitable countermeasure to normalize the bone microvascular flow during spaceflight.

bone microvascular flow; head-down tilt; spaceflight; photoplethysmography; oxygenation; limb girth

PROLONGED SPACEFLIGHT results in the loss of cancellous and cortical bone of tibia at the rate of 1–2%/mo in astronauts (9, 44). In addition, the estimated reduction in strength of bone after long-duration spaceflight is comparable to the estimated mean lifetime losses associated with aging in women (16, 27). Loss of bone mineral density in astronauts is progressive during 6 mo spaceflight and is estimated to require at least 9 mo to 3 yr for recovery (30, 44). The osteopenic effect of spaceflight was first observed in cosmonauts who had increased levels of urinary calcium excretion after the Vostok 2 and 3 space missions (8). Later investigations on the astronauts and cosmonauts from the Gemini, Soyuz, Apollo, and Skylab missions confirmed significant reductions in bone mass when exposed to weightlessness (16). In case of the bone loss in space, skeletal changes occur in the absence of disease, thereby providing new data on how the lack of gravity affects bone tissue.

The mechanisms of bone loss during spaceflight in the absence of disease are poorly understood. The cephalic fluid shifts that occur in space may affect the bone microvascular flow and alter bone interstitial fluid pressures (8). Previous studies with hindlimb unloading (HU) in rats have shown that HU alters muscle perfusion pressure and blood flow (13, 15, 40, 45). Therefore, these blood supply alterations may also occur in bone, thereby affecting the osteoprogenitor, osteoblast, and osteoclast cell populations, ultimately changing the balance between bone formation and bone resorption. Bone loss at the hip and calcaneus in older healthy women is linked to reduced blood flow to the lower extremities (50). The result of this prospective community-based study provides the first evidence of the association of vascular flow in the lower extremities and an increased rate of bone loss in humans. The normal intraosseous blood flow rate for all the bones studied to date ranges from 5 to 20 ml·min⁻¹·100 g⁻¹ of bone in human and animals (29). The change in the bone blood flow rates in response to mechanical unloading has not been investigated in detail because of the complexity of the bone and lack of efficient detection methods in humans.

Among different bones in the body, the tibia is a uniquely placed weight-bearing bone that is affected by mechanical unloading in space. The vascular flow in the tibia adapts to the blood pressures on Earth and in space supine and upright positions. Upright body posture has profound effects on cardiovascular hemodynamics because of the hydrostatic pressure (7). For example, upright posture causes an increase in lower-body blood pressure (21) and pulse rate, reduction in brachial blood pressure (52), and venous flow velocity decreases in the lower limbs (48, 55), reducing the cardiac output by ~20% relative to supine position (47). Exposure to microgravity in space removes the blood pressure gradient associated with upright posture from head to feet (22). The mean arterial pressure in the feet reduces from 200 mmHg to ~100 mmHg, and the mean arterial pressure within the head increases from 70 mmHg in an upright posture on Earth to ~100 mmHg in space (2). Reduced local pressure in the tibial cancellous bone and the reduced blood volume may be responsible for the bone loss observed in astronauts during their spaceflight. The large blood vessels in the tibia experience lower than normal upright 1 g blood pressure during spaceflight and respond appropriately to local pressure conditions (1). The blood shifts from the tibia to the thorax in seconds, whereas tibial interstitial fluid movement back into the circulation may take 2–5 h. The initial abrupt change in bone microvascular flow with transition to microgravity has not been investigated so far.

Head-down tilt provides a model of mechanical unloading of the tibia while stimulating fluid shift effects similar to that
induced by actual microgravity (4, 11, 21, 39). Head-down tilt studies in rats document that mechanical unloading diminishes bioavailability of nitric oxide, which may account for the lack of vasodilation and shear stress induced constriction of arteries (40, 54). Recent evidence suggests that skeletal adaptation to mechanical loading is controlled by the interstitial fluid flow. Microfluidics studies in mice suggest that dynamic pressure loading of the intramedullary compartment for 3 min/day significantly eliminates the losses in trabecular and cortical bone mineral density in hindlimb-unloaded mice and enhances the structural integrity and bone formation (28). Increase in intramedullary pressure enhances the cortical interstitial fluid flow, which results in bone remodeling and prevention of bone loss.

There are very few techniques available to influence local blood pressures and monitor the microvascular blood flow in vivo. Photoplethysmography is a noninvasive device to measure local tissue perfusion in real time. The changes in the local perfusion due to head-down tilt and corresponding changes in the blood flow are measured by placing the photoplethysmography probe on the anteromedial compartment of the tibia. The tibia is covered sparsely by soft tissue, and hence most of blood flow measurements can be expected from bone vascular structures. Although it has been applied extensively to measure skin perfusion, recently it has been established to measure perfusion of deeper tissues such as muscles and bone because of its frequency properties (37, 42).

Lower-body negative pressure provides load bearing and may influence local blood pressure (23). As early as 1834, positive or negative pressure has been used as a therapeutic agent and is applied to different regions of the body (3, 25a). When lower-body negative pressure is applied, the fluid shifts from the upper body to lower body and to the extravascular fluid space (1, 18, 36). Lower-body negative pressure simulates the gravitational environment by generating weight bearing as well as gravitational blood pressures in the body (20). Previous studies record that supine lower-body negative pressure at −25 mmHg reduces leg muscle blood flow by 14% and increases leg volume by 2.5% (47). This study used previously-validated photoplethysmography device to measure changes in tibial bone blood flow noninvasively with tilt and lower-body negative pressure (33). Strain-gauge plethysmography is used to measure the changes in leg circumference and near-infrared spectroscopy is used to measure tibial oxygenation with head-down tilt and lower-body negative pressure. We hypothesize that 1) lower-body negative pressure counters increased tibial microvascular flow during simulated microgravity, 2) lower-body negative pressure reduces the head-down tilt-associated increase in tibial oxygenation, and 3) lower-body negative pressure restores the head-down tilt-induced reduction in leg circumference.

**MATERIALS AND METHODS**

**Subjects**

Eleven, nonsmoking subjects, 3 women and 8 men, age range 20–40 yr, volunteered, without pay, for the study. Among the 11 subjects, 3 were considered as “athletes” based on their participation in sports (>3 days/wk and >40 min/session of aerobic exercise), and 8 were considered as “nonathletes” (exercised <3 days/wk). Subjects were given written and verbal explanations of the testing protocol and signed documentation indicating understanding and consent. The study was approved by the Institutional Research Board of the University of California, San Diego. On the day of the experiment, the subjects were asked to wear loosely fit clothes to prevent any mechanical compression of the leg.

**Instrumentation and Measurements**

**Tibial and skin blood flow.** Tibial microvascular flow measurements were acquired using a previously validated photoplethysmography (PPG) system with a custom-made probe (33). There are three main parts of the probe: a green light-emitting diode (LED), an infrared LED, and a photodetector. The green and infrared lights operate at 560 and 800 nm, respectively, which are the isobestic points of oxygenated and deoxygenated hemoglobin, respectively. These lights penetrate the skin and the photodetector senses the reflected light that is attenuated by blood after being absorbed and scattered by the underlying tissue. Green light has a shorter penetration depth and was used to measure the skin blood flow. The infrared, with its greater penetration ability, was used to measure the tibial microvascular flow. This PPG method to measure bone and skin microvascular flows was first demonstrated by Mateus and Hargens (33) and Sandberg (42). This system was connected to a LabView data-acquisition system. The pulsatile nature of the PPG waveform corresponds to the rhythmic heartbeat, and the AC component of the PPG signal represents the changes in the microvascular blood flow. The PPG probe was placed on anteromedial surface of the left tibia, secured in place with tape, and covered with a foil bandage to reduce external light interference of the probe photodetector.

**Tibial oxygenation.** A near-infrared spectroscopy (NIRS) instrument (Somanetics INVOS Oximeter, model 5100C) was used to measure the tibial regional oxygenation saturation (rSO2). The NIRS probe is similar to that of the PPG in that it contains an LED and a photodetector. However, the NIRS probe transmits infrared light of two different wavelengths, 760 and 850 nm, which are used to measure the levels of deoxygenated and oxygenated hemoglobin, respectively. The ratio of the two measurements is used to determine the rSO2. Two probes were placed on the upper medial surface of the right and left tibia (Fig. 1). Fresh, new NIRS probes were used for each subject.

**Calf circumference.** The relative calf circumference was measured with a mercury strain gauge connected to a Hokanson photoplethysmograph, which was also connected to the LabView data-acquisition system. The strain gauge was calibrated to 0 V for the baseline measurement. All subsequent measurements were relative to the baseline. The sizes of the strain gauge varied with each subject so that it could be securely wrapped circumferentially around the middle of the right calf.

**Head-down tilt table/flower-body negative pressure chamber.** A head-down tilt table combined with a lower-body negative pressure (LBNP) chamber was used to tilt the subjects head-down and to apply negative pressure to their lower body (31) (Fig. 1). There is an opening at one end through which the subject can enter the chamber and rest their legs on a height adjustable wooden block. The opening is fitted with a neoprene waist seal that is secured around the subject’s waist. The other end has a small opening where a vacuum tube is inserted. An external vacuum system generates negative pressure in the chamber. The chamber is secured onto the table with adjustable belts. An inclinometer is placed on both the sides of the bed to measure the tilt angle. The amount of negative pressure can be monitored using a pressure gauge connected to the chamber.

**Experimental Protocol**

The study was carried out in a quiet room with an ambient temperature of 22°C, and minimum electronic and light interference. The measurements with the PPG, NIRS, and strain gauge were taken with the subjects in the following positions: sitting, supine, head-down tilt (HDT), head-down tilt with 25 mmHg lower-body negative pressure at −25 mmHg side-lying (HDL), and supine side-lying (HDL). The sessions were undertaken in random order. Each subject took part in a minimum of three sessions. The order of the test sessions was fixed for each subject, and the same order was followed for all subjects. The order of the test session was fixed for all subjects. Before each session, the subjects were given written and verbal explanations of the testing protocol and signed documentation indicating understanding and consent. The study was approved by the Institutional Review Board of the University of California, San Diego. On the day of the experiment, the subjects were asked to wear loosely fit clothes to prevent any mechanical compression of the leg.
pressure (HDT + LBNP), supine and sitting. Blood pressure and heart rate were determined during the last minute of measurements in each position using a blood pressure monitor (Deluxe Automatic Blood Pressure, Microlife).

Probe placement. The PPG probe was placed on the antero-medial surface of the left tibia. NIRS probe was placed on the medial surface of the tibial bone, about 4–6 cm distal to the tibial tuberosity. There was no interference between the probes. The strain gauge was positioned around the right calf below the NIRS probe. The equipments used for all the parameters were grounded. Subjects were instructed to keep conversation and movement to a minimum throughout the experiment. Signals were recorded simultaneously.

Sitting. The subjects were asked to sit on the edge of the tilt table for 5 min to obtain a control baseline. The legs of the subjects were supported by an adjustable foot rest.

Supine. After the first sitting position, subjects were then instructed to lie supine and position their lower bodies in the pressure chamber. Care was taken to see that the probes did not shift from their initial positions. As a precaution, the position of strain gauge on the tibia was marked to check if it moved when the subjects changed positions. If the strain gauge moved the string gauge was readjusted and the experiment repeated. The neoprene seal at the opening of the chamber was snugly fit around the subject's waist and their feet were flat against a height-adjustable platform at the end of the chamber. The feet were slightly raised with foam pads so the strain gauge did not come into contact with the floor of the chamber (Fig. 1). Once the subject was relaxed in supine position, readings were taken for 5 min.

HDT. The subjects were then tilted head-down 15 degrees. A pillow was placed under their heads for additional comfort and to prevent them from slipping. They remained in this position while readings were taken for 5 min.

HDT + LBNP. Twenty-five millimeters Hg of LBNP was then applied by means of an industrial vacuum pump to the chamber (Fig. 1). The subjects remained in the tilted position when the LBNP was administered. Readings were recorded for 10 min.

Data Analysis

Photoplethysmography recordings. The intensity of the light affects the value of the PPG amplitude. The PPG recordings of tibial and skin blood flow were taken and the Labview peak to peak analysis algorithm of root mean squares averages in each position were calculated over 1 min. The infrared light can penetrate an average depth of 13 mm depending on the skin color and can record the skeletal blood flow, whereas the green light can penetrate a depth of 6 mm and can record skin microvascular blood flow (33). The intensity of the PPG lights was adjusted for each subject to maximize the PPG signal quality, but not to saturate the photo detector. To ensure that the infrared primarily recorded bone blood flow, the PPG probe was placed on the medial surface of the tibia where there is no musculature. The soft tissue consists mainly of skin and subcutaneous fat and is ~2 mm thick. The body mass index (BMI) of all the subjects was within a range of 23–25 kg/m² and hence the skin and fat thickness was minimized.

Signal acquisition and analysis. The data were recorded at a sampling frequency of 300 Hz and processed using LabView 7 Express 2003 (National Instruments, Austin, TX). The signal processing eliminates artifact disturbances before extracting the relevant...
numbers from the raw data. The AC signal was high pass filtered in the software at 0.5 Hz to eliminate the oscillations due to respiration and then the peak-to-peak amplitude was calculated using the repeated mean squares algorithm and averaged over a window of 5 min. The peak-to-peak average values for the last minute of each position was entered manually into an Excel sheet. Similarly the strain-gauge data were imported to Excel and the last minute of measurements for each position was averaged.

The NIRS data were given as absolute rSO2 values. NIRS data were imported into an INVOS Analytics Tool Program (Covidien, Mansfield, MA), then exported to Excel for post analysis processing. The average rSO2 values from the last minute of data for each position were averaged.

**Statistics.** The data are expressed as means ± SD compared with baseline (supine). The sitting and supine values at the beginning and at the end of the experiments were averaged as one sitting and one supine. The comparisons within four positions (sitting, supine, HDT, HDT and LBNP) were made using repeated-measures ANOVA (RMANOVA). One of subject’s skin blood flow was nonresponsive and hence was dropped from the final analysis. If a significant main effect was determined, pairwise comparisons were conducted to determine individual differences among all conditions using the SPSS software (SPSS, Chicago, IL). Nonparametric Wilcoxon signed ranked test was used to determine significant differences by age and fitness level. The statistics were conducted on the raw data. Bonferroni was used to control for multiple comparisons. Significance was set at $P < 0.05$.

**RESULTS**

All 11 subjects were able to complete the experiment protocol with minimal discomfort and no adverse effects. Heart rate and blood pressure did not change significantly during all test conditions. The mean arterial pressures in supine, HDT, and HDT + LBNP were 88.4 ± 6.8, 87.4 ± 9.4, and 88.0 ± 6.7 mmHg, respectively; and the mean heart rates were 61.4 ± 7.7, 59.8 ± 6.1, and 60.5 ± 5.5 beats/min, respectively.

**Tibial microvascular flow changes in different positions.** The last 1 min of raw data of tibial microvascular flow, skin blood flow and calf circumference from a median responder to LBNP are depicted in Fig. 2. Tibial microvascular flow increased significantly from the sitting to the supine (from 0.7 ± 0.5 to 1.4 ± 0.8 V, $P = 0.003$) posture (Fig. 2B). After 5 min, tibial microvascular flow further increased from the supine to the HDT position (from 1.4 ± 0.8 to 2.2 ± 1.1 V, $P < 0.001$). However, LBNP (25 mmHg) for 10 min significantly reduced tibial microvascular flow (50%) compared with HDT values (from 2.2 ± 1.1 to 1.1 ± 0.5 V, $P < 0.001$) and was not significantly different from those in the supine position. When the bone microvascular flow data were segmented based on age (<30 yr and >30 yr), there was no significant difference in the magnitude of the lower-body negative pressure response compared with the HDT condition, $P = 0.285$ (see Fig. 6). In addition, when the data were segmented based on physical activity (“athletes” and “nonathletes”), no significant difference was observed in the magnitude of the LBNP response compared with the HDT condition (see Fig. 7).

**Skin microvascular flow changes in different positions.** Skin microvascular flow followed the same trend as the tibial microvascular flow. The skin microvascular flow increased significantly from sitting to supine (0.5 ± 0.7 V, 1.1 ± 0.8 V, $n = 11$) posture. There were significant increases in skin microvascular flow when transitioning supine to HDT (from 0.5 ± 0.7 to 1.1 ± 0.8 V, $P = 0.004$). With addition of lower-body negative pressure to HDT, there was a 45% decrease in skin microvascular flow (from 1.1 ± 0.8 to 0.8 ± 0.7 V, $P = 0.001$). The normalized values are depicted for graphical clarity (Fig. 3C).

**Tibial oxygenation changes in different positions.** Tibial oxygenation measured using the NIRS showed no significant difference in oxygenation levels from sitting to supine position (79.0 ± 5.5 to 81.3 ± 5.5 rSO2, $P = 0.615$). From supine to HDT, there is a 1.8% decrease in tibial oxygenation (81.3 ± 5.5 to 79.8 ± 5.4 rSO2, $P = 0.123$). From HDT to HDT + LBNP, there is a further 3.8% decrease (79.8 ± 5.4 to 76.8 ± 5.8 rSO2, $P < 0.001$) (Fig. 4).

**Calf circumference changes in different positions.** Changes in calf circumference measured using Hokanson strain-gauge plethysmograph are shown in Fig. 5 ($n = 11$). Each subject began with a baseline calf circumference of 0 V. Significant decreases in calf circumference were observed at position changed from sitting to supine (from 0 to −0.5 ± 0.4 V, $P < 0.013$). From supine to HDT there was a further decrease (from −0.5 ± 0.4 to −0.7 ± 0.4 V, $P < 0.003$). The addition of LBNP resulted in a significant increase in calf circumference to supine levels (from −0.7 ± 0.4 V to −0.3 ± 0.4 V, $P < 0.002$).

**DISCUSSION**

The primary findings of this study are that LBNP (25 mmHg) restores tibial microvascular flow to the supine levels within 10 min. Tibial oxygenation also returns to sitting levels with LBNP. Moreover, leg calf circumference returns to supine levels with LBNP. The results support our hypothesis that bone microvascular flow, tissue oxygenation, and changes in the calf volume with HDT are normalized using LBNP. Thus LBNP may provide gravity-like skeletal stress to counteract early vascular changes with simulated microgravity in the tibia.

**LBNP Restores the Tibial Bone Microvascular Flow to Supine Levels**

Starling’s equation describes the relation between capillary pressure and transudation of fluids into the tissue. Starling postulated that tissue colloidal osmotic pressure decreases with an increase in capillary pressure and provides opposing forces to limit transudation of fluid into interstitial spaces. The fluid filtration across the capillary wall to the interstitium ($J_c$) is given by Starling-Landis equation $J_c = L_p A_t [(P_c - P_t) - \sigma_r (\pi_c - \pi_t)]$, where $J_c$ is the net transcapillary fluid transport, $L_p$ is the hydraulic conductivity of the capillary wall, $A_t$ is the capillary surface area, $P_c$ is the capillary pressure, $P_t$ is interstitial fluid pressure, $\sigma_r$ is the reflection coefficient for protein, $\pi_c$ is the capillary blood colloid osmotic pressure, and $\pi_t$ is the interstitial fluid colloid osmotic pressure. There is mounting evidence that bone cells can sense the interstitial fluid flow by two mechanisms, one mechanical and the other electrokinetic. Reich and Frangos (41) propose that the bone cells respond to fluid shear stress similar to endothelial cells. Mechanical loading of the bone using LBNP in the HDT position may change the pressure in the intramedullary compartment. The intramedullary pressure measured previously in the human tibia is 30 mmHg in supine and increases to 85 mmHg during standing (35). LBNP normalizes microvascular flow to supine levels during simulated microgravity based on...
our present data. LBNP simulates cardiovascular effects of gravity and has been proposed as a potential countermeasure to microgravity (53).

Increase in tibial microvascular flow with HDT is consistent with previous studies on blood flow (6, 32). The loss of hydrostatic pressure gradient during the HDT may result in the decrease in large artery flow but increases in tibial microvascular flow. In bone, the transcortical gradient produced by hydrostatic pressure may decrease the large artery flow and interstitial fluid flow (24). However, local regulatory factors such as precapillary sphincters contraction and decreased capillary surface area may increase the microvascular flow during HDT. When LBNP is applied, the elevated transmural pressure across the vessels results in precapillary contraction of the sphincters, which in turn prevents edema in vascular beds exposed to hydrostatic loads. As the transmural pressure increases with LBNP, vasoconstriction occurs and the blood flow increases with HDT as seen in Fig. 3A. The vasoconstriction may occur through both myogenic and sympathetic mechanisms (49). The myogenic response involves constriction of the intrasosseous arteries in response to an increase in transmural pressure gradient.

The exact mechanisms of the contractile response are not clear, although membrane potential changes, and endothelial vasodilators such as nitric oxide (NO), modulate myogenic activity (12). Apart from myogenic responses there are sympathetic mechanisms such as baroreflex control mediated centrally by the adrenergic activation in response to unloading of cardiopulmonary baroreceptors (53). The myogenic response is more pronounced in the leg with upright posture and at higher tilt angles compared with non-weight-bearing regions such as the head. In our case the tibial microvascular flow changes are most likely a myogenic response as the heart rate did not change significantly. Moreover, the distance from head to head is shorter than the distance from the heart to the feet. Consequently, the capillary blood pressure above the heart level is

**Fig. 2.** Visual representation of PPG, NIRS, and SG data output of the median responder of the group and comparisons with each position. Pulsatile waveforms of tibial (A) and skin (B) blood flows from PPG from which peak-to-peak amplitude is measured. Voltage output from strain gauge (C). HDT, head-down tilt; LBNP, lower-body negative pressure.
lower than capillary pressure below the heart level. The microvasculature in the tibia is more adapted to vascular pressures during microgravity than the head (2, 43). Thus we see an increase in microvascular flow in the tibia in the HDT condition primarily because of the local regulatory mechanisms and distance of the tibia from the heart.

A previous study with 6° HDT for 3 h resulted in a 427% increase in the microvascular flow in the tibia compared with baseline measured by laser-Doppler flowmetry (46). Our PPG data demonstrate a 36% increase in bone microvascular blood flow from supine with 15° HDT (Fig. 3B). From supine to HDT position, the hydrostatic increases in the head and transmural pressure decreases in the leg. HDT induces a more rapid and pronounced head-ward fluid shift in a short, measurable period of time compared with horizontal posture (26). The local hydrostatic pressures in the leg are reduced with HDT, thus increasing microvascular flow (5). Interstitial fluid pressure of tibialis anterior muscle also drops with tilt, thus increasing microvascular blood flow (21).

**Tibial Skin Microvascular Flow Response to LBNP**

Laser-Doppler flowmetry, which is considered as a “gold standard” to measure regional skin blood flow, showed that at -60 mmHg, the leg skin blood flow reduces to ~36% of supine baseline levels (53). Jacobson and co-workers demonstrate that 10 mmHg of LBNP decreases blood flow by 17% in subcutaneous tissue and 2% in skeletal muscle in the human leg (40).
circumference probably due to precapillary vasoconstriction, thus reducing capillary pressure and flow (Fig. 5). But when the LBNP was added, calf circumference returns to supine levels. Moore and Thornton detected 11.6% loss in blood volume of the leg of astronauts due to a head-ward fluid shift (36). In bed rest studies, Nixon and coworkers established that 5° HDT induces 900 ml fluid shift from both limbs after 30 min of HDT bed rest, probably due to the acute shift of the pooled venous blood away from the limbs (38). Hargens and coworkers (21) detected a decrease in calf circumference from 36.9 to 36.7 cm when subjects shifted from upright to horizontal position (21). From the above studies the lower limb girth decreases about ~4% during HDT (10). A 4% decrease in circumference represents 8% decrease in area consistent with our results (Fig. 5). The change in the calf circumference is due to LBNP and increased venous volume followed by increased capillary filtration. Since LBNP was restricted to only 10 min, the increase in calf circumference was due to filling of venous spaces along with minor capillary fluid filtration in the extravascular space (1).

**Limitations**

The vasculature associated with the bone is not an exclusive component of the bone; rather it is contiguous with the vasculature of the connective tissue, muscle, and marrow (14). The PPG (infrared) and NIRS techniques measure vessels in the bone cortical region, 13 mm deep. Also the PPG detects scattered light not only from the hemoglobin component of the blood, but the PPG signal can also be affected by the red blood cell orientation, vessel wall movement, and blood volume. Blood flow rate depends on the flow velocity and cross sectional area of the vessels which was not measured in the current study. PPG values are only relative measurements of the microvascular flow and not absolute numbers.

**Conclusions**

The previous notion of bone being immune to gravity and external pressure changes is dispelled to some extent from our
present results. The bone microvascular flow can be measured using a light based photoplethysmography device. This is one step closer to measuring bone circulation in real time, regionally and noninvasively. Since the large vessels and the microvascular respond differently to stimulus, further studies are required to compare the responses to LBNP in different animal and human models. The main finding of this work is that short-term LBNP can reduce the HDT-induced increase in microvascular blood flow in the tibia to values consistent with sitting posture. Acute exposure to LBNP might provide gravity-like skeletal stress and prevent head-ward fluid shifts. It is possible that extended exposure to LBNP may maintain Earth-like skeletal perfusion dynamics during long-duration space missions and thus reduce bone loss and fracture risk to the lower limb. Future studies are warranted to determine the effects of age, sex, and physical activity on the tibial microvascular flow responses to LBNP. Long-term effects of LBNP on tibial microvascular flow and bone quality measures have to be investigated for LBNP to be used as a suitable countermeasure for long-term space missions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.H.S., B.R.M., and A.R.H. conceived and designed of research; J.H.S. and P.C.L. prepared experiments; J.H.S., B.R.M., and A.R.H. interpreted the results of experiments; J.H.S. performed experiments; J.H.S., B.R.M., and A.R.H. edited and revised manuscript; J.H.S., P.C.L., B.R.M., and A.R.H. approved final version of manuscript; P.C.L. analyzed data.

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