Tibia Bone Microvascular Flow Dynamics as Compared to Anterior Tibial Artery Flow During Body Tilt

Rachel L. Becker; Jamila H. Siamwala; Brandon R. Macias; Alan R. Hargens

BACKGROUND: We compared microvascular and macrovascular blood flows of the tibia and anterior tibial artery during graded whole-body tilt. We hypothesized equal responses for bone microvascular and macrovascular blood flows during varying angles of tilt.

METHODS: There were 18 volunteers who were randomly positioned in the following postures: supine, 15° head-up tilt, 6° head-up tilt, 6° head-down tilt, and 15° head-down tilt using an inversion table with reference to seated posture (baseline control). Ultrasonography quantified anterior tibial arterial diameter and peak systolic velocity, enabling calculation of macrovascular blood flow to the tibia. Tibial bone microvascular blood flow was measured noninvasively using photoplethysmography in the same leg.

RESULTS: Transitioning from a seated position to a supine position, macrovascular blood flow did not change significantly (1.81 ± 1.18 to $2.80 \pm 1.74 cm^3 \cdot s^{-1}$). However, bone microvascular flow increased significantly (0.36 ± 0.23 to 1.11 ± 0.79 V) from the seated to the supine position. Transitioning from a seated posture to 15° head-down tilt, both arterial macrovascular and bone microvascular flows increased significantly (1.81 ± 1.18 to $3.32 \pm 2.08 cm^3 \cdot s^{-1}$ and 0.36 ± 0.23 V to 2.99 ± 2.71 V, respectively). The normalized flow for microvascular blood flow as a function of body tilt was significantly greater than that for macrovascular blood flow at 6° and 15° head-down tilt.

DISCUSSION: These data do not support our hypothesis that bone microvascular flow and arterial macrovascular flow share equal responses to altered body tilt. Therefore, for a given decrease in local blood pressure in the leg with head-down body tilt, the magnitude of increase in blood flow is greater in the microcirculation as compared to the feeding artery.

KEYWORDS: posture, head up tilt, head down tilt, simulated microgravity, microcirculation, spaceflight, blood flow, weightlessness.

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Among physiological adaptations to microgravity, bone loss is an important manifestation that draws considerable interest because decreased bone mass may increase the risk of fracture upon return to Earth or upon landing on other planets such as Mars.[10,12] Bone mineral density decreases by approximately 1–2% each month during spaceflight at weight-bearing bone sites such as the tibia.[25] The primary contributor to bone loss during spaceflight is reduced mechanical loading without weight bearing.[29] However, spaceflight alterations of bone remodeling may also be due to fluid shifts as well as altered blood and interstitial perfusion.[7,30]

Upon exposure to microgravity, it has been hypothesized that a cephalad fluid shift occurs due to the removal of the blood pressure gradient, normally present on Earth, which occurs from head to feet.[10] The loss of this gradient causes a decrease of mean arterial pressure (MAP) of the feet from approximately 200 mmHg to 100 mmHg, and an increase in MAP in the head from approximately 70 mmHg to 100 mmHg.[10] Previous evidence supports the role of cephalic fluid shifts in bone remodeling. According to Zhang and coworkers, load-stress placed on bone cells is primarily due to intraosseous fluid movement, a product of normal positional changes

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and muscle contractions, thus promoting fluid flow to induce bone interstitial sheer stress.[30] The increased intraosseous fluid movement from muscle contraction and positional changes may stimulate higher osteoblast activity which stimulate bone formation.[30] However, with consistent unloading and reduced MAP, increased vascular resistance and reduced blood-perfusion pressure can occur, causing vascular adaptations that alter mechano-transduction pathways (release of nitric oxide and prostaglandin E) as well as decreasing nutrient delivery to bone, thus decreasing bone mineral density.[27] This implies that unloading of bones and reduced MAP may reduce intraosseous and interstitial fluid transport, preventing osteoblasts from receiving mechanical signals for remodeling/adaptation, and potentially causing a decrease in bone mineral density.[13,19] Overall, a decrease in MAP and bone microvascular flow experienced by the tibia and lack of weight bearing load are possible explanations for bone loss in microgravity. However, it is not clear if altered bone microvascular flow or input arterial blood flow play a significant role in microgravity induced bone loss.

Input arterial flow to bone originates in muscle, enters the central portion of a long bone, and is regulated by perfusion pressure and vascular conductance.[16] The anterior tibial artery is an example of a well-regulated nutrient artery. Nutrient arteries for bone may represent as much as 30–50% of total resistance to blood flow through skeletal tissues.[26] As the resistance of arterial vasculature significantly regulates the downstream intravascular pressures, changes in blood flow through the arterial system into the surrounding microvasculature embedded in muscle and bone tissues can impact interstitial fluid flow in bone. It is therefore important to study the relationship between the blood flow in bone nutrient arteries and the microvasculature to which these large arteries feed. However, little is known about the relationship between microvascular bone blood flow and input macrovascular (arterial) blood flow with different body positions. In this regard, head-down tilt (HDT) serves as a useful model to simulate mechanical unloading of bone and head-ward fluid shifts in order to understand the regulation of macro- and microvascular flows with posture.[8] Although an increase in tibia microvascular bone blood flow with higher degrees of head-down tilt has been previously demonstrated, the role of macrovascular blood flow that feeds into the bone microcirculation is not well understood.[24]

Both photoplethysmography (PPG) and ultrasound technology are validated methods to measure microvascular blood flow and macrovascular blood flow, respectively.[17,21] This study is designed to use PPG and ultrasound technology simultaneously in order to investigate altered tibial bone microvascular blood flow relative to anterior tibial arterial blood flow during various angles of HDT and head-up tilt (HUT) with reference to seated posture. Seated posture was chosen for reference because it is the most common posture related to upright activity on Earth. Because macrovascular blood flow supplies the bone microcirculation, we hypothesized that there will be equivalent responses of blood flow in the input artery and the tibia bone during various degrees of body tilt.

METHODS

Subjects

This study was approved by the Institutional Research Board of the University of California, San Diego. We recruited 18 healthy subjects, 10 women and 8 men, with a mean ± SD age of 25 ± 7 yr for the study. Four subjects were above age 30 and ranged to age 45. The average subject height was 170.2 ± 10.1 cm. All subjects were healthy nonsmokers with no history of cardiovascular disease. Subjects were given detailed written and verbal explanations of the experimental protocol, devices used, and aims of the study. All subjects provided written and verbal consent to participate in the study. Subjects were asked to wear loose fitted clothing to prevent any compression of the leg.

Procedure

The study was conducted in a quiet room at a temperature of 23°C. All lights were turned off to reduce light interference and subjects were asked to remove any electronic devices on them due to electrical interference potentially causing an alteration of the PPG waveform. All subjects had their medial surfaces of the lower right tibias cleaned with an alcoholic wipe to remove any sweat or lotion to prevent interference with the PPG signal. Subjects were placed in the following positions sitting, and in random order supine: 15° HUT, 6° HUT, 6° HDT, and 15° HDT. A 79-cm tall chair was used for the seated baseline position, while a Teeter Hang Ups F500 Inversion Table was used to tilt subjects randomly in the remaining five positions. Subjects spent 5 min in each position. In the seated position, the subjects' hips and knees were in 90° of flexion with their feet planted flat. After the baseline sitting position, subjects were assisted into their remaining positions. Subjects were asked to refrain from talking and to minimize movement to maintain the quality of the signal. During the last minute of every position, measurements with the PPG and ultrasound were recorded simultaneously (**Fig. 1**). The PPG device remained on the subject and recorded data for 5 min in each position. Only the last 60 s of PPG data were analyzed for each position. The ultrasound transducer head was only used during the last 30 s of data collection in each position. Blood pressure and heart rate were recorded during the 3rd min of each position using noninvasive oscillometry on the brachial artery (Automatic Blood Pressure Monitor, Welch Allyn Inc., NY).

Equipment

Anterior Tibial Artery Ultrasound Measurements. A GE Medical Systems, Logiq 9 Ultrasound device (GE Medical Systems, Milwaukee, WI) noninvasively measured macrovascular blood flow and diameter and in the right anterior tibial artery. The ultrasound device was equipped with a 2D imaging system,

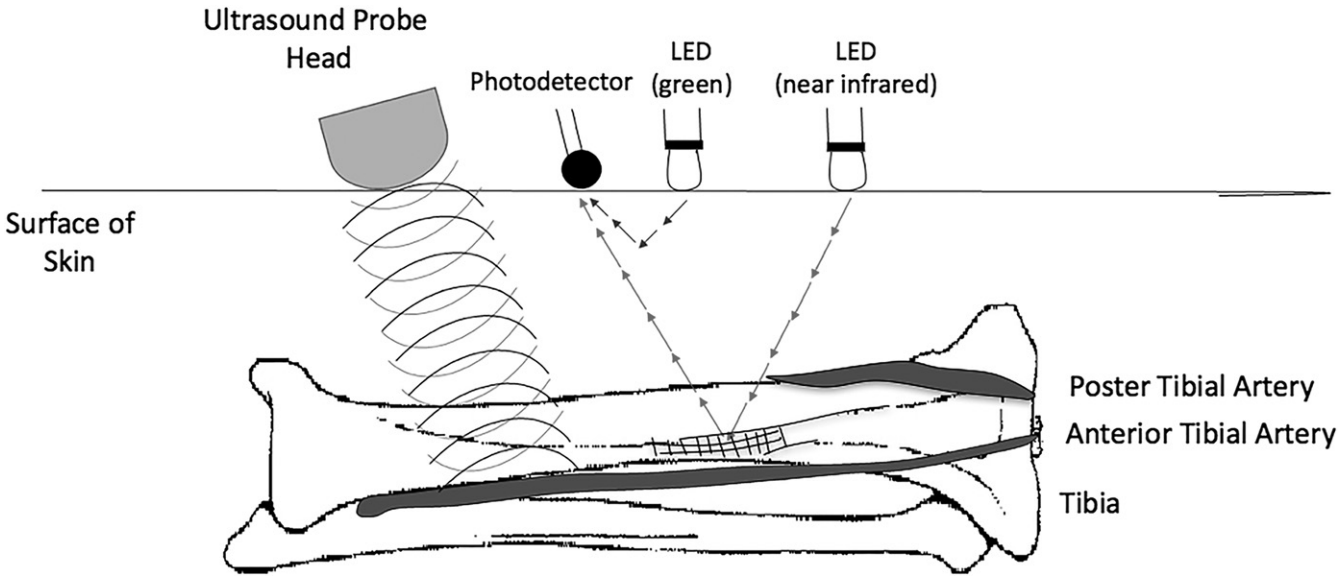

Fig. 1. Visual schematic of anterior tibial artery macrovascular blood flow detection using ultrasound technology and tibial bone microvascular blood flow detection using the photoplethysmography (PPG) device.

color and spectral Doppler, an internal electrocardiogram, and a high frequency transducer head. The sampling frequency was 2300 Hz, which provided high resolution of measured changes in blood flow. A 7–12 MHz linear array transducer head was used to acquire the images with sufficient resolution for accurate analysis. The 2D image was generated by placing the transducer approximately 10 cm below the right tibial plateau. Before each trial, the anterior tibial artery was interrogated and compared to other anatomical landmarks such as veins to insure consistent probe placement. All ultrasound measurements were taken by simultaneously using B-mode, pulsed wave, color flow imaging at a 60° angle of insonation to insure the perpendicular direction of the beam emitted from the probe for accurate measurement of the peak systolic velocities, time averaged maximum velocities, and accurate gray-scale imaging for a picture of the vessel for diameter measurement to manually calculate flow. The average values and standard deviations of peak systolic velocities, time averaged maximum velocities, and vessel diameters were calculated by manually inputting three frame-by-frame values from every third peak for the 30-s period into an Excel™ worksheet. Macrovascular blood flow was measured as a product of average cross sectional area of the vessel and average peak systolic velocity in each position.

Tibial bone microvascular flow measurements. Tibial bone microvascular blood flow was measured noninvasively using a customized PPG device. The PPG is a validated optical technique that emits light onto various tissues (skin, muscle, or bone) and monitors relative change in microvascular volumetric flow from the attenuated light reflected back from the underlying tissue.[15] The PPG contains a photodetector and two light emitting diodes (LEDs). The photodetector measured the attenuation in light intensity received from the changes in volumetric flow of the underlying skin and the microcirculation of the bone. Two LEDs emit light onto the tissue at different wavelengths, which was reflected back from the red blood cells in the tibia.[15] Some light is absorbed by blood, but other portions of light will eventually get reflected back and reach the photodetector. The green LED operated at wavelengths of 560 nm while the near infrared LED operated at an 800-nm wavelength. Both LEDs operate within a biological window where skin and bone are transparent to light. The green LED did not penetrate as deeply as the near infrared LED and was used to measure change in perfusion of the skin. The green LED penetrated approximately 2–3 mm, while the infrared LED penetrated approximately 13 mm.[15] For this experiment, we only utilized the data from the near infrared LED used to scatter light into the medial surface of the lower right tibia. The near infrared LED projected light into the tibia to measure relative changes of bone blood flow and was placed approximately 5-6 cm above the medial malleolus. The PPG device was connected to a BioPac Systems MP150 on the AcqKnowledge 4.4 software program. The probe was secured with tape and wrapped with a cloth bandage to reduce the external light interference. All subjects were grounded during the experiment. The intensity of the LED was adjusted for every subject to insure optimum signal strength. The PPG recordings of tibial blood flow were taken and analyzed using AcqKnowledge software. The AcqKnowledge software conducted a peak-to-peak analysis algorithm of root mean squares averages in every position for each subject over a 60-s period. The average PPG waveform for each position is depicted in **Fig. 2**. The PPG was placed on the medial surface of the lower right tibia where there is no musculature. PPG placement insured the infrared was primarily recording bone blood flow as opposed to skin blood flow.[15]

Statistical Analysis

Data are presented as means ± SD. Measurements were made relative to the sitting baseline. Raw values were used for statistical analysis except for comparisons between tibial bone

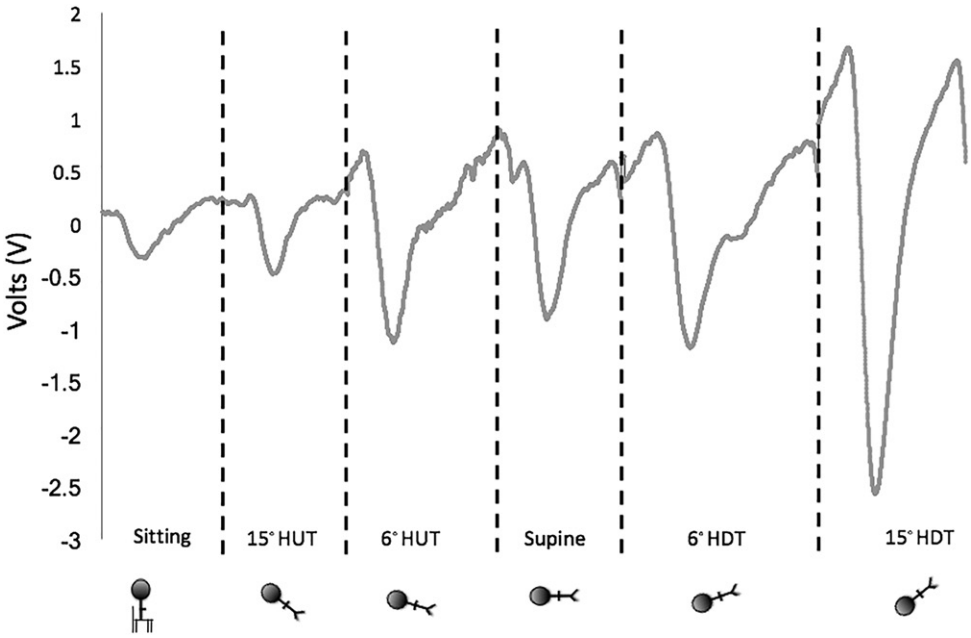

Fig. 2. A visual representation of the average PPG waveform output being compared during each position.

microvascular blood flow and anterior tibial arterial blood flow. Both anterior tibial arterial blood flow and tibial bone microvascular blood flow values were normalized to the sitting position (control). The comparisons within all positions were determined with a two-way RMANOVA. A Dunnett's post hoc test was used to determine significant differences for within group comparisons. A Holm-Sidak post hoc test was performed to determine significant differences between the normalized values for microvascular and macrovascular blood flow groups. A Bonferroni post hoc test was used to determine significance for arterial diameter. Significance level was set at $P < 0.05$ using Prism Software (GraphPad, La Jolla, CA).

RESULTS

All 18 subjects completed the study without any adverse effects and were included in the data analyses. Average blood pressure (BP), heart rate (HR), and MAP were within the range for normal healthy volunteers. The averages for HR, systolic pressure, diastolic pressure, and MAP remained within the same range during each position and showed no significant differences (**Table I**). Additionally, there were no significant differences in microvascular blood flow or macrovascular blood flow between the age group above 30 ($N = 4$) and the age group below 30 ($N = 14$) in all positions.

Relative changes in bone microvascular blood flow as compared to anterior tibial macrovascular blood flow. There were significant differences between normalized microvascular blood flow and normalized macrovascular blood flow at 6° HDT (normalized macro: 1.69 to normalized micro: 5.67, $P = 0.02$) and 15° HDT (macro: 2.25 to micro: 10.52, $P < 0.0001$). Normalized blood flow was determined by the following equation:

$$normalized\,blood\ flow = \frac{measured\,blood\ flow}{seated\,blood\ flow}.$$

When examining the normalized blood flow, tibial bone microvascular blood flow increased more per angle of tilt and was more variable and responsive than relative anterior tibial artery blood flow (**Fig. 3A**).

Anterior tibial macrovascular blood flow in different positions. There was a significant main effect of body posture on anterior tibial macrovascular blood flow ($P < 0.001$). Anterior tibial arterial blood flow showed a significant difference between the sitting and 15° HDT positions (sitting: $1.81 \pm 1.18\ cm^3 \cdot s^{-1}$ to 15° HDT: $3.32 \pm 2.08\ cm^3 \cdot s^{-1}$, $P = 0.036$) (**Fig. 3B**). However, there were no other significant differences of anterior tibial arterial blood flow between other positions. Additionally, anterior tibial artery vessel diameter was not significantly different between various positions of tilt (**Fig. 3C**).

Tibial bone microvascular blood flow in different positions. There was a significant main effect of body posture on tibial bone microvascular blood flow ($P < 0.001$). Tibial bone microvascular blood flow was significantly higher in the supine

Table I. Average ± SD for all Subjects' Heart Rate, Blood Pressure, and Mean Arterial Pressure During the 3rd Minute for All Postures.

AVERAGE SUBJECT BLOOD PRESSURE AND HEART RATE				
POSITIONS	**HR (bpm)**	**SYSTOLIC PRESSURE (mmHg)**	**DIASTOLIC PRESSURE (mmHg)**	**MAP (mmHg)**
Sitting	76 ± 13	109 ± 13	65 ± 9	80 ± 10
15HUT	65 ± 17	113 ± 11	69 ± 5	83 ± 5
6HUT	65 ± 12	114 ± 12	68 ± 5	83 ± 6
Supine	62 ± 16	117 ± 21	67 ± 7	81 ± 6
6HDT	64 ± 12	111 ± 10	67 ± 5	82 ± 6
15HDT	63 ± 12	115 ± 10	66 ± 6	82 ± 6

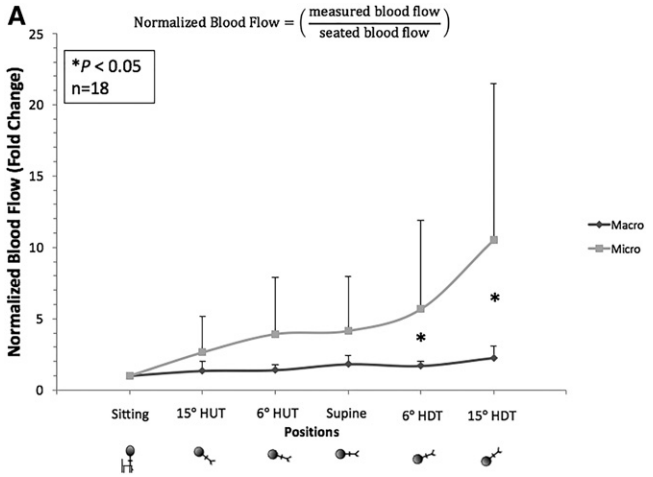

Fig. 3A. The effect of varying postures on tibial bone blood flow, anterior tibial artery blood flow, and vessel diameter. Normalized average anterior tibial artery macrovascular blood flow and tibial bone microvascular blood flow values for all subjects during sitting, 15° head-up tilt (HUT), 6°HUT, supine, 6° head-down tilt (HDT), and 15°HDT. Comparisons were made using two-way RMANOVA followed by a Holm-Sidak post hoc test. Significant differences were found between microvascular and macrovascular blood flow during 6°HDT ($P = 0.02$) and 15°HDT ($P < 0.0001$).

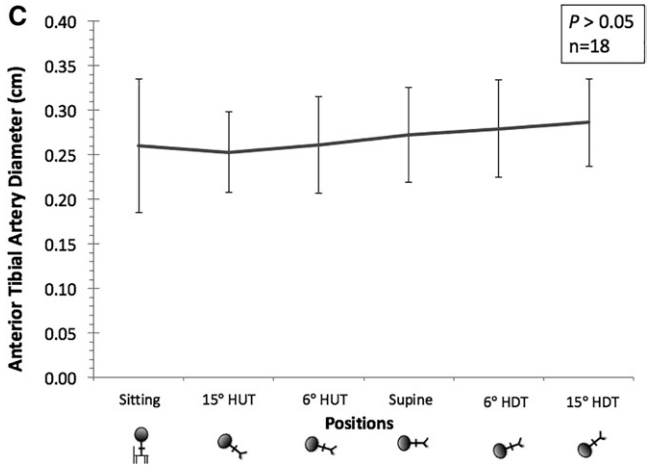

Fig. 3C. Average anterior tibial artery diameter (cm) during sitting, 15°HUT, 6°HUT, supine, 6°HDT, and 15°HDT. Comparisons were made using two-way RMANOVA followed by Bonferroni correction. There were no significant differences between diameters in any position.

(1.11 ± 0.79 V, $P = 0.026$), 6° HDT (1.59 ± 1.32 V, $P = 0.003$), and 15° HDT (2.99 ± 2.71 V, $P = 0.001$) postures when compared to sitting (0.36 ± 0.23 V) (**Fig. 3D**).

DISCUSSION

The novel finding of this study is that the magnitude of change in tibial bone microvascular blood flow is greater for the same incremental change in local blood pressure when compared to anterior tibial arterial blood flow during supine and HDT postures. In addition, anterior tibial arterial blood flow increases significantly from sitting to 15° HDT and not in 6° HDT, whereas bone microvascular blood flow increases significantly in supine, 6° HDT, and 15° HDT positions compared to sitting. Thus, the results of this study do not support our hypothesis that anterior tibial macrovascular blood flow and tibial bone microvascular flow increase or decrease with equal responses during altered body posture.

Bone is a highly vascularized tissue consisting of an extensive network of capillaries and large vessels.[18] Bone perfusion is important in homeostasis and repair. Ramasamay and colleagues investigated in detail the arrangement of arteries, veins, and capillaries in the tibia to understand bone blood flow in mice.[18] The arterial blood in the bone enters the long bone through the distal end and flows through the capillaries into the

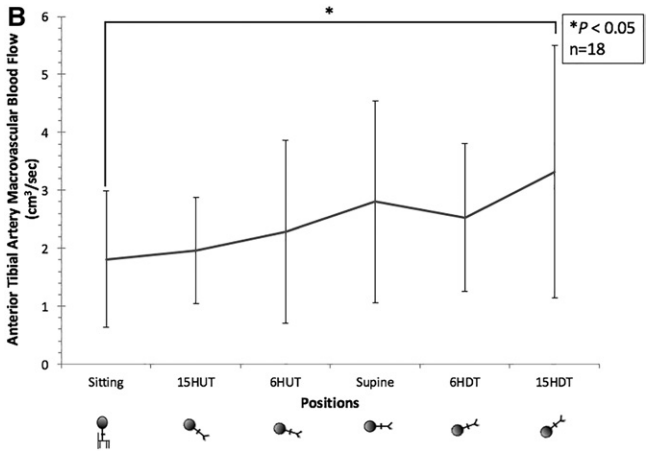

Fig. 3B. Average anterior tibial arterial macrovascular blood flow during sitting, 15°HUT, 6°HUT, supine, 6°HDT, and 15°HDT. Comparisons were made using two-way RMANOVA followed by a Dunnett's post hoc test. There was a significant increase in anterior tibial arterial macrovascular blood flow from sitting to 15°HDT (*$P = 0.04$).

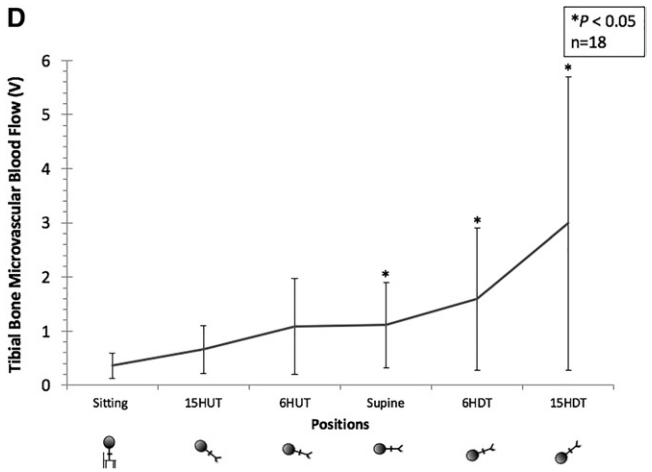

Fig. 3D. Average tibial bone microvascular blood flow during sitting, 15°HUT, 6°HUT, supine, 6°HDT, and 15°HDT. Comparisons were made using two-way RMANOVA followed by a Dunnett's post hoc test. There was a significant increase of tibial bone microvascular blood flow from the sitting to supine ($P = 0.03$), 6°HDT ($P = 0.003$), and 15°HDT ($P = 0.001$) positions. Error bars for all graphs indicate standard deviation.

highly branched sinusoidal vasculature. The blood is then drained into the vein of a lumen size less than 100 μm. The blood velocity in the capillaries is much higher (0.98 ± 0.1 mm $\cdot$ s^{-1}) than in the vessels (0.16 ± 0.04 mm $\cdot$ s^{-1}). Further changes in bone blood flow impairs bone osteogenesis.[18] This implies that microgravity induced fluid shifts from the tibial bone may affect the bone osteogenesis due to changes in the bone blood flow. In our study in humans, we show that head down tilt results in greater increases in bone blood flow in the microcirculation compared to input arterial blood flow into the tibia. We cannot attribute our measures specifically to the arterioles/capillaries. These differences may have implications in fracture management both on Earth and in space.

Our previous work has demonstrated similar increases in tibial bone blood flow with HDT.[1,24] Siamwala and coworkers investigated the effects of lower body negative pressure (LBNP) as a potential countermeasure to spaceflight. They measured tibial bone blood flow, tibial skin blood flow, and calf circumference during sitting, supine, and 15° HDT. They found increased tibial bone blood flow of approximately 69% during 15° HDT when compared to the sitting position, whereas we found tibial bone blood flow increased 91% during 15° HDT when compared to the sitting position. Both studies show increased percentages of microvascular bone blood flow with HDT. However, differences in percentage increases are probably attributed to variation in the two subject populations with differences in height, weight, age, and habitual levels of physical activity.

The reported increase in tibial bone blood flow in this paper differs from the pioneering studies of Colleran and coworkers' on rats.[2] They employed the direct, gold standard method using radiolabeled microspheres to measure microvascular flow in bones of hind-limb unloaded rats at 10 min, 7 d, and 28 d. They found that bone microvascular flow decreased in the proximal end and shaft at all time points compared to baseline. The reason for the differences in results is unknown, but it could be due to differing local microvascular flow regulatory mechanisms of the lower extremities in rats compared to humans during altered posture. Another possible mechanism is that during acute periods of HDT, the bone may vasodilate to greater degrees initially, and respond more readily compared to surrounding vasculature, such as the anterior tibial artery and skin, for short periods of time to adjust to the bone's rate of metabolism or need for nutrients.[5,11,26] Bone may adjust more readily during acute periods to maintain production of red and white blood cells and other functions. Adequate blood perfusion is necessary to provide nutrients to bone cells to maintain these important functions during acute postural changes. During initial transition to HDT, we suggest that higher levels of perfusion are needed in bone. This could help explain why our data in humans displayed an initial increase in tibial bone microvascular flow.

The potential mechanism of increased bone blood flow in the HDT positions is probably due to sympathetic mechanisms and the myogenic responses.[1,27] The sympathetic mechanisms that regulate the increased bone blood flow response are the baroreflexes and the cardiopulmonary reflexes, otherwise known as the orthostatic reflexes.[14] These reflexes help to maintain blood pressure at relatively constant levels in the body. According to Convertino and associates, HDT increases blood pressure at the atrial and carotid baroreceptors, and increases baroreceptor loading to relax arterial tone.[3] This could potentially explain why there was an increase in microvascular blood flow of the arterioles with decreased regulation. However, another mechanism that could contribute to an increase in the arterioles of microvascular bone blood flow is the myogenic response. The myogenic response mechanism causes vasodilation of the intraosseous arteries in the tibia from a decrease in the transmural pressure gradient in arterioles of the leg during HDT. The myogenic response mechanism is controlled by intrinsic smooth muscle membrane potential, involving L-type calcium channels and potassium channels. However, when endothelial vasodilators such as nitric oxide are released from vascular endothelial cells, they help to regulate myogenic activity in response to induced sheer stress such as fluid shifts causing vasodilation.[4] Myogenic tone may also decrease during HDT due to inhibition of arterial and cardiopulmonary baroflexes.[14] Based on our data, bone blood flow changes in the microcirculation are probably due to the combined effects of reduced sympathetic vasoconstrictor tone and a myogenic response because heart rate did not change significantly. Overall, due to reduction of the hydrostatic blood-pressure gradient in the leg, arteriolar transmural pressures decrease during HDT, thus increasing microvascular flow.

In contrast, our anterior tibial arterial blood flow data show a relatively constant macrovascular flow with supine, HUT, and HDT postures, suggesting that the arterial vasculature is regulated less than that of tibial bone microvasculature. A study conducted by Villar and coworkers compared central cardiac vascular response to the peripheral vascular response during various positions of body tilt.[28] The results document that during HDT popliteal arterial blood flow remains constant with 15° HDT when compared to the supine position. The anterior tibial artery is preceded by the popliteal artery, allowing us to compare the two studies because both share a similar experience under simulation to microgravity because they are below heart level. Therefore, the potential mechanistic response for maintenance of arterial blood flow and diameter during various degrees of tilt could be due to inhibition of decreased sympathetic vasoconstrictor tone and inhibition of the vasoconstrictor response.[20,22] The decreased activation of sympathetic vasoconstrictor tone would result in the ability of the artery to remain in a dilated state as opposed to being constricted. This would also mean that there could be maintained regulation of other vasodilatory mechanisms in the artery to prevent the arterial diameter from reducing in size. Potentially other vasodilatory mechanisms that do not include nitric oxide.[6] Another potential reason why anterior tibial arterial blood flow did not change could be due to changes in blood flow from other vascular sources that feed into the tibia, for example the posterior tibial artery. The posterior tibial artery could have provided increased blood flow to the tibia bone during head down tilt.

However, posterior tibial artery blood flow was not measured. Furthermore, the anterior tibial artery does not solely feed into the tibial bone, it feeds other sources such as the muscles of the anterior compartment of the leg as well as the dorsalis pedis artery. The need to maintain anterior tibial artery blood flow to other sources is a potential reason as to why vessel diameter does not change. Local vasodilatory effects could help to maintain oxygen supply to all tissues that the anterior tibial artery supplies.

These data suggest that other biomechanical mechanisms are more important for bone loss in space as opposed to bone microvascular flow. Previous studies support the concept that decreased bone mass during spaceflight, as well as during head-down tilt bed rest studies, is probably due to reduced mechanical loading and reduced signaling of mechanotransduction pathways.[9,13,23,30] Our data further confirm the more important role of reduced mechanical loading as primarily being responsible for bone loss during spaceflight because these data potentially downplay decreased bone microvascular flow as the main factor in bone loss.

Overall, when comparing relative changes in tibial bone microvascular blood flow to relative changes in anterior tibial artery blood flow, we find that tibial bone microvasculature is more responsive to local blood pressure alterations when compared to the feeding arterial vasculature which is less responsive to tilt. We can ultimately conclude that the microcirculation must meet the needs of the local bone tissues during altered posture.

The skin and bone under investigation have common vascularity near each other, meaning that the tissues being served are not exclusively bone. The region of interest interrogated by the IR light is close to the surface and has little to no muscle tissue in between the skin and the surface of the bone, meaning there is likely to be limited interference from subcutaneous tissue.[15] Therefore, the data presented primarily represent relative changes in bone blood flow. The PPG device represents relative and not absolute values, but the application for this experiment is used to measure relative change in perfusion from a baseline condition. Additionally, the PPG AC signal can respond to changes in both blood flow velocity as well as pulsatile blood volume changes. Microvascular perfusion from all tissues is dependent on blood flow velocity as well as vasomotor tone of vessels. If a blood vessel is fully dilated, but has low blood flow velocity, nutrient supply to the tissue is compromised. Nutrient supply to tissue is also compromised when a blood vessel is vasoconstricted with high blood flow velocity. When increases in PPG signal are seen, they can be represented as an increase in blood flow velocity, vasodilation of the tissue being sampled, or most likely both. Either way, an increase in tissue perfusion is being demonstrated. The reverse can also be argued. When the PPG signal decreases, this could represent decreases in blood flow velocity, vasoconstriction, or both, all of which can represent decreased tissue perfusion. The difference between these two effects on PPG signal have been previously demonstrated by Mateus and Hargens with their arterial occlusion and cold pack study validating the PPG device.[15] Another limitation is that anterior tibial artery measurements are using ultrasound technology, which requires strategic hand placement in order to maintain the correct visibility of the vessel without having partial vessel sampling. This was overcome by having one person conduct all measurements and having their arm resting on a stable platform to reduce the amount of variability and improve data quality. Both the PPG and ultrasound have high variability in measurements of blood flow, but significant differences were still found despite high variability in the data. In addition to various limitations with technology, this study is not a long-term acute head-down tilt study and only examines an initial window of time to investigate early vascular responses to altered posture. Furthermore, other feed arteries, such as the posterior tibial artery, were not measured.

Overall, there are very few studies that compare macrovasculature to microvasculature flows, especially in bone. In conclusion, this study disproved our original hypothesis by demonstrating that for a given incremental increase in local blood pressure, the magnitude of change is greater at the microvascular level in bone, as compared to one of the macrovascular feed arteries. These findings may have important implications for the development of countermeasures for bone loss in space. With greater understanding of bone loss related to altered bone microvascular flow, we may identify more specific countermeasures to enhance bone development, maintenance, and healing in space.

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