Effects of hindlimb unloading and ionizing radiation on skeletal muscle resistance artery vasodilation and its relation to cancellous bone in mice

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Prisby RD, Alwood JS, Behnke BJ, Stabiley JN, McCullough DJ, Ghosh P, Globus RK, Delp MD. Effects of hindlimb unloading and ionizing radiation on skeletal muscle resistance artery vasodilation and its relation to cancellous bone in mice. J Appl Physiol 120: 97–106, 2016. First published October 15, 2015; doi:10.1152/japplphysiol.00423.2015.—Spaceflight has profound effects on vascular function as a result of weightlessness that may be further compounded by radiation exposure. The purpose of the present study was to assess the individual and combined effects of hindlimb unloading (HU) and radiation (Rad) on vasodilator responses in the skeletal muscle vasculature. Adult male C57BL/6J mice were randomized to one of four groups: control (Con), HU (tail suspension for 15 days), Rad (200 cGy of 137Cs), and HU-Rad (15-day tail suspension and 200 cGy of 137Cs). Endothelium-dependent vasodilation of gastrocnemius feed arteries was assessed in vitro using acetylcholine (ACH, 10−6 – 10−4 M) and inhibitors of nitric oxide synthase (NOS) and cyclooxygenase (COX). Endothelium-independent vasodilation was assessed using DEA/NO (10−6 – 10−4 M). Endothelium-dependent and -independent vasodilator responses were impaired relative to Con responses in all treatment groups; however, there was no further impairment from the combination of treatments (HU-Rad) relative to that in the HU and Rad groups. The NOS-mediated contribution to endothelium-dependent vasodilation was depressed with HU and Rad. This impairment in NOS signaling may have been partially compensated for by an enhancement of PGIII-mediated dilation. Changes in endothelium-dependent vasodilation were also associated with decrements in trabecular bone volume in the proximal tibia metaphysis. These data demonstrate that the simulated space environment (i.e., radiation exposure and unloading of muscle and bone) significantly impairs skeletal muscle artery vasodilation, mediated through endothelium-dependent reductions in NOS signaling and decrements in vascular smooth muscle cell responsiveness to NO.

radiation; spaceflight; endothelium-dependent vasodilation; microgravity; bone remodeling

SPACE EXPLORATION comes at a substantial physiological cost to the astronaut, including muscle atrophy (39), bone loss (40), and visual impairments (42). Upon return to earth, among the detrimental cardiovascular consequences of adaptations to weightlessness are diminished aerobic capacity (65, 83, 86) and orthostatic intolerance (10, 48, 86), which are attributed in part to modifications in cardiovascular regulation (7, 86). Additionally, short- and long-term exposure to weightlessness result in increased calcium excretion (23) and loss of bone mass (12, 23) and density (67, 68) due to reduced mechanical loading of the skeleton and possible alterations in skeletal per fusion (11, 84, 85). During spaceflight, not only are astronauts subjected to weightlessness, but they are also exposed to ionizing radiation (4), extreme temperature fluctuations (22), vibration and mechanical stress (43), and alterations in the environmental fraction of CO2 (82), each of which pose a risk to astronaut health. In particular, space radiation exposure may predispose astronauts to ailments such as stroke (47), heart disease (13), cardiovascular degeneration (25), and osteopenia (50, 57).

Both microgravity and radiation exposure have profound effects on vascular function. For example, spaceflight-induced orthostatic intolerance has been attributed to a diminished capacity to adequately elevate peripheral vascular resistance to maintain mean arterial pressure (10, 53), implicating the resistance vasculature as a site of dysfunction. Additionally, severe disruptions in the cytoskeleton of cultured endothelial cells during spaceflight is thought to contribute to the development of the endothelium-dependent vasodilator dysfunction accompanying microgravity exposure (33). Indeed, in ground-based animal models (e.g., hindlimb unloading) designed to mimic the physiological effects of spaceflight (28, 52, 54), aortas isolated from 14-day hindlimb unloaded (HU) rats exhibited impairment of vasoconstrictor (17, 19) and vasodilator responses (17). Moreover, 14 days of hindlimb unloading results in a 20–60% reduction in endothelium-dependent vasodilation of feed arteries and 1A arterioles isolated from rat slow-twitch muscle (18, 31, 69). These data demonstrate dysfunction in both vasodilator and constrictor pathways of multiple vascular beds with simulated microgravity.

Vascular endothelial cells are also particularly sensitive to the effects of radiation, showing signs of dysfunction within days of radiation exposure (49, 64) and persisting years later (1). Following radiation exposure, endothelial cells undergo greater incidences of apoptosis (24, 38), reductions in the production and release of nitric oxide (29, 63, 76), and morphological changes characterized by cell shrinkage, wider gaps between cell-junctions, and detachment from the basement membrane (63). Capillaries and arterioles isolated from the cremaster muscles of irradiated hamsters exhibited smaller vessel diameters, concomitant with reduced red blood cell velocity through the capillaries, signifying a reduction in blood flow (64). Isolated large artery studies from various animals (1, 49, 56, 63, 74, 75, 79) and results from isolated rat submucosal
resistance arterioles (29) further indicate that these changes in vessel diameter may be initially due to a radiation-induced impairment of endothelium-dependent vasodilation. However, many studies examining the effects of radiation on vascular endothelial cells use doses in the range of 10–65 Gy (29, 49, 56, 63, 64, 79) which are relevant to cancer radiotherapy, but which are an order of magnitude or greater than the anticipated radiation exposure in space (24, 75, 76).

Although there are published studies demonstrating HU- and radiation-induced reductions in endothelium-dependent vasodilation, there are no studies to date that have examined the combined effects of HU and radiation on vascular function. The primary hypothesis of this investigation is that both HU and radiation will impair endothelium-dependent vasodilation and that combined HU and radiation will potentiate endothelium-dependent vasodilator dysfunction of skeletal muscle resistance arteries. In addition, since impaired endothelium-dependent vasodilation has been previously shown to be related to bone loss in HU rats (59), a secondary aim is to determine the effects of HU and radiation on cancellous bone volume and other indexes of bone microarchitecture, and to determine whether there is a relation between changes in endothelium-dependent vasodilation and bone loss. We hypothesized that the magnitude of the impairment of endothelium-dependent vasodilation elicited through HU and radiation alone and in combination will be directly related to the magnitude of the loss of trabecular bone volume.

MATERIALS AND METHODS

Experiment Design

To investigate the effects of simulated spaceflight, specifically mechanical unloading and radiation exposure, on vascular function and cancellous tissue, a 2 × 2 experiment was designed with a previously established timeline (35), using treatments and durations known to elicit bone loss (34, 87, 89). Individually housed C57BL/6J male mice (The Jackson Laboratory, Sacramento, CA, n = 40, 16 wk of age) served as normally ambulating controls (Con, n = 10) or were HU (n = 10) for a total of 15 days as previously described (35). On the third day of unloading, two additional groups of conscious Con and HU mice were irradiated (Rad, n = 10 and HU-Rad, n = 10, respectively) with 200 cGy (84 cGy/min over 2.38 min) or 137Cs gamma-rays at NASA Ames Research Center with a JL Sheppard Mark I irradiator (San Gabriel, CA). This dose, which is approximately the total dose exposure during a Solar Particle Event (over several days), a space mission outside Earth’s magnetosphere (over several years), and a single fraction delivered during radiotherapy (87, 88), was chosen as it has been previously shown to effectively induce tibial bone loss (34, 35, 89) and aortic vasodilator dysfunction (74–76). During irradiation, mice were housed in custom cages that allowed the HU mice to remain unladen. Con and HU animals were sham-irradiated for the same amount of time. The treatment schedule was staggered to realize tissue harvests of 4 mice per day that included 1 mouse from each treatment group. Mice were provided food (5001 LabDiet, St. Louis, MO) and water ad libitum and experienced a 12:12-h light/dark cycle. To monitor animal health, body mass was measured at least twice weekly during the experiment. The Institutional Care and Use Committee of NASA Ames Research Center approved all procedures.

In Vitro Vessel Experiments

Mice were anesthetized with isoflurane (5%/O2) and euthanized by an intracardiac injection of potassium chloride. Vessels were then dissected free from surrounding tissue via dissecting microscope (Olympus, Japan) and placed into a bath of cold (4°C) physiological saline solution (PSS) containing 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.17 mM MgSO4, 1.2 mM Na2HPO4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 ml BSA, pH 7.4. Using a dissecting microscope (Olympus SX710), gastrocnemius feed arteries were isolated, removed from the muscles before the muscles were weighed, and transferred to a Lucite chamber containing PSS equilibrated to room air. Vessels were then cannulated to glass microper- pettes and secured using nylon suture (Alcon 11-0 nylon microfilament) at each end.

Once cannulated, the microvessel chamber was transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310) and video caliper (Microcirculation Research Institute, Texas A&M University) to measure intraluminal diameter. Vessels were pressurized to 90 cmH2O through two independent hydrostatic pressure reservoirs. Any vessels displaying leaks were discarded. Those vessels free of leaks were warmed to 37°C and allowed to develop spontaneous tone over a 30- to 60-min equilibration period, during which the bathing PSS was changed every 20 min. Vessels were then preconstricted with 10−4 M phenylephrine to elicit steady tone prior to evaluation of vasodilator responses.

Evaluation of Vasodilator Responses

Endothelium-dependent and -independent vasodilator responses were assessed through the cumulative addition of acetylcholine (ACH, 10−8–10−4 M) and DEA-NONOate (10−9–10−4 M), respectively. To determine the endothelial pathway(s) through which potential endothelium-dependent vasodilator changes may occur, ACH-induced vasodilation was also evaluated following a 20-min incubation with the nitric oxide synthase (NOS) inhibitor Nω-nitro-l-arginine methyl ester (l-NAME, 10−5 M), and the combination of l-NAME with the cyclooxygenase (COX) inhibitor indomethacin (10−5 M) finally. Maximal intraluminal diameter was determined after two 15-min incubations in Ca2+-free PSS supplemented with sodium nitroprusside (SNP, 10−4 M) to achieve complete smooth muscle relaxation. Responses were recorded as intraluminal diameters.

eNOS mRNA Expression

RNA isolation and reverse transcription. Gastrocnemius feed arteries and principal nutrient arteries to the femur from each mouse were dissected free from surrounding tissue via dissecting microscope (Olympus SZX12). Arteries were immediately frozen in liquid N2 and placed into a −80°C freezer. The RNAqueous-Micro Kit (Ambion, Carlsbad, CA) was used to isolate total RNA from the vessels. Total RNA quantity and concentration were assessed by spectrophotometry (Biotek Synergy 2, Biotek, Winsoski, VT). SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) was combined with 20 ng of total RNA, 250 ng of random primers, 10 mM dNTP Mix, and RNaseOUT Recombinant Ribonuclease Inhibitor (Invitrogen) according to the manufacturer’s protocol.

Real-time polymerase chain reaction. Complementary DNA (cDNA) was used in the 10-µl real-time PCR (StepOnePlus, Applied Biosystems, Carlsbad, CA) to determine mRNA expression for endothelial nos (eNOS) by way of a TaqMan Gene Expression Assay (Mm00435217_m1, Applied Biosystems). TaqMan Ribosomal RNA Control Reagents (Applied Biosystems) were used to determine 18S rRNA expression. eNOS gene expression was quantified relative to 18S gene expression using the comparative method (i.e., ΔΔCt) as previously described (77).

Cancellous Microarchitecture of the Proximal Tibia Metaphysis

At the time of tissue harvest, tibias were collected for 3D structural analyses by microcomputed tomography (SkyScan 1174, Bruker-MicroCT, Kontich, Belgium; 6.7-µm pixel size). Tibias were wrapped
with saline-soaked paper and imaged in air with a 3,500 ms integration time using X-rays generated by a 50 kV- and 800 μA-powered source. Images were binarized with a global threshold and the bone volume fraction (BV/TV, the percent of mineralized tissue within a given region of interest in cancellous bone tissue), trabecular thickness (μm), separation (μm), and number (1/mm) were measured in the region 0.2–1.2 mm distal to the proximal metaphysis using semi-autonomous selection of the cancellous tissue (35).

Statistical Analysis

Vasodilation was calculated according to the formula: vasodilation (% maximal response) = (IDm – Idb)/(IDm – Idb) × 100, where IDm is the maximal inner diameter obtained after incubation in calcium-free PSS with 10^-4 M SNP, Idb is the steady-state baseline diameter, and Ids is steady-state diameter recorded after a 3-min incubation with each dose of ACh or Dea-NONoate. Vascular sensitivity, the concentration of ACh or Dea-NONoate exhibiting 50% maximal relaxation (IC50), was determined by logarithmic curve-fitting equations.

Comparisons of body mass, muscle mass, maximal diameter, vessel tone, IC50, mRNA expression, and trabecular properties across conditions were made using one-way ANOVA and Tukey’s post hoc tests. Vasodilator responses were evaluated using repeated-measures ANOVAs to detect differences between groups and doses. To examine the relation between peak endothelium-dependent vasodilation, NO-mediated vasodilation or muscle mass and trabecular bone volume or other indexes of trabecular bone microarchitecture, as well as between gastrocnemius feed artery eNOS mRNA and femoral PNA eNOS mRNA, linear regression analyses were performed. All values are presented as means ± SE. A value of P ≤ 0.05 was considered statistically significant.

RESULTS

Body Mass, Muscle Mass, and Vessel Characteristics

Body mass both before treatment (Con, 26.0 ± 0.4 g; HU, 25.6 ± 0.4 g; Rad, 26.3 ± 0.4 g; HU-Rad, 26.2 ± 0.6 g) and after (Con, 26.4 ± 0.3 g; HU, 25.1 ± 0.5 g; Rad, 26.3 ± 0.3 g; HU-Rad, 25.0 ± 0.4 g) did not differ among groups. Soleus muscle mass in the HU and HU-Rad groups were lower than that in the Con and Rad groups (Con, 11.9 ± 0.6 mg; HU, 9.1 ± 0.5 mg; Rad, 12.1 ± 0.6 mg; HU-Rad, 9.3 ± 0.6 mg); there was no difference between HU and HU-Rad soleus muscle mass or between Con and Rad mass. Likewise, gastrocnemius muscle mass was lower in the two groups that included HU (Con, 188 ± 4 mg; HU, 171 ± 4 mg; Rad, 197 ± 3 mg; HU-Rad, 170 ± 5 mg) with no difference between HU and HU-Rad gastrocnemius muscle mass or between Con and Rad muscle mass.

Maximal diameter of the gastrocnemius feed artery tended to be lower in groups that included the HU treatment (Con, 177 ± 7 μm; HU, 166 ± 4 μm; Rad, 178 ± 5 μm; HU-Rad, 164 ± 3 μm), but these differences did not reach statistical significance (P = 0.061). Preconstricted vessel tone did not differ among groups (Con, 28 ± 2%; HU, 33 ± 4%; Rad, 24 ± 3%; HU-Rad, 32 ± 3%).

Endothelium-Dependent and -Independent Vasodilatory Responses

Both HU and Rad impaired ACh-mediated endothelium-dependent vasodilation relative to that in Con gastrocnemius muscle feed arteries (Fig. 1A). The combination of treatments (HU-Rad) also impaired ACh-mediated vasodilator responses relative to that in Con arteries, but did not demonstrate an additive effect compared with that of HU and Rad alone. There was no difference in ACh IC50 among groups. During NOS inhibition with L-NAME, endothelium-dependent vasodilation was lowest in arteries from Con mice and highest in arteries from HU-Rad mice (Fig. 1B). The combined effects of NOS and COX inhibition eliminated statistical differences in vasodilation among groups (Fig. 1C).

Endothelium-independent vasodilation was not different among groups (Fig. 2). However, the IC50 for all treatment groups was higher than that in Con arteries (IC50: Con, 5.82e-8; HU, 2.64e-7 ± 8.00e-8; Rad, 8.59e-7 ± 2.24e-7; HU-Rad, 7.25e-7 ± 2.28e-7), indicating vascular smooth muscle sensitivity to NO was lower in all groups relative to that in Con.

eNOS Expression

Neither HU nor Rad alone altered eNOS mRNA expression in gastrocnemius feed arteries from mice (Fig. 3A). However, vessels isolated from the HU-Rad group exhibited greater...
eNOS mRNA expression than those from Con, HU, and Rad groups. Changes in eNOS expression in the principal nutrient artery to the femur showed a similar pattern to that in the gastrocnemius feed artery, and there was a significant correlation between gastrocnemius muscle feed artery eNOS and principal nutrient artery eNOS expression (Fig. 3B).

**Bone**

Although HU did not alter the BV/TV, trabecular separation or trabecular number in the proximal tibia metaphysis, it diminished trabecular thickness relative to that in Con mice (Table 1). Conversely, radiation exposure reduced BV/TV and trabecular number while increasing trabecular separation, but did not alter trabecular thickness (Table 1). The combination of HU-Rad diminished BV/TV, trabecular thickness and trabecular number while increasing trabecular separation in the proximal tibia metaphysis relative to that in Con, HU and Rad mice (Table 1). There was a significant relation between cancellous bone volume and peak endothelium-dependent vasodilation (Fig. 4A). When the portion of endothelium-dependent vasodilation is limited to that which is sensitive to NOS inhibition, the relation between cancellous bone volume and NO-mediated vasodilation is stronger (Fig. 4B). Likewise, trabecular thickness (Fig. 5A), separation (Fig. 5B), and number (Fig. 5C) demonstrated a significant relation with peak endothelium-dependent vasodilation, but this relation was more robust when endothelium-dependent vasodilation is expressed using the NO-mediated portion of the vasodilation (Fig. 6, A–C). Cancellous bone volume and gastrocnemius muscle mass or gastrocnemius-soleus muscle mass did not demonstrate a significant relation (Fig. 7).

**DISCUSSION**

Spaceflight has been shown to alter the intrinsic vasomotor properties of resistance arteries (2, 30, 73, 82), including those in skeletal muscle (78). Additionally, spaceflight (30, 73), ground-based simulations of microgravity (17, 18, 31, 59, 62, 69) and ionizing radiation (24, 29, 74–76, 79) each diminish endothelium-dependent vasodilation and, in the case of spaceflight, disrupt the endothelial cell cytoskeleton (33). In this context, the primary hypothesis of the present study was that both hindlimb unloading and radiation would impair endothelium-dependent vasodilation in the skeletal muscle microcirculation, and that the combined effects of these treatments would further diminish endothelium-mediated vasodilation. The results demonstrate that hindlimb unloading and radiation individually elicit endothelial dysfunction; however, contrary to our hypothesis, the combination of treatments does not further impair endothelium-dependent vasodilation (Fig. 1A). The impairment of vasodilator responses occurred predominantly through the NO signaling mechanism (Fig. 1B), although other mechanisms mediating endothelium-dependent vasodilation (e.g., PGI2) appear to be upregulated with HU and Rad, and to an even greater extent with HU-Rad (Fig. 1C). eNOS mRNA levels were unchanged by HU and Rad, but were elevated 71% by the combination of the 15-day HU and Rad treatments (Fig. 3A). HU, Rad, and HU-Rad also impair the sensitivity of smooth muscle cells in the skeletal muscle resistance vasculature to NO (Fig. 2). Finally, a secondary hypothesis of the present study was that the magnitude of the impairment of endothelium-dependent vasodilation elicited through HU and Rad alone and in combination will be directly associated with the magnitude of the loss of cancellous bone volume and changes in cancellous bone microarchitecture. The relations between impairment of endothelium-dependent vasodilation mediated by HU, Rad, and HU-Rad and changes in trabecular bone volume and microarchitecture were significant (Figs. 4A, 5, A–C). However, the relations were stronger when the NO component of the endothelium-dependent vaso-
dilation was regressed with cancellous bone properties (Figs. 4B and 6, A–C). In contrast to this evidence for the coupling of endothelial cell signaling with bone remodeling, neither gastrocnemius muscle mass (Fig. 7A) nor the combined gastrocnemius and soleus muscle masses (Fig. 7B) were significantly associated with cancellous bone volume.

The present findings show that HU reduced maximal endothelium-dependent vasodilation in mouse gastrocnemius muscle feed arteries by \( \frac{1}{101} \) 33%, which is similar to that previously reported to occur with HU in rat soleus muscle resistance vessels (18, 31, 69). NOS inhibition significantly diminished ACh-induced vasodilation in Con arteries, but had less effect on arteries from HU, Rad, and HU-Rad mice (Fig. 1B). This suggests that the contribution of NO to the vasodilator response is less in arteries from HU, Rad, and HU-Rad mice relative to that in Con animals, and that other signaling pathways mediating endothelium-dependent vasodilation may be upregulated. The lack of significant differences in the vasodilator responses among groups in the presence of both NOS and COX inhibition (Fig. 1C) suggests that it is a COX signaling pathway that is upregulated in the three treatment groups. A similar pattern of adaptation has been reported in cerebral arteries of rats exposed to HU, where endothelium-dependent vasodilation through the NO signaling pathway is impaired, but this impairment is partially compensated for through an upregulation of an endothelium-dependent hyperpolarizing factor (62).

The mechanism through which endothelium-dependent vasodilation is impaired in the skeletal muscle resistance vascu-

### Table 1. Morphometric indices of cancellous bone in the proximal tibia metaphysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Bone volume fraction, %</th>
<th>Trabecular thickness, µm</th>
<th>Trabecular separation, µm</th>
<th>Trabecular number, 1/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Con)</td>
<td>16.8 ± 0.5</td>
<td>54.1 ± 0.16</td>
<td>170 ± 3</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Hindlimb Unloading (HU)</td>
<td>15.5 ± 0.5</td>
<td>51.0 ± 0.4*</td>
<td>176 ± 4</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Radiation (Rad)</td>
<td>13.9 ± 1.1*</td>
<td>53.5 ± 0.15†</td>
<td>185 ± 6*</td>
<td>2.6 ± 0.2†</td>
</tr>
<tr>
<td>Hindlimb Unloading and Radiation (HU-Rad)</td>
<td>10.2 ± 0.4**†‡</td>
<td>48.8 ± 0.6*†‡</td>
<td>200 ± 3**†‡</td>
<td>2.1 ± 0.1*†‡</td>
</tr>
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Values are means ± SE. *Significant difference from that of Con group; †significant difference from that of HU group; ‡significant difference from that of Rad group; \( P < 0.05 \).

Fig. 4. Relation between the percent trabecular bone volume in the proximal tibia metaphysis and peak percent endothelium-dependent vasodilation (A) and NO-mediated vasodilation of gastrocnemius muscle feed arteries (B) from Con, HU, Rad, and HU-Rad mice.

Fig. 5. Relation between peak percent endothelium-dependent vasodilation of gastrocnemius muscle feed arteries and trabecular thickness (A), trabecular separation (B), and trabecular number in the proximal tibia metaphysis (C) from Con, HU, Rad, and HU-Rad mice.
The present study is the first to investigate the combined effects of unloading and radiation exposure on arterial vasodilation. Contrary to our hypothesis, the combination of HU-Rad did not further impair endothelium-dependent or -independent vasodilator responses compared with either treatment alone. Previously, the combination of radiation and weightlessness may be attributed to the radiosensitivity of vascular endothelial cells (21, 38). However, to our knowledge, the present study is the first investigation to examine the effects of radiation on the skeletal muscle vascular function. Similar to results presented from other vascular beds (29, 74, 79, 81), declines in endothelium-mediated vasodilation occurred via a NO signaling pathway. Radiation-induced endothelial dysfunction may be a product of greater NO scavenging rather than due to decrements in NO production and release. Using in vitro fluorescence microscopy, investigators have shown a significant increase in reactive oxygen species (ROS) and decrease in NO production in vessels from irradiated rats (29, 76). Incubation with a superoxide dismutase mimetic (29) and xanthine oxidase inhibitor (76) restored NO and reduced ROS fluorescence, not only implicating greater ROS production as a modulator of radiation-induced endothelial dysfunction, but also suggesting a weaker antioxidant capacity of vessels after radiation exposure. Increased ROS production following radiation exposure could also potentially contribute to radiation-induced vascular smooth muscle vasodilator dysfunction, whereby ROS scavenges Dea-NONOate-donated NO, as has been shown to occur in thoracic aortas isolated from atherosclerotic rabbits (51). However, further investigation into the effects of radiation on mediators of ROS in the skeletal muscle vasculature is warranted.

Vascular alterations observed in response to radiation include retardation of blood vessel development and structural loss within mature blood vessels (24). Functional vascular consequences of radiation exposure are similar to those seen with hindlimb unloading in that diminished endothelium-dependent vasodilation is commonly observed, and...
has been shown to delay recovery from pathologies affecting bone and skeletal muscle to a greater extent than either insult alone (55, 58). Thus, it is possible that repeating these experiments after a period of recovery may result in distinctive differences in vasodilator responses of vessels from the combined HU-Rad group vs. the HU or Rad only groups. Additionally, a large contributor to the absorbed dose of radiation when in space is from heavy ions (55), which possess and impart larger amounts of energy to the materials they traverse compared with low-energy gamma rays. Heavy ion radiation can result in more extensive damage to biological tissue, as evidenced by larger clusters of DNA damage (27, 70), greater incidences of apoptosis (3), and higher levels of ROS (14, 29, 41). Therefore, it may also be the case that use of heavy ion radiation as opposed to gamma-rays in future studies would lead to more immediate damage to the skeletal muscle vasculature, which when combined with unloading, would illustrate differences in vasodilator responses from either treatment alone. As such, the only signs of an interaction with the combined treatments are 1) the increase in eNOS mRNA expression (Fig. 3A), and 2) a possible increased reliance on PGI2-mediated endothelium-dependent dilation (Fig. 1, B and C). Data from the present study revealed that eNOS mRNA expression in the gastrocnemius feed artery was unchanged in the HU and Rad groups, but was 71% greater in the HU-Rad mice relative to that in Con animals. This latter response showing an increase in eNOS mRNA expression, but a decrease in endothelium-dependent NO-mediated vasodilation (Fig. 1), suggests a possible uncoupling of the NOS signaling pathway, as has been shown to occur with aging in the skeletal muscle microcirculation due to the greater presence of ROS (16, 71, 72). Further work is necessary to clearly establish whether the putative eNOS uncoupling occurs with the combined HU-Rad treatments. Finally, the shift toward enhanced PGI2-mediated dilation with the HU-Rad treatment is indicated by the greater ACh-induced vasodilation compared with that in Con, HU and Rad arteries in the presence of NOS inhibition (Fig. 1B), but no difference in vasodilator response among groups with the addition of COX inhibition (Fig. 1C). As indicated above, this greater ACh-induced vasodilation in the presence of t-NAME may be a compensatory effect for a larger decline in NO-mediated dilation. Previous studies, however, have divergent results regarding the effects of radiation exposure on PGI2-mediated vasodilation, with indications of both no change (74) or impaired PGI2 signaling (79). These discrepancies may be related to differences in the type of vessels studied (i.e., large conduit arteries vs. small resistance arteries) or the vascular beds from which the vessels were isolated (aorta and neck arteries vs. skeletal muscle arteries).

Reports in the human and animal literature suggest a coupling between the vascular system and bone. For example, endothelium-dependent vasodilation of a forearm conduit artery was positively associated with bone mineral density of the lumbar spine in postmenopausal women (66, 80). Mechanisms relating bone vascular function to bone metabolism, and ultimately bone mass, are gaining considerable attention in the medical and scientific communities (37). Bone vascular endothelial cells have the capacity to release endothelium-derived factors (e.g., NO and PGI2) and influence bone cellular activity (9, 15). In addition, bone resistance arterioles regulate skeletal blood flow and the significance of this control lies in its impact on bone interstitial fluid flow and pressure (5, 9, 11, 32, 44). In light of these control mechanisms, there is evidence in the literature to indicate that the bone vascular system has the capacity to modulate bone metabolism (5, 6). There are correlates in the literature regarding osteopenia and age-related declines in bone and marrow blood flow (8, 20, 26, 36, 61), which were related to reduced bone vascular endothelial cell function (20, 60, 61). In the present study, decrements in endothelium-dependent vasodilation of the gastrocnemius muscle feed artery from HU mice are similar to those reported to occur in the femur principal nutrient artery from HU rats (59). Further, changes in eNOS expression across all groups in the gastrocnemius feed arteries in the present study are significantly correlated to those of the femur principal nutrient artery (Fig. 3B). Therefore, using the gastrocnemius muscle feed artery as a surrogate to the bone nutrient artery, results from the current investigation are in support of the concept of a bone-vascular coupling in bone remodeling. Regression analyses indicate a relation between bone volume and peak endothelium-dependent vasodilation (Fig. 4A), as well as between bone volume and NO-mediated endothelium-dependent vasodilation (Fig. 4B). Other morphometric indexes of cancellous bone structure also correlate with peak endothelium-dependent vasodilation (Fig. 5, A–C) and NO-mediated vasodilation (Fig. 6, A–C). The strength of these relations, as indicated by the coefficient of determination (R²), suggests that while endothelium-dependent vasodilation is significantly correlated with bone volume (Fig. 4A) and trabecular thickness (Fig. 5A), separation (Fig. 5B), and number (Fig. 5C), it is the NO component of the endothelium-mediated vasodilation that is the important factor coupling vascular signaling with bone remodeling (Figs. 5B and 6, A–C). According to these data, the NO signaling from the vascular endothelium accounts for approximately 30–60% of the variance in the bone volume and indexes of trabecular bone structure from these groups. Other nonvascular factors are also considered important determinants of bone volume and structure, including the loading of the skeleton and the level of muscle force exerted on bone. However, the reduced gastrocnemius and soleus muscle masses and the presumed lower muscle-associated mechanical loads to the tibia were not significantly related to cancellous bone volume (Fig. 7).

In conclusion, the results of the current investigation demonstrate that environmental conditions evident in spaceflight (i.e., weightlessness and radiation exposure) have the capacity to impair vascular function. More specifically, both HU and Rad cause decrements in endothelium-dependent vasodilation of the skeletal muscle microcirculation (Fig. 1A) that are attributed to declines in the NO signaling pathway (Fig. 1, B and C). Interestingly, the combined effects of HU-Rad did not further reduce endothelium-dependent vasodilation compared with HU and Rad alone; however, the mechanism of vasodilation shifted from one reliant primarily upon production of NO to one presumably more reliant upon PGI2 signaling. Finally, impairment of endothelium-dependent vasodilation mediated by HU, Rad, and the combined effects of these treatments was strongly associated with changes in trabecular bone volume and microarchitecture (Figs. 4A and 5, A–C). However, it appears that it is the NO component of the endothelium-dependent vasodilation that serves to couple vascular signaling to bone remodeling (Figs. 4B and 6, A–C).
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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


