Simulated microgravity effects on the rat carotid and femoral arteries: role of contractile protein expression and mechanical properties of the vessel wall

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Hwang S, Shelkovnikov SA, Purdy RE. Simulated microgravity effects on the rat carotid and femoral arteries: role of contractile protein expression and mechanical properties of the vessel wall. J Appl Physiol 102: 1595–1603, 2007. First published January 11, 2007; doi:10.1152/japplphysiol.01020.2006.—The goal of this study was to determine the effects of microgravity on myofilament protein expression and both passive and active length-force relationships in carotid and femoral arteries. Microgravity was simulated by 20-day hindlimb unweighting (HU) in Wistar male rats, and carotid and femoral artery segments were isolated from both HU and control (CTL) rats for Western blot and length-force analysis. Western blots revealed that HU significantly decreased myosin light chain-20 (MLC-20) protein levels in both carotid and femoral arteries and revealed that HU significantly decreased myosin light chain-20 (MLC-20) protein levels in both carotid and femoral arteries and decreased myosin heavy chain (MHC) in femoral artery. Western blots revealed that HU significantly decreased myosin light chain-20 (MLC-20) protein levels in both carotid and femoral arteries and decreased myosin heavy chain (MHC) in femoral artery. α-Actin levels were not altered by HU treatment in either artery. Length-force analysis demonstrated that HU did not change either passive or active length-force relationships in the femoral artery. HU-treated arterial rings developed significantly less force to 100 mM KCl than CTL, but optimal lengths were identical. In the carotid artery, length-active force curves were identical for both CTL and HU; however the length-passive force curve for HU-treated rings exhibited a steeper slope than CTL, suggesting decreased compliance of the artery wall. In conclusion, our data suggest that the HU-induced decreases in both MLC-20 and MHC in femoral artery are responsible for the decreased contraction to 100 mM KCl in HU-treated femoral artery rings. In the carotid artery, the HU-induced decrease in vessel wall compliance may counter any decrease in contractility caused by the decreased MLC-20 levels.

Hindlimb unweighting; deoxyribonucleic acid; actin; myosin; length-force relationship

The gravity of Earth produces a blood pressure gradient that is 70 mmHg at the head and 200 mmHg at the feet in the standing human. However, in microgravity, there is a cephalad fluid shift and an elimination of this gradient, yielding a uniform blood pressure of 100 mmHg throughout the body (42). These hemodynamic changes are thought to trigger adaptive changes in the cardiovascular system such that on return to the gravity of Earth, astronauts adapted to microgravity experience cardiovascular deconditioning effects, the most serious of which is orthostatic intolerance (29). Orthostatic intolerance is a condition in which subjects suffer from lightheadedness, dizziness, palpitations, and even syncope on assuming the upright position. Orthostatic intolerance afflicts not only space-flew astronauts but also ~500,000 Americans who experience a chronic form of the syndrome (36) and an even greater population in patients confined to long-term bed rest. The mechanisms underlying orthostatic intolerance are unknown. However, it is clear that on landing day, all astronauts exhibit hypovolemia, decreased stroke volume, and resting tachycardia (3, 18, 25). The parameter that distinguishes those astronauts experiencing presyncopal symptoms is a decreased capacity to elevate total peripheral resistance (3, 28, 43). There is evidence that this could be due to either a reduced sympathetic response to orthostasis, or an impaired vascular smooth muscle contraction to sympathetic stimulation (18, 25).

Hindlimb unweighting (HU) in the rat was used in the present study to simulate microgravity. This model mimics many of the effects of both real and simulated microgravity in humans. For example, HU causes hypovolemia (39), decreased stroke volume (32), resting tachycardia (24), and impaired baroreceptor reflex (26). HU also causes a decreased vasoconstrictor response in many isolated vessels, including the feed arterioles of the gastrocnemius muscle (7), the abdominal aorta (9, 35), and the carotid and femoral arteries (21, 35, 36). In gastrocnemius feed arterioles, the depressed vasoconstrictor response has been attributed to a reduction in medial cross-sectional area (8), while in the abdominal aorta it was argued that such structural changes do not occur (33, 35) and that HU-induced changes at the cellular or molecular level are likely to be important (13, 33).

In the carotid and femoral arteries, several HU-induced adaptations have been proposed to underlie the vasoconstrictor hyporesponsiveness. Sangha and coworkers (38) found that HU treatment increased nitric oxide (NO) vasodilator mechanisms in the carotid artery. In agreement, Ma and coworkers (19) reported an increased expression of endothelial NO synthase in this vessel. Endothelial mechanisms do not appear to play a role in the femoral artery, although Sangha et al. (38) obtained functional evidence for a role for the inducible isoform of NO synthase (iNOS) in this vessel (but see Ref. 19). Mao and coworkers (21) found HU produced structural changes in these two vessels. Namely, medial cross-sectional area was increased in the carotid but decreased in the femoral artery after 28 days of HU. Although these studies revealed effects of HU on endothelium and vascular structure, the impact of HU treatment on vascular smooth muscle cell number or intracellular contractile mechanisms is unknown.

The goal of the present study was to identify possible mechanisms responsible for the HU-induced vasoconstrictor deficit. The carotid and femoral arteries were chosen for two reasons. First, these vessels experience differential changes in blood flow and pressure during HU. These parameters are increased in the carotid artery (25, 45) and decreased in the femoral artery (25, 38). The consequences of changes in flow...
for endothelial function, described above, have been addressed previously (19, 38), and endothelial denudation was performed in the present study to remove this parameter from consideration. Thus the present study focused on the possible consequences of differential changes in intravascular distending pressure. Second, carotid and femoral arteries were chosen to determine whether the vasoconstrictor deficits observed in our laboratory at 20 days of HU could be explained exclusively by structural changes in the vascular wall as proposed by Mao et al. (21) for 28-day HU (for review, see Ref. 45).

In our previous study, we found that 20-day HU had no effect on wet or dry weights of carotid or femoral arteries (35). This led to the hypothesis that 20-day HU has no effect on the morphological or mechanical properties of carotid or femoral arteries. Because of previous preliminary evidence that HU decreases the expression of vascular myofilament proteins (13, 14, 22), it was also hypothesized that 20-day HU decreases the expression of α-actin and myosin. To test these hypotheses, we explored the effects of HU on 1) the expression of the contractile proteins α-actin and myosin, 2) passive and active length-force relationships, 3) medial cross-sectional area, and 4) DNA levels as a measure of cell number. A preliminary report of these findings has been presented elsewhere (14).

MATERIALS AND METHODS

HU. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. To simulate the cardiovascular deconditioning effects of microgravity, we used the rat HU model as previously described (19). Briefly, male Wistar rats weighing 250–300 g (Charles River Laboratories, Wilmington, MA) were caged individually in a room maintained in a 12:12-h light-dark cycle and were allowed free access to water and standard laboratory chow. Rats were randomly assigned to a control or HU group (n = 62 control group; n = 61 HU group), and those assigned to the HU group were subjected to hindlimb-unweighting for 20 days. HU was achieved by using a tail harness tethered to the top of the cage and shortened sufficiently to elevate the hindlimbs above the cage floor ~1 cm when fully extended. This produced a head-down tilt of ~35° with respect to the cage floor. To apply the tail harness, the tail of the rat was cleaned and dried, and a coat of benzoin tincture (Smith and Nephew, Memphis, TN) was applied and allowed to air dry until tacky. Adhesive strips of Moleskin Plus (Dr. Scholl’s, St. Louis, MO) ~1 cm were placed laterally along the proximal two-thirds of the length of the tail, beginning ~2 cm distal from the base of the tail. The Moleskin strips were secured by three, 1-cm-wide adhesive strips (Beiersdorf-Jobst, Rutherford College, NC) wrapped circumferentially at three equidistant sites along the length of the tail. The secured tail harness was then tethered to a swivel and loop, which moved freely along the length of a horizontal tube attached to the top of the cage. This allowed the animal 360° rotation and full freedom of movement throughout the cage.

Collection of tissue samples. Following the 20-day period, animals were deeply anesthetized by exposure to 100% CO2 (11) and euthanized by opening of the chest and removing the heart. Carotid and femoral arteries were rapidly removed, cleaned of extraneous fat and connective tissue under a microscope, weighed and either used in subsequent measurements of circumference. Carotid and femoral arteries were rapidly removed, cleaned of extraneous fat and connective tissue under a microscope, weighed and either used in subsequent measurements of circumference. In some experiments, stretched vessels were fixed in 10% formalin, and the resulting supernatant was collected. Protein concentration of the supernatant was measured using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Tissue lysates were then mixed with Tris-glycine SDS sample buffer (containing β-mercaptoethanol), and proteins were denatured by boiling for 4 min. Sample protein from both control and HU lysates (10–20 µg/lane) were loaded equally onto 4–20% Tris-glycine gels and size-fractionated by SDS-PAGE at 125 V for 2–3 h in a minigel apparatus (Invitrogen, Grand Island, NY). The proteins were then transferred to nitrocellulose membrane (Amersham, Piscataway, NJ) at 500 mA for 90 min and subsequently blocked with 6.5% nonfat dry milk in T-PBS (0.01 mol/l PBS, 0.1% Tween-20) and incubated overnight at 4°C. Membranes were drained and incubated with the primary antibody of interest for 30 min at 37°C. They were then washed in T-PBS for 30 min, changing the T-PBS every 5 min. Membranes were incubated in secondary antibody for 1 h at room temperature and then washed in T-PBS for 30 min as above. Membranes were immediately subjected to analysis using a Licor Odyssey system for quantitative detection of fluorescent signal (LI-COR, Lincoln, NE). Quantitation of band density was performed using a densitometry analysis program, UN-SCAN-IT (Silk Scientific, Orem, UT). Levels of α-actin were used to confirm equal loading of the lanes. The following dilutions of primary antibodies were used: rabbit polyclonal anti-myosin light chain-20 (MLC-20), 1:500; mouse anti-myosin heavy chain (MHC), 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA); mouse monoclonal anti-α-actin, 1:5,000 (Sigma Chemical Company, St. Louis, MO). The following dilutions of secondary antibodies were used: IRDye 800 conjugated anti-mouse IgG 1:20,000 (Rockland, Gilbertsville, PA); Alexa Fluor 680 goat anti-rabbit IgG 1:20,000 (Molecular Probes, Eugene, OR).

Length-force relationships. Optimal length was determined in each vessel type in both carotid and femoral arteries according to the method of Elliott and Pearce (10). A vessel ring length of 1 mm was used to minimize isotonic contraction and more closely approximate true isometric conditions. Namely, in preliminary experiments, it was found that the displacement of the transducer arm was <0.1 mm at forces <10% or less than 4 g and that the maximum force developed by 1-mm rings fell within this limit. Vessel rings were endothelium denuded by gentle mechanical rubbing with stainless steel wire. Absence of endothelium was confirmed at the end of the experiments by the failure of phenylephrine-precontracted vessels to relax to 1 µm acetylcholine. Vessel rings were mounted on wire supports, one fixed and the other manipulated by a micrometer to adjust and measure vessel length. In all experiments, every vessel ring was subjected to the following protocol. Vessel rings were bathed in zero-calcium Krebs solution containing 2 mM EGTA to eliminate spontaneous tone. Each vessel ring was placed under near-zero stretch (between 8 and 22 mg of force, depending on vessel and treatment), just sufficient to reshape the vessel segments from cylindrical to two parallel sheets. The resulting unstretched length was called L0. Each vessel was set to its L0 and stretched incrementally to determine passive force. In other experiments, tissues were placed at L0 switched to regular calcium-containing Krebs solution, and exposed to 100 mM K+ after each increment, and the resulting active force development was measured. The tissues were washed with Krebs solution between each increment, and the resulting active force development was measured. The composition of the Krebs solution was 119.2 mM NaCl, 4.9 mM KCl, 1.3 mM CaCl2, MgSO4, 1.2 mM MgSO4, 25 mM NaHCO3, 11.1 mM glucose, 0.114 mM ascorbic acid, and 0.03 mM tetradsodium EDTA. At the end of these experiments, stretched vessels were fixed in 10% formalin, removed from the wire supports, and saved in 10% formalin for subsequent measurements of circumference. In some experiments, fresh vessel rings were used for the measurement of circumference immediately after isolation and cleaning. Rings were cut open, laid flat on a microscope slide in a drop of Krebs solution, and cover-
shorts, and the length was measured by videomicroscopy using a stage level calibration grid. Sodium nitroprusside, 100 μM, was included in the Krebs solution in some experiments. However, this had no effect on tissue length, and all measurements in the presence and absence of sodium nitroprusside were pooled.

Vessel wall dimension analysis. Following 20 days of HU, control and HU-treated carotid and femoral arteries were removed from rats, and extraneous fat and connective tissue was removed. Arteries were then immediately cannulated and pressurized to 100 mmHg in oxygenated Krebs maintained at 37°C and a pH of 7.4 and fixed in 10% formalin solution buffered in Krebs for 30 min. Arteries were then dehydrated, wax-embedded, thin-sectioned, stained with trichrome, and mounted on microscope slides. Medial thickness and cross-sectional area were measured using a videomicroscope and UTHSCSA Image Tool 3.00 imaging software (University of Texas Health Science Center in San Antonio, San Antonio, TX).

DNA content determination. Arterial DNA concentration was measured in arterial homogenates by using a fluorometric assay with the DNA-binding fluorochrome Hoechst 33258 (Calbiochem/EMD Biosciences, San Diego, CA) (17). Frozen carotid and femoral arteries were homogenized in ice-cold PBS (Sigma Chemical, St. Louis, MO), and fluorescence was measured using a TKO 100 mini fluorometer (Hoeffer Scientific, San Francisco, CA) with calf thymus DNA (Sigma Chemical) as a standard. All results were normalized to arterial wet weight.

Statistical analysis for Western blots and DNA quantification. Values are presented as means ± SE, and unpaired t-tests were made between groups. P < 0.05 was required for significance.

RESULTS

Results presented in Table 1 provide data on the effects of HU on soleus muscle and body mass. Absolute soleus muscle weight and soleus weight corrected for differences in body weight decreased by 40% and 29%, respectively, after 20 days HU. These findings confirm the efficacy of the unweighting treatment (27).

Experiments were carried out in this study to measure the effects of HU on α-actin, MLC-20, and MHC in the carotid and femoral arteries. The levels of α-actin obtained in the carotid and femoral arteries are shown in Figs. 1 and 2, respectively. HU treatment did not significantly change the levels of α-actin in either artery. However, HU caused a marked decrease in MLC-20 in both the carotid (Fig. 3) and femoral arteries (Fig. 4). MHC expression was unchanged by HU in the carotid artery (Fig. 5) but was significantly decreased by HU in the femoral artery (Fig. 6).

Experiments were carried out to determine length-force relationships in carotid and femoral arteries. First, vessel rings 1 mm in length were placed under near-zero force to reshape them to two parallel sheets of tissue. The forces required to achieve this unstretched length, L_{zero}, were measured, and the results are shown in Fig. 7. HU caused a nearly threefold increase in the force required to reach L_{zero} in the carotid artery, indicating an increase in vessel stiffness. In contrast, HU reduced the force required to reach L_{zero} in the femoral artery to less than half that in the control, indicating a decrease in vessel stiffness.

The length-passive force relationship was also determined, and the results in the carotid artery are shown in Fig. 8A. The HU rings exhibited significantly higher forces than control rings at all suprathreshold lengths. Thus both the force required to achieve L_{zero} and the length-passive force measurements indicated that HU increased carotid artery stiffness. The length-passive force relationships in the femoral artery are shown in Fig. 8B. While passive force increased with increasing length, HU had no effect compared with control.

Table 1. Body weights at day 0 and day 20 and soleus weights at day 20 in control and HU rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HU</th>
<th>P Values (HU vs. Control)</th>
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<tbody>
<tr>
<td>Day 0 body weight, g</td>
<td>349.1 ± 7.4</td>
<td>346.5 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Day 20 body weight, g</td>
<td>430.1 ± 7.6*</td>
<td>369.0 ± 6.4†</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Day 20 soleus weight, mg</td>
<td>249.8 ± 7.3</td>
<td>149.9 ± 13.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Day 20 soleus weight, mg/g body weight</td>
<td>0.5827 ± 0.015</td>
<td>0.4160 ± 0.042</td>
<td>&lt; 0.0003</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 62 control and 61 hindlimb-unweighted (HU) rats. NS, not significant. *Different from control day 0 body weight, P < 0.0001; †different from HU day 0 body weight, P < 0.02.
Artery rings were placed at $L_{zero}$, lengthened incrementally, and exposed to 100 mM K/H11001 at each increment to determine the length-active force relationship. The results in carotid artery are shown in Fig. 9A. In this vessel, there was no contraction to 100 mM K/H11001 at $L_{zero}$. However, the magnitude of force development increased with increasing length up to 0.6 mm and remained at a plateau thereafter. Moreover, there were no significant differences between control and HU vessels.

The results in the femoral artery are shown in Fig. 9B. In contrast to the carotid artery, the femoral artery exhibited a contraction to 100 mM K/H11001 at $L_{zero}$. This vessel also exhibited increases in active force with increasing length to a peak at 0.6 mm. Thereafter, there was a decrement in force with each further increase in length. The control and HU force curves mirrored each other on the x-axis. On the other hand, the magnitude of force on the y-axis was significantly less in HU compared with control at all lengths.

The unstretched length was determined by cutting both fresh and formalin-fixed rings open and measuring the length of the ring, representing the circumference. Diameters were determined by dividing the circumference by π. Neither zero-calcium Krebs solution nor 100 μM sodium nitroprusside influenced the length. As shown in Table 2, there were no...
significant differences between control and HU carotid or femoral diameters in fresh tissue. Similar results were obtained in the formalin-fixed samples with the exception that the HU femoral artery diameter was 4% less than that of the control femoral artery. It is also of interest that the fresh and formalin-fixed tissues had nearly identical diameters. The tissues fixed in formalin at maximum stretch returned to their original un-stretched lengths during the 2-mo storage in formalin.

Medial thickness and medial cross-sectional area were measured in both control and HU carotid and femoral arteries that had been isolated, pressurized to 100 mmHg, formalin-fixed, and prepared for histological analysis (Table 3). HU had no significant effect on these parameters in either vessel. Shrinkage and other fixation and treatment artifacts may have occurred, decreasing the reliability of measurements of vessel circumference. This parameter was measured in separate ex-

Fig. 7. Force (mg) required to achieve $L_{zero}$ in carotid and femoral arterial rings from control and HU rats ($n = 7$). *$P < 0.0001$.

Fig. 8. A: length-passive force curves generated from 1-mm carotid artery rings from control and HU rats ($n = 7$). *$P < 0.05$, control vs. HU carotid artery. B: length-passive force curves generated from 1-mm femoral artery rings from control and HU rats ($n = 7$).

Fig. 9. A: length-active force curves generated from stimulating 1-mm carotid artery rings from control and HU rats with 100 mM KCl ($n = 7$). B: length-active force curve generated from stimulating 1-mm femoral artery rings from control and HU rats with 100 mM KCl ($n = 7$). Active force was significantly greater at each 0.2-mm increment in length. *$P < 0.0001$. 

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periments (see above). On the other hand, the measurements of wall thickness and medial cross-sectional area were considered more reliable because of the identical treatment to which all vessels were subjected and because of the noncompressible nature of the vessel wall. Thus we conclude that HU had no effect on either medial thickness or cross-sectional area.

The total DNA content of the carotid and femoral arteries in both control and HU-treated rats is shown in Figs. 10 and 11, respectively. HU has no effect on DNA. This suggests that cell number did not change with HU treatment.

**DISCUSSION**

One of the goals of the present study was to investigate the effects of HU treatment on the vascular smooth muscle contractile proteins in the carotid and femoral arteries. We hypothesized that HU would decrease protein levels of α-actin, MHC, and MLC-20. The present findings revealed that HU had no effect on α-actin expression but reduced the expression of MLC-20 in both arteries by approximately 45% and 63%, respectively. MHC was reduced in the femoral artery but not in the carotid artery by HU.

Myosin is a hexamer composed of both light-chain (MLC-17, MLC-20) and heavy-chain (SM1, SM2) proteins (12). It is the phosphorylation of MLC-20 that triggers myosin activation, leading to the actin-myosin cross-bridge cycling that constitutes smooth muscle contraction. Given the decisive role for MLC-20 in contraction, we hypothesized the decreased expression of this protein, and of MHC, contributed to the HU-induced vasoconstrictor deficit in femoral artery (35, 38). While this was not addressed in the present study, two interventions decreased the likelihood that an NO mechanism played a role in the present experiments. First, all contractions were elicited with a high concentration of K⁺, 100 mM. It is resistant to NO-mediated vasodilation (1, 2, 4, 30). In the present study, the contractions of HU femoral artery to 100 mM K⁺ were markedly less than those of control femoral artery throughout the length-force curve (Fig. 9B). We propose that the HU-induced reduction in both MLC-20 and MHC expression accounted for this decreased contraction to 100 mM K⁺. HU could also alter myofilament sensitivity to calcium. However, this would not have played a role in the present experiments. The present study does not address an alternative possibility, namely, that HU could decrease calcium entry through voltage-dependent calcium channels. Future experiments are required to test this possibility.

In a previous study, functional evidence was obtained suggesting that iNOS activity leading to the production of NO could contribute to the HU-induced hyporesponsiveness of the femoral artery (38). While this was not addressed in the present study, two interventions decreased the likelihood that an NO mechanism played a role in the present experiments. First, all vessels were endothelium denuded. Second, all contractions were elicited with a high concentration of K⁺, 100 mM. It is well known that K⁺-induced vasoconstriction with a very high concentration of K⁺ is resistant to NO-mediated vasodilation because it abolishes NO-induced hyperpolarization (16, 34).

Table 3. Medial thicknesses and cross-sectional areas of carotid and femoral arteries pressurized to 100 mmHg, formalin-fixed, and prepared for histological analysis

<table>
<thead>
<tr>
<th>Carotid</th>
<th>Femoral</th>
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<tbody>
<tr>
<td>Control</td>
<td>HU</td>
</tr>
<tr>
<td>Medial thickness, mm</td>
<td>0.00817±0.00075</td>
</tr>
<tr>
<td>Medial cross-sectional area, mm²</td>
<td>0.0383±0.0031</td>
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Values are means ± SE; n = 6 for carotid and n = 5 for femoral arteries.
Thus the most likely explanation for the HU-induced vasoconstrictor hyporesponsiveness was a reduction in MHC and MLC-20 expression. HU did not affect the length-passive force relationship, medial thickness or cross-sectional area, or DNA content in femoral artery. Thus, with no change in either compliance or morphology, the HU length-active force curve was identical to that for control femoral artery on the x-axis. In turn, the HU-induced reduction in MLC-20 and MHC expression was unopposed, with the consequence that the magnitude of the contraction on the y-axis was markedly reduced.

It must be noted that the force required to achieve $L_{\text{zero}}$ in the HU femoral artery was significantly less than that in the control vessel. This suggests that HU may have increased the compliance of the femoral artery. However, the magnitude of this effect was small as HU had no effect on the length-passive force curve (Fig. 8B).

HU did not alter the contractile capacity of the carotid artery to 100 mM K$^+$. This was surprising since HU did cause a significant reduction in the expression of MLC-20. HU also did not cause a change in carotid artery medial thickness, cross-sectional area, or in DNA content, a surrogate for cell number. On the other hand, HU decreased carotid artery compliance. Thus it is conceivable that the decreases in MLC-20 and vessel compliance had opposing effects. The following argument is a hypothetical basis for this proposition. Smooth muscle cell length may influence vessel force development. For example, stretching a cell to a greater length may optimize the contractile units within in a manner analogous to optimizing sarcomere length by stretching cardiac or skeletal muscle cells. The elastic elements are important as well. The shortening of smooth muscle cells during contraction may be absorbed by the in-series elastic elements, decreasing the measured force development. The increased stiffness of the HU carotid artery, compared with control, could influence both the cellular and elastic elements in the following manner. At any given length, the cells of the HU carotid artery could be stretched to a greater length, i.e., closer to optimal length, than control. Moreover, the elastic elements could be stretched closer to their limit and therefore less able to absorb the active shortening of the cells. If this speculation is correct, the increased stiffness of the HU carotid artery would yield a more efficient conversion of muscle shortening to measured force development in the HU carotid artery compared with the control. In turn, this greater efficiency of conversion could have offset any reduction in actual contraction conferred by the HU-induced reduction in MLC-20.

An alternative possibility must also be considered. HU caused a contractile deficit in the femoral but not the carotid artery. HU also decreased both MHC and MLC-20 in femoral artery but only MLC-20 in the carotid artery. Perhaps the reduction of MLC-20 alone in carotid artery was insufficient to produce a contractile deficit. Future experiments are required to explore the differential HU effects on contractile capacity in femoral versus carotid arteries.

The present finding that HU had no effect on the carotid artery contraction to 100 mM K$^+$ agrees with our previous observation that HU had no significant effect on the contraction of endothelium-denuded carotid artery to NE (38). In the femoral artery, we found previously that HU decreases the contraction to both NE and 60 mM K$^+$ (38).

Mao and coworkers (21) reported that HU increased the medial thickness and cross-sectional area of carotid artery and decreased both of these parameters in the femoral artery. Mao and coworkers (21) treated their rats with HU for 28 days. Thus failure to find morphological changes in the present study may be explained by our use of 20-day HU. On the other hand, the study by Mao and workers (21) and the present study taken together point to the importance of the hemodynamic consequences of simulated microgravity. As reviewed by Zhang (45), HU increases flow (20) and pressure (44) in vessels in the upper body including the carotid artery and decreases flow (37) and pressure in the vessels of the lower body (6, 24), including the femoral artery. Through shear stress, these hemodynamic changes yield an increased endothelial vasoconstrictor contribution in the upper body, including the carotid (38) and pulmonary (31) arteries. The opposite hemodynamic effect has been identified in the lower body, at least in the feed arteries of the soleus muscle (7, 15, 23). In addition, the present results and those of Mao and coworkers (22) are consistent with the suggestion that the elevated pressure in the upper body can cause the carotid artery to become stiffer at 20 days HU and actually manifest medial morphological changes at 28 days. In contrast, the lower pressure in the lower body may initiate a decrease in compliance in the femoral artery at 20 days (Fig. 7) and exhibit morphological changes at 28 days. This hemodynamic explanation for the effects of simulated microgravity is clearly important. However, the present finding that MLC-20 is reduced in both carotid and femoral artery suggests that non-hemodynamic factors are at work as well.

In the present study, 20-day HU had no effect on either wall thickness or medial cross-sectional area in the two vessels studied. Vessel diameter was measured in both fresh tissues and in stretched tissues fixed in formalin at the end of the length-force measurements as subsequently stored in formalin. Similar results were obtained in the fresh and formalin-fixed tissues. HU had no effect on vessel diameter in the carotid artery. However, there was a trend toward a decrease in the fresh HU femoral artery, which became significant in the formalin-fixed rings. Thus 20-day HU appears to be a threshold for femoral artery remodeling because significant decreases in diameter have been reported at 28 days (21) and 10 wk (5) of HU.

In conclusion, the present findings demonstrate that the carotid artery becomes stiffer and MLC-20 expression de-
creases after 20 days of HU. We propose that these two changes may have equal and opposite effects, possibly explaining the lack of an HU effect on contraction. In contrast, the femoral artery did not experience any morphological or compliance changes that could influence force development. Thus we propose that the decrease in the expression of both MHC and MLC-20 after 20 days of HU contributed to the decreased contraction of this vessel to 100 mM K+ . If decreases in contractile protein expression are found to be generalized in the vasculature in both simulated (13) and real microgravity, these proteins could become sites for therapeutic intervention in the prevention and reversal of orthostatic intolerance. Furthermore, in addition to the field of microgravity, our findings hold additional relevance to fields of study concerning vascular adaptations to local hypertension/hypotension and their resultant pathologies. This new information is not just limited to changes in specific myofilament proteins and the time course over which they occur in the carotid and femoral arteries but may also provide further insight into the adaptive vascular responses of medium-sized arteries.

GRANTS
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