Structural and functional remodeling of skeletal muscle microvasculature is induced by simulated microgravity

MICHAEL D. DELP,1,2 PATRICK N. COLLERAN,1 M. KEITH WILKERSON,1 MATTHEW R. McCURDY,1 AND JUDY MULLER-DELP1
Departments of 1Health and Kinesiology and 2Medical Physiology and Cardiovascular Research Institute, Texas A&M University, College Station, Texas 77843

Delp, Michael D., Patrick N. Colleran, M. Keith Wilkerson, Matthew R. McCurdy, and Judy Muller-Delp.

Structural and functional remodeling of skeletal muscle microvasculature is induced by simulated microgravity. Am J Physiol Heart Circ Physiol 278: H1866–H1873, 2000.—Hindlimb unloading of rats results in a diminished ability of skeletal muscle arterioles to constrict in vitro and elevate vascular resistance in vivo. The purpose of the present study was to determine whether alterations in the mechanical environment (i.e., reduced fluid pressure and blood flow) of the vasculature in hindlimb skeletal muscles from 2-wk hindlimb-unloaded (HU) rats induces a structural remodeling of arterial microvessels that may account for these observations. Transverse cross sections were used to determine media cross-sectional area (CSA), wall thickness, outer perimeter, number of media nuclei, and vessel luminal diameter of feed arteries and first-order (1A) arterioles from soleus and the superficial portion of gastrocnemius muscles. Endothelium-dependent dilation (ACh) was also determined. Media CSA of resistance arteries was diminished by hindlimb unloading as a result of decreased media thickness (gastrocnemius muscle) or reduced vessel diameter (soleus muscle). ACh-induced dilation was diminished by 2 wk of hindlimb unloading in soleus 1A arterioles, but not in gastrocnemius 1A arterioles. These results indicate that structural remodeling and functional adaptations of the arterial microvasculature occur in skeletal muscles of the HU rat; the data suggest that these alterations may be induced by reductions in transmural pressure (gastrocnemius muscle) and wall shear stress (soleus muscle).

acetylcholine; arteriole; endothelium; shear stress; smooth muscle

The human body is exquisitely adapted for maintaining an upright posture on Earth. However, when the force of gravity is removed during spaceflight, there is a cephalic fluid shift and an elimination of the head-to-foot hydrostatic pressure gradient (34, 36). This change in the fluid pressure distribution has been hypothesized to trigger adaptations within the cardiovascular system that are subsequently rendered inappropriate on return to the Earth’s gravitational environment (36). These microgravity-induced alterations of the cardiovascular system are primarily manifested as a diminished aerobic capacity (30, 36) and orthostatic intolerance (36). Although several factors clearly contribute to the postflight orthostatic intolerance (36), one of the most prominent is orthostatic hypotension and a corresponding inability to elevate peripheral vascular resistance (2, 22). Whether this compromised ability to raise peripheral vascular resistance results from alterations in neurally mediated vascular tone, a diminished ability of resistance arteries to constrict, or a combination of the two remains to be determined.

To study these phenomena on Earth, the tail-suspended hindlimb-unloaded (HU) rat has been used to simulate the effects of microgravity. This model induces the cephalic fluid shift (10, 19, 28) and postural muscle unloading (24) that occur in microgravity. Additionally, the HU animals manifest many of the adaptations that are characteristic of exposure to microgravity, including postural muscle atrophy (24), hypovolemia (23, 32), a diminished capacity to elevate vascular resistance (20, 26, 38), orthostatic hypotension (18), and a reduced aerobic capacity (7, 27). Previous work with conduit arteries (5, 29) and skeletal muscle arterioles (4) from HU rats indicates that at least part of the inability to elevate vascular resistance results from a blunting of myogenic autoregulation and a diminished responsiveness to vasoconstrictor stimuli. The attenuated vasoconstrictor responsiveness was not due to alterations in the receptor-second messenger signal transduction mechanism but was hypothesized to result from smooth muscle atrophy or hypoplasia and the corresponding loss of contractile proteins (4, 5). In addition, reductions in myogenic and vasoconstrictor reactivity did not occur in arterioles isolated from postural muscles of the hindlimb, such as the soleus muscle, indicating that several factors may be involved in initiating adaptations in the skeletal muscle vasculature (4). Therefore, the purpose of the present study was to test the hypothesis that alterations in the mechanical environment of arterial microvessels, i.e., reduced fluid pressure and blood flow, induce a structural remodeling of the resistance vasculature in hindlimb skeletal muscles from HU rats and correspondingly alter the endothelium-dependent dilatory properties of these vessels.

METHODS

Animals. All procedures performed in this study were approved by the Texas A&M University Institutional Animal...
Thirty-seven male Sprague-Dawley rats weighing 350 g were randomly assigned to either HU or cage control groups. The HU animals were placed in a head-down position by elevating the hindlimbs to an approximate spinal angle of 40–45° from horizontal. This was done with a harness attached to the tail as previously described (4, 5). Briefly, a harness consisting of curved, molded plastic cast (X-Lite splint material; AOA/Kirschner) was placed around the wrapped (Co-Flex bandage, Andover) proximal two-thirds of the animal’s tail. Moleskin adhesive material was placed as contact points between the tail skin and the cast to allow for adequate blood flow. Two hooks attached to opposite ends of the cast were connected by a small chain to a swivel apparatus fixed at the top of the cage. The length of the chain was adjusted to prevent the hindlimbs of the animal from touching any supportive surfaces while the forelimbs maintained contact with the cage floor. This allowed the animal free range of movement about the cage. Control animals were individually housed and maintained in a normal cage environment. Both groups were kept in their respective condition for 2 wk. This time period has been shown to be sufficient to induce cephalic fluid shifts (10, 19, 28) and produce cardiovascular alterations in HU animals (4, 7, 18, 20, 23, 26, 27, 38). After the experimental period, HU and control animals were weighed and injected with pentobarbital sodium (30 mg/kg ip) to induce deep anesthesia without allowing the hindlimbs of HU rats to become weight bearing. The animals were then decapitated, and the gastrocnemius-plantaris-soleus and triceps brachii muscle and the hindlimb superficial arteries and 1A arterioles was set at 60 cmH2O, and the vessels were allowed to equilibrate for 1 h at 37°C before endothelium-dependent vasodilation was characterized; the bathing solution was replaced every 15 min during the equilibration period. Internal diameter was continuously measured throughout the experiment with the use of videomicroscopic techniques (4, 8). To assess endothelium-dependent vasodilation, we determined concentration-

\[ \tau = 4\eta \dot{Q} / r^3 \]  

where \( \eta \) is the blood viscosity (0.035 Poise) (17) and \( \dot{Q} \) is the blood flow rate through the vessel. \( r \) during the three conditions was derived by dividing previously published (20) total blood flow (ml/min) to gastrocnemius (summed flows to red, white, and mixed portions) and soleus muscles by the number of feed arteries leading to the muscles (2 feed arteries to gastrocnemius muscle and 3–5 feed arteries to soleus muscle in control and HU rats). To calculate shear stress in each of the three conditions, it was necessary to consider the relative state of the artery in vivo during standing and after 10 min and 2 wk of hindlimb unloading. The \( r \) for soleus feed artery during standing was considered to be equivalent to \( r_{\text{max}} \), because the soleus muscle is actively recruited in maintaining posture and, correspondingly, the soleus muscle vascular conductance can approach near maximal levels during standing (16). However, because the soleus muscle is quiescent during the two unloaded conditions, and the superficial and middle portions of the gastrocnemius muscle are inactive during each of the three conditions (13, 16, 24), \( r \) was adjusted to reflect the amount of intrinsic tone these vessels develop in vitro (Ref. 4 and present study). Soleus feed artery \( r \) was estimated to be 70% of \( r_{\text{max}} \) with 10 min of hindlimb unloading because control soleus vessels develop ~30% spontaneous tone (Ref. 4 and present study). Soleus feed artery \( r \) after 2 wk of hindlimb unloading was assumed to be 65% of \( r_{\text{max}} \), because soleus vessels from 2-wk HU rats develop ~35% spontaneous tone. The \( r \) for the superficial gastrocnemius feed arteries during standing and 10 min of hindlimb unloading was estimated to be ~65% of \( r_{\text{max}} \) after 2 wk of hindlimb unloading, it was assumed to be ~80% of \( r_{\text{max}} \).

In vitro studies. In a second set of animals (HU, \( n = 8 \); control, \( n = 7 \)), isolated 1A arterioles from soleus and gastrocnemius muscles were transferred to a Lucite vessel chamber containing PSS and cannulated as described in Microvessel preparation. After cannulation, each isolated vessel in the tissue chamber was transferred to the stage of an inverted microscope (Olympus IX70) coupled to a video camera (Panasonic BP310), video micrometer (Microcirculation Research Institute, Texas A&M University), video recorder (Panasonic AG-1300), and data-acquisition system (Macintosh/MacLab). Intraluminal pressure was set at 60 cmH2O, and the vessels were allowed to equilibrate for 1 h at 37°C before endothelium-dependent vasodilation was characterized; the bathing solution was replaced every 15 min during the equilibration period. Internal diameter was continuously measured throughout the experiment with the use of videomicroscopic techniques (4, 8). To assess endothelium-dependent vasodilation, we determined concentration-
response relationships to the cumulative addition of Ach (10^{-9}–10^{-4} M).

Because Ach-induced dilation was diminished in soleus muscle arterioles after 2 wk of hindlimb unloading, even though calculated shear stress was normalized by the 2-wk period of unloading, a third group of rats was hindlimb unloaded for 4 wk (4-wk HU, n = 6) to determine whether endothelium-dependent dilation is normalized in soleus muscle arterioles following a longer period of tail suspension. Soleus muscle 1A arterioles were isolated, cannulated, and treated identically to the previous two groups of HU and control rats. Ach-mediated dilation was induced in a dose-dependent (10^{-9}–10^{-4} M) manner.

Data analysis. Student’s t-tests were used to determine the significance of differences in the morphological parameters of resistance arteries, body mass, soleus muscle mass, and the soleus muscle-to-body mass ratio between control and HU groups. A one-way ANOVA was used to compare wall shear stress during standing and after 10 min and 2 wk of hindlimb unloading. The Student-Newman-Keuls method was used as a post hoc test to determine the significance of differences among means. Ach concentration-response curves were evaluated using repeated-measures ANOVA with one within-treatment (Ach concentration) and one between-treatment (experimental groups) factor. Planned contrasts were conducted at each molar concentration level to determine whether differences existed among groups. All values are presented as means ± SE. A P < 0.05 was required for significance.

RESULTS

Body and soleus muscle mass. Body mass of control rats (436 ± 12 g) tended to be greater than that of 2-wk HU (407 ± 7 g) and 4-wk HU rats (418 ± 9 g) (P = 0.071). Hindlimb unloading reduced soleus muscle mass of HU rats (2-wk HU: 148 ± 7 mg; 4-wk HU: 127 ± 6 mg) relative to control soleus muscle mass (230 ± 9 mg). Similarly, the soleus-to-body mass ratio of 2-wk (0.362 ± 0.015 mg/g) and 4-wk HU rats (0.305 ± 0.007 mg/g) was lower than that of control rats (0.530 ± 0.023 mg/g). Soleus muscle atrophy, which is characteristic of reduced skeletal muscle weight-bearing activity, confirms the effectiveness of the hindlimb unloading intervention.

Vessel morphology. Hindlimb unloading elicited structural adaptations in resistance arteries that differed for each of the three skeletal muscles studied. However, the pattern of adaptation induced in feed arteries and 1A arterioles was similar within the same muscle (Figs. 1 and 2). In the forelimb triceps muscle, unloading resulted in a tendency (P = 0.064) for the media CSA of the feed artery to increase (Fig. 3). This apparent increase in media CSA resulted from a significant increase in vessel diameter (control: 140 ± 27 µm; HU: 212 ± 25 µm), no change in media wall thickness (Fig. 4), and a tendency for media outer perimeter (Fig. 5) to increase (P = 0.086). Hindlimb unloading did not induce a change in the number of nuclei in the media (control: 60 ± 4 nuclei; HU: 54 ± 6 nuclei) in the triceps muscle feed artery.

In the two hindlimb muscles, unloading resulted in a decrease in the media CSA of feed arteries and 1A arterioles (Figs. 1–3). The number of smooth muscle cell nuclei between control and HU rats was not

---

Fig. 1. Cross-sectional view of gastrocnemius muscle feed artery (A and B) and first-order (1A) arteriole (C and D) from a control (A and C) and a 2-wk hindlimb-unloaded (B and D) rat. Bar, 20 µm.
different in gastrocnemius muscle feed arteries (control: 69 ± 10 nuclei; HU: 55 ± 9 nuclei) and 1A arterioles (control: 30 ± 4 nuclei; HU: 29 ± 4 nuclei) and in soleus muscle feed arteries (control: 17 ± 3 nuclei; HU: 16 ± 2 nuclei) and 1A arterioles (control: 10 ± 1 nuclei; HU: 10 ± 1 nuclei), indicating that the decrease in media CSA resulted from smooth muscle atrophy rather than hypoplasia. The decrease in media CSA from gastrocnemius muscle resistance vessels resulted from the thinning of the media wall (Figs. 1 and 4) and not a change in the outer media perimeter (Fig. 5) or luminal diameter of feed arteries (control:

Fig. 2. Cross-sectional view of soleus muscle feed artery (A and B) and 1A arteriole (C and D) from a control (A and C) and a 2-wk hindlimb-unloaded (B and D) rat. Bar, 20 µm.

Fig. 3. Media cross-sectional area (CSA) of gastrocnemius, soleus, and triceps brachii muscle resistance arteries from control (n = 8) and 2-wk hindlimb-unloaded (n = 8) rats. Values are means ± SE. *Significantly different from control (P < 0.05).

Fig. 4. Media wall thickness of gastrocnemius, soleus, and triceps brachii muscle resistance arteries from control (n = 8) and 2-wk hindlimb-unloaded (n = 8) rats. Values are means ± SE. *Significantly different from control (P < 0.05).
294 ± 24 μm; HU: 243 ± 33 μm) and 1A arterioles (control: 88 ± 8 μm; HU: 93 ± 9 μm). In contrast, the decrease in media CSA from soleus muscle resistance arteries resulted from a reduction in the outer media perimeter (Fig. 2 and 5) and diameter of feed arteries (control: 157 ± 17 μm; HU: 101 ± 13 μm) and 1A arterioles (control: 48 ± 6 μm; HU: 28 ± 4 μm).

Shear stress. Acute (10 min) or chronic (2 wk) hindlimb unloading (Fig. 6) did not significantly alter calculated wall shear stress in gastrocnemius muscle feed artery during standing. However, in soleus muscle feed artery, acute unloading diminished shear stress relative to that occurring during control standing. By 2 wk of hindlimb unloading, shear stress returned to levels similar to that during standing.

DISCUSSION

The primary purpose of the present study was to determine whether the reduction of hydrostatic fluid pressure in the hindlimbs of tail-suspended rats and reductions in muscle blood flow would induce a structural remodeling of the resistance vasculature in skeletal muscle. The results demonstrate that the media CSA of feed arteries and 1A arterioles from both gastrocnemius and soleus muscles is diminished by hindlimb unloading (Figs. 1–3), whereas the media CSA of the feed artery from the forelimb triceps brachii muscle is unaltered (Fig. 3). The decrease in media CSA...
appears to be due to smooth muscle cell atrophy, as indicated by the decreased CSA without a change in the number of media nuclei. The reduction in media CSA of resistance arteries from gastrocnemius muscle resulted from a decrease in media thickness (Figs. 1 and 4), whereas the reduction in media CSA of resistance arteries from soleus muscle resulted from a decrease in the media outer perimeter (Figs. 2 and 5) and vessel diameter.

Two primary mechanical forces are thought to act on the vasculature to induce structural adaptations: 1) the shear stress that arises from the blood acting on endothelial cells, and 2) the stress and strains that cause deformations within the artery wall (13, 14). Calculated shear stress in the arteries from gastrocnemius muscle during both acute and chronic hindlimb unloading was not different from that during standing (Fig. 6). Thus it does not appear that changes in shear stress stimulate the media thinning that occurs in gastrocnemius resistance arteries with hindlimb unloading. However, elevation of the hindlimbs during the unloading procedure produces a reduction in transmural pressure within the hindlimb arterial vasculature by inducing a cephalic fluid shift and increasing diuresis (10, 19, 23, 28). In addition, increases in central blood volume engage cardiopulmonary receptors and elicit reflexive decreases in efferent sympathetic nerve activity (35). Thus the reduction in transmural pressure and a putative attenuation of sympathetic nerve activity may diminish both myogenic and sympathetically mediated smooth muscle contractile activity in resistance arteries from gastrocnemius muscle. The data suggest that the persistent decrease in vasomotor tone is an adequate stimulus to induce a structural remodeling of the feed artery and 1A arteriole. The remodeling does not involve a change in vessel diameter but rather consists of a decrease in media thickness that appears to occur as a result of radial atrophy of smooth muscle cells (i.e., a decrease in smooth muscle cell thickness). This type of vascular adaptation is in fact the inverse of that reported to occur with hypertension (9) and hindlimb unloading in cerebral (basilar) resistance arteries (37), where increases in transmural pressure elevate the circumferential stress within the arterial wall. The elevation of circumferential stress leads to radial hypertrophy of smooth muscle cells and increased media wall thickness (9, 11, 25, 37).

The same decrease in vasomotor tone may not occur in resistance arteries from soleus muscle. For example, soleus muscle is actively recruited in the conscious rat and receives a relatively constant perfusion of 70–140 ml·min⁻¹·100 g⁻¹ (16, 20). When hindlimb unloading eliminates the weight-bearing activity of soleus muscle, blood flow immediately decreases to ≤10 ml·min⁻¹·100 g⁻¹ (20). Although the diminished intravascular fluid pressure resulting from the elevation of the hindlimbs would tend to induce myogenic relaxation (4, 6, 21), this effect is presumably offset in soleus muscle by a reduction in vasodilatory metabolite release when the muscle becomes unloaded (6, 20). In other words, we speculate that the soleus muscle resistance vasculature goes from a metabolite-induced relaxed state during standing to a myogenic-induced relaxed state during unloading. Thus the circumferential stress within the wall of soleus muscle resistance arteries may not be greatly altered by hindlimb unloading and, therefore, would not appear to be the stimulus for structural adaptation in these vessels. Rather, it appears that the chronic decrease in blood flow to soleus muscle may provide the stimulus for adaptation. Acute unloading of the hindlimb reduces calculated shear stress in the feed arteries from soleus muscle (Fig. 6). Although blood flow to soleus muscle remains low through 2 wk of unloading (20), the estimated decrease in shear stress appears to be normalized to presuspension levels by a structural reduction in vessel diameter. The data suggest that this decrease in diameter is the result of circumferential atrophy of smooth muscle cells (i.e., a decrease in smooth muscle cell length), because the outer media perimeter is decreased and media thickness is unaltered.

Previous investigators have demonstrated that sustained reductions in blood flow for ≥2 wk through large conduit arteries of rabbits (1, 14, 15) and dogs (12) reduce vessel diameter without altering media wall thickness. This process was shown to be endothelium dependent and serves to restore vessel wall shear stress (1, 12, 14, 15). The present study demonstrates that a similar remodeling can also occur in the arterial microvasculature to maintain relatively constant levels of shear stress. In addition, data from the present study suggest that reductions in shear stress precede the diminution of endothelium-dependent dilation in skeletal muscle arterioles and that the structural remodeling that occurs to normalize shear stress precedes the normalization of endothelium-dependent dilation.

The functional consequences of changing the structure of resistance arteries are profound. For example, the thinning of the medial layer of resistance arteries from the superficial portion of the gastrocnemius muscle results in the blunting of myogenic autoregulation and a diminished responsiveness of these vessels to vasoconstrictor stimuli (4). These adaptations to hindlimb suspension undoubtedly contribute to the diminished ability to elevate vascular resistance in other hindlimb muscles that have activity levels or fiber composition similar to that of the superficial gastrocnemius muscle (20). Furthermore, the decrement in myogenic and vasoconstrictor responsiveness of resistance arteries and arterioles is consistent with the inability of HU rats to maintain arterial pressure during an acute orthostatic challenge (90° head-up tilt) (18).

Although the structural remodeling that occurs in soleus muscle resistance arteries does not affect vascular responsiveness to vasoconstrictor stimuli (4), the reduction in vessel diameter and endothelium-dependent vasodilation does appear to have important functional consequences. After a 2-wk period of unloading, blood flow to rat soleus muscle is reduced both at rest and during exercise (20, 38). This reduction in the blood flow capacity in soleus and other highly oxidative muscles presumably results, at least in part, from the
Reductions in wall shear stress appear to induce layer (Figs. 1 and 4) with no change in vessel diameter. Muscle cells that results in the thinning of the medial wall pressure appear to induce radial atrophy of smooth muscle tissue. Remodeling of the microvasculature occurs in skeletal muscles of the HU rat, apparently as a result of reductions in transmural pressure and wall shear stress. Reductions in transmural pressure appear to induce radial atrophy of smooth muscle cells that results in the thinning of the medial layer (Figs. 1 and 4) with no change in vessel diameter. Reductions in wall shear stress appear to induce circumferential atrophy of smooth muscle cells, resulting in a reduction in vessel diameter with no change in media thickness (Figs. 2 and 4), and 2) reductions in endothelium-dependent dilation (Fig. 8). Furthermore, estimates of wall shear stress suggest that the reduction in vessel diameter serves to normalize intraluminal shear stress (Fig. 6) and subsequently restore endothelium-dependent dilation (Fig. 8). It is possible that the altered fluid pressure gradients and the unloading of postural muscles that occur in humans residing in space or during prolonged bed rest may also induce similar changes in the mechanical forces acting on the resistance arteries. If this does occur, then arterial vascular remodeling may underlie the compromised ability to elevate peripheral vascular resistance that leads to orthostatic hypotension (2, 22, 33) and the decrements in maximal aerobic power (30, 31) in humans.

This study was supported by National Aeronautics and Space Administration (NASA) Grants NAGW-4842 and NAGS-3754 (to M. D. Delp), National Space and Biomedical Research Institute Grant NCC9–58-H (to M. D. Delp), and two NASA Space Physiology Research Grants awarded through the American College of Sports Medicine Foundation (to P. N. Colleran and M. K. Wilkerson).

Address for reprint requests and other correspondence: M. D. Delp, College of Education, Dept. of Health and Kinesiology, Texas A&M University, College Station, TX 77845 (E-mail: mdd@hkn.tamu.edu).

Received 11 August 1999; accepted in final form 7 December 1999.

REFERENCES


