Effects of hindlimb unweighting on the mechanical and structure properties of the rat abdominal aorta

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Papadopoulos, Anthony, and Michael D. Delp. Effects of hindlimb unweighting on the mechanical and structure properties of the rat abdominal aorta. J Appl Physiol 94: 439–445, 2003. First published October 4, 2002; 10.1152/japplphysiol.00734.2002.—Previous studies have shown that hindlimb unweighting of rats, a model of microgravity, reduces evoked contractile tension of peripheral conduit arteries. It has been hypothesized that this diminished contractile tension is the result of alterations in the mechanical properties of these arteries (e.g., active and passive mechanics). Therefore, the purpose of this study was to determine whether the reduced contractile force of the abdominal aorta from 2-wk hindlimb-unweighted (HU) rats results from a mechanical function deficit resulting from structural vascular alterations or material property changes. Aortas were isolated from control (C) and HU rats, and vasoconstrictor responses to norepinephrine (10⁻⁹–10⁻⁴ M) and AVP (10⁻⁹–10⁻⁵ M) were tested in vitro. In a second series of tests, the active and passive Cauchy stress-stretch relations were determined by incrementally increasing the uniaxial displacement of the aortic rings. Maximal Cauchy stress in response to norepinephrine and AVP were less in aortic rings from HU rats. The active Cauchy stress-stretch response indicated that, although maximum stress was lower in aortas from HU rats (C, 8.1 ± 0.2 kPa; HU, 7.0 ± 0.4 kPa), it was achieved at a similar hoop stretch. There were also no differences in the passive Cauchy stress-stretch response of the gross vascular morphology (e.g., medial cross-sectional area: C, 0.30 ± 0.02 mm²; HU, 0.32 ± 0.01 mm²) between groups and no differences in resting or basal vascular tone at the displacement that elicits peak developed tension between groups (resting tension: C, 1.71 ± 0.06 g; HU, 1.78 ± 0.14 g). These results indicate that HU does not alter the functional mechanical properties of conduit arteries. However, the significantly lower active Cauchy stress of aortas from HU rats demonstrates a true contractile deficit in these arteries.

indeed, previous reports indicate that alterations in vascular structure are induced by hindlimb unweighting in femoral arteries (3) and gastrocnemius muscle arterioles (12), which also show diminished contractile function (9, 32). However, the effects of unweighting on the material and, consequently, mechanical properties of arteries have yet to be determined. Alterations in

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arterial wall mechanics can have profound effects on tension development of arterial ring segments (5, 6, 7, 14, 35). For example, alterations in both the contractile elements of arterial smooth muscle and the passive elastic structures parallel to, and in series with, the contractile elements can affect the length-maximal active force relation ($L_{\max}$), as well as the initial ring length ($L_o$). Differences in $L_o$ of as little as 10% can result in as much as a 100% difference in force development (35). Therefore, the diminished contractile response of peripheral arteries from HU rats to the broad array of vasoconstrictor agonists acting through various mechanisms could result from alterations in the mechanical properties of arteries.

The purpose of the present study was to determine whether hindlimb unweighting alters the structure and material properties of the abdominal aorta, an arterial segment that has been shown in several studies from different laboratories to have a contractile deficit to all vasoconstrictor agonists thus far tested (11, 13, 25, 32, 43, 44). We hypothesized that the reductions in arterial pressure that occur in the hindquarter region of HU rats (4) would induce a decrease in arterial segment that has been shown in several studies from different laboratories to have a contractile deficit to all vasoconstrictor agonists thus far tested (11, 13, 25, 32, 43, 44). We hypothesized that the reductions in arterial pressure that occur in the hindquarter region of HU rats (4) would induce a decrease in arterial compliance would be increased by hindlimb unweighting, so that $L_{\max}$ would be attained at a greater uniaxial displacement.

MATERIALS AND METHODS

Animals

All procedures performed in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee and conform to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

Thirty-nine male Sprague-Dawley rats weighing ~400 g were obtained (Harlan) and housed in a temperature-controlled (23 ± 1°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. The rats were randomly assigned to a control (C) or HU group after arrival from the breeder. After an habituation period of at least 1 wk to the animal housing facility, the hindlimbs of the HU animals were elevated to an approximate spinal angle of 40–45° from horizontal. Briefly, the animals were injected with pentobarbital sodium (Nembutal, Abbott Laboratoriss, 30 mg/kg ip) to induce anesthesia. While anesthetized, the animals’ tails were washed and dried, and a length of breathable nonelastic adhesive tape (Curity Porous tape, Kendall) with a hook attached to the end was placed on the proximal two-thirds of the tail, which allowed the end of the tail to remain unattached. The ends of the adhesive tape were further bonded to the tail with an additional adhesive (Goop) and allowed to dry for 20 min before suspension. The hook attached to the adhesive tape was connected by a small chain to a swivel apparatus fixed at the top of the cage. Inspection of the animals was performed daily. Adjustments to the length of the chain were made as necessary to prevent the rat hindlimbs 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. C animals were maintained in a normal cage environment while HU rats were unweighted for 2 wk. After the 2 wk unweighting period, the rats were anesthetized with pentobarbital (35 mg/kg ip) and euthanized by decapitation, and the abdominal aorta and soleus muscle were excised.

Vessel Preparation

The segment of the abdominal aorta between the renal artery and bifurcation of the iliac arteries was carefully exposed, excised, and placed in chilled (4°C) Krebs physiological saline solution. With the aid of a stereomicroscope (Olympus SZX12), six large (~3 mm in axial length) and six small (~1 mm in axial length) rings were cut from each aortic segment by a scalpel. Each ring was cut and used to measure outside diameter (OD) and inside diameter (ID) with a Filar calibrated micrometer eyepiece as previously described (11, 13). These measurements were used to calculate the wall thickness (H) for each aortic ring by using the following formula: $H = (OD - ID)/2$. The small rings were subsequently discarded after measurement, and the large rings were used for the vasoconstrictor and mechanical studies.

Experimental Design

Three separate protocols were performed in this study. Protocol I. The purpose of protocol I was to determine the peak of the length-developed tension (DT) relation ($L_{\max}$) and the active Cauchy stress responses of aortic segments from C ($n = 6$) and HU ($n = 7$) rats to several vasoconstrictor agonists. The vascular rings were mounted on two stainless steel wires passed through the vessel lumen. One wire was attached to a force transducer (model FT03, Grass Instruments), and the other to a micrometer microdrive (Stoebling/Prior Microdrive, Stoebling) to permit the vessel to be stretched by known increments. The six vessel rings were immersed in a 20-ml tissue bath (Harvard Apparatus) containing Krebs buffer solution equilibrated at 37°C with 95% O2-5% CO2. Isometric tensions were measured and recorded by using a computer and data-acquisition system (MacLab Electronic Data Acquisition System). All segments started from a reference position designated as $L_o$. This length represents the ID of the segments at their unloaded or unstretched state. Rings from both groups were individually stretched by increments of 20% of their initial ID. The increments of stretch in protocol I are referred to as increments of uniaxial displacement, which is the distance between the loading pins. The length-DT or the active tension was determined by repeated test exposures to 80 mM KCl after each increment of uniaxial displacement. The DT at each uniaxial displacement was calculated by subtracting the resting tension from the contractile tension. Uniaxial displacement was continued past the point at which peak DT was reached. All subsequent pharmacological responsiveness studies were conducted with vessels at the stretch that elicited peak DT ($L_{\max}$). The abdominal aortic rings were allowed 30–40 min to equilibrate at $L_{\max}$ before further study.

The active Cauchy stress responses of aortic rings to the vasoconstrictors NE and AVP were tested in three vessel rings from each animal. Responses of the three rings from each animal were averaged and counted as one observation. NE and AVP were chosen as vasoconstrictors to allow comparison of contractions resulting from $\alpha_1$- and $\alpha_2$-adrenergic receptor (NE) and $V_1$-receptor (AVP) mechanisms. Concentration-response relationships were determined by the cumulative addition of NE (10$^{-9}$–10$^{-4}$ M) or AVP (10$^{-9}$–10$^{-5}$ M).
STRESS ANALYSIS. Although tension is commonly used to express resting and isometric contractile force of vessel rings, it is limited because it does not take into account potential differences in cross-sectional area or the material properties (e.g., distensibility) of vessels. Stress responses will, however, account for these potential differences. The Cauchy stress is defined as the actual force or tension acting over an area in the present (deformed) configuration (23). The active Cauchy stress was calculated as (7, 23)

\[ t = \frac{\lambda L}{2HD} \]  

where \( t \) is the one-dimensional Cauchy stress, \( \lambda \) is the circumferential stretch ratio or hoop stretch, defined from continuum mechanics as the ratio of the deformed hoop length over the undeformed hoop length, \( D \) is the axial length, and \( L \) is the applied load (applied tension), which is equal to DT.

Protocol II. The purpose of protocol II was to determine the active and passive mechanics (i.e., stress-strain relations) of abdominal aortic rings by evaluating the active and passive Cauchy stress-stretch responses. The active stress-stretch relation of aortas from C (\( n = 6 \)) and HU (\( n = 6 \)) rats was delineated by an extension protocol identical to that of the length-DT relation as described in protocol I. The increments of deformation in protocol II are referred to as the hoop stretch (\( \lambda \)), which is a dimensionless measure of strain for these arteries. The passive stress-stretch relation (C, \( n = 6 \); HU, \( n = 6 \)) was calculated by using Eq. 1 and was performed similarly to the active response described in protocol I, except that KCl was not administered and the stretch response was performed in Ca\(^{2+}\)-free Krebs solution. The active and passive Cauchy stress were each determined in three aortic rings per animal and were averaged as one observation.

STRAIN ANALYSIS. Motion, which comes from a measure of strain or deformation, can be described as the position of a particle contained in a particular configuration of a body and how it is altered relative to its position in a reference configuration (15, 23). Because Eq. 1 is defined in the present configuration, a one-dimensional strain in the present configuration can only be used to measure the deformation associated with the stress. Because the deformations of aortic rings are large, principal stretch ratios are appropriate measures of strain for these arteries (23). Thus the hoop stretch (\( \lambda \)) was calculated from measures of undeformed and deformed inner wall hoop lengths. These lengths were measured by photographing (Olympus SC35 camera and SZX12 stereomicroscope) the aortic rings at each increment of uniaxial displacement and measuring the inner circumference from the vessel image with the use of a Bioquant image analysis system.

Protocol III. Vessel dimensions measured in protocols I and II were of freshly cut aortic rings in buffer solution. Although measures of \( H \) showed no differences between groups, it is not possible to detect differences in thickness or cross-sectional area of the medial or adventitial layers from this type of analysis. Therefore, the purpose of protocol III was to determine the structural morphology of the abdominal aortic smooth muscle (medial) layer from C (\( n = 7 \)) and HU (\( n = 7 \)) rats. Because of the possibility that arterial pressure, a stimulus for vascular remodeling, may vary along the length of the abdominal aorta in HU rats (i.e., the proximal end being near the hydrostatic indifference point and the distal end being exposed to decreases in arterial pressure (4)), the rings were cut from the most proximal and distal regions of the abdominal aorta and compared to determine whether hindlimb unweighting might have preferentially altered one of these regions. The undeformed vessels were placed in Ca\(^{2+}\)-free Krebs solution for 1 h and then fixed in 10% neutral buffered formalin. The aortas were embedded in paraffin, and transverse cross sections were cut (6-μm thickness) and stained with Vierhoff-Van Geison to distinguish elastin and smooth muscle fibers. The inner and outer medial layer circumferences and medial cross-sectional area were examined by light microscopy and measured with a Bioquant image analysis system.

Solutions and Drugs

The Krebs solution contained (in mM) 131.5 NaCl, 5 KCl, 1.2 NaH\(_2\)PO\(_4\), 1.2 MgCl\(_2\), 2.5 CaCl\(_2\), 11.2 glucose, 13.5 NaHCO\(_3\), 0.003 propranolol, and 0.025 EDTA. In the Ca\(^{2+}\)-free solution, 2 mM EDTA was added, and CaCl\(_2\) was replaced with 2.5 mM NaCl. Solutions were aerated with 95% O\(_2\)-5% CO\(_2\) (pH 7.4) and maintained at 37 °C ± 0.05°C. Concentrated stock solutions of vasoconstrictor agents (NE and AVP) were prepared in distilled water.

Statistical Analysis

Concentration-response and stress-stretch relations were evaluated by using repeated-measures analysis of variance with one within (drug concentration or stretch) and one between (experimental groups) factor. To determine whether difference existed between experimental groups (C vs. HU), planned contrasts were conducted at each molar concentration or level of stretch. Data for individual vessel rings from each animal were averaged and counted as one observation. Unpaired t-tests were used to determine the significance of differences in soleus muscle-to-body weight ratio and aortic ring dimensional characteristics between C and HU rats. All values are means ± SE. A \( P < 0.05 \) was required for significance.

RESULTS

Soleus Muscle-to-Body Weight Ratio

Hindlimb unweighting resulted in a 38% lower soleus muscle mass (C, 197 ± 3 mg; HU, 122 ± 9 mg) and a 35% reduction in the soleus muscle-to-body mass ratio (C, 0.46 ± 0.02 mg/g; HU, 0.30 ± 0.02 mg/g). A characteristic of reduced skeletal muscle weight-bearing activity is muscle atrophy, which confirms the efficacy of the unweighting intervention.

Vessel Characteristics

There were no significant differences in the abdominal aortic vessel segments excised from C and HU rats for OD, ID, or \( H \) (Table 1). In addition, hindlimb unweighting did not alter the inner and outer circumferential and cross-sectional areas of these rings. The percentage differences in cross-sectional area or the material properties (e.g., distensibility) of vessels. Stress responses will, however, account for these potential differences.

Table 1. Aortic vessel characteristics

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD, mm</td>
<td>1.04 ± 0.03</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>ID, mm</td>
<td>0.75 ± 0.03</td>
<td>0.80 ± 0.03</td>
</tr>
<tr>
<td>WT, mm</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Resting tension, g</td>
<td>1.70 ± 0.10</td>
<td>1.73 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. There were no significant differences between control (C) and hindlimb-unweighted (HU) rats for any of these parameters. Measures were made of fresh tissue in Krebs buffer solution.
ence, thickness, or cross-sectional area of the medial layer in either the proximal or distal portions of the abdominal aorta (Table 2). Resting tension was also not different between experimental groups (Table 1).

**Length-Active Tension Relationship**

As illustrated in Fig. 1, DT of aortic rings from HU rats was significantly lower than that from C rats. However, $L_{\text{max}}$ was achieved in both groups with $\sim 0.64\, \text{mm}$ of stretch or $80\%$ beyond their unstretched ID.

**Vasoconstrictor Responses**

NE and AVP produced concentration-related increases in contractile force in the ring segments (Fig. 2). Stress responses to NE and AVP were lower in arterial segments from HU rats.

**Mechanical Responses**

The active force developments (Figs. 1 and 3A) were different between groups. However, maximum DT and maximum active stress were achieved at similar uniaxial displacement or hoop stretch, respectively. The passive stress-stretch response (Fig. 3B) was also not different between groups.

**DISCUSSION**

One of the predominant effects of microgravity on the cardiovascular system is a compromised ability to elevate peripheral vascular resistance (2, 28, 39). Studies of simulated microgravity also indicate that the ability to elevate peripheral vascular resistance is diminished in HU rats (27, 30). This inability to adequately elevate vascular resistance is, at least in part,

| Table 2. Medial layer dimensions of the proximal and distal portions of the abdominal aorta |
|---------------------------------|--------|--------|--------|--------|
| Outer circumference, mm         | C      | HU     | C      | HU     |
|                                 | 1.28 ± 0.05 | 1.28 ± 0.03 | 1.25 ± 0.03 | 1.28 ± 0.03 |
| Inner circumference, mm         | 1.12 ± 0.05 | 1.11 ± 0.03 | 1.10 ± 0.04 | 1.10 ± 0.02 |
| Thickness, mm                   | 0.081 ± 0.01 | 0.084 ± 0.01 | 0.075 ± 0.01 | 0.086 ± 0.03 |
| Cross-sectional area, mm$^2$    | 0.31 ± 0.01 | 0.31 ± 0.02 | 0.30 ± 0.02 | 0.32 ± 0.01 |

Values are means ± SE. There were no significant differences between C and HU rats for any of these parameters. Measures were made from aortic rings after induced relaxation and formalin fixation (see MATERIALS AND METHODS for details).

![Fig. 1. Length-developed tension relation of abdominal aortic rings from control ($n = 6$) and hindlimb-unweighted ($n = 7$) rats in response to 80 mM KCl. Values are means ± SE. Maximal developed tension was achieved at a uniaxial displacement of $\sim 0.64\, \text{mm}$ or $180\%$ of the inner diameter for both groups. *Group responses are significantly different ($P < 0.05$).](image)

![Fig. 2. Isometric Cauchy stress responses of rat abdominal aortic rings from control ($n = 6$) and hindlimb-unweighted ($n = 7$) rats to norepinephrine (A) and arginine vasopressin (B). Values are means ± SE. *Group responses are significantly different ($P < 0.05$).](image)
due to a diminished ability of arteries to vasoconstrict (10, 22). Because this contractile defect occurs with various vasoconstrictor agonists acting through different mechanisms (11, 13, 25, 32, 43, 44), it was hypothesized that the generalized depression of the contractile response in the abdominal aorta was the result of mechanical alterations, possibly resulting from changes in vascular structure. Contrary to our hypothesis, the results demonstrate that vascular structure (Tables 1 and 2) and the material properties (Fig. 3B) of the abdominal aorta are not altered by hindlimb unweighting. Although previous studies have shown that hindlimb unweighting diminishes DT of abdominal aortic segments (11, 13, 25, 32, 43, 44), this computation of force development is a measure that is influenced by vessel wall cross-sectional area, the material properties of the vessel, and the contractile function of the smooth muscle cells. In contrast, the active Cauchy stress is a measure that factors out or normalizes for possible differences in vessel wall dimensions and material properties. Therefore, the present study is the first to demonstrate definitively that hindlimb unweighting diminishes the contractile function of abdominal aortic vascular smooth muscle.

It was originally hypothesized that the hindlimb unweighting-induced depression of arterial contractile force was the result of 1) alterations in the mechanical properties of the vessels, i.e., increase compliance; 2) enhanced release of a vasodilator substance(s) within the vascular tissue; or 3) an alteration in the smooth muscle contraction signal transduction pathway beyond the receptor-second messenger system (11, 13). The first possibility that hindlimb unweighting alters the mechanical properties of arteries was based on the work of Hargens and colleagues (18, 19) demonstrating that anatomic variations in arterial transmural pressure correspond to differences in vascular structure and the suggestion that fluid shifts induced by microgravity may alter arterial transmural pressure and, consequently, vessel structure and mechanics (1, 17, 21). The evidence that localized changes in arterial pressure and blood flow induced by hindlimb unweighting alter arterial structure and contractile function has recently been reviewed by Zhang (42). Zhang points out that the hindlimb unweighting of rats creates an arterial pressure gradient in the animal so that there is an increase in arterial pressure in the head and an incremental decrease in pressure toward the hindlimbs (4, 40, 41). He suggests that there are graded alterations in arterial contractile function from the head to the hindlimbs related to these changes in arterial pressure and blood flow, so that there are increased contractile responses of cerebral arteries, no change in carotid arteries, decreases in vasoconstriction of mesenteric arteries and abdominal aortas, and profound reductions in the contractile responses of femoral arteries. Zhang (42) further proposes that these alterations are based on changes in arterial structure or, more specifically, due to changes in the medial cross-sectional area.

There is sufficient evidence to support a conditional conclusion that hindlimb unweighting-induced changes in arterial pressure and blood flow provide a stimulus for vascular remodeling and, consequently, alterations in vasomotor responses. For example, there is an increase in arterial pressure in the head of HU rats (4, 40, 41), which is associated with an increase in cerebral artery medial cross-sectional area (41, 43, 44) and an enhanced myogenic (16) and agonist-induced vasoconstrictor response (45). Conversely, in the hindlimb of the HU rat, there is a decrease in arterial pressure and blood flow (4, 27, 33), which is associated with a decreased medial cross-sectional area of gastrocnemius muscle resistance arteries (12) and diminished vasconstrictor responsiveness (9). However, the suggestion that there are graded alterations in arterial contractile function from the head to the hindlimb that are related to hindlimb unweighting-induced arterial remodeling is an oversimplification that does not fit all of the experimental data. For example, there are deficits...
in vasconstrictor responses of similar magnitude in the thoracic aorta (13), which is exposed to an increase in arterial pressure with hindlimb unweighting (4, 27, 41), and the abdominal aorta (13), which is exposed to a decrease in arterial pressure (4). In the femoral artery, vascular remodeling has been reported to occur (3, 43), but, in at least one report, hindlimb unweighting has been shown to decrease contractile responsiveness without significant vascular remodeling (44). Mesenteric arteries have also been shown to have diminished unweighting-induced contractile responses (24, 30) without corresponding changes in vascular structure (24, 41). The present study demonstrates that contractile responses of the abdominal aorta are diminished without parallel changes in medial cross-sectional area and that the diminished contractile response is specifically the result of a deficit in smooth muscle contractile function. Therefore, whereas it does appear that arteries in the extreme portions of the HU rat (i.e., head and hindlimbs) undergo vascular remodeling that affects the vasconstrictor responsiveness and presumably the arterial mechanics, arteries at or relatively near the hydrostatic indifference point show similar decrements in contractile function without evidence of vascular remodeling and, specifically, without changes in medial cross-sectional area.

A second possibility that has been proposed to account for the diminished vasconstrictor responsiveness of arteries such as the abdominal aorta, which are in proximity to the hydrostatic indifference point, is an enhanced release of a vasodilator substance(s) within the vascular tissue (13). Only a few vasodilator substances are presently known to be released directly from the vasculature. These include endothelium-derived vasodilator substances (e.g., nitric oxide, prostacyclin, and hyperpolarizing factor) and vasodilators originating within the smooth muscle layer (e.g., nitric oxide through an inducible nitric oxide synthase mechanism). Delp et al. (13) and Sangha et al. (34) have demonstrated that the deficit in contractile function of the abdominal aorta from HU rats is evident, both when the endothelium is intact and after endothelial cell removal. It has also been shown that the diminished vasoconstrictor response of the abdominal aorta from HU rats is not the result of an enhanced release of nitric oxide through an inducible nitric oxide synthase mechanism (31, 34). Thus current evidence indicates that enhanced release of vasodilator substances cannot account for the diminished contractile response of this arterial segment with hindlimb unweighting.

In conclusion, the present study demonstrates that 2 wk of hindlimb unweighting do not alter vascular structure (Tables 1 and 2) or the material properties (Fig. 3B) of the abdominal aorta. Therefore, these results demonstrate definitively that previous reports of reductions in DT of aortic segments from HU rats (11, 13, 25, 32, 43, 44) are due to a smooth-muscle contractile deficit and are not the result of alterations in arterial mechanics.
24. Looft-Wilson RC and Gisol.[53x700] and Gisol.[53x700]