Increased Vascular Cell Adhesion Molecule–1 Was Associated with Impaired Endothelium–Dependent Relaxation of Cerebral and Carotid Arteries in Simulated Microgravity Rats

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Abstract: The aim of the present study was to investigate whether an expression of vascular cell adhesion molecule–1 (VCAM-1) was upregulated in 3-week simulated microgravity rat cerebral and carotid arteries and whether impaired endothelium–dependent relaxation was concomitant with VCAM-1 expression. Male Sprague-Dawley rats were randomly divided into control (CON) and hindlimb unweighting (HU) groups. After 3 weeks, the expression of the VCAM-1 protein and the vasodilation of the basilar artery and common carotid artery were determined. Immunohistochemical results revealed positive staining of VCAM-1 on endothelial cells in these arteries from HU compared with CON rats. Western blot analysis confirmed an upregulated expression of VCAM-1 protein in these arteries from HU rats. Acetylcholine induced concentration-dependent vasodilatation in all artery rings, but with significantly smaller amplitude in the basilar artery ($P < 0.01$) and the common carotid artery ($P < 0.05$) from HU than those from CON rats. The data suggested that the expression of VCAM-1 protein was upregulated in cerebral and common carotid arteries of simulated microgravity rats, and the upregulation of VCAM-1 may contribute to impaired endothelium–dependent relaxation in simulated microgravity rat vasculature.

Key words: orthostatic intolerance, simulated microgravity, vascular remodeling, VCAM-1, endothelium-dependent relaxation, hindlimb unweighting.

Low gravitational stress in space induces postflight orthostatic intolerance that manifests a series of cardiovascular symptoms in astronauts; however, the molecular mechanisms underlying these manifestations are still not fully understood. Orthostatic intolerance remains a touchy medical problem in aerospace cardiovascular medicine, and no single mechanism can account for its occurrence [1, 2]. Recent works have demonstrated that it is associated with vascular functional and structural changes, including endothelial dysfunction, altered contractility, and vascular remodeling [2].

The relevance of inflammation and its mediators in cardiovascular diseases has received intense investigation in the past decade [3–5]. Vascular wall inflammation has been regarded as an initial event of cardiovascular disorders. Elevated arterial pressure is believed to be a strong stimulus of inflammation that is characterized by increased serum levels of cytokines, chemokines, or adhesion molecules [3]. Epidemiological studies have demonstrated higher serum levels of inflammatory cytokines in hypertensive patients [3] and have confirmed the linkage of inflammation and hypertension that enables circulating inflammatory molecules to well predict the onset and development of hypertension [5, 6]. The expression of vascular cell adhesion molecule–1 (VCAM-1), a cell surface protein expressed by endothelial cells (ECs), mediates lymphocyte recruitment and is upregulated in both hypertensive patients and hypertensive rats [7, 8]. VCAM-1 is also referred to inducible cell adhesion molecule CD106 with a molecule weight of 100–110 kDa. Increased cyclic strain [9] and oscillatory flow [10] upregulate the expression of VCAM-1, which is mediated by a variety of inflammatory signals through the NK-κB pathway [7, 11], and it mediates lymphocyte adherence via interaction with very late antigen-4 [12, 13]. The lymphocytes release cytokines such as IL-1β, TNF-α, and IL-6 which are believed to result in vascular remodeling [14] and higher vascular reactivity [15]. Therefore the expression of VCAM-1 in vascular ECs determines proinflammatory cytokine production, which modulates vascular tone and remodeling. Moreover, angiotensin II (ANG II) can upregulate the expression of adhesion molecules and induce...
oxidative stress, which is also related to vascular inflammation [16]. Previous studies [17, 18] in our lab detected a marked upregulation of angiotensin and angiotensin- converting enzyme (ACE) mRNA and protein expression in simulated microgravity rat cerebral arteries, which strongly suggested that the local renin-angiotensin system (L-RAS) was activated in simulated microgravity rat. However, whether VCAM-1 expressed in simulated microgravity rat vasculature was previously seldom studied and whether VCAM-1 expression was related to endothelium-dependent relaxation was not stated in published studies.

Microgravity induces a redistribution of transmural pressures and flows within the arterial vasculature as described in the work by Hargens et al. [19], and in humans, an exposure to microgravity shifts the mean arterial pressure of the head from 70 mmHg in the upright posture on Earth to 100 mmHg in space. Head-down hindlimb unweighting rats in the present study can increase cephalic blood flow [2, 19, 20] and therefore better simulate the hemodynamic changes that occur when humans are exposed to microgravity, which often results in orthostatic intolerance. Microgravity increases headward hydrostatic pressure; therefore it elevates arterial pressure and cyclic strain [21, 22]. Based on the above premises, the present study tended to verify the hypothesis that VCAM-1 protein was highly expressed in cerebral and carotid arteries from simulated microgravity rat because of their increased headward blood flow and activated L-RAS in the arteries. The endothelium-dependent relaxation of basilar artery and common carotid artery from 3-week simulated microgravity rat was also investigated in the present study to evaluate the linkage between arterial inflammation and endothelium-dependent relaxation.

MATERIALS AND METHODS

Animal treatment. The protocols and procedures used in this study were in accordance with Guidelines for the Care and Use of Animals in the Field of Physiological Sciences and proved by the Animal Care and Use Committee of the Fourth Military Medical University.

Male Sprague-Dawley rats were randomly assigned to 3-week hindlimb unweighting simulated microgravity rat (HU) and control (CON). The head-down hindlimb unweighting model described in detail previously [23] was used to simulate microgravity in rats. The rats in the HU group were maintained in about 30° head-down tilt position and housed individually under a 12:12 h light-dark circle with food and water available ad libitum at 24 ± 1°C.

Tissue preparation. After 3 weeks of treatment, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and dissected. Basilar arteries and common carotid arteries were rapidly removed and placed in Krebs solution consisting of (in mmol/l): NaCl, 118.3; KCl, 14.7; KH$_2$PO$_4$, 1.2; MgSO$_4$, 7H$_2$O, 1.2; CaCl$_2$, 2H$_2$O, 2.5; NaHCO$_3$, 25; dextrose, 11.1; and EDTA, 0.026; pH 7.40. The parallel weight of soleus muscle-to-body mass ratio was used to test the efficacy of simulated microgravity. A small part of the arteries was fixed in 4% paraform for immunohistochemistry. Artery rings were prepared for isometric force measurement, and the remains were kept at ~70°C for Western blot analysis.

Isometric force measurement. Basilar arteries (2 mm long, n = 8) were carefully dissected free from the brain and mounted on two 40 µm stainless wires in the jaws of Dual Wire Myograph System (Danish Myo Technology A/S, Denmark) for the recording of changes in isometric force. The bathing chamber contained 8 ml of Krebs buffer solution maintained at 37°C and bubbled continuously with 95% O$_2$ + 5% CO$_2$ to maintain pH 7.40. Individual arteries were allowed to equilibrate for 60 min and were then loaded to optimal resting tension. Following loading, they were washed by Krebs buffer every 10 min in the myograph bath and left to equilibrate until they were at a steady baseline. Common carotid artery segments (3 mm long, n = 8) were mounted vertically on two stainless steel hooks and placed on stainless steel holders in tissue baths (Isolated Tissue Bath, Radnoti, USA) for vasoreactivity recordings with isolated tissue bath (ADInstruments, Australia). After the artery mounting and preparations were concluded, they were challenged with 60 mmol/l KCl to test vascular activity. Then KCl (60 mmol/l) was used to induce arterial contraction and the arteries were then relaxed by the cumulative concentration (10$^{-9}$–10$^{-5}$ mol/l) of acetylcholine (Ach) and sodium nitroprusside (SNP). Cumulative concentration response curves were generated. Ach can dilate arteries through vascular endothelial cells, and SNP releases nitric oxide (NO) directly when it enters arteries; Ach-induced vasodilatation can then be used to identify endothelium-dependent relaxation compared with SNP-induced endothelium-independent relaxation of arteries. Vasodilatation is represented by percentages of maximal contraction to KCl at 60 mmol/l. 50% effective concentration (EC$_{50}$) was used to evaluate the sensitivity to vasoactive substances.

Immunohistochemical localization. Arterial segments from HU and CON rats to be examined for VCAM-1 expression were immediately frozen in Tissue-Tek O.C.T. embedding medium. Serial tissue sections (8 µm) were mounted on polyllysine-coated slides. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min and washed three times for 5 min each time in TPBS (phosphate buffered saline with 0.5% Tween-20 in PBS, pH 7.40). The sections were then subjected to Triton ×100 for 30 min. After preincubation with 20% normal bovine serum for 30 min at room temperature to block the nonspecific antigens, the sections were incubated with rabbit polyclonal antibody against rat VCAM-1 (1:50, Santa Cruz Biotechnology) at 4°C overnight in a
moist chamber. After all the sections were washed with PBS, they were incubated with a biotinylated secondary antibody (1:700, Santa Cruz Biotechnology) in PBS for 2 h at 37°C. After another three washes in PBS, the horseradish peroxidase was developed with 3,3-diaminobenzidine (DAB) (Roche Diagnostics, Mannheim, Germany) as the chromogen substrate. The sections were rinsed, dehydrated in ethanol, cleared in xylene, and mounted.

**Western blot analysis.** Arteries frozen at −70°C in a refrigerator were prepared in lysis buffer (Invitrogen Life Technologies, Carlsbad, USA). Thereafter it was homogenized. The Lysates were then centrifuged at 12,000 × g for 10 min at 4°C. Protein concentrations were determined by the bicinchoninic acid (BCA) assay kit (Pierce, USA). Equal amount of extracts (50 µg) were subjected to 4%–12% Bis-Tris PAGE gels under denaturing conditions using the NuPAGE Bis-Tris System (Invitrogen Life Technologies, Carlsbad, USA), and the fractionated proteins were electropheretically transferred to nitrocellulose membranes (Amersham Life Science, USA) in a tank transfer system (Invitrogen Life Technologies, Carlsbad, USA). The membranes were blocked in 5% nonfat milk for 1 h at room temperature and then incubated with rabbit polyclonal antibody against VCAM-1 (1:500) at 4°C overnight, washed three times with TPBS, then incubated for 2 h with a polyclonal goat anti-rabbit conjugated with horseradish peroxidase. After a washing with TPBS, the ECL detection reagents (Amersham Life Science, USA) were added, and the membranes were exposed to HyperfilmTM (Amersham Life Science, USA). In all instances, the membranes were stained with ponceau stain to verify the uniformity of protein load and transfer efficiency across the test samples.

**Drugs and reagents.** All drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO). Rabbit polyclonal anti-rat VCAM-1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase (HRP)–conjugated anti-rabbit IgG was the product of Invitrogen.

**Statistical analysis.** The results are expressed as mean ± SEM. Concentration response curves were evaluated by repeated measures analysis (two-way ANOVA), and differences between individual concentration points were analyzed by post hoc test. Student’s *t*-test was used to assess the differences of body weight, soleus muscle mass, or soleus muscle-to-body mass ratio between groups. The values of *P* < 0.05 were considered significant.

**RESULTS**

**General data**

The animals from both groups grew well in 3-week experimental period. The initial rat body weight from CON and HU were 305.38 ± 3.37 g and 308.13 ± 4.52 g, respectively. Simulated microgravity induced by hindlimb unweighting resulted in insignificant (*P* > 0.05) differences of rat body weight in HU (388.38 ± 4.38 g) versus CON (406.88 ± 9.35 g) and lower soleus muscle mass (CON, -9 -8 -7 -6 -5 100 80 60 40 20 0 **** Relaxation (% of 60 mM KCl Contraction) Acetylcholine (log[mol/L]) CON HU ** ** ** A Fig. 1. Concentration-response curves for acetylcholine (Ach)- and sodium nitroprusside (SNP)–induced vasodilation in basilar artery (A, B) and common carotid artery (C, D) from control (CON, open circles) and hindlimb unweighting simulated microgravity rat (HU, filled circles). Statistical significance was tested (Values are means ± SEM. *P* < 0.05, **P** < 0.01 vs. CON, *n* = 8).
165.01 ± 6.58 mg; HU, 82.63 ± 4.51 mg) (P < 0.001). The soleus muscle-to-body mass ratio (CON, 0.40 ± 0.01 mg/g; HU, 0.21 ± 0.01 mg/g) (P < 0.001) was significantly reduced in HU rats, which confirmed the efficacy of simulated microgravity and the reliability of animal models used in this set of experiments.

**Measurement of vasodilatation**

KCl (60 mM) was used in this set of experiments to precontract the arteries. Acetylcholine (Fig. 1, A and C) and sodium nitroprusside (Fig. 1, B and D) produced concentration-related vasodilatation of the artery rings from CON and HU rats. The amplitude of vasodilatation in response to Ach in basilar artery and common carotid artery from HU rats was significantly (basilar artery, P < 0.01; common carotid artery, P < 0.05) less than that from CON rats (Fig. 1, A and C), which confirmed the impaired endothelium–dependent relaxation of basilar and carotid arteries from HU rats. However, concentration-response to SNP was similar between groups (Fig. 1, B and D). Also, no significant differences in EC50 between groups were noted. The data were summarized in Table 1.

**Immunohistochemical localization**

As shown in Fig. 2, VCAM-1 was undetectable in artery segments of basilar artery and common carotid artery from the CON group (Fig. 2, A and C). A 3-week simulated microgravity induced a significant increase of VCAM-1 expression localized to the EC surface characterized by strong staining in the basilar artery (Fig. 2B) and common carotid artery (Fig. 2D).

**Table 1.** EC50 (–log [mol/l]) of arterial response to acetylcholine (Ach) and sodium nitroprusside (SNP) in basilar artery, common carotid artery from control (CON), and hindlimb unweighting simulated microgravity rat (HU).

|                | Ach       | SNP       |
|----------------|-----------|-----------|
| Basilar artery | CON 6.61 ± 0.48 | 6.68 ± 0.91 |
|                | HU 6.77 ± 0.17 | 6.72 ± 0.31 |
| Common carotid artery | CON 6.82 ± 0.20 | 6.73 ± 0.61 |
|                | HU 6.80 ± 0.70 | 6.70 ± 0.50 |

Values are means ± SEM. EC50: 50% effective concentration. There were no significant differences between groups.

Fig. 2. Representative photomicrograph of immunohistochemical examination for VCAM-1 expression in basilar artery (A, B) and common carotid artery (C, D) from control (CON) (A, C) and hindlimb unweighting simulated microgravity rat (HU) (B, D). Bar: 100 µm. A: No staining is detectable in rat basilar artery from CON rats. B: VCAM-1 protein expression in basilar artery from HU rats was localized predominantly to the endothelium. C: No staining is detectable in rat common carotid artery from CON rats. D: VCAM-1 protein expression in common carotid artery from HU rats was localized predominantly to the endothelium (n = 5).
From the HU rats. However, a significantly upregulated expression of VCAM-1 protein was detected in these arteries from CON rats. However, no detectable expression of VCAM-1 protein has been observed in cerebral arteries and common carotid arteries from CON rats. However, a significantly upregulated expression of VCAM-1 protein was detected in these arteries from the HU rats. As shown in Fig. 3, no detectable expression of VCAM-1 protein has been observed in cerebral arteries and common carotid arteries from CON rats. However, a significantly upregulated expression of VCAM-1 protein was detected in these arteries from the HU rats.

Fig. 3. Representative Western blots depicting VCAM-1 expression in cerebral arteries (A) and common carotid arteries (B) from control (CON) and hindlimb unweighting simulated microgravity rat (HU) (n = 4).

Expression of VCAM-1 protein
To confirm the expression of VCAM-1 in arteries from HU rats, we used Western blot in the present study to detect the expression of VCAM-1 protein. As shown in Fig. 3, no detectable expression of VCAM-1 protein has been observed in cerebral arteries and common carotid arteries from CON rats. However, a significantly upregulated expression of VCAM-1 protein was detected in these arteries from the HU rats.

DISCUSSION
The data we obtained in this study showed that VCAM-1 protein has been overexpressed in cerebral and common carotid arteries from 3-week simulated microgravity rats, and the Ach-induced endothelium-dependent relaxation of the basilar artery and the common carotid artery has been impaired by 3-week hindlimb unweighting.

The relationship between blood pressure and inflammation has been widely studied for several years. Chae et al. [24] reported that increased blood pressure was a stimulus of inflammation in hypertension. Cell adhesion biology research suggests that increased blood pressure may promote inflammation through the modulation of biomechanical stimuli from pulsatile blood flow, such as increased hydrostatic pressure and cyclic stretch, which in turn upregulates proinflammatory gene expression and function [25]. The renin-angiotensin system (RAS) in arteries activated by high blood pressure has now been considered to be involved in vascular inflammation by modifying several inflammatory responses [16]. Recent studies have demonstrated that ANG II, the key effector of RAS, is a central mediator of vascular inflammation and remodeling beyond modulating vascular tone [26]. In animal and human studies, proinflammatory properties of ANG II have been demonstrated not only in the large conduit and small arteries, but also in the kidney and the heart [27]. ANG II modulates cytokine release and inflammatory gene NF-κB expression, which in turn regulates the expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) [16]. Oxidative stress in the vasculature induced by ANG II has been reported to be involved in the ANG II–induced inflammation process by modulating NAD (P)H oxidase and the expression of NAD(P)H oxidase subunits [28], and it then enhances the expression of VCAM-1 by activating NK-κB [29]. The hindlimb unweighting model is a widely used way to simulate the physiological effects of microgravity on rat. Hindlimb unweighting and space flight induce similar physiological responses in many systems, including immune and cardiovascular systems [30]. Although direct evidence is still insufficient, several studies have indicated that hindlimb unweighting treatment changes the gradients of pressure, alters intravascular hydrostatic pressure and increases cephalic blood flow, which may result in elevated headward arterial blood pressure [2, 19–21]. For instance, Wilkerson and associates reported that 2-week hindlimb unweighting elevated the mean arterial pressure of the aorta nearly 21 mmHg, and therefore the mean blood pressure in the basilar artery would be increased by 17 mmHg during hindlimb unweighting compared with standing [22]. Furthermore, previous studies in our lab [17, 18] demonstrated that L-RAS was activated in simulated microgravity rat vasculature. Thus we selected basilar artery and common carotid artery with higher blood pressure and activated L-RAS in simulated microgravity rat arteries to determine the effects of simulated microgravity on VCAM-1 expression. As expected, strong endothelial VCAM-1 staining by immunohistochemistry and the expression of VCAM-1 protein by Western blot were detected in cerebral and carotid arteries from HU rats; however, in femoral arteries with decreased blood flow and thereby decreased hydrostatic pressure [31], VCAM-1 was undetectable in another experiment (data not shown here).

Endothelium-dependent relaxation was significantly impaired in rats following a 3-week simulated microgravity in the present study, especially in basilar arteries from HU rats. The results seem contrary to our earlier study [23], which found no impaired endothelium–dependent relaxation of basilar arteries in simulated microgravity rats. However, a much higher concentration of KCl used to precontract the arteries, much more easily manipulated myograph system, and consequent better protection of the endothelium function in the present study may be the underlying cause. A NO-dependent or cGMP-dependent mechanism has been confirmed important in endothelium dysfunction in microgravity and simulated microgravity [2], but its underlying mechanisms remain to be established. A recent study [32] indicated diminished endothelium-dependent vasodilatation through alterations in the endothelial NO synthase (NOS) signaling pathway and a decreased expression of caveolin-1 protein, an inhibitor of

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endothelial NOS activity, in 2-week HU rat middle cerebral arteries. These data suggest a more complex relation among the variables involved in the NOS signaling pathway that results in impaired endothelium–dependent relaxation. Although inconsistent opinions remained on the alterations of the NOS content of different cerebral arteries after different durations of HU in rat [33, 34], the decreased expression of caveolin-1 protein [32] and the elevated content of nitrate and nitrite [34] suggest that impaired endothelium–dependent relaxation might not be ascribable to altered NOS activity. Therefore we hypothesized that an alternative mechanism might be involved in modulating NO levels in HU rat cerebral arteries besides the change in NOS. We cannot directly conclude from the present study that the expression of VCAM-1 contributes to impaired endothelium–dependent relaxation by modulating NO levels, but several lines of evidence in the other cardiovascular disorders may provide insight into the possible linkage between arterial inflammation and impaired endothelium–dependent relaxation in HU rats. Because inflammation such as VCAM-1 expression contributes to cytokine release, cytokines may therefore be involved in endothelium injury in cardiovascular diseases. Cytokines induce the upregulation of vasoactive substances in the cytokine family and in several other mediators, such as prostanoids, leukotrienes, NO, bradykinin (BK), reactive oxygen species, and platelet-activating factor, all of which can affect vascular function [35]. They may also induce vasoconstriction or increase the response to vasoconstrictor agents and impaired endothelium–dependent relaxation in several cardiovascular diseases [35]. Proinflammatory cytokines such as IL-6, TNF-α, and IL-1β and anti-inflammatory cytokines such as TNF-β, IL-8, and IL-10 have been identified. TNF-α and IL-1β decreased NO-related endothelium-dependent relaxation in response to Ach by increasing the levels of superoxide anions (O2·−) and the ability of O2·− to inactivate NO [15, 36]. IL-6 induced the inhibition of vascular relaxation and the enhancement of contraction in systemic vessels, which provided direct evidence that IL-6 as one mediator of the increased vascular resistance associated with hypertension [37]. IL-10 protected endothelium-dependent relaxation by reducing xanthine oxidase-derived O2·− production [38, 39]. Circulating lymphocytes adhering to vascular endothelium by binding to VCAM-1 is the initial step in inflammation, and anchored lymphocytes release a spectrum of cytokines. Therefore the expression of vascular adhesion molecules is important in immunological response in arterial inflammation. That is, the overexpression of VCAM-1 attracts many more lymphocytes to artery tissues and releases many more proinflammatory cytokines (such as IL-6, TNF-α, and IL-1β), which in turn modulate vascular tone and vasoreactivity. Furthermore, the expression of inducible NOS induced by cytokines such as IL-1β and TNF-α has been reported to be upregulated in simulated microgravity rat common carotid arteries [34], which provides the evidence of excessive proinflammatory cytokines in simulated microgravity rat vasculature. Now that inflammation occurred in simulated microgravity rat vasculature in our study and also in another study [40], it was bound to be of important significance in arterial function because of the relationships between arterial inflammation and vasoreactivity [35]. Therefore, although further direct studies are needed to clarify the effects and mechanisms of vascular inflammation on vasoreactivity, impaired Ach-dependent vasodilation and concurrent VCAM-1 expression deliver the information that enables us to associate arterial inflammation with arterial vasoreactivity in simulated microgravity rat.

In conclusion, the current work demonstrated that simulated microgravity upregulated the expression of VCAM-1 protein and impaired the endothelium–dependent relaxation of cerebral and common carotid arteries in rat. Further research should be carried out on the mechanism of inflammation, and the common signaling pathway between arterial inflammation and endothelium-dependent relaxation in simulated microgravity rat should be determined.

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REFERENCES

1. Convertino VA. Insight into mechanisms of reduced orthostatic performance after exposure to microgravity: comparison of ground-based and space flight data. J Gravit Physiol. 1998;5:65-8.
2. Zhang LF. Vascular adaptation to microgravity: what have we learned? J Appl Physiol. 2001;91:2415-20.
3. Paulotto P, Rattazzi M. Inflammation and hypertension: the search for a link. Nephrol Dial Transplant. 2006;21:850-3.
4. Mangge H, Hubmann H, Pflz S, Schauensee K, Renner W, Marz W. Beyond cholesterol–inflammatory cytokines, the key mediators in atherosclerosis. Clin Chim Acta. 2004;42:467-74.
5. Ogwara F, Takahashi M, Ikeda U. Inflammatory markers and cytokines in cardiovascular disease. Rinsho Byori. 2004;52:686-92.
6. Bautista LE. Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. J Hum Hypertens. 2003;17:223-30.
7. Tummala PE, Chen XL, Sundell CL, Larussen JB, Hammes CP, Alexander RW. Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: A potential link between the renin-angiotensin system and atherosclerosis. Circulation. 1999;100:1223-9.
8. Ferri C, Desideri G, Valenti M, Bellini C, Pasin M, Santucci A. Early upregulation of endothelial adhesion molecules in obese hypertensive men. Hypertension. 1999;34:568-73.
9. Sung HJ, Yeo A, Eskin SG, McIntire LV. Cyclic strain and motion control produce opposite oxidative responses in two human endothelial cell types. Am J Physiol Cell Physiol. 2007;293:87-94.
10. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. Circ Res. 1998;82:532-9.
11. Sanz-Rosano D, Obuna MP, Cediel E, de Las Heras N, Vegazo O, Jimenez J. Effect of AT1 receptor antagonism on vascular and circulating inflammatory mediators in SHR: role of NF-kappaB/I kappaB system. Am J Physiol Heart Circ Physiol. 2005;288:111-5.
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oxygen species. Mol Immunol. 2002;39:499-508.
13. Abdala-Valencia H, Cook-Mills JM. VCAM-1 signals activate endothelial cell protein kinase Calpha via oxidation. J Immunol. 2006;177:6379-87.
14. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. Hypertension. 2005;46:1118-22.
15. Jimenez-Altayo F, Briones AM, Giraldo J, Planas AM, Salacies M, Villa E. Increased superoxide anion production by interleukin-1beta impairs nitric oxide-mediated relaxation in resistance arteries. J Pharmacol Exp Ther. 2006;316:42-52.
16. Cheng ZJ, Vapaatalo H, Mervaala E. Endothelium-dependent vasodilation of cerebral arteries is altered with simulated microgravity through nitric oxide synthase and EDHF mechanisms. J Appl Physiol. 2006;101:348-53.
17. Bao JX, Zhang LF, Ma J. Angiotensin and AT1R expression in cerebral and femoral arteries during hindlimb unloading in rats. Aviat Space Environ Med. 2007;78:852-8.
18. Meng QJ, Zhang LF, Zhang LN, Ma J. Changes of angiotensinogen expression in arteries of tail-suspended rats. J Gravit Physiol. 2002;9:87-8.
19. Hargens AR, Steskai J, Johansson C, and Tipton CM. Tissue fluid shift, forelimb loading, and tail tension in tail-suspended rats. Physiologist. 1984;27:37-8.
20. Colleran PN, Wilkerson MK, Boomfield SA, Suva LJ, Turner RT, and Delp MD. Alterations in skeletal perfusion with simulated microgravity: a possible mechanism for bone remodeling. J Appl Physiol. 2000;89:1046-54.
21. Gottho TM, Fujiki N, Matsuura T, Gao S, Monta H. Cerebral circulation during acute microgravity induced by free drop in anesthetized rats. Jpn J Physiol. 2003;53:223-9.
22. Wilkerson MK, Muller-Delp J, Colleran PN, Delp MD. Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology. J Appl Physiol. 1999;87:2115-2121.
23. Zhang LN, Zhang LF, Ma J. Simulated microgravity enhances vasoconstrictor responsiveness of rat basilar artery. J Appl Physiol. 2001;90:2296-305.
24. Chae CJ, Lee RT, Riffat N, Ridker PM. Blood pressure and inflammation in apparently healthy men. Hypertension. 2001;38:399-403.
25. Gmbrone MA Jr, Nagel T, Toper BJ. Mechanical activation: an emerging paradigm in endothelial adhesion biology. J Clin Invest. 1997;99:1809-13.
26. Savoia C, Schifflin EL. Inflammation in hypertension. Curr Opin Nephrol. Hypertens. 2006;15:152-8.
27. Theuer J, DeChend R, Muller DN, Park JK, Fiebeler A, Barta P, Angiotensin II induced inflammation in the kidney and in the heart of double transgenic rats. MC Cardiovasc Discor. 2002;2:23.
28. Lasssegue B, Clempsus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol. 2003;285:277-97.
29. Puype ME, Gonzalez W, Nicoletti A, Savoei F, Amal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol. 2000;20:645-651.
30. Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: technical aspects. J Appl Physiol. 2002;92:1367-77.
31. Roer RD, Dillaman RM. Decreased femoral arterial flow during simulated microgravity in the rat. J Appl Physiol. 1994;76:2125-9.
32. Prisby RD, Wilkerson MK, Sokoya EM, Bryan RM Jr, Wilson E, Delp MD. Endothelium-dependent vasodilation of cerebral arteries is altered with simulated microgravity through nitric oxide synthase and EDHF mechanisms. J Appl Physiol. 2006;101:348-53.
33. Wilkerson MK, Lesniewski LA, Golder EM, Bryan RM Jr, Amin A, Wilson E, Delp MD. Simulated microgravity enhances cerebral artery vasoconstriction and vascular resistance through endothelial nitric oxide mechanism. Am J Physiol Heart Circ Physiol. 2005;288:1652-61.
34. Ma J, Kajitwai CI, Ni Z, Vaziri ND. Purdy RE. Effects of simulated microgravity on arterial nitric oxide synthase and nitrate and nitrite content. J Appl Physiol. 2003;94:83-92.
35. Villa E, Salaries M. Cytokines and vascular reactivity in resistance arteries. Am J Physiol Heart Circ Physiol. 2005;288:1016-21.
36. Wimalasundera R, Fexdy S, Regan L, Thom SA, Hughes AD. Effect of tumour necrosis factor-alpha and interleukin 1beta on endothelium-dependent relaxation in rat mesenteric resistance arteries in vitro. Br J Pharmacol. 2003;138:1285-94.
37. Orshal JM, Khalil RA. Interleukin-6 impairs endothelium-dependent NO-cGMP-mediated relaxation and enhances contraction in systemic vessels of pregnant rats. Am J Physiol Regul Integr Comp Physiol. 2004;286:1013-23.
38. Gunnnett CA, Heistad DD, Berg DJ, Faraci FM. IL-10 deficiency increases superoxide and endothelial dysfunction during inflammation. Am J Physiol Heart Circ Physiol. 2000;279:1555-62.
39. Gunnnett CA, Heistad DD, Faraci FM. Interleukin-10 protects nitric oxide dependent relaxation during diabetes: role of superoxide. Diabetes. 2002;51:1931-7.
40. Jungs CK, Chung S, Lee YY, Hwang SH, Kang CS, Lee KY. Monocyte adhesion to endothelial cells increases with hind-limb unloading in rats. Aviat Space Environ Med. 2005;76:720-5.