Contrasting effects of simulated microgravity with and without daily $-G_x$ gravitation on structure and function of cerebral and mesenteric small arteries in rats

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HUMANS EXPOSED TO MICROGRAVITY often exhibit signs of cardiovascular deconditioning marked by orthostatic intolerance and reduced exercise capacity on re-exposure to gravity. The impaired cardiovascular response to standing after return from space might be among the highest risks to the safety, well-being, and performance of astronauts (34, 43). In addition to hypovolemia, postflight cardiovascular deconditioning has also been proposed to be associated with diminished cardiac and vascular function (1, 4, 5, 15, 38, 43, 50, 61). Furthermore, currently used, exercise-based countermeasures seem insufficient to prevent the occurrence of cardiovascular dysfunction in future long-duration, exploration class mission. In the past two decades, intermittent artificial gravity (IAG) by incorporating a short-arm centrifuge into the spacecraft has been suggested as a gravity-based countermeasure for future spaceflight (8, 48, 49, 58). Essentially, no data are currently available on the effectiveness of IAG in preventing cardiovascular deconditioning in humans during spaceflight. Nevertheless, ground-based studies using intermittent centrifugation (8, 16, 25, 49), or standing and walking without (48) or with lower body negative pressure (LBNP) (1, 15, 50) have provided promising data.

Animal studies have their own advantages over human studies not only in elucidating the underlying mechanisms but also in developing new countermeasures. The tail-suspended, head-down tilt (SUS) rat model (32) has been used to investigate the mechanisms underlying postflight cardiovascular dysfunction (for review, see Refs. 56, 59, 61). In the past decade, depression in myocardial contractility (61) and region-specific adaptation of vessels in different anatomic regions (12, 13, 56, 61) due to SUS have been well documented. It has been speculated that microgravity-induced adaptation in structure and function of myocardium and vessels might be among the most important mechanisms responsible for postflight cardiovascular dysfunction (12, 13, 50, 61). Our work has further shown that daily short-duration exposure to $-G_x$ (dorsoven- tral) gravitation by standing (STD) to restore the rat’s orthostatic posture, or $-G_x$ with $+G_z$ component by $+45^\circ$ head-up tilt, which mimics the IAG countermeasure, is surprisingly effective in preventing myocardial contractility depression (60) and vascular changes (44, 58). For example, it has been demonstrated that daily 1-h $-G_x$ by STD is sufficient to prevent the differential changes in two kinds of medium-sized conduit arteries that might occur due to a 28-day simulated microgravity alone (44, 58).

Although the inability to adequately elevate peripheral resistance (4, 5) and the altered autoregulation of cerebral vasculature (23) have been considered, respectively, as a critical and a plausible contributing factor to postflight orthostatic intolerance (50, 61), adaptation changes in mesenteric and cerebral small resistance arteries are seldom investigated. And only a few functional studies have been reported on various results. For example, Looft-Wilson and Gisolfi (28) reported that a medium-term (28 day) SUS resulted only in an attenuation of the myogenic responses in small mesenteric arteries...
with their responses to vasoconstrictors and arterial dimensions being unaffected. However, Behnke et al. (3) and Colleran et al. (10) recently reported that 14-day SUS resulted in only lowered responsiveness to norepinephrine (NE), but with their myogenic tone and responses and passive wall thickness being not affected. On the basis of their dimension data, all these authors have suggested lack of change in structure of mesenteric small arteries (3, 10, 28). For cerebral small arteries, it has been well documented that SUS induces augmented myogenic tone and responses in middle cerebral arteries (MCA) through both NOS-dependent (19, 51) and -independent mechanisms (19); but it remains unclear on their structural adaptation, although the wall thickness of MCAs was greater after a 14-day SUS (40, 51). Furthermore, our previous work (55) demonstrated that a medium-term SUS can up- and downregulate the current and protein expression of L-type calcium channel (CaL) in cerebral and mesenteric small arterial vascular smooth muscle cells (VSMCs), respectively. However, the intervention of daily 1-h \( G_x \) exposure by STD has differential effect on the CaL current and protein expression in these two kinds of small arteries. In mesenteric arterial VSMCs, it prevents the current decrease and expression reduction of the CaL; whereas, in cerebrovascular myocytes, the augmented current and increased expression of CaL are not prevented by such an intervention. The unresponsiveness of CaL channel in cerebral arterial VSMCs to 1 h/day \( G_x \) seems to be an important mechanism to ensure an increased \( \text{Ca}^{2+} \) influx for the maintenance of an increased myogenic tone whenever the rat is subjected to simulated microgravity. On the basis of the previous findings (19, 28, 44, 51, 52, 55), we hypothesized that a medium-term simulated microgravity could induce hypertrophic change and augmentation of myogenic response in the middle cerebral arteries (MCAs), whereas atrophic change and decrement of myogenic response in the mesenteric third-order (3A) arterioles. Also, we further hypothesized that, in addition to the enhanced myogenic tone and increased vasoactivity in the MCAs, the differential structural remodeling changes in both kinds of small resistance arteries and the myogenic tone decrement in the mesenteric 3A arterioles due to SUS alone could be prevented by the intervention of daily 1-h STD.

The purpose of the present study was threefold: 1) to determine whether a medium-term simulated microgravity can induce hypertrophic and atrophic change in the MCAs and mesenteric 3A arterioles, 2) to examine whether it can induce an enhancement and depression of the myogenic responses to pressure increases and the vasoconstrictor responses to nonreceptor- and receptor-mediated agonists in these two kinds of small resistance arteries, respectively, and 3) to clarify whether the intervention of 1 h/day STD can fully prevent the structural adaptation changes in both kinds of small arteries and the decrement of myogenic tone and vasoactivity in the mesenteric 3A arterioles, but not the enhanced myogenic tone and increased vasoactivity of the MCAs.

**MATERIALS AND METHODS**

**Animal Model and Experimental Design**

Tail-suspended, head-down tilt rat model. The technique of tail suspension (32) with modification from our laboratory has been described in detail previously (62). Briefly, the rats were attached by tape to a plastic bar in a swivel mounted at the top of the cage allowing free 360° rotation. The rats were maintained in about \(-30°\) head-down tilt position with their hindlimbs unloaded to simulate cardiovascular effect of microgravity. The control animals were housed in identical Plexiglas cages, except that the tail suspension device was removed. All animals received standard lab chow and water ad libitum and were caged individually in a room maintained at 23°C on a 12:12-h light/dark cycle.

Model of daily short-duration \( G_x \), gravitation. Daily stationary ground support in normal orthostatic posture or standing (STD) for 1 h was adopted to simulate the countermeasure effect of IAG as previously described (44, 60). For daily short-duration STD, the suspended rat was released from suspension and then placed into a 50-cm-long, tubelike metallic mesh cage maintained in horizontal position for 1 h. The rat could move forward and backward, but it could not turn around. Food and water were provided ad libitum at the front end of the cage. The gravity vector was \(-G_x\).

**Experimental design.** All protocols and procedures were reviewed and approved by the Animal Care and Use Committee of the Fourth Military Medical University. Two separate protocols were carried out. In protocol 1, changes in myogenic tone and reactivity and vasoconstrictor responsiveness of middle cerebral and mesenteric small arteries of rats subjected to tail suspension with and without STD over 28 days were examined and compared with those of control rats. Twenty-four male Sprague-Dawley rats weighing between 240 and 250 g were randomly assigned to three experimental groups (\( n = 8 \) rats/group): control (CON), tail suspension (SUS), and daily suspension for 23 h plus standing for 1 h (S+D). During the 28-day period, daily STD intervention was conducted between 0800 and 0900.

In protocol 2, histomorphological changes of the middle cerebral and mesenteric small arteries of SUS, S+D, and CON rats were examined under carefully controlled conditions by electron microscopy and compared. Eighteen male Sprague-Dawley rats weighing between 240 and 250 g were randomly assigned to three experimental groups (\( n = 6 \) rats/group): CON, SUS, and S+D. In addition, some specimens from protocol 1 (1 CON, 2 SUS, and 1 S+D rats) were also used for histomorphometry and prefixed on completion of arteriograph experiments. Daily STD intervention was conducted between 0800 and 0900.

**Pressure Arteriography**

**General.** After 28 days of simulation, rats from protocol 1 were anesthetized with pentobarbital sodium (50 mg/kg, ip) and killed by exsanguination via the abdominal aorta. The entire brain was rapidly removed and placed in a dissecting dish with cold physiological salt solution (PSS) containing (in mM): 119.0 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 2.5 CaCl\(_2\), 5.5 glucose, and 0.026 EDTA (pH 7.4). Simultaneously, a segment of small intestine (the proximal part of jejunum) with attached mesentery was excised and placed on a separate Petri dish containing cold PSS. Because vasoactivity experiments with middle cerebral arteries (MCA) require 4–6 h, mesenteric small arteries were stored for 6 h in PSS (4°C). This experimental approach allowed us to conduct functional analysis of these two kinds of small resistance arteries from the same animals. A pilot study demonstrated that myogenic responses to pressure increase and contractile responses to PE and isotonic KCl of mesenteric small arteries were similar after 6-h storage and on freshly prepared vessel segments.

**Vessel isolation and cannulation.** During sampling of the arteries, we tried to maintain the in vivo length of the arterial segments in the following ways to reduce errors in wall dimension and structure measurements (6). While isolating MCAs, the length of the excised segment determined with an eyepiece micrometer of the dissecting microscope was used as an estimate of the in vivo length (L\(_0\)), since there is basically no dissection retraction. While isolating the mesenteric small arteries, the mesentery was first pinned flat to the transparent bottom of the dish and then a third-generation (3A) arteriole
was isolated from the mesentery and surrounding fat. The length between the two cutting marks previously made was first determined with an eyepiece micrometer and then the retraction ratio was calculated from the length of the excised segment determined with an eyepiece micrometer and then the \( L_0 \) was estimated.

The isolated arterial segment was transferred to a vessel chamber containing PSS. Two pipettes were used to cannulate the arterial segment. Cannulation involved sliding the proximal end of the vessel onto the primary pipette tip and then securing it with 10-0 nylon ophthalmic suture. Any residual blood in the vessel lumen was gently flushed out. The distal end of the vessel was then cannulated with the secondary pipette and secured. After cannulation, the chamber was transferred to the stage of an inverted microscope coupled to a video camera, a video micrometer, and a data-acquisition system (Pressure Myograph System P110, DMT, Denmark).

The isolated arteriole was perfused under a pressure of 25 mmHg for 5–10 min to remove any blood or debris and check for leaks and then changed to no-flow condition and the axial length of the arteriole was set to its in vivo length and the longitudinal force was adjusted to 0 mN (9). The intraluminal pressure was increased to 125 mmHg to further detect the leaks and to remove any buckle from the vessel by adjusting the axial length from \( L_0 \) to \( L_1 \) (35, 36). The vessel was then warmed slowly to 37°C and allowed to equilibrate at 50 mmHg for 1 h. The superfusion PSS gassed with 21% O2-5% CO2-74% N2 was replaced every 15 min during the equilibration period. After equilibration, the viability of the arteriole was assessed by its reactivity to 100 mM KCl, PE, or 5-HT were represented as percentage contraction relative to the baseline internal diameter (28) according to the formula:

\[
\text{luminal diameter change} = \frac{D_i - D_o}{D_i} \times 100
\]

where \( D_i \) is the baseline internal diameter measured in Ca\(^{2+}\)-free PSS and \( D_o \) is the active internal diameter at a particular intraluminal pressure.

Vasoconstrictor responses of the vessel segments to cumulative superfused KCl, PE, or 5-HT were represented as percentage contraction relative to the baseline internal diameter (28) according to the formula:

\[
\text{vasoconstrictor response} = \frac{D_i - D_o}{D_i} \times 100
\]

where \( D_i \) is the baseline internal diameter measured in active state at a pressure of 75 mmHg and \( D_o \) is the steady-state internal diameter measured to each subsequent change in agonist concentration at the same pressure.

Statistics. Values are means ± SE. One vessel per rat was used for analyses unless otherwise stated. Two-way ANOVA with repeated measures was used to determine the overall differences of myogenic and vasoconstrictor responsiveness among different groups and different intraluminal pressures or agonist concentrations of the same group, and then the Student-Newman-Keuls post hoc test was used to determine the significance of differences among means. Comparisons of body weight and soleus wet weight and myogenic tone (%) and dimensions of vessel segments at a particular pressure were analyzed by Student’s \( t \)-test. The 0.05 level of probability was chosen as significant for all analyses.

RESULTS

Body Weight and Soleus Wet Weight

The data are summarized in Table 1. There were no significant differences in final body weight among the three groups of the two protocols. The wet weight of soleus of 28-day SUS rats was 53% and 46% less than that of the CON rats, respectively, in protocols 1 and 2 (\( P < 0.01 \)). However, the soleus wet weight of S-D rats was 26% and 18% less than CON rats, respectively, in protocols 1 and 2 (\( P < 0.01 \)), indicating the countermeasure effectiveness of 1 h/day STD in attenuating muscle atrophy (58–60).

Vessel Characteristics

MCA. As shown in Table 2, the passive lumen diameter (Di,p) determined in Ca\(^{2+}\)-free PSS at 100 mmHg showed no significant differences among the three groups. However, the passive wall thickness (Wp) was significantly greater (\( P < 0.01 \)) in MCAs from SUS and S+D than CON rats. In PSS containing Ca\(^{2+}\), the active lumen diameter (Di,a) at 100 mmHg was significantly less (\( P < 0.01 \)) in MCAs from SUS and S+D than CON rats. Hence the myogenic tone at 100 mmHg was significantly greater (\( P < 0.01 \)) in SUS and S+D
than CON groups. The active wall thickness (Wa) and wall/radius ratio ([W/r]a) were also significantly greater (P < 0.01) in SUS and S+D than CON groups.

**Mesenteric small artery.** The passive lumen diameter (Di,p) at 100 mmHg showed no significant differences among the three groups. However, the passive wall thickness (Wp) was significantly less (P < 0.01) in mesenteric 3A arteries from SUS and S+D rats and the difference between CON and S+D was nonsignificant. In PSS containing Ca²⁺, the active lumen diameter (Di,a) of mesenteric small arteries from SUS was significantly greater (P < 0.01) than that from CON and S+D rats. Hence the myogenic tone at 100 mmHg was significantly less (P < 0.01) in SUS than CON and S+D groups. The active wall thickness (Wa) and wall/radius ratio ([W/r]a) were also significantly less (P < 0.01) in SUS than the other two groups (Table 2).

### Table 1. Body weight and wet weight of left soleus of CON, SUS, and S+D rats

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Wet Weight of Left Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td><strong>CON</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>247.5±4.2</td>
<td>392.3±4.9</td>
</tr>
<tr>
<td><strong>SUS</strong></td>
<td>250.8±4.3</td>
<td>385.2±5.2</td>
</tr>
<tr>
<td><strong>S+D</strong></td>
<td>245.3±6.2</td>
<td>387.6±4.6</td>
</tr>
<tr>
<td><strong>Protocol 1 (n = 8)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CON</strong></td>
<td>249.5±4.8</td>
<td>396.3±4.7</td>
</tr>
<tr>
<td><strong>SUS</strong></td>
<td>246.8±4.6</td>
<td>389.2±4.4</td>
</tr>
<tr>
<td><strong>S+D</strong></td>
<td>241.3±5.2</td>
<td>381.6±3.9</td>
</tr>
<tr>
<td><strong>Protocol 2 (n = 6)</strong></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE. n = 8 and 6 animals/group in Protocol 1 and Protocol 2, respectively. CON, control; SUS, 4-wk tail suspended; S+D, 23 h/day suspension +1 h/day standing. *P < 0.01 vs. CON; †P < 0.01 vs. SUS.

**Myogenic Tone and Reactivity to Pressure Change**

MCA. Figure 1A shows the luminal diameters of MCAs to step changes in intraluminal pressures under passive and active conditions. There were no significant differences in passive pressure-diameter relationship among the three groups. In active conditions, diameters of MCAs from the three groups were obviously less than passive diameters due to the presence of myogenic tone. Furthermore, between 25 and 125 mmHg, MCAs from SUS and S+D maintained an essentially constant and significantly smaller diameter compared with that of CON rats (P < 0.01). However, there was no significant differences between SUS and S+D groups. Figure 1B, depicting myogenic tone developed, further reveals that the myogenic tone of MCAs from SUS and S+D gradually increased between 0 and 75 mmHg and then

### Table 2. Dimensions of segments of small resistance arteries isolated from CON, SUS, and S+D rats

<table>
<thead>
<tr>
<th>Vessel Data at 100 mmHg</th>
<th>Group Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON versus SUS</td>
</tr>
<tr>
<td><strong>Middle cerebral artery (n = 8–10)</strong></td>
<td></td>
</tr>
<tr>
<td>Di, μm</td>
<td>168.1±16.0</td>
</tr>
<tr>
<td>Di,a</td>
<td>229.5±7.7</td>
</tr>
<tr>
<td>Di,p</td>
<td>237.6±7.7</td>
</tr>
<tr>
<td>W, μm</td>
<td>21.1±0.7</td>
</tr>
<tr>
<td>Wp</td>
<td>16.8±0.9</td>
</tr>
<tr>
<td>Wa</td>
<td>21.1±0.7</td>
</tr>
<tr>
<td>W/r</td>
<td>0.13±0.0</td>
</tr>
<tr>
<td>W/p</td>
<td>0.25±0.0</td>
</tr>
<tr>
<td>Tone, %</td>
<td>46.9±3.5</td>
</tr>
<tr>
<td><strong>Mesenteric third-order small artery (n = 8)</strong></td>
<td></td>
</tr>
<tr>
<td>Di, μm</td>
<td>201.6±6.3</td>
</tr>
<tr>
<td>Di,a</td>
<td>192.1±3.1</td>
</tr>
<tr>
<td>Di,p</td>
<td>132.2±5.0</td>
</tr>
<tr>
<td>W, μm</td>
<td>21.1±1.25</td>
</tr>
<tr>
<td>Wp</td>
<td>23.1±1.7</td>
</tr>
<tr>
<td>Wa</td>
<td>23.1±1.7</td>
</tr>
<tr>
<td>W/r</td>
<td>0.17±0.0</td>
</tr>
<tr>
<td>W/r,a</td>
<td>0.23±0.0</td>
</tr>
<tr>
<td>Tone, %</td>
<td>7.5±2.4</td>
</tr>
</tbody>
</table>

Measurements were made on cannulated vessels pressurized to 100 mmHg. n = number of rats. Di, lumen diameter; W, wall thickness; W/r, wall/radius ratio. Subscripts p and a represent passive and active state, respectively; r represents radius. NS, not significant. *P < 0.01.
reached a plateau until 125 mmHg, and there were no significant differences between the two groups. In contrast, MCAs from CON maintained a nearly constant but significantly smaller myogenic tone compared with that of SUS and S/H11001D rats (P<0.01).

**Fig. 2.** Pressure-diameter relationship (A) and myogenic tone developed (B) in mesenteric small arteries isolated from CON, SUS, and S/H11001D rats at intraluminal pressure of 0–125 mmHg. Both active pressure-diameter and pressure-myogenic tone curves showed no significant differences between SUS and S+D groups; however, these 2 curves from the respective group were significantly different from that of the CON group. n = 8 animals per group. **P<0.01; NS, not significant (2-way ANOVA); ##P<0.01, CON vs. SUS; ++P<0.01, CON vs. S+D.

Mesenteric small artery. Figure 2A shows that the passive pressure-diameter relationship was not significantly different among the three groups. In active conditions, diameters of mesenteric 3A arteries from CON and S+D rats maintained a nearly constant level between 25 to 125 mmHg and the active diameters were significantly greater in S+D compared with CON group (P<0.01). In contrast, mesenteric arterioles from SUS behaved as though they were in passive state with their active diameters to each pressure increase being significantly greater compared with those of CON and S+D rats (P<0.01). The myogenic tone of mesenteric small arteries from CON and S+D rose beyond 50 mmHg and approached a plateau until 125 mmHg and was significantly greater compared with that from SUS rats (Fig. 2B, P<0.01).

**Agonist-Induced Vasoconstrictor Responsiveness**

MCA. Increases in KCl (15–90 mM) and 5-HT (10−10−10−5 M) induced dose-dependent decreases in luminal diameter of MCAs from rats of the three groups. However, vasoconstrictor responsiveness of MCAs to both agonists was significantly augmented in SUS and S+D groups compared with that of CON (P<0.01). There was no significant difference between SUS and S+D groups (Fig. 3, A and B).
The EC₅₀ was not significantly different among three groups.

**Mesenteric small artery.** KCl (15–90 mM) and PE (10⁻¹⁰–10⁻⁵ M) induced dose-dependent decreases in luminal diameter of mesenteric 3A arteries from rats of the three groups. However, the vasoconstrictor responsiveness of mesenteric arterioles to both agonists was significantly attenuated in SUS compared with that of CON and S+D groups (P < 0.01). The difference in vasoconstrictor response to KC1 was significant between CON and S+D groups (Fig. 4, A and B). The EC₅₀ was not significantly different among three groups.

**Spontaneous Vasomotion in Middle Cerebral Arteries**

In PSS containing Ca²⁺, vasomotion was observed almost exclusively in MCAs when pressure was >75 mmHg (Fig. 5A). MCAs isolated from SUS and S+D had significantly greater amplitude and frequency of vasomotion compared with that of MCAs from CON rats (Fig. 5, B and C).

**Structural Adaptation**

Representative electron micrographs of longitudinal sections of the middle cerebral and mesenteric small arteries from the three groups are shown in Fig. 6. The histomorphometric data are summarized in Table 3. SUS for 28 days resulted in differential structural changes in middle cerebral arteries and mesenteric 3A small arteries. Compared with that of CON group, TW and TM of MCAs from SUS rats increased by 56.4% (P < 0.01) and 63.0% (P < 0.01), respectively, whereas the TW and TM of mesenteric small artery from SUS decreased by 20.0% (P < 0.01) and 28.6% (P < 0.01), respectively (Table 3). The media CSA of MCAs increased by 50.1% (P < 0.01), but the CSA of mesenteric small artery decreased by 38.5%
(P < 0.01) after SUS. Moreover, for the NCL in the media of MCAs, the SUS vessels contained 3.2 cell layers, 23.1% more than the CON vessels that contained 2.6 cell layers (P < 0.01). Whereas for the mesenteric small artery, SUS vessels contained 2.4 cell layers, which was 33.3% less than the CON vessels that contained 3.6 cell layers (P < 0.01). However, daily 1-h STD completely prevented these structural changes in these two kinds of small resistance arteries that might occur due to a 28-day SUS alone. Thus there were no significant differences in TW, TM, CSAm, and NCL between S+D and CON groups (Table 3). Nevertheless, it should also be noted that both D and the CSA of smooth muscle cell (Ac) of these...
two kinds of small vessels showed no significant differences among CON, SUS, and S + D groups (Table 3).

**DISCUSSION**

The purpose of the present study was to test the hypothesis that a medium-term simulated microgravity induces upward and downward regulations in both the structure and function of the cerebral and mesenteric small resistance arteries and that functional adaptation changes in the cerebral resistance arteries cannot be prevented by the intervention of 1 h/day functional adaptation changes in the cerebral resistance arteries and downward regulations in both the structure and function of D groups (Table 3).

### Table 3. Histomorphometric data of the wall of middle cerebral arteries and mesenteric 3A small arteries from CON, SUS, and S + D rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>CON (n = 6)</th>
<th>SUS (n = 6)</th>
<th>S + D (n = 6)</th>
<th>CON vs. SUS</th>
<th>SUS vs. S + D</th>
<th>CON vs. S + D</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_W )</td>
<td>( \mu m )</td>
<td>3.9 ± 0.1</td>
<td>6.1 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>( T_M )</td>
<td>( \mu m )</td>
<td>2.7 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>( CSAm )</td>
<td>( \mu m^2 )</td>
<td>1711 ± 75</td>
<td>2569 ± 236</td>
<td>1631 ± 72</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>( A_C )</td>
<td>( \mu m^2 )</td>
<td>2.6 ± 0.2</td>
<td>2.9 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>( NCL )</td>
<td></td>
<td>2.6 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>( D )</td>
<td>( \mu m )</td>
<td>164.5 ± 6.5</td>
<td>167.0 ± 5.8</td>
<td>165.0 ± 6.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
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</table>

### Middle cerebral artery

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Unit</th>
<th>CON (n = 6)</th>
<th>SUS (n = 6)</th>
<th>S + D (n = 6)</th>
<th>CON vs. SUS</th>
<th>SUS vs. S + D</th>
<th>CON vs. S + D</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_W )</td>
<td>( \mu m )</td>
<td>6.0 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>6.5 ± 0.4</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( T_M )</td>
<td>( \mu m )</td>
<td>4.2 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>4.4 ± 0.3</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( CSAm )</td>
<td>( \mu m^2 )</td>
<td>2309 ± 123</td>
<td>1421 ± 68</td>
<td>2598 ± 292</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( A_C )</td>
<td>( \mu m^2 )</td>
<td>2.3 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( NCL )</td>
<td></td>
<td>3.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( D )</td>
<td>( \mu m )</td>
<td>164.0 ± 6.4</td>
<td>169.8 ± 6.4</td>
<td>168.4 ± 3.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

### Mesenteric small artery

Values are means ± SE; \( n \) number of rats per group. \( T_W \), wall thickness; \( T_M \), media thickness; \( CSAm \), media cross-sectional area; \( A_C \), CSA of smooth muscle cell; \( NCL \), number of smooth muscle cell layers; \( D \), lumen diameter; NS, not significant. * \( P < 0.01 \).

anatomic location (distance from the hydrostatic indifference level), vessel types (elastic vs. muscular, large vs. small arteries/arterioles, and conduit vs. resistive), functional individualities of vessels in different vascular beds (e.g., cerebral, renal, or others), duration of exposure (short, medium, vs. long term), and systemic factors to be elucidated. (3, 50, 56, 57).

Although the decrements in splanchnic vascular conductance could account for about one-third of the total peripheral resistance (TPR) adjustment (41), other than a few earlier studies on mesenteric vascular bed (for review, see Ref. 56), the first direct observation on mesenteric small arteries isolated from SUS rats was made by Looft-Wilson and Gisolfi (28). They showed that a 28-day SUS resulted in only an attenuation of myogenic tone of mesenteric 2A and 3A small arteries, but no differences in responsiveness to vasoconstrictors and shear stress and vessel dimensions were found (28). The findings of the present study on myogenic tone and responses to pressure increase are in agreement with the previous study (28). However, our findings do indicate that the vasoconstrictor responsiveness to both KCl and PE is significantly decreased. As the duration of simulated microgravity and the generation of the arterioles are exactly the same in these two studies, therefore, this discrepancy might be related to some technical details in the experimental assessment of vasoreactivity. According to Coats and Hillier (9), for isobaric-mounted rat 3A mesenteric arteries, degree of longitudinal stretch can significantly affect their responses to PE and a ≥20% stretching will provide optimal experimental conditions. Appropriate measures have been taken and described in *Vessel isolation and cannulation*. Our findings on vasoreactivity are consistent with those of rats after a 14-day SUS (3, 10) and of rats after an 18-day spaceflight reported by Hatton et al. (22). An on-board experiment showed for the first time that after spaceflight, maximal contractility of rat’s mesenteric 2A/3A arteries to NE was significantly attenuated, but the vessel wall thickness and medial volume was not affected (22). Nevertheless, it should be noted that in the flight experiment, the rat strain was spontaneously hypertensive rats (SHR) and the technique for assessing vaso-
reactivity was wire myography (22). In addition, regarding the results on myogenic tone and responses, there remains a discrepancy between the present study and those reported by Behnke et al. (3) and Colleran et al. (10). Our results showed that after a 28-day SUS, the active myogenic responses of the isolated mesenteric 3A arterioles to pressure increases (25 to 125 mmHg) almost disappeared, behaving just as in their passive state (Fig. 2A) and their spontaneous tone was decreased by 75% compared with CON (Table 2). However, Colleran et al. (10) reported that there were no significant differences in myogenic response in mesenteric small arteries between SUS and CON rats within the physiological range of pressures (80–132 mmHg), but the myogenic responses to subphysiological levels (22–81 mmHg) was less in the SUS group. Behnke et al. (3) also reported no difference in initial myogenic tone between the two groups. The reasons for this discrepancy remain unclear. It seems not to be related to simulation duration, since a 3-day SUS has resulted in a decrease of myogenic tone by 82% (29). Some differences in experimental protocols between these two studies merit further consideration. For example, in our study, the in vivo length of excised vessel segments was measured and the degree of longitudinal stretch was controlled, vessel’s mechanical hysteresis was reduced (19, 35), and the isolated mesenteric arterioles were sensitized by incubating vessel segments with a subthreshold dose of PE (28, 45). Finally, the present study has provided the first evidence to demonstrate that a medium-term SUS does induce structural adaptation in mesenteric small arteries. We found that the passive wall thickness (Wp) of mesenteric 3A small arteries measured at 100 mmHg was significantly decreased after a 4 wk SUS compared with that of CON. Compared with our previous short-term (3-day) study (29), it strongly suggests that these remodeling changes in mesenteric small arteries can occur only after a medium-term SUS. However, according to the four previous studies (3, 10, 22, 28), simulated or real microgravity did not affect passive wall thickness. In addition, Wilkerson et al. (53) reported that the CSA of isolated MSAs measured by histological method was also not affected by SUS. The discrepancy might be related to the experimental procedure adopted. In such kinds of studies, how to sample from the same site and maintain their in vivo length or a standard degree of stretching during fixation is of prime importance, since the vessel wall thickness and CSA are easily affected by the length of the isolated arterial segment (6). In the present study, precautions put forward by Bund and Lee (6) have been taken to avoid possible errors in dimension measurement and further histological examination. To obtain more precise information about the structural properties of vessels, histomorphometric study was made on these arterial segments on completion of each functional experiment according to Mulvany et al. (33). The histomorphometric data of the present study further demonstrate that a medium-term SUS induces structural adaptation in mesenteric small arteries, characterized by a decrease in the wall T, media T and CSA, and N_{CL}, which also verifies the findings on passive Wp obtained from functional experiments. There has been a debate as to whether SUS may induce a “true contractile deficit” in arteries without concomitant structural modification, and the results reported by Looft-Wilson and Gisolfi (28) have been cited as an example to support the view of functional alteration with no structural correlate (37, 57). It is apparent that VSMC’s hypoplasia/atrophy and the corresponding loss of contractile proteins with concomitant functional deficit of the splanchic resistance arteries and arterioles undoubtedly contribute to diminished ability to raise the TPR on returning to Earth’s gravity (4, 5). In addition, these adaptation changes may also result in impairment in the redistribution of blood flow to active muscles during exercise, which might contribute to the diminished aerobic capacity after flight (3, 10, 30).

We also sought to elucidate whether structural and functional adaptations do occur concomitantly in proximal cerebral resistance arteries. Results from the present study demonstrate that the functional adaptation of the middle cerebral arteries during a mid-term SUS is very striking. It includes augmented myogenic tone and responses to intraluminal pressure-increased vasoconstrictor responsiveness to KCl and 5-HT and greater vasomotion. Our results are consistent with the previous work by Geary et al. (19) and Wilkerson et al. (51). These authors have showed that these functional differences between SUS and CON groups could be eliminated by inhibition of NOS or endothelium removal (19, 51). Wilkerson et al. (51) further showed that an endothelial NOS (eNOS) signaling mechanism might be involved. However, at low pressure, greater vasomotion of middle cerebral arteries due to SUS was not affected by NOS inhibition, which suggests functional adaptation changes in VSMCs (19). Our previous study with the basilar artery has observed similar results (62). It seems that endothelium plays an important modulatory role in functional adaptation of cerebral arteries to SUS, although myogenic autoregulation is considered to rely on the intrinsic ability of VSMCs (17, 24, 27, 39). On the contrary, in rat mesenteric small arteries, myogenic responsiveness is not significantly affected by the endothelium (45). Region-specific adaptation of vasodilatory function also suggests the important modulatory role of endothelium in cerebral resistance vessels. For example, 3-day SUS resulted in a diminished vasodilatory function only in MCAs with that of mesenteric 3A arteries unaffected (29). Prisby et al. (40) further showed that 2-wk SUS may induce diminished endothelium-dependent vasodilation of MCAs via an eNOS signaling mechanism. Moreover, the present study has also provided the first direct evidence concerning structural adaptation of cerebral proximal resistance arteries due to SUS. The passive wall thickness (Wp) measured at 100 mmHg was significantly greater after a 4-wk, but not a 3-day (29), SUS. These results are also consistent with the data from 2-wk SUS rats (40, 51). Our morphometric data further demonstrate that a medium-term SUS results in medial hypertrophy of the middle cerebral arteries, characterized by an increase in the number of VSM cell layers (N_{CL}) with the average cross-sectional area of VSMCs (A_{CS}) unchanged, which suggests that the medial hypertrophy is due to hyperplasia of VSMCs. Nevertheless, the possibility of simultaneous hypertrophy in smooth muscle cells still cannot be excluded, because we only measured and calculated the cross-sectional area of smooth muscle cells in two dimensions rather than in three dimensions (33). Dickhout and Lee (14), using morphometric protocols and confocal microscopy, have reported that increased smooth muscle cell length, and not hyperplasia, in prehypertensive SHR is responsible for the observed increase in the number of smooth muscle cell layers in the mesenteric arteries. SUS-induced augmentation of myogenic responses makes the small cerebral resistance arteries...
can withstand higher pressures due to HDT without losing myogenic tone and to autoregulate cerebral blood flow despite a chronic elevation in cerebral perfusion pressure during SUS or microgravity. Augmented vasomotion in MCAs of SUS rats may assist the myogenic control of cerebral blood flow (CBF) by making VSMCs hyperreactive to neural or hormonal stimuli (36) and increasing the overall gain of the vessel network (24). It is apparent that these structural and functional alterations in MCAs of SUS rats are due to chronic elevation of transmural pressure instead of any increase in local blood flow or volume associated with cephalic fluid shift due to HDT, since the functional consequence of this autoregulation is a decrement in CBF associated with an increased cerebral vascular resistance (CVR; 51, 52). The cerebral circulation is not actively involved in PVR adjustment and blood redistribution during orthostasis and exercise (24, 41). However, inappropriate cerebral vasomotor failure due to maladaptation in myogenic autoregulation (5, 23, 40, 50, 56, 61) might also lead to orthostatic intolerance not associated with a clear hypotensive episode. In addition, animal studies have also suggested the possibility of a new kind of adverse effect on cerebral vessels due to a sustained enhancement of vascular local renin-angiotensin system during a prolonged existence in microgravity (2, 18).

Taken as a whole, the present findings support the hypothesis that redistribution of transmural pressure is the primary factor that initiates differential structural and functional adaptations in cerebral and mesenteric small resistance vessels during simulated/real microgravity exposure. Differential changes in responsiveness of small arteries in fore and hind-limb muscular small arteries from the same animal have also been shown in SUS rats (12, 47). There are several reasons to consider this possibility. First, during real/simulated microgravity, the primary local hemodynamic change is the redistribution of transmural pressure across the vasculature and the changes in flow and volume seem to be a secondary consequence (21, 50, 55–57). In cerebral circulation, the blood flow is well autoregulated (50, 51, 52, 56), thus, we can speculate that the pressure, not flow and volume, is the only factor that initiates structural adaptation. Second, the redistribution of transmural pressure during simulated or real microgravity is not only a prediction based on physical principles like G-vector change due to tilting or loss of hydrostatic pressure gradients due to the removal of gravity during weightlessness, but it has also been supported by the data obtained from ground-based human (26) and animal studies during tilting (42) or during real microgravity induced by free fall (20) or parabolic flight (46). Third, it should be noted that the hydrostatic pressure gradient is a constant and depends on physical parameters only, such as specific gravity of the blood, angle of tilt, and G, therefore, the differences in body size seem not to influence the basic conclusions thus obtained with small rodents. The dramatic influence of a very small change in hydrostatic pressure gradient has also been well demonstrated with veins. For example, Monos et al. (31) have shown that chronic 45°HUT resulted in a doubling of local venous pressure, enhancement of myogenic tone, and sympathetic hyperinnervation in perivascular nerves of the saphenous vein; whereas chronic 45°HUT induced a diminution of myogenic tone in the vein. Finally, the findings of the present study on SUS-induced structural and functional adaptation in cerebral proximal resistance arteries are quite consistent with those reported for genetic and nongenetic hypertensive rats (19, 35, 36, 53, 54, 62). Our data on mesenteric small arteries are similar to that report by Buus et al. (7) that long-term heart failure resulted in a reduced media thickness in mesenteric small arteries of rats, which has been speculated to be associated with the small blood pressure reduction. Overall, these findings have demonstrated that pressure-induced increase/decrease in circumferential stress can account for hypertrophic/atrophic structural adaptation in small resistance arteries. Also, VSM plays a key role in this adaptation process not only by adjusting its degree of tone during the acute phase, but also by means of its synthetic or proliferative activity during chronic sustained change in pressure (17, 24, 27, 39). However, there are yet other local and systemic factors that could not be excluded (3, 10, 56, 57) that need further studies to elucidate and integrate. Nevertheless, currently there is no evidence to support vascular plasticity during microgravity in humans (11, 43).

Differential Responsiveness of Cerebral and Mesenteric Small Resistance Arteries to 1 h/day $-G_x$ Intervention

The present results extend our previous findings on medium-sized conduit arteries (44, 58), providing further support to the hypothesis that daily short-duration $-G_x$ exposure can also fully prevent the structural adaptation changes in resistance arteries from different anatomic regions. It is surprising that daily $-G_x$ gravitation for 60 min, or just 4% of the total unloading time, is sufficient to maintain the structural homeostasis of the cerebral and splanchic resistance vessels at their normal 1-G level in rats subjected to HDT for 23 h/day during a 28-day period. Given that the redistribution of transmural pressure is the primary stimulus that initiates region-specific structural adaptations in resistance vessels, then present findings also support the hypothesis that a daily short-duration restoration of earth-like distribution of transmural pressures is essential and sufficient to counteract cardiovascular deconditioning during real/simulated microgravity (21, 50, 58, 60). The reason for the limited success of exercise-based countermeasure in preventing or alleviating postflight orthostatic intolerance might be that in microgravity environment exercise alone cannot produce an acceleration field and hence cannot restore the vascular hydrostatic pressure gradients (21, 50, 58, 60).

A surprising but expected result of the present study is that daily 1-h $-G_x$ over 28 days can prevent the augmented myogenic tone and increased vasoreactivity in the MCAs due to SUS, although the increased vasoreactivity of the basilar artery due to a 28-day SUS can be totally prevented (44). This result met the expectation raised in our previous work (55), which indicated that 1 h/day $-G_x$ exposure does not show any counteracting effect in preventing the augmentation of $C_{aL}$ function and protein expression during a 28-day SUS. The unresponsiveness of $C_{aL}$ channel in cerebral arterial VSMCs to such an intervention is crucial for the maintenance of an augmented myogenic tone and vasoconstrictor responsiveness whenever the rat is subjected to HDT. This seems also to be an example for the wisdom of the body. Enhanced $C_{aL}^{2+}$-dependent vascular tone is an important protective mechanism against an elevated cerebral perfusion pressure induced during SUS to reduce the risk of excessive capillary filtration, cerebral edema, and possible stroke (19, 21, 50, 51, 56). These findings
also suggest that, for cerebral small resistance arteries, functional adaptation can be dissociated from the structural adaptation during microgravity exposure.

In contrast, 1 h/day —Gx can fully prevent functional adaptation change in the mesenteric 3A arterioles, which is not only consistent with our previous work on CaH in mesenteric VSMCs (55) but also is in agreement with our previous result regarding the functional adaptation of femoral arteries (44). It also provides a mechanistic explanation to the potential benefit of IAG in preventing post-spaceflight cardiovascular deconditioning (8, 25, 48–50, 58), since splanchnic and muscular vascular beds are the main contributors to the maintenance of peripheral resistance.

Taken together, differential responsiveness of cerebral and mesenteric small resistance arteries to 1 h/day —Gx intervention has further revealed the complexity of vascular adaptation to microgravity and its gravity-based countermeasure. Our observations show the importance of not extrapolating from medium-sized conduit arteries to small resistance vessels, or from one kind of vascular bed to another, as functional individualities abound among the vessels to satisfy the individual needs of disparate organs. Although the present study together with our previous work (44, 58–60) have provided a preliminary mechanistic explanation for the potential benefit of IAG (8, 15, 16, 25, 48–50), it remains a big challenge to elucidate the underlying cellular and molecular mechanisms that account for “switching on” the structural adaptation during continuous 0 G exposure and “switching off” it during 0 G with daily short-duration restoration to 1 G.

In conclusion, the present study demonstrates that 28 days of simulated microgravity induces hypertrophy and atrophy in middle cerebral arteries and mesenteric 3A arterioles of rats, and, correspondingly, augmentation and depression of myogenic tone and agonist-induced vasoactivity in the two kinds of resistance arteries. Furthermore, the differential structural changes in the two kinds of arteries and the functional decrement in the mesenteric resistance arteries can be fully prevented by daily 1-h —Gx gravitation, but the functional enhancement in the cerebral resistance arteries cannot be prevented by such an intervention. These results constitute an important addition to vascular adaptation to microgravity and provide new mechanistic insight into the IAG.

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