Microgravity-induced changes in aortic stiffness and their role in orthostatic intolerance

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Microgravity-induced changes in aortic stiffness and their role in orthostatic intolerance. J Appl Physiol 102: 853–858, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.00950.2006.—Microgravity (μG)-induced orthostatic intolerance (OI) in astronauts is characterized by a marked decrease in cardiac output (CO) in response to an orthostatic stress. Since CO is highly dependent on venous return, alterations in the resistance to venous return (RVR) may be important in contributing to OI. The RVR is directly dependent on arteriovenous compliance (Cav), where aortic compliance (Ca) contributes up to 60% of Cav. We tested the hypothesis that μG-induced changes in Ca may represent a protective mechanism against OI. A retrospective analysis on hemodynamic data collected from astronauts after 5- to 18-day spaceflight missions revealed that orthostatically tolerant (OT) astronauts showed a significant decrease in Ca after spaceflight, while OI astronauts showed a slight increase in Ca. A ground-based animal model simulating μG, hindlimb-unweighted rats, was used to explore this phenomenon. Two independent assessments of Cav in vivo pulse wave velocity (PWV) of the thoracic aorta and in vitro pressure-diameter squared relationship (PDSR) measurements of the excised thoracic aorta, were determined. PWV showed a significant increase in aortic stiffness compared with control, despite unchanged blood pressures. This increase in aortic stiffness was confirmed by the PDSR analysis. Thus both actual μG in humans and simulated μG in rats induces changes in Cav. The difference in Cav in OT and OI astronaut suggests that the μG-induced decrease in Cav is a protective adaptation to spaceflight that reduces the RVR and allows for the maintenance of adequate CO in response to an orthostatic stress.

microgravity; arterial compliance; hindlimb unweighting; resistance to venous return

Microgravity (μG)-induced orthostatic intolerance (OI) is a common consequence of manned spaceflight, occurs frequently in astronauts, and poses a significant risk (3, 18, 27). It is clear that the μG-induced cardiovascular changes contributing to OI are due to a collective maladaptive response involving cardiac, neural, and hormonal deficiencies (3, 16, 18). Large postural decreases in venous return, cardiac output, and stroke volume are thought to be major contributors to OI (3). It is axiomatic that cardiac output is dependent on venous return, and therefore it is possible that a μG-induced increase in the resistance to venous return could, in part, account for the large postural decreases in venous return and cardiac output seen in OI. In addition, the venous return is dependent on the mean circulatory filling pressure, which is directly dependent on the blood volume. A lack of blood volume in the large veins (either trapped in compliant vessels, blocked by a constriction, or combination of both) for delivery to the right atrium would also decrease the venous return and consequently cardiac output via the Frank-Starling relationship. We contend that an increase in the resistance to venous return is an important determinant contributing to OI.

Using either a three-element model (2 capacitors, 1 resistor) of the vasculature (25) or a four-element model (2 capacitors, 2 resistors) (10), the resistance to venous return (RVR) is either

$$RVR = \frac{C_a R_v}{C_v + C_a}$$

or

$$RVR = \frac{C_a R_v}{C_v + C_a} + R_v$$

where Ca is arterial compliance, Cv is venous compliance, Rv is arterial resistance, and Rv is venous resistance for a three-element or four-element model, respectively. In either case, the RVR is directly dependent on Ca. The importance of Ca in the maintenance of normal values of the resistance to venous return was demonstrated by Hatanaka et al. (11). This study demonstrated that by decreasing the Cav, the resistance to venous return was effectively decreased. The maintenance of cardiac output by the carotid sinus baroreceptors was demonstrated by Potts et al. (22), who showed that increases in Cav attenuated the overall baroreceptor reflex gain. More importantly, the reflex control of cardiac output was impaired by increases in Cav (22). Thus a decrease in Cav may represent a mechanism by which one may be protected against OI by lowering the RVR and allowing for the maintenance of adequate cardiac output during an orthostatic stress.

While the adaptive responses to μG occur in all people exposed to a period of weightlessness, not all astronauts develop OI. Thus astronauts may be stratified as having orthostatic tolerance (OT) or OI, depending on their response to an orthostatic challenge (18). Given that a decrease in Cav (increase in stiffness) may decrease the resistance to venous return, we tested the hypothesis that μG-induced decreases in Cav may represent a protective mechanism and that OI develops, in part, as a consequence of a failure of this adaptive response. Thus we predict that OT astronauts have a decreased Cav. The goal of this study was to determine if spaceflight induces a change in Cav and if there is indeed a difference in Cav between OI and OT astronauts.
astronauts. To address this question, we have performed a retrospective analysis of previously collected hemodynamic data from astronauts to estimate $C_a$. In addition, a rat model simulating the cardiovascular effects of $\mu G$ was used to further investigate this effect on aortic compliance ($C_{ao}$), which accounts for up to 60% of the compliance for the entire arterial bed (26).

**METHODS**

**Humans.** All human astronaut data were from previously published studies, with permission from the lead researcher, conducted by Meck et al. (18). The data set consisted of supine hemodynamic measures taken from 57 astronauts usually 10 days before launch, on landing day, and 3 days after landing. Tilt tests were used to determine whether astronauts were orthostatically intolerant before and after spaceflight. Detailed methods are available from Meck et al. (18) and Fritsche-Yelle et al. (8); briefly, blood pressure was measured every minute using an automated arm cuff concurrently with beat-to-beat wrist cuff. Aortic cross-sectional area, determined using two-dimensional echocardiography, and ascending aortic flow, sampled with pulsed Doppler, were used to determine the stroke volume. OT was evaluated using a 10-min tilt protocol that brought the subject to an 80° upright position from supine. The subjects remained in a vertical position for 10 min or until presyncope symptoms manifested. A subject was determined to be intolerant if the tilt was prematurely ended due to the manifestation of presyncope symptoms. $C_a$ was estimated as the stroke volume divided by the arterial pulse pressure (systolic minus diastolic blood pressure).

**Animals.** The following protocol was approved by the Institutional Animal Care and Use Committee at Johns Hopkins University School of Medicine. Six male Wistar rats underwent hindlimb unweighting (HLU) for 7 days. The HLU method is described in detail in Ref. 19. Briefly, the tail was placed in traction tape, which unweighting (HLU) for 7 days. The HLU method is described in the Additional Animal Care and Use Committee at Johns Hopkins University.

Blood pressure. The blood pressure was measured by using the XBP1000 noninvasive Tail Blood Pressure System from Kent Scientific. Ten measures were taken and averaged while the rat was anesthetized and body temperature was held at 37°C.

**Pulse wave velocity.** The pulse wave velocity (PWV) was measured using a Doppler Signal Processing Workstation by Indus Instruments (24). The PWV for the thoracic aorta was obtained by measuring the aortic blood velocity via ultrasonic Doppler signals at two different points (one proximal and one distal) on the thoracic aorta, with the distance between the two points being called the separation distance (SPD). For each aortic blood velocity profile, a time-synchronized electrocardiogram (ECG) was recorded. At each point, pulse wave arrival time (PAT) was measured and is defined as the time lapsed from the occurrence of the R-wave on the ECG until the foot of the blood velocity profile. From two measures, the pulse transit time (PTT) can be calculated from the equation

$$\text{PTT} = \text{PAT2} - \text{PAT1}$$

From PTT and SPD, the PWV for the thoracic aorta can be calculated from the equation

$$\text{PWV} = \frac{\text{SPD}}{\text{PTT}}$$

The PWV for the abdominal aorta was calculated in a similar manner, using the distal thoracic aorta point as PAT1 and a third measure on the distal abdominal aorta as PAT2.

The PWV for the thoracic and abdominal aorta was measured before HLU or the control period and measured again 7 days later. The data were analyzed using a paired t-test for intergroup comparisons and a standard t-test for intragroup comparisons. All data are reported as means ± SE.

**Diameter-pressure relationships.** The diameter-pressure relationship was measured and analyzed in the thoracic aorta from the same rats that underwent the PWV protocol using a procedure similar to that described by Dunbar et al. (6). An incision was made down the entire midline of the rat; the thoracic aorta was dissected free from surrounding tissue and promptly removed. The vessel was constantly bathed in HEPES buffer. Once the aorta was removed, a section of aorta to be pressurized was selected, and any branching arteries from that section were tied off using sutures. The vessel was then cannulated with a 30-gauge, three-hole needle (one hole at each end and one in the middle). The vessel was then tied to the needle such that the middle hole was encapsulated by the vessel, but the two end holes were not. The ends of the needle were then connected to pressure reservoirs filled with HEPES buffer via plastic tubing. This allowed for the pressurizing of the vessel. The vessel was imaged with a microscope and projected onto a video monitor via a CCD camera. The outer diameter of the vessel was continuously monitored using a video-dimension analyzer (Instrumentation for Physiology and Medicine, San Diego, CA). The data were collected using an analog-to-digital conversion computer interface system (Biopac Systems, Goleta, CA).

**Diameter-pressure protocol.** The thoracic aortas from six HLU rats and six control rats were pressurized in intervals of 10 cmH2O from 0 to 150 cmH2O. The pressure was maintained at each step for 30 s or until the vessel diameter remained stable. Through the whole procedure, the vessel preparation was monitored for any signs that the vessel wall or any of the sutures were compromised.

**Diameter-pressure data analysis.** Diameter squared vs. pressure relationships were obtained because the changes of the diameter squared more closely approximate volume changes. The slope of the diameter squared-pressure relationship was determined using a linear regression least squares-fit algorithm. The slope of the diameter squared-pressure relationship is a measure of the compliance of the vessel ($C = \Delta V/\Delta P$, where $\Delta V$ and $\Delta P$ are change in volume and pressure, respectively). The inverse of the slope is a measure of the stiffness of the vessel. The data are reported as stiffness, or the inverse of the slope of the diameter squared-pressure relationship.

All data are reported as means ± SE, and significant differences were evaluated using $t$-tests of the mean. Paired $t$-tests were used when comparing pre- and postspaceflight and HLU data; otherwise unpaired tests were implemented.

**RESULTS**

Astronaut $C_a$. Figure 1 summarizes the $C_a$ in astronauts preflight, on landing day, and postlanding (in general, preflight measures were taken 10 days before flight, missions lasted between 5 and 18 days, and postlanding measures were taken 3 days after landing). As demonstrated in Fig. 1A, and consistent with our hypothesis, there is a significant decrease in $C_a$ in OT astronauts between preflight and landing day ($P = 0.0011; n = 40$). The $C_a$ then returns toward normal 3 days following landing ($P = 0.097$). In marked contrast, there is no significant decrease in compliance in OL astronauts at landing day ($P = 0.13$ to

$$\text{PWV} = \frac{\text{SPD}}{\text{PTT}}$$
2.3 ± 0.39 ml/mmHg; \( P = 0.36; n = 17 \) (Fig. 1B). In fact, although not statistically significant, there is a trend toward an increase in OI astronaut \( C_a \) between preflight and landing day. Interestingly, there is no significant difference between OI and OT \( C_a \) preflight [1.9 ± 0.13 (\( n = 17 \)) and 2.0 ± 0.097 ml/mmHg (\( n = 40 \)), respectively; \( P = 0.53 \)]. However, on landing day, the \( C_a \) values are dramatically different [2.3 ± 0.39 (\( n = 17 \)) and 1.7 ± 0.083 ml/mmHg (\( n = 40 \)), respectively; \( P = 0.046 \); Fig. 1C].

**Rat studies in vivo and in vitro.** We next tested the hypothesis that \( C_a \) is altered in a rat model simulating \( \mu G \). We measured both blood pressure and PWV, a well-validated in vivo measure of large artery stiffness (a greater PWV indicates a greater vessel stiffness and a lower \( C_a \)) (20), to address whether there are demonstrable changes in \( C_a \) in HLU rats. The blood pressures of the rats did not differ significantly between the groups or days (HLU: 111/85 ± 6/6 mmHg pre-HLU and 117/91 ± 3/7 mmHg post-HLU; control: 117/88 ± 6/5 mmHg precontrol and 115/85 ± 2/2 mmHg postcontrol; \( P > 0.40 \) for all comparisons).

**Rat aortic PWV.** The PWV in the rat thoracic aorta increased significantly, indicating a decreased \( C_a \), after 7 days of HLU compared with pre-HLU measurements (4.05 ± 0.05 to 7.23 ± 0.14 m/s, \( P < 0.0001 \)). The control group did not demonstrate a change in thoracic PWV during an equivalent period. Additionally, the post-HLU PWV was significantly greater than that of the postcontrol PWV (4.07 ± 0.19 m/s) (\( P < 0.0001 \)) (Fig. 2).

The PWV in the rat abdominal aorta did not show any significant changes either within groups HLU or the control group or between groups. (Fig. 3).

**Rat proximal aortic diameter squared-pressure response.** Given the findings, we wished to verify if indeed the alterations in PWV represented alterations in the passive properties of the aorta. We therefore measured the diameter squared-pressure response.
Therefore, it is reasonable to conclude that the decrease in Ca decreases the resistance to venous return. Thus a stiffer, or less compliant, arterial bed will allow for less accumulation of blood volume in the arterial circulation and consequently shift the extra volume to the venous side, aiding in venous return.

This is demonstrated by the fact that an estimate of Ca was seen to change in astronauts (Fig. 1), and two independent measures of proximal aortic stiffness was greatly increased after 7 days of HLU compared with the control group (\(P = 0.02\)).

**DISCUSSION**

The data presented are consistent with the hypothesis that exposure to actual or simulated \(\mu G\) induces a change in \(C_a\). This is demonstrated by the fact that an estimate of \(C_a\) was seen to change in astronauts (Fig. 1), and two independent measures of \(C_{ao}\) showed changes after HLU in rats (Figs. 2 and 5). The data also support the hypothesis that a decrease in \(C_a\) (increase in stiffness) maybe an adaptive change that protects astronauts from OI by decreasing the resistance to venous return.

The astronaut hemodynamic data indicates that while the OI astronauts had a slight, nonsignificant increase in \(C_a\), the OT astronauts demonstrated a large decrease in \(C_a\) after space-flight. This may explain why these astronauts were able to maintain adequate cardiac output and blood pressure during an orthostatic challenge. On the other hand, OI astronauts failed to decrease their \(C_a\). Potts et al. (22) showed that a reduction of \(C_a\) was partly responsible for minimizing a baroreflex-evoked increase in resistance to venous return. Additionally, Hatanaka et al. (11) demonstrated that a decrease in \(C_a\) effectively decreases the resistance to venous return. Thus a stiffer, or less compliant, arterial bed will allow for less accumulation of blood volume in the arterial circulation and consequently shift the extra volume to the venous side, aiding in venous return. Therefore, it is reasonable to conclude that the decrease in \(C_a\) seen in the OT astronauts is, in part, responsible for maintaining OT, while failure to decrease compliance contributes to OI.

Experiments in our laboratory have demonstrated that 8–14 days of HLU caused a significant decrease in stroke volume and cardiac output (\(5–15%\)) but no change in mean arterial pressure in rats \((n = 4)\) during a 75° head-up tilt test (E. M. Brooks-Asplund, unpublished observations). Additional studies of HLU rats undergoing head-up tilts have demonstrated a decrease in mean arterial pressure (\(11%\) decrease of supine levels) and other symptoms of OI (15, 28). These results suggest that HLU does induce cardiovascular alterations that are consistent with OI, supporting the idea that rats are susceptible to orthostatic stress. However, the decreases in stroke volume, cardiac output, and mean arterial blood pressure are not as great as reported from OI astronauts during an upright tilt test (\(30–50%\) decrease for stroke volume and cardiac output and \(25%\) for mean arterial blood pressure (3, 18)). The decrease in \(C_{ao}\) in rats in response to HLU may represent a protective mechanism (similar to OT astronauts) that attenuates the decrease in stroke volume, cardiac output, and mean arterial blood pressure associated with head-up tilt through a decrease in the resistance to venous return. This may result in the rats exhibiting greater tolerance to an orthostatic stress than OI astronauts, despite demonstrating some vulnerability to such a stress.

It is apparent that the changes in the rat model simulating \(\mu G\) are consistent with the changes seen in the OT astronauts with regard to \(C_{ao}\). After 7 days of HLU, the rat thoracic aorta demonstrated an increased PWV, which indicates stiffer vessels (decreased compliance) (Fig. 2). The blood pressure of the rats at the time of PWV measurement did not differ between groups, and the unstressed aortic diameter (determined in vitro) did not differ significantly between HLU and control either. Considering this, it is likely that the increase in PWV was due to vessel stiffening rather than an increased blood pressure or a decreased vessel diameter. The diameter squared-pressure relationship determined from thoracic aorta in vitro shows that the thoracic aorta of the HLU animals was stiffer than that of the control group, providing an independent confirmation of the PWV data (Fig. 5). The in vitro testing of the vessel eliminates any acute neural or nonlocal hormonal influence that may affect the vessel stiffness. Combining the in vivo and in vitro data, it can be concluded that the proximal aorta does undergo stiffening during HLU. Furthermore, it can be hypo-

![Fig. 4. Representative proximal aorta diameter squared (mm²)-pressure responses for a single control and a single HLU animal. The slope of the curves is a measure of compliance. The inverse of the slope is therefore a measure of the stiffness.](http://example.com/fig4.png)

![Fig. 5. Average stiffness, inverse slopes of the diameter squared (mm²)-pressure relationships, of the control and HLU groups after the 7-day control or HLU period, respectively. \(P = 0.0216\). Data are reported as means ± SE.](http://example.com/fig5.png)
recognized that the vessel stiffening is due to a change in vessel wall composition, rather than acute dynamic neurohormonal signals. These changes arise possibly as a result of changes in the ratio of collagen to elastin, altered collagen cross-linking, or alterations in vascular matrix (2, 9, 17). This hypothesis is supported by the finding that HLU increases the expression of both endothelial and inducible nitric oxide synthase in the thoracic aorta of Wistar rats (14). This could contribute to smooth muscle relaxation, thereby eliminating the smooth muscle as a possible contributor to the decreased Cao and leaving the composition of the vessel wall as the prime candidate for change in response to HLU. The change in the vessel wall may result from differences in mechanical forces experienced by the vessel wall, which in turn could contribute to vascular remodeling. It has been previously demonstrated that a differential pressure gradient exists along the aorta during HLU. As a result of HLU, a greater than normal pressure is present in the proximal aorta (4, 29) and a less than normal pressure is present in the caudal artery (4). This establishes a pressure gradient along the whole of the aorta. This differential pressure gradient may induce the increased stiffness of the proximal aorta, which experiences a greater than normal blood pressure during HLU. This idea is supported by previous observations of pressure-dependent remodeling of vessel walls in hypertension (12). This may also explain why no change in abdominal aorta stiffness was observed following HLU. Since the abdominal aorta is close to the hydrostatic indifference point in the HLU-induced gradient, it is not exposed to a significant change in pressure during HLU. The stiffness therefore remains unaltered.

The data presented in our study are in agreement with other studies of the rat aorta. Previous studies have shown that the rat thoracic aorta has diminished vasoconstrictor abilities following HLU (5). It is possible that a stiffening of the vessel wall contributes to the decrease in vasoconstrictor response seen after HLU. However, the same study also demonstrated a deficit in abdominal aorta contractile responses, while our study did not demonstrate any evidence of stiffening of the abdominal aorta. This suggests that the deficit in contractile response of the abdominal aorta is due to other factors, such as smooth muscle dysfunction, and may not contribute to vessel stiffening. Subsequent studies on the mechanical properties of the rat abdominal aorta in Sprague-Dawley rats have found no evidence that the vascular structure is changed, despite vascular contractile dysfunction (21). Rat abdominal aorta vasoconstrictor hyperresponsiveness has also been found in Wistar rats (23). Since the PWV of the rat abdominal aorta does not change following HLU (Fig. 3), this is consistent with the data that vessel wall structure is not altered. Since blood pressure is not different between animal groups, it is possible that the unstressed diameter in the abdominal aorta (not measured) is different. This is believed to be unlikely because previous research has shown no change in the vessel diameter after HLU (21). Our data suggest that the rat abdominal aorta does not experience any vessel wall stiffening as a result of HLU and support the idea that altered regional loading (differential blood pressure) explains the selectivity of stiffness changes on the thoracic aorta.

One issue that arises is the assumption that proximal Cao accurately reflects changes in the overall compliance of the arterial bed. It has been demonstrated that Cao contributes up to 60% of overall Ca in dogs (26). It is possible that the other major contributors to Ca change in an opposite manner, thereby canceling the effect of the decrease in Cao on the resistance to venous return, as suggested by another study from our laboratory (7), where it was found that the overall total Ca does not change in response to HLU. This is supported by the finding that both active and passive force-generating mechanisms of the rat arterial system are impaired by HLU (4, 18, 20), suggesting that these components of the arterial system may counteract the decrease in Cao by an increase in local compliance, resulting in no overall change in Ca. This also explains why rats demonstrate a degree of OI.

With regard to orthostatic stress, an increase in arterial stiffness would be considered a positive adaptation. While the increase in systolic and diastolic pressure, as well as the increase in pulse pressure that accompanies arterial stiffening, is seen as protective in terms of OI and hypotension, it can also have deleterious effects on human health. The increased pulse pressure results in an increased afterload on the left ventricle, increasing the workload on the heart, and can eventually lead to hypertrophy and heart failure. Additionally, the increased pulse pressure can alter coronary perfusion and increase the risk of myocardial infarction (1). Increased pulse pressure also raises the risk of cardiovascular diseases such as stroke and heart failure (27). Additionally, it could be hypothesized that altered vascular properties due to μG (or HLU) could exacerbate conditions such as atherosclerosis and hypertension in which arterial stiffening preexists (13, 30), although the true effects of μG in astronauts with silent or known preexisting disease are likely to remain unknown.

In conclusion, this study observed a μG-induced decrease in Ca in OT astronauts. The HLU rat model also demonstrated similar decreases in Cao when measured by two independent methods: PWV and diameter squared-pressure measurements. Given the influence of the Cao on the overall Ca, such changes will significantly affect parameters such as the resistance to venous return. It is possible that such decreases in Cao and Cao can sufficiently lower the resistance to venous return as to be a protective mechanism against μG-induced OI by maintaining cardiac output.

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