Blood pressure and mesenteric resistance arterial function after spaceflight

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Blood pressure and mesenteric resistance arterial function after spaceflight. J Appl Physiol 92: 13–17, 2002.—Ground studies indicate that spaceflight may diminish vascular contractility. To examine that possibility, vascular function was measured in spontaneously hypertensive rats immediately after an 18-day shuttle flight. Isolated mesenteric resistance arterial responses to cumulative additions of norepinephrine, acetylcholine, and sodium nitroprusside were measured using wire myography within 17 h of landing. After flight, maximal contraction to norepinephrine was attenuated \( P < 0.001 \) as was relaxation to acetylcholine \( P < 0.001 \) and sodium nitroprusside \( P < 0.05 \). At high concentrations, acetylcholine caused vascular contraction in vessels from flight animals but not in vessels from vivarium control animals \( P < 0.05 \). The results are consistent with data from ground studies and indicate that spaceflight causes both endothelial-dependent and endothelial-independent alterations in vascular function. The resulting decrement in vascular function may contribute to orthostatic intolerance after spaceflight.

spontaneously hypertensive rats; microgravity; vascular contraction; vascular relaxation

Ground-based studies indicate that exposure to simulated weightlessness impairs vascular contractility. There have been several reports of attenuated vascular contraction and relaxation in vessels isolated from animals exposed to head-down tilt \( (6, 7, 9, 30, 31) \). If vascular function is similarly impaired after spaceflight, it may diminish the ability to make adjustments necessary to meet hemodynamic challenges. This possibility is suggested by the observations of McDonald et al. \( (25) \), who found that rats exposed to hindlimb un-weighting for 15 days were unable to divert blood flow from the viscera during exercise. In humans, impaired vascular function may contribute to the high incidence of orthostatic intolerance that has been reported after spaceflight. Recent research in humans indicates that failure to adequately increase total peripheral resistance is the fundamental cause of increased risk for orthostatic intolerance after spaceflight \( (4, 12) \). An unresponsive vasculature coupled to an impaired baroreflex arc \( (12, 26) \) may explain the failure to increase total peripheral resistance.

In this report, data are presented indicating that mesenteric resistance arterial function is impaired after an 18-day space shuttle flight.

METHODS

The data presented in this report represent a subset of data from NIH.R4, a life sciences mission flown on a NASA space shuttle \( (STS-80) \). That mission was designed to examine the influence of dietary calcium on calcium metabolism and cardiovascular function in microgravity \( (17) \). The subset of data presented here describes the effects of spaceflight on vascular function. Because there was no interaction between dietary calcium and spaceflight on vascular function, data are collapsed across the dietary calcium variable in this report for purposes of clarity and focus.

Institutional animal care and use approval. The protocols used in these experiments were approved by the Institutional Animal Care and Use Committees at Oregon Health Sciences University, Ames Research Center, Johnson Space Center, Kennedy Space Center.

Animals. Male spontaneously hypertensive rats \( (SHR) \) were selected for use because they have been used extensively for the study of diet-related alterations in cardiovascular function and their blood pressure is known to be sensitive to variations in dietary calcium \( (16) \). The SHR were obtained from Taconic Farms \( (Germantown, NY) \) at 21 days of age. On arrival at Kennedy Space Center, the animals were placed on either a high- \( (2.0\%) \) or low-calcium \( (0.2\%) \) diet \( (Teklad, Madison, WI) \) for the duration of the experiment. The animals were assigned to flight and vivarium control groups based on receipt date from the vendor, as described in Hatton et al. \( (17) \).

Procedure. Two animal enclosure modules with seven animals each were flown on STS-80 when the animals were 7 wk of age. One animal enclosure module contained high-calcium diet, and the other contained low-calcium diet animals. The flight lasted 18 days. Three hours after landing, the animals were available for experimentation.

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The animals were anesthetized with halothane (2% in O₂), and the mesenteric vascular bed was collected for harvesting of resistance vessels after exsanguination. One rat was killed every 0.5 h for the first eight rats, followed by a break for 2.5 h. The final six rats were also killed every 0.5 h. The order of testing alternated between high- and low-calcium-fed rats. From beginning to end, it took a total of 14 h to finish vessel testing. Experiments on the vivarium control animals were conducted on exactly the same schedule as that for the flight animals, only on a separate day.

**Vessel protocol.** The vascular tests were performed using branch II or III mesenteric resistance arteries (~250 μm). On isolation, the vessels were placed in ice-cold gassed physiological saline solution.

For testing, the vessels were mounted on a dual-channel wire myograph and bathed in physiological saline solution of the following composition (in mmol/l): 130 NaCl, 4.7 KCl, 14.9 NaHCO₃, 1.1 NaH₂PO₄, 0.1 Na₂EDTA, 1.17 MgSO₄·7H₂O, 1.6 CaCl₂, and 5.5 glucose, with a pH of 7.4 when gassed with 95% O₂ and 5% CO₂. Resting diameter and wall thickness were determined for each vessel by using a filar micrometer eyepiece in the field of a ×40 objective. These values were then used to calculate the volume of the media. The vessel was then stretched to a circumference equivalent to 90% of the diameter that it would have with an intraluminal pressure of 100 mmHg (28). The normalized medial thickness was then calculated from values of diameter of the segment, its axial length, and medial volume, which was assumed to remain constant.

After the morphometric measurements were made, the contractile response of each vessel to a challenge with 100 mmol/l KCl (NaCl substituted) was determined three times, followed by two challenges with 100 mmol/l KCl + 10 μmol/l norepinephrine (NE). The vascular response to cumulative additions of NE was measured 30 min later. The apparent sensitivity of each vessel to NE was assessed by using the concentration of the agonist that elicited EC₅₀. The EC₅₀ values were determined by using nonlinear curve fitting. The force generated by each vessel was normalized to the cross-sectional area of the vessel and reported as active stress (mN/mm²).

The chamber was flushed twice, and the vessel was allowed 20 min to equilibrate with indomethacin (3 × 10⁻⁶ M) before relaxation responses to acetylcholine were tested. Relaxation was determined by cumulative addition of acetylcholine to vessels that were precontracted to 80% of maximal contraction with NE. The procedure was repeated for testing relaxation to sodium nitroprusside. Relaxation was expressed as a percentage of the original response to NE.

**Data analysis.** Analysis of variance, with repeated measures where appropriate, was conducted by using a statistical package, SPSS for Windows. Post hoc comparisons were done by using Tukey’s tests. Trend analysis, using time of testing as the independent variable, was used to determine whether there were any linear or higher order trends over time in vessel responses as a consequence of adaptation to normal gravity. A probability of 0.05 was used to establish statistical significance.

**RESULTS**

Mesenteric vessel wall thickness was not different for the flight and control groups (30.83 ± 4.07 vs. 31.33 ± 5.58 μm). Axial length, diameter, and medial volume of the vessels are provided in Table 1.

The contractile responses to NE are shown in Fig. 1. Maximal contraction was significantly reduced in the flight animals relative to the control animals (P < 0.001). There was no difference in the EC₅₀ between flight and control animals (6.65 ± 0.11 vs. 6.61 ± 0.17).

The relaxation response to acetylcholine is presented in Fig. 2. The relaxation response was significantly attenuated in the flight animals relative to the control animals (P < 0.001), and, at the higher doses, there was vasoconstriction to acetylcholine (P < 0.05). There was a significant main effect for flight condition (P < 0.05) for sodium nitroprusside because of attenuated relaxation in the flight group relative to the control group, but there was no interaction between flight condition and the dose of sodium nitroprusside (Fig. 3; P > 0.05).

**DISCUSSION**

The finding that contractility to NE was impaired in mesenteric resistance arteries from the flight animals is consistent with the observations of Purdy and colleagues (30, 31) and Delp et al. (6–9) from rats exposed to hindlimb elevation and provides validation of head-down tilt as a model for studying the effects of microgravity on vascular function. The combined results suggest that diminished contractility is a consistent finding after actual or simulated microgravity in many but not all vascular beds.

Delp (6) reported that resistance vessels from the soleus muscle did not have attenuated responses to NE after exposure to head-down tilt, whereas vessels from

<table>
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<th>Table 1. Vessel dimensions</th>
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Values represent means ± SD; n, no. of animals.
gastrocnemius muscles did. Looft-Wilson and Gisolfi (22) found no attenuation in response to vasoconstrictors in mesenteric resistance vessels from rats exposed to head-down tilt, although they did report a significant deficit in myogenic tone. In contrast, Geary et al. (14) reported that microgravity increases cerebral arterial myogenic tone, suggesting that adaptations to microgravity are dictated by the local conditions in each vascular bed.

The disparity between the results of Looft-Wilson and Gisolfi (22) and those reported in the present study regarding mesenteric resistance arteries may be related to differences in technique, rat strains, or experimental conditions. Looft-Wilson and Gisolfi used a pressurized system to examine vessels from normotensive Sprague-Dawley rats after head-down tilt. Whereas there are differences in vascular function between SHR and normotensive strains, there are more similarities than differences. Shaw and colleagues (32) found that there was no difference between SHR and normotensive Wistar-Kyoto rats with regard to sensitivity of the contractile apparatus to Ca\(^{2+}\) or agonist-induced Ca\(^{2+}\) sensitization in vascular smooth muscle in either 5- or 20-wk-old rats. Moreover, Izzard et al. (19) found no evidence of increased contractility of mesenteric resistance vessels to NE under isobaric conditions when studied at physiological distending pressure. Thus it would seem less likely that a difference in strain could account for the disparate outcomes and more likely that it was related to either technique or experimental treatment. One possible explanation for the disparate outcomes is the length of exposure to treatment. Looft-Wilson and Gisolfi (22) exposed animals to head-down tilt for 28 days, whereas animals in the present study were exposed to microgravity for 18 days. Delp et al. (8) observed a reduction in acetylcholine-induced dilation in soleus muscle arterioles after 14 days, but not 28 days, of head-down tilt, suggesting that vessels continue to adapt beyond 14 days of exposure to treatment.

Attenuated vascular responses are not limited to contractile responses to NE. Delp et al. (7, 9) reported that aortic contractile responses to NE, phenylephrine, KCl, CaCl, and arginine vasopressin were diminished by hindlimb suspension. Similarly, Purdy et al. (30) observed diminished contractility to potassium. The generality of the phenomena across agonists suggests that the decrement in responsiveness is not due to a change in a specific receptor population. Instead, the phenomena appear to be relatively nonspecific.

Delp et al. (9) suggested that an inability to mobilize intracellular calcium could result in the kind of non-specific decrement in contractility that is observed in animals exposed to hindlimb suspension. Data on platelet intracellular Ca\(^{2+}\) concentration levels from the present study (17) show that both basal and thrombin-stimulated intracellular Ca\(^{2+}\) concentration were reduced in flight animals with no change in ionomycin-releasable calcium stores. These data are similar to those from hindlimb-suspended rats that showed an attenuated increase in cytosolic calcium content of portal vein myocytes after exposure to vasoactive compounds (27). This raises the possibility that mobilization of calcium may be hampered in flight animals, resulting in attenuated contractility.

Hargens and Watenpaugh (15) suggested that changes in hydrostatic pressure gradients may result in atrophy of vascular smooth muscle during exposure to microgravity, leading to a reduction in maximal force generated by the vessel. In the present study, there was no difference between flight animals and control animals in terms of media thickness and thus no evidence of smooth muscle atrophy that could account for the results. Similarly, Wilkerson et al. (37) found no evidence of a change in structural morphology in mesenteric or splenic resistance arteries after head-

Fig. 2. Relaxation responses of mesenteric resistance arteries to acetylcholine. Data are for SHR flight and vivarium control groups. Values represent means ± SE. Flight animals had significantly attenuated relaxation relative to control animals (P < 0.001). Significant difference between flight and control animals, *P < 0.05 for post hoc test.

Fig. 3. Relaxation responses of mesenteric resistance arteries to sodium nitroprusside. Data are for SHR flight and vivarium control groups. Values represent means ± SE. Flight animals had significantly attenuated relaxation relative to control animals (P < 0.05).
down tilt. There have been mixed results in other vessels. Neither Delp et al. (7) nor Purdy et al. (30) found any indication of atrophy in conduit vessels from rats exposed to head-down tilt. However, Delp et al. (8) have found evidence of atrophy in arterioles from gastrocnemius and soleus muscle after head-down tilt.

There is evidence that nitric oxide activity may be involved in the decrement in vascular contractility after head-down tilt. Sangha et al. (31) reported an increase in inducible nitric oxide synthase (NOS) and endothelial NOS in animals exposed to head-down tilt. Removal of the endothelium corrected the diminished contractility in vessels with elevated endothelial NOS, whereas treatment with aminoguanidine normalized contractility in vessels with elevated inducible NOS. These findings are consistent with reports that nitric oxide interferes with the contractile response to NE as well as a variety of other vasoconstrictors (36). However, they also observed a decrement in contractility in aortic rings that appeared to be independent of NOS activity.

Increased NOS activity may provide a partial explanation for the impaired contractile responses to NE observed in the present study, although the relaxation responses to acetylcholine shown in Fig. 2 do not support this possibility. There is also a possibility that increased β-adrenergic activity may have contributed to the decrement in contractility because both neuronal and extraneuronal uptake processes were functional as were β-adrenergic receptors in the present protocol. However, it is likely that both uptake processes were saturated at NE concentrations that provoked contraction. Moreover, there is little evidence that β-adrenergic-receptor function is altered in rats exposed to simulated (3) or actual microgravity (11); although Ohira et al. (29) did observe a decrease in β-receptor density in plantaris muscle from rats exposed to 14 days of spaceflight.

Considering that relaxation to sodium nitroprusside was also diminished in the present study, it seems likely that the impaired relaxation to acetylcholine was related, in part, to a change in vascular smooth muscle function. Delp et al. (7) observed a similar impairment in both nitroprusside and acetylcholine-induced relaxation in aortic rings from rats subjected to head-down tilt and suggested that the deficit in relaxation may be due to alterations in the cGMP-mediated dilatory mechanism within the vascular smooth muscle cell.

The presence of vascular contraction at high levels of acetylcholine in the flight animals suggests that the release of an endothelium-derived vascular contracting factor may have contributed to the impaired relaxation. Arteries from SHR, as well as other hypertensive models, have impaired relaxation responses to acetylcholine when they are precontracted with NE because of a cyclooxygenase-derived pressor substance released from the endothelium (e.g., Refs. 21, 24). Indomethacin has been shown to prevent the contraction to acetylcholine (10, 23). However, indomethacin failed to prevent the contraction in the present study, suggesting that the dosage used was insufficient or some other mechanism was responsible for the contraction, such as a direct effect of acetylcholine on the vessel (13, 20).

The extent to which alterations in vascular function might alter blood pressure regulation and contribute to orthostatic intolerance is difficult to discern. Blood pressure regulation is highly complex and involves a number of interacting systems, many of which have been shown to be altered by exposure to microgravity (15), such as baroreflex control of blood pressure (12, 26). However, considering that the vasculature represents the primary effector mechanism for neurohormonal regulation of peripheral resistance, a decrement in responsiveness to vasoactive compounds could seriously hamper the ability of the organism to make adjustments to hemodynamic challenges.

A critical question that remains to be answered is whether or not the vascular impairments that have been observed in rats also apply to humans and, if so, whether or not they contribute to orthostatic intolerance after spaceflight. Although there is evidence of a lack of increase in lower limb vascular resistance and abnormal flow redistribution during orthostatic tests in humans (2, 18) that may be due to an unresponsive vasculature, there are also data indicating an inadequate increase in sympathetic nervous system outflow in individuals susceptible to orthostatic intolerance (12, 35). Data regarding the relative roles of the nervous system and the vasculature in orthostatic intolerance are sparse. Convertino et al. (5) found no indication of an alteration in the blood pressure response to graded infusions of NE at days 16 and 29 of 6° head-down tilt and thus no evidence of an alteration in vascular response to NE in humans. On the other hand, Shoemaker et al. (33) measured muscle sympathetic nerve activity and forearm blood flow after 14 days of head-down bed rest and found that resting burst frequency was reduced, whereas forearm vascular resistance and total peripheral resistance were unchanged. They concluded that central modulation of sympathetic discharge and peripheral vascular adaptations may have occurred concurrently during the bed-rest period to maintain blood pressure, despite reductions in sympathetic discharge. In a subsequent study, Shoemaker et al. (34) found that the peak forearm vasodilatory response to ischemia was attenuated after 14 days of head-down bed rest as was the ability to constrict a dilated vascular bed. The authors suggested that simultaneous reductions in dilatory and constrictor responses in forearm muscle vascular tissue develop during prolonged bed rest.

Clearly, additional research will be needed to clarify the relative roles of the nervous system and the vasculature in orthostatic intolerance in humans. In the absence of human data, data from animal work provide strong evidence that there are functional and structural vascular adaptations to microgravity that appear to be dictated by normal physiological responses to local environmental conditions. It seems likely that those conditions would apply to humans as well as animals.

In summary, the data from the present study represent the first direct observation in animals that vascular function is compromised after exposure to spaceflight. This finding verifies data from ground-based studies in ani-
VASCULAR FUNCTION AFTER SPACEFLIGHT


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