

# Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect?

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<sup>1</sup>*Noll Physiological Research Center and Department of Kinesiology, The Pennsylvania State University, University Park, Pennsylvania 16802; and* <sup>2</sup>*Institute for Exercise and Environmental Medicine, University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas, Dallas, Texas 75231*

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**Pawelczyk, James A., and Benjamin D. Levine.** Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect? *J Appl Physiol* 92: 2105–2113, 2002. First published December 21, 2001; 10.1152/jappphysiol.00979.2001.—Unlike quadrupeds, the legs of humans are regularly exposed to elevated pressures relative to the arms. We hypothesized that this “dependent hypertension” would be associated with altered adrenergic responsiveness. Isoproterenol (0.75–24 ng·100 ml limb volume<sup>-1</sup>·min<sup>-1</sup>) and phenylephrine (0.025–0.8 µg·100 ml limb volume<sup>-1</sup>·min<sup>-1</sup>) were infused incrementally in the brachial and femoral arteries of 12 normal volunteers; changes in limb blood flow were quantified by using strain-gauge plethysmography. Compared with the forearm, baseline calf vascular resistance was greater (38.8 ± 2.5 vs. 26.9 ± 2.0 mmHg·100 ml·min<sup>-1</sup>·ml<sup>-1</sup>;  $P < 0.001$ ) and maximal conductance was lower (46.1 ± 11.9 vs. 59.4 ± 13.4 ml·ml<sup>-1</sup>·min<sup>-1</sup>·mmHg<sup>-1</sup>;  $P < 0.03$ ). Vascular conductance did not differ between the two limbs during isoproterenol infusions, whereas decreases in vascular conductance were greater in the calf than the forearm during phenylephrine infusions ( $P < 0.001$ ). With responses normalized to maximal conductance, the half-maximal response for phenylephrine was significantly less for the calf than the forearm ( $P < 0.001$ ), whereas the half-maximal response for isoproterenol did not differ between limbs. We conclude that  $\alpha_1$ - but not  $\beta$ -adrenergic-receptor responsiveness in human limbs is non-uniform. The relatively greater response to  $\alpha_1$ -adrenergic-receptor stimulation in the calf may represent an adaptive mechanism that limits blood pooling and capillary filtration in the legs during standing.

vasoconstriction;  $\alpha$ -adrenergic receptor;  $\beta$ -adrenergic receptor

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AT REST OR DURING ORTHOSTATIC stress in humans, sympathetic activity targeted to arm and leg skeletal muscle vasculature is largely homogenous (22, 29). However, at rest, the release of norepinephrine in these limbs is decidedly specific, with greater norepinephrine spillover from the arms than the legs (13, 16). Yet, with some exceptions (e.g., Refs. 5, 9, 26), the typical pattern of regional vascular resistance is directionally opposite (i.e., legs greater than arms) (8, 13, 16, 21, 28). These

results suggest that the effects of sympathetic nerve stimulation are qualitatively and/or quantitatively different in the arms and legs of humans.

In this paper, we consider the possibility that differentiation of adrenergic receptors contributes to the high degree of specialization of sympathetic nerve transduction in the legs and arms. Pressure (i.e., transmural wall stress) is known to regulate growth and adrenergic responsiveness in vascular smooth muscle (23), and bipeds such as humans afford an unusual opportunity to study the prolonged effect of increased pressure on vascular regulation. During much of the day, hydrostatic pressure elevates leg blood pressure, causing the legs to be exposed to intermittent hypertension. To the contrary, the arms, lying near the approximate level of the heart, are less gravitationally dependent and are exposed to pressures closer to that experienced in the aorta. Thus one might theorize that human arms and legs exhibit differential hemodynamics and adrenergic responsiveness as a long-term consequence of the upright posture, exposing arm and leg vasculature to different loading conditions.

In the present investigation, we studied both forearm and calf vascular responses to  $\alpha_1$ - and  $\beta$ -adrenoceptor agonists. We hypothesized that legs would exhibit greater responsiveness to an infused  $\alpha_1$ -adrenergic agonist than arms as a long-term consequence of intermittent exposure to “dependent hypertension” (i.e., the sum of hemodynamic and hydrostatic pressures while standing), whereas  $\beta$ -mediated vasodilation would not differ between limbs. Using local arterial infusions, we were able to characterize the functional effects of adrenergic-receptor stimulation in vascular smooth muscle while minimizing confounding reflex responses that would result from changes in systemic arterial pressure. Our hypotheses were largely confirmed in the present investigation, revealing a heterogeneous distribution of  $\alpha_1$ -adrenergic responsiveness in human limbs previously unappreciated.

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## METHODS AND PROCEDURES

### Subjects

Twelve healthy subjects (11 men, 1 woman) were studied in the morning after an overnight fast. Physical characteristics included the following: mean age of  $24 \pm 2$  yr (range 18–35 yr), height  $185 \pm 23$  cm, and weight  $79 \pm 3$  kg. No subject smoked, used recreational drugs, or had significant chronic medical problems. Subjects were screened with a history and physical examination, resting electrocardiogram, and resting echocardiogram; potential subjects with vascular abnormalities were excluded. All subjects provided voluntary, informed consent. The Institutional Review Boards of both the University of Texas Southwestern Medical Center and the Presbyterian Hospital of Dallas approved the protocol.

### Measurements

**Arterial pressure and heart rate.** With the use of local anesthesia and modified Seldinger technique, a 5-cm, 3-Fr catheter (Cook Medical) was placed in the brachial artery of the nondominant arm, and a 12-cm, 4-Fr catheter (Cook Medical) was placed in the femoral artery of the nondominant leg at the level of the inguinal ligament. Pressure waveforms were transduced (Transpac IV, Abbott), amplified (Hewlett-Packard 78534A), and displayed on a strip-chart recorder (Astromed MT 95000) with at least 0.5-mmHg resolution. The zero for both pressure transducers was set at 5 cm below the sternal angle in the supine position.

After catheter insertion, at least 1 h elapsed before pressure measurement commenced. Each pressure wave was sampled at 200 Hz and integrated in real time with customized software. Heart rate (HR) was recorded from lead II of the electrocardiogram and sampled at 1,000 Hz. Both pressures and HR were stored in beat-to-beat format during the experiment; mean HR and blood pressure were calculated from the time-weighted average of these data.

**Limb volume.** The volume of the infused arm and leg was determined by anthropometry. Limbs were modeled as a series of truncated cones that were summed to estimate the limb volume (27). Two sections were used to approximate the forearm, and 11 sections were used to approximate the leg. This method compares favorably with measurements made with water displacement (19).

**Limb blood flow.** Calf and forearm blood flows were determined by venous occlusion plethysmography. Flows were measured in both calves or both forearms during infusions, with the noninfused limb serving as a control for the infused limb. Changes in limb volume were transduced by using dual-strand mercury-in-Silastic strain gauges (12), amplified (Hokanson EC-4), and displayed on a strip-chart recorder (Astromed MT-95000) for post hoc analysis. Strain gauges were sized to approximate the largest circumference of the calf or forearm. Blood flow to the feet or hands was excluded from the measurement, with ankle or wrist arresting cuffs inflated to 250 mmHg just before flow measurements at each stage of the infusion and deflated between stages. Venous occlusion pressures of 50 mmHg were employed; the lower legs or forearms were elevated 20 cm above the subjects' midaxillary line to facilitate venous drainage between determinations. Limb vascular conductance, calculated as the quotient of limb blood flow and mean arterial pressure (MAP), was used to compare responses to local infusion, as this variable is linearly related to blood flow when perfusion pressure is not changing (17).

### Procedures

**Arterial infusion.** To assess  $\alpha_1$ -adrenergic responsiveness, phenylephrine HCl (Neo-Synephrine, Sanofi Winthrop) was infused intra-arterially at rates of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8  $\mu\text{g} \cdot 100 \text{ ml tissue volume}^{-1} \cdot \text{min}^{-1}$ .  $\beta$ -Adrenergic responsiveness was determined with intra-arterial infusions of isoproterenol HCl (Isuprel, Sanofi Winthrop) at rates of 0.075, 1.5, 3, 6, 12, and 24  $\text{ng} \cdot 100 \text{ ml tissue volume}^{-1} \cdot \text{min}^{-1}$ . Phenylephrine was diluted in sterile, buffered, isotonic saline to a concentration of 16.7  $\mu\text{g/ml}$  for arm infusions, and 50  $\mu\text{g/ml}$  for leg infusions, whereas isoproterenol was diluted to concentrations of 500 and 1,500  $\text{ng/ml}$  for arm and leg infusions, respectively. On the basis of these concentrations and the typical volumes of the limb segments ( $\sim 1$  liter for the arm and 10 liters for the leg), the maximal volume infused was  $\sim 0.5$  ml/min in the arm and 1.6 ml/min in the leg, or  $\sim 1\%$  of the resting flow in the brachial or femoral arteries.

Infusions were maintained for a period of 5 min and were conducted sequentially in order of lowest to highest concentration. After a 3-min equilibration period at each infusion rate, at least five measurements of limb blood flow were made at 20-s intervals. The mean of these data was used as the estimate of limb blood flow for that trial. The order of trials was fixed as follows: isoproterenol arm, isoproterenol leg, phenylephrine arm, and phenylephrine leg. Isoproterenol infusions were preceded by an infusion of vehicle only. The protocol was designed to minimize any potential interaction between arm and leg infusions or between  $\alpha_1$ - and  $\beta$ -adrenergic-receptor stimulation; thus  $>1$  h separated infusions in a given limb. Baseline resistance was determined before each drug infusion; the mean of these values was used to compare baseline resistance between the forearm and calf.

**Maximal limb conductance.** On a separate day, maximal conductance of the forearm and calf was determined by using a modification of published procedures (25). Maximal flows for both limbs were determined in the supine position by using venous occlusion plethysmography after an exhausting bout of ischemic exercise. For the forearm, subjects performed wrist flexion, extension, pronation, and supination, whereas, for the calf, subjects performed heel and toe raises. For either limb, fatigue occurred in 2–4 min. Occlusion pressures of 300 mmHg were used on the upper arm or leg; subjects were repositioned supine before release of the cuffs. Flow determinations commenced within 6 s of cuff release and remained elevated at maximal levels up to 90 s thereafter. Blood flow was determined at 10-s intervals; usually the highest value was obtained with the first or second measurement after cuff release. Simultaneously, blood pressure was determined with an automated auscultatory device (Suntech 4240) for the calculation of conductance as the quotient of maximal limb blood flow and MAP.

**Dose-response curves.** To determine adrenergic responsiveness in the calf and forearm, individual responses to infusion of each agonist were normalized to each subject's maximal conductance for the respective limb. The averages of these data, plotted as a function of the log-transformed infusion rates, were used to construct dose-response curves based on a four-parameter logistic model. From these equations, we calculated the maximal response ( $E_{\text{max}}$ ) and the dose of each agonist that produced the half-maximal response ( $ED_{50}$ ) in each limb. Because the vasomotor responses were normalized to maximal conductance and the infusion rates were normalized to limb volume, we were able to compare adrenergic sensitivity between the forearm and calf while avoiding the problems with interpretation caused by unequal local concentrations of agonist (13).

### Statistical Analyses

To determine whether a systemic effect of drug infusion had occurred, HR responses were compared within each of the four local infusions by using one-factor, repeated-measures analysis of variance. Local hemodynamic responses to infusion (infused vs. control limb or forearm vs. calf) were compared by using two-factor, repeated-measures analyses of variance (dose  $\times$  limb). Significant differences were probed post hoc by using *t*-tests with a Bonferroni correction for multiple comparisons. Between-limb differences in baseline resistance and maximal conductance were assessed with paired *t*-tests. Logistic models were fit iteratively with non-linear regression (SigmaPlot 2.1, Jandel Scientific); unpaired *t*-tests were used to identify between-limb differences in the  $E_{\max}$  and  $ED_{50}$  for each agonist. The probability of rejecting the null hypothesis (no difference from infusion or between limbs) was set at 5%.

## RESULTS

### Systemic Drug Effects

Figures 1 and 2 illustrate the HR responses to infusion of adrenergic agonists. During arm infusions of isoproterenol, the baseline HR was  $63.9 \pm 1.3$  beats/min and did not change throughout the infusion protocol. To the contrary, HR increased during leg isoproterenol infusions from a baseline of  $65.9 \pm 2.6$  to  $93.3 \pm 1.3$  beats/min at the highest rate of infusion ( $P < 0.0001$  vs. baseline).

Systemic MAP (i.e., leg MAP during arm infusions, arm MAP during leg infusions) did not change from baseline during arm infusions but was significantly less than baseline ( $-6.7 \pm 1.4$  mmHg) during leg isoproterenol infusion at  $12 \text{ ng} \cdot 100 \text{ ml tissue volume}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.0003$ ). The HR and MAP changes, coupled with the observation that limb blood flow was increasing in the contralateral (control) limb at the highest rate of leg isoproterenol infusion ( $P = 0.01$  vs. control), led us to conclude that systemic spillover of isoproterenol was occurring during leg isoproterenol infusions. We therefore terminated the highest rate of leg isoproterenol infusions in 7 of the 12 subjects and excluded the highest infusion rate from subsequent analysis. In contrast, no rate of phenylephrine infusion altered HR, MAP, or blood flow in the control limb.

### Regional Resistance to Blood Flow During Resting and Maximally Vasodilated Conditions

Resting limb resistance for each subject is depicted in Fig. 3 based on the mean of three determinations at different times during the experiment. Resting calf vascular resistance was greater than forearm vascular resistance in 11 of 12 subjects, with a mean value of  $26.9 \pm 2.0$  vs.  $38.8 \pm 2.5$  mmHg  $\cdot 100 \text{ ml} \cdot \text{min} \cdot \text{ml}^{-1}$  ( $P < 0.001$  by paired *t*-test). These findings are echoed in the differences in maximal vascular conductance as shown in Fig. 4. Maximal calf conductance was less than forearm conductance in 10 of 12 subjects, with a mean value of  $46.1 \pm 11.9$  vs.  $59.4 \pm 13.4$  ml  $\cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$  ( $P < 0.03$  by paired *t*-test). Thus resistance to flow was greater in the calf, both at rest and during maximally vasodilated conditions.

### Regional Drug Effects

The responses of limb vascular conductance to  $\beta$ -adrenergic stimulation are shown in Fig. 5. Proper positioning of the brachial arterial catheter could not be obtained in one subject; thus the results from agonist infusion are limited to a total of 11 subjects. During isoproterenol infusions, flow and conductance steadily increased, and conductance was significantly increased above the level in the corresponding control limb at the four highest levels of isoproterenol infusion ( $P < 0.0001$ ). Conductance did not differ between the forearm and calf at either rest or any of the five levels of infusion compared. Analysis of the determinants of conductance (shown in Table 1) revealed that changes in pressure contributed only modestly to the changes in conductance. Local MAP did not change during arm isoproterenol infusion. However, it fell progressively during leg isoproterenol infusion and was significantly lower than baseline at the four highest rates of infusion, reaching a maximum level of  $-10.0 \pm 1.3$  mmHg ( $P < 0.0001$ ). As a result, the pressure difference between local (leg) and systemic (arm) pressure widened and was significantly different from zero at two of the three highest infusion rates ( $P < 0.05$ ).

With infusion of phenylephrine (Fig. 6), vascular conductance fell progressively in both limbs and was

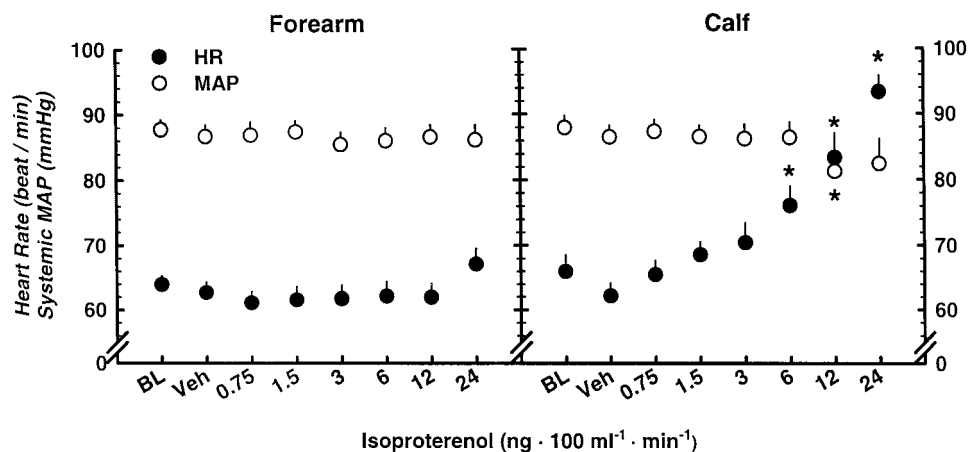
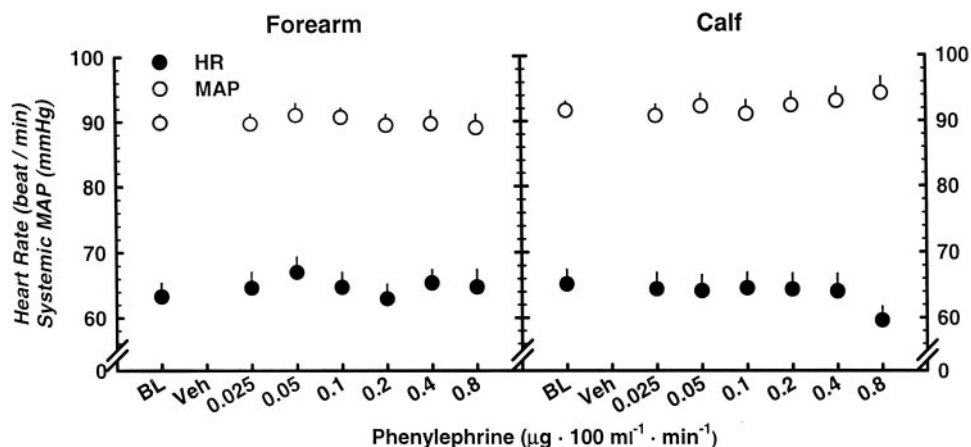


Fig. 1. Heart rate (HR) and systemic blood pressure responses to isoproterenol infusion. Data are means  $\pm$  SE. MAP, mean arterial pressure; BL, baseline; Veh, vehicle. There were no statistically significant differences in HR during arm infusion; however, HR increased during leg infusions. Systemic MAP was not changed from control at any level of isoproterenol infusion in either limb. Values are means  $\pm$  SE. \* $P < 0.05$  vs. BL.

Fig. 2. HR and systemic MAP responses to phenylephrine infusion. Neither HR nor MAP was different at any level of phenylephrine infusion in either limb. Values are means  $\pm$  SE.



statistically decreased from baseline in the forearm at the three highest infusion rates ( $P < 0.0001$ ) and in the calf at the five highest infusion rates ( $P < 0.0001$ ). Unlike the responses to isoproterenol infusion, a statistically significant interaction between infusion rate and limb was noted, such that the calf responses were significantly greater than the corresponding forearm responses for four of the doses of phenylephrine ( $P < 0.0001$ ). Decreases in conductance could be attributed mainly to reductions in blood flow (Table 1). Local, but not systemic, pressure was modestly, but significantly, elevated above baseline during the highest rate of phenylephrine infusion in the leg ( $+4.5$  mmHg,  $P = 0.02$ ), whereas significant changes in either local or systemic pressure were not detected during arm infusion.

#### Adrenergic-receptor Sensitivity

Results from nonlinear regression of the normalized dose-response data are shown in Figs. 7 and 8. Neither the  $ED_{50}$  (forearm:  $6.9$   $\mu\text{g} \cdot \text{min}^{-1} \cdot 100$   $\text{ml}^{-1}$ ; calf:  $9.9$   $\mu\text{g} \cdot \text{min}^{-1} \cdot 100$   $\text{ml}^{-1}$ ) nor the  $E_{\text{max}}$  (forearm:  $50.2\%$ ; calf:

$57.4\%$ ) differed between limbs during isoproterenol infusions.

In contrast, analysis of the responses to phenylephrine infusion revealed that the  $ED_{50}$  was significantly different between limbs. The  $ED_{50}$  was more than threefold higher for the forearm ( $0.157$   $\mu\text{g} \cdot \text{min}^{-1} \cdot 100$   $\text{ml}^{-1}$ ) compared with the calf ( $0.045$   $\mu\text{g} \cdot \text{min}^{-1} \cdot 100$   $\text{ml}^{-1}$ ;  $P < 0.001$ ), whereas the  $E_{\text{max}}$  was virtually indistinguishable between limbs (forearm:  $2.4\%$ ; calf:  $2.3\%$ ).

#### DISCUSSION

The major finding of the present investigation is that, for an equal concentration of phenylephrine, decreases in vascular conductance were greater in the calf compared with the forearm. To our knowledge, this is the first observation that  $\alpha_1$ -adrenergic responsiveness is heterogeneous in human skeletal muscle arterial vasculature. To the contrary, when  $\beta$ -adrenergic receptors were stimulated with infusions of isoproterenol, vasodilatory responses were similar in the forearm and calf circulations. Thus the heterogeneity of

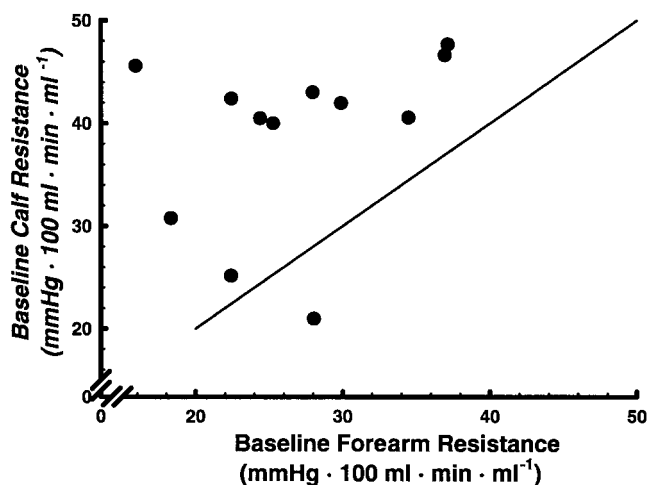


Fig. 3. Individual BL vascular resistance for each limb. Resting supine calf vascular resistance was greater than that of the forearm in 11 of 12 subjects with a mean value of  $26.9 \pm 2.0$  vs.  $38.8 \pm 2.5$   $\text{mmHg} \cdot 100$   $\text{ml} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$  ( $P < 0.001$  by paired  $t$ -test).

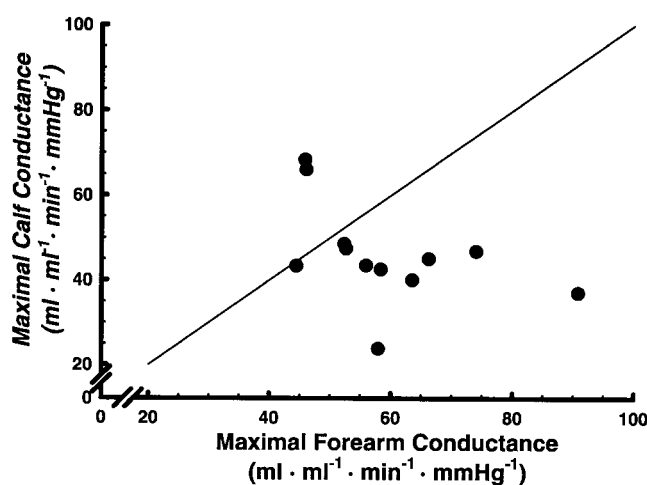


Fig. 4. Individual maximal conductance for each limb. Maximal calf conductance was less than forearm conductance in 10 of 12 subjects, with a mean value of  $46.1 \pm 11.9$  vs.  $59.4 \pm 13.4$   $\text{ml} \cdot \text{min}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$  ( $P < 0.03$  by paired  $t$ -test).

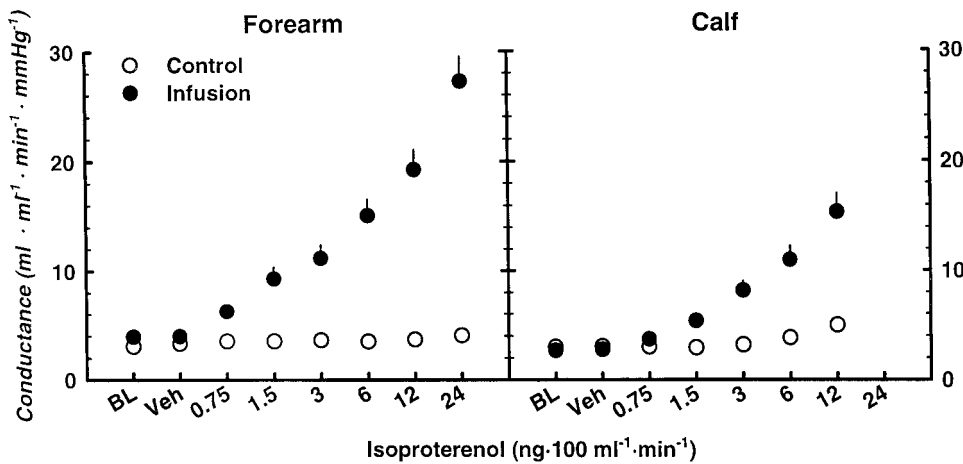


Fig. 5. Limb vascular conductance responses to isoproterenol infusion. Values for the control limb (○) and the infused limb (●) are shown. Calf vascular conductance for the highest level of isoproterenol has been omitted because the infusion was terminated when systemic responses were observed in 7 of 12 subjects. There was no significant difference between limbs at any dose. Values are means ± SE.

adrenergic responses in human limbs that we identified was confined to α<sub>1</sub>-adrenergic receptors.

Unique to this investigation is the use of local arterial infusions normalized to limb volume to produce equal concentrations of agonist in the forearm and calf vasculature. This approach holds distinct advantages over the alternative approach (i.e., systemic infusion). First, determination of local pressure in the infused limb afforded more accurate calculations of changes in limb vascular tone. A modest, although statistically significant, pressure disparity between the infused limb and control limb was noted during local infusions. For example, pressure in the leg decreased by ~6–7 mmHg relative to the control limb (arm) during calf isoproterenol infusions, whereas the opposite effect tended to occur during phenylephrine infusions. Thus local arterial pressure was affected by changes in downstream vascular tone independent of systemic arterial pressure, but we can only speculate to what extent these changes propagated through the forearm and calf circulations.

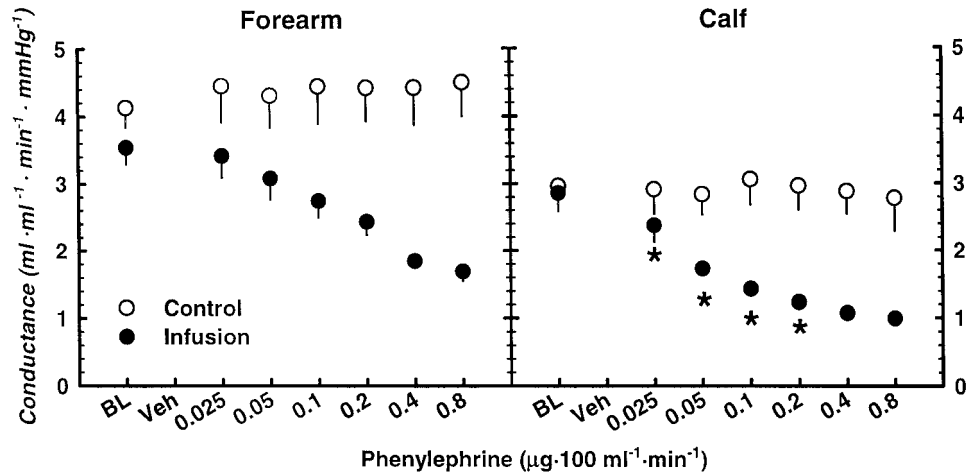
Second, using intra-arterial infusions rather than a systemic infusion allowed us to determine regional vascular responses while minimizing confounding baroreflex-mediated effects on HR and/or sympathetic activity. Despite this approach, HR increased, systemic MAP decreased, and the contralateral calf vasodilated in the majority of subjects during the highest rate of leg isoproterenol infusion. Each of these subjects reported sensations associated with an increase in cardiac contractility (palpitations). The other infusion trials (arm and leg infusion of phenylephrine, arm infusion of isoproterenol) appeared to be free of such changes. We are, therefore, confident that some systemic spillover of isoproterenol occurred during leg infusions, resulting from the combined effect of hyperemia and the total dose of isoproterenol needed to achieve the desired normalized dose. (The leg dose, if given systemically, was within the therapeutic range for a cardiac effect.) Because we cannot completely exclude the possibility that reflex effects associated with systemic spillover of isoproterenol from the leg elicited increases in sympa-

Table 1. Local flow and pressure responses to adrenergic agonists

	Forearm			Calf	
	Dose	Flow	MAP	Flow	MAP
Isoproterenol	BL	3.4 ± 0.3	87.1 ± 1.4	2.4 ± 0.3	90.1 ± 2.2
	Veh	3.5 ± 0.3	86.7 ± 1.6	2.4 ± 0.2	87.8 ± 1.6
	0.75	5.5 ± 0.8	87.0 ± 1.8	3.2 ± 0.3	87.4 ± 2.0
	1.5	8.1 ± 0.9†	87.1 ± 1.7	4.6 ± 0.4	86.2 ± 2.1
	3	9.6 ± 1.1†	85.4 ± 1.8	7.0 ± 0.8†	85.6 ± 2.2†
	6	13.0 ± 1.3†	85.7 ± 1.7	9.4 ± 1.1†	85.9 ± 2.3†
	12	16.4 ± 1.4†	85.2 ± 1.9	12.4 ± 1.3†	81.0 ± 2.1*†
	24	22.7 ± 1.8†	83.9 ± 2.4	13.5 ± 1.6*†	78.7 ± 3.7*†
Phenylephrine	BL	3.2 ± 0.3	90.0 ± 1.7	2.7 ± 0.3	91.6 ± 1.5
	0.025	3.1 ± 0.3	89.8 ± 1.6	2.2 ± 0.2	91.5 ± 1.9
	0.05	2.9 ± 0.3	91.6 ± 2.0	1.6 ± 0.1*†	92.9 ± 2.1
	0.1	2.5 ± 0.2	90.4 ± 1.6	1.3 ± 0.1*†	92.6 ± 2.2
	0.2	2.2 ± 0.2	89.6 ± 1.7	1.2 ± 0.1*†	93.2 ± 2.2
	0.4	1.7 ± 0.1†	90.6 ± 2.1	1.0 ± 0.1*†	94.3 ± 2.1
	0.8	1.5 ± 0.1†	90.0 ± 1.9	1.0 ± 0.1*†	96.1 ± 2.5*†

Values are means ± SE. Phenylephrine and isoproterenol doses are in units of μg · 100 ml tissue volume<sup>-1</sup> · min<sup>-1</sup> and ng · 100 ml tissue volume<sup>-1</sup> · min<sup>-1</sup>, respectively. MAP, mean arterial pressure; BL, baseline; Veh, vehicle only. \* Different from corresponding arm value, P < 0.05. † Different from baseline, P < 0.05.

Fig. 6. Limb vascular conductance responses to phenylephrine infusion. Data are presented identically to Fig. 5. Infusions of Veh were not repeated because the same Veh was used for isoproterenol and phenylephrine infusions. Calf vascular conductance was significantly less than that of the forearm at the 4 lowest levels of infusion. Values are means  $\pm$  SE. \* $P < 0.0001$  vs. forearm.



thetic activity that opposed the vasodilation evoked by direct  $\beta$ -adrenergic-receptor stimulation, the isoproterenol data should be interpreted judiciously.

Finally, by normalizing the infusions to limb volume, pharmacological models could be constructed to compare the forearm and calf responses at the same agonist concentrations, obviating the problems caused by infusing a fixed dose of agonist in limbs with different vascular volumes (13). Because the measured leg volume was  $\sim 10$  times larger than forearm volume,  $\sim 10$ -fold more phenylephrine was infused into the femoral artery compared with the brachial artery. Although the same point holds true for the isoproterenol infusions, responses to isoproterenol were similar between the limbs. This observation suggests that our findings are not an artifact of procedure (i.e., infusing a larger total dose of agonist in the leg), rather that there exists a true difference between upper and lower limbs in response to  $\alpha_1$ -adrenergic stimulation that is not manifested with  $\beta$ -adrenergic stimulation. Assuming that

Poiseuille's law can be applied to the limb circulations under these steady-state conditions, the 50–60% decrease in conductance in response to phenylephrine and the 400–500% increase in conductance in response to isoproterenol are consistent with  $\sim 30\%$  decreases and  $\sim 50\%$  increases in mean vessel diameter, respectively. These estimates are comparable to data obtained from isolated rat skeletal muscle microvasculature (e.g., Refs. 4, 18).

*Why Is the Response to Phenylephrine Greater in the Calf?*

One interpretation of our primary finding is that  $\alpha_1$ -adrenergic receptors are distributed unevenly in human limbs, with relatively greater numbers and/or sensitivity found in the lower leg compared with the forearm, leading to greater release of calcium in vascular smooth muscle in response to sympathetic activation. Additionally, two other explanations should be considered. First, it is hypothetically possible that a

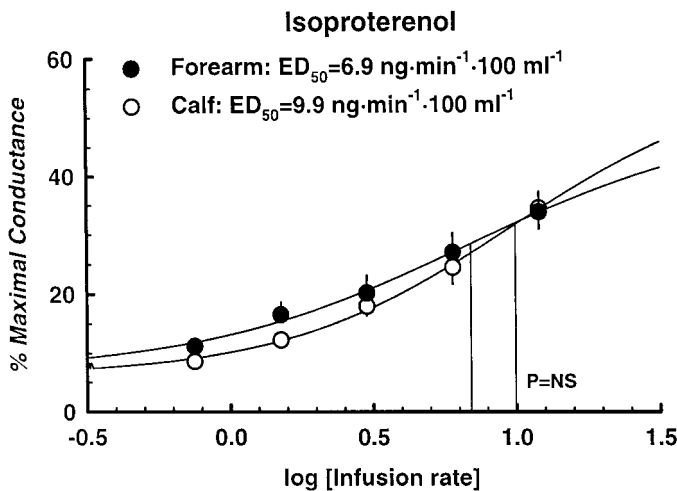


Fig. 7. Dose-response curve of the limb vascular responses to isoproterenol infusion presented as a percentage of maximal conductance. Vertical lines indicate the calculated dose that produced half-maximal response ( $ED_{50}$ ), which did not differ between limbs. Values are means  $\pm$  SE. NS, not significant.

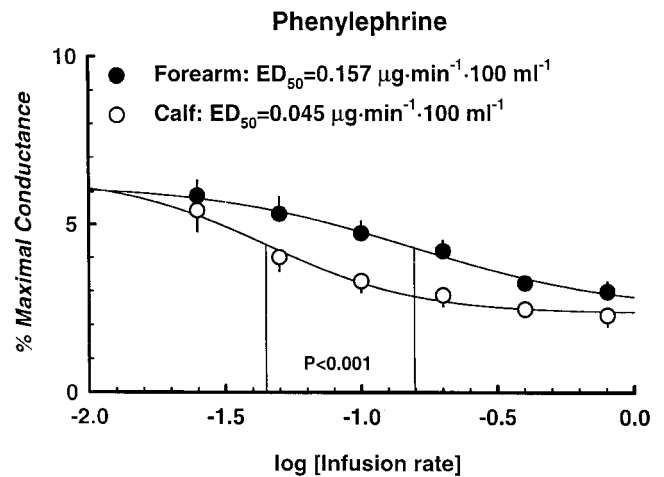


Fig. 8. Dose-response curve of the limb vascular responses to phenylephrine infusion presented as a percentage of maximal conductance. Data are presented identically to Fig. 7. Values are means  $\pm$  SE. The  $ED_{50}$  for the calf was significantly less than that of the forearm,  $P < 0.001$ .

smaller response to phenylephrine in the forearm circulation could reflect greater presynaptic  $\alpha_2$ -adrenergic inhibition of endogenous norepinephrine release, masking the response to phenylephrine. Although the presynaptic mechanism plays an important role in the human forearm (10, 15), no data exist that investigate the presence or magnitude of this mechanism in the human calf. However, we believe this mechanism cannot fully explain the relative difference in adrenergic responsiveness that we observed, as the contribution of endogenous norepinephrine spillover to vascular resistance is relatively small in the resting state (13, 16).

Alternatively, it is possible that greater accumulation of vascular smooth muscle in the calf led to a greater contractile response without a difference in  $\alpha_1$ -adrenergic-receptor sensitivity. Such an effect could explain both the lower resting and maximal calf vascular conductance (Figs. 2 and 3). Other notable examples from nature implicate this mechanism. In an extreme case, the ratio of wall thickness to lumen radius of arteries from the lower legs of giraffes is >400% greater than that found in arteries from the neck (11)! Thus vascular hypertrophy could affect resting and maximal blood flow and play a role in amplifying the response to  $\alpha_1$ -adrenergic stimulation.

#### *Why Would Vascular Hypertrophy Predominate in the Legs?*

$\alpha_1$ -Adrenergic receptors belong to the heptahelical family of receptors that share common features with angiotensin II and endothelin-1A receptors, among others. These receptors are linked to the phosphatidylinositol pathway via  $G_q$ . The products of this pathway (inositol trisphosphate and diacylglycerol) elicit two major types of responses. The first is contractile, mobilizing intracellular  $Ca^{2+}$  through inositol trisphosphate-stimulated release from sarcoplasmic reticulum. This response plays an important role in orthostatic regulation, controlling the rate at which blood is redistributed to capacitance vessels in peripheral extremities. If this mechanism is blocked, rapid pooling of blood in dependent regions of the circulation causes profound orthostatic intolerance (1). The second response is synthetic, where diacylglycerol activates several transcriptional pathways via protein kinase C, causing hypertrophy and further expression of  $\alpha$ -adrenergic receptors.

With some clinical conditions, altered pressure gradients are associated with lasting effects on microvessel architecture. For example, greater  $\alpha_1$ -receptor sensitivity has been reported in pulmonary and "essential" hypertension (23). In a unique "experiment of nature," Gidding et al. (8) studied the vasoconstrictor responses to norepinephrine infused in the arms and legs of patients after surgical correction of coarctation of the aorta. As the stenosis is characteristically located in the descending or abdominal aorta, the typical pressure gradient between the upper and lower limbs is reversed, causing upper body hypertension often greater than the lower body-dependent hypertension.

Despite the 6-yr average length of time from surgery until study, residual hypertension existed in the arms of the patients (arm-to-leg systolic pressure gradient of  $8.5 \pm 4.3$  and  $75 \pm 13.5$  mmHg at rest and after exercise, respectively). In patients, responses to graded, systemic infusion of norepinephrine were greater in the forearm than the calf, whereas control subjects exhibited directionally opposite results (i.e., calf responses were greater than forearm, much like in the present study). These results indicate that perfusion pressure is an important stimulus for vascular hypertrophy and  $\alpha$ -adrenergic responsiveness. In humans, this effect appears to exhibit long-term effects, being present even when the pressure stimulus is removed for extended periods.

If pressure is the primary stimulus for these events, can acute reversal of the hydrostatic pressure gradients typically encountered in the cardiovascular system change the pattern of vascular hypertrophy and  $\alpha$ -adrenergic responsiveness? Several reports support this assertion. Prolonged head-down tilting in tail-suspended rats causes atrophy in dependent arteries (2) and attenuates constriction resulting from adrenergic stimulation (4). Conversely, the basilar artery hypertrophies in this model because of the increase in hydrostatic pressure cephalad to the heart (30). However, in humans, the attenuation of hydrostatic gradients by bed-rest deconditioning failed to affect vasoconstriction in the lower limb in response to systemic phenylephrine infusion after 14 days of head-down tilt (3) or to norepinephrine in the upper limb after 12 days of bed rest (24). Although the discrepancy between the human and animal investigations is not yet resolved, we speculate that the time course of vascular smooth muscle adaptation to bed-rest deconditioning may be longer than that produced by hindlimb unloading in tail-suspended rats.

#### *Limitations to Interpretation*

*Measurement of vascular responses.* We quantified vascular responses as changes in conductance, rather than resistance, because the former is linearly related to flow when pressure remains relatively constant (17, 20). Because the relationship between resistance and conductance is reciprocal, large changes in resistance yield proportionally smaller changes in conductance if flow is low. This "floor effect" may have limited our ability to discern statistical differences in conductance at very high doses of phenylephrine. Although we believe that the pharmacological parameters  $ED_{50}$  and  $E_{max}$  best portray the physiological differences between the forearm and calf, the reader is provided with the primary data in Table 1 so that either variable may be calculated.

*Adrenergic-receptor subtypes.* No attempt was made in this investigation to discriminate between  $\alpha_1$ - and  $\alpha_2$ -adrenergic receptors, which vary in density and proportion through the vascular tree. For example, Flavahan et al. (7) reported the responses of isolated conduit and peripheral arteries obtained from ampu-

tated legs or arms for nonvascular complications. After pretreatment with yohimbine, a greater loss of constriction was observed in the peripheral arteries, suggesting that relatively more  $\alpha_2$ -receptors were located in the peripheral arteries. The authors reported that the proportions of  $\alpha_1$ - and  $\alpha_2$ -receptors were not different between arms and legs; however, no comparisons of the absolute differences between limbs are available from this study (J. T. Shepherd, personal communication). An important follow-up to the present study would be to identify differences in  $\alpha$ -receptor distribution at successive generations of the arterial network in upper and lower limbs of humans.

### Perspectives

Several stimuli have been reported to produce heterogeneous regional vascular responses in skeletal muscle. For example, orthostatic stress (5, 6), cold stress (13), and ventricular chemoreceptor activation (14) may elicit differential responses. Despite lower norepinephrine spillover from legs compared with arms (13, 16), neural traffic to arm and leg skeletal muscle during orthostatic stress appears to be relatively uniform (22, 29). The results from the present investigation suggest that the greater efficacy of  $\alpha_1$ -adrenergic stimulation in the lower legs of humans may offset the effects of lower norepinephrine spillover, thus normalizing or magnifying the vascular response in the legs to changes in sympathetic activity.

The lower limbs of bipeds routinely experience large changes in pressure resulting from the combined effect of arterial and hydrostatic gradients. Results from the present investigation suggest that accentuated  $\alpha_1$ -adrenergic responses mitigate this effect in daily life. Moreover, chronic exposure of the legs to such pressures may be associated with functional or morphological differences between the legs and arms that are manifested in higher resting vascular resistance and lower maximal conductance in the calf compared with the forearm. These adaptations would act synergistically to serve two important roles: first, to limit the extent of venous pooling during quiet standing when muscle pumping is not active, and second, to regulate capillary hydrostatic pressure so that capillary filtration in the legs does not become excessive.

In summary, results from this investigation reveal that adrenergic responsiveness in human limbs is not uniform. Whereas the dilator responses to  $\beta$ -receptor stimulation do not differ between the calf and forearm, the constrictor responses to phenylephrine are more efficacious in the calf compared with the forearm. We hypothesize that the long-term combination of arterial and elevated hydrostatic pressure leads to this greater response. The extent to which acute reductions in hydrostatic pressure gradients can modify this effect in humans remains unclear.

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### REFERENCES

1. **Brown GE, Wood EH, and Lambert EH.** Effects of tetraethyl-ammonium chloride on the cardiovascular reaction in man to changes in posture and exposure to centrifugal force. *J Appl Physiol* 2: 117–132, 1949.
2. **Chew HG Jr and Segal SS.** Arterial morphology and blood volumes of rats following 10–14 weeks of tail suspension. *Med Sci Sports Exerc* 29: 1304–1310, 1997.
3. **Convertino VA, Polet JL, Engelke KA, Hoffler GW, Lane LD, and Blomqvist CG.** Evidence for increased  $\beta$ -adrenoceptor responsiveness induced by 14 days of simulated microgravity in humans. *Am J Physiol Regulatory Integrative Comp Physiol* 273: R93–R99, 1997.
4. **Delp MD.** Myogenic and vasoconstrictor responsiveness of skeletal muscle arterioles is diminished by hindlimb unloading. *J Appl Physiol* 86: 1178–1184, 1999.
5. **Essandoh LK, Duprez DA, and Shepherd JT.** Postural cardiovascular reflexes: comparison of responses of forearm and calf resistance vessels. *J Appl Physiol* 63: 1801–1805, 1987.
6. **Essandoh LK, Houston DS, Vanhoutte PM, and Shepherd JT.** Differential effects of lower body negative pressure on forearm and calf blood flow. *J Appl Physiol* 61: 994–998, 1986.
7. **Flavahan NA, Cooke JP, Shepherd JT, and Vanhoutte PM.** Human postjunctional  $\alpha_1$  and  $\alpha_2$  adrenoceptors: differential distribution in arteries of the limbs. *J Pharmacol Exp Ther* 241: 361–365, 1987.
8. **Gidding SS, Rocchini AP, Moorehead C, Schork MA, and Rosenthal A.** Increased forearm vascular reactivity in patients with hypertension after repair of coarctation. *Circulation* 71: 495–499, 1985.
9. **Greenleaf JE, Montgomery LD, Brock PJ, and van Beaumont W.** Limb blood flow: rest and heavy exercise in sitting and supine positions in man. *Aviat Space Environ Med* 50: 702–707, 1979.
10. **Grossman E, Chang PC, Hoffman A, Tamrat M, and Goldstein DS.** Evidence for functional  $\alpha_2$ -adrenoceptors on vascular sympathetic nerve endings in the human forearm. *Circ Res* 69: 887–897, 1991.
11. **Hargens AR, Gershuni DH, Danzig LA, Millard RW, and Petterson K.** Tissue adaptations to gravitational stress: newborn versus adult giraffes. *Physiologist* 31: S110–S113, 1988.
12. **Hokanson DE, Sumner DS, and Strandness DE.** An electrically calibrated plethysmograph for direct measurement of limb blood flow. *IEEE Trans Biomed Eng* 22: 25–29, 1975.
13. **Jacob G, Costa F, Shannon J, Robertson D, and Biaggioni I.** Dissociation between neural and vascular responses to sympathetic stimulation: contribution of local adrenergic receptor function. *Hypertension* 35: 76–81, 2000.
14. **Jacobsen TN, Lange RA, Olivari MT, Yancy CW, Horn VP, Ring WS, Cooley RA, and Victor RG.** Differential reflex control of forearm and calf resistance vessels by chemosensitive cardiac afferent activation. *J Appl Physiol* 76: 1123–1129, 1994.
15. **Jie K, van Brummelen P, Vermey P, Timmermans PB, and van Zwieten PA.** Modulation of noradrenaline release by peripheral presynaptic  $\alpha_2$ -adrenoceptors in humans. *J Cardiovasc Pharmacol* 9: 407–413, 1987.
16. **Karlsson AK, Elam M, Lonnroth P, Sullivan L, and Friberg P.** Differentiated norepinephrine spillover in human skeletal muscle. *Am J Physiol Regulatory Integrative Comp Physiol* 273: R16–R21, 1997.
17. **Lautt WW.** Resistance or conductance for expression of arterial vascular tone. *Microvasc Res* 37: 230–236, 1989.
18. **McCurdy MR, Colleran PN, Muller-Delp J, and Delp MD.** Effects of fiber composition and hindlimb unloading on the vasodilator properties of skeletal muscle arterioles. *J Appl Physiol* 89: 398–405, 2000.

19. **Moore TP and Thornton WE.** Space shuttle inflight and post-flight fluid shifts measured by leg volume changes. *Aviat Space Environ Med* 58: A91–A96, 1987.
20. **O'Leary DS.** Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol Heart Circ Physiol* 260: H632–H637, 1991.
21. **Pawelczyk JA, Zuckerman JH, Blomqvist CG, and Levine BD.** Regulation of muscle sympathetic nerve activity after bed-rest deconditioning. *Am J Physiol Heart Circ Physiol* 280: H2230–H2239, 2001.
22. **Rea RF and Wallin BG.** Sympathetic nerve activity in arm and leg muscles during lower body negative pressure in humans. *J Appl Physiol* 66: 2778–2781, 1989.
23. **Salvi SS.** Alpha-1 adrenergic hypothesis for pulmonary hypertension. *Chest* 115: 1708–1719, 1999.
24. **Schmid PG, McCally M, Piemme TE, and Shaver JA.** Effects of bed rest on forearm vascular responses to tyramine and norepinephrine. In: *Hypogravic and Hypodynamic Environments*. Washington, DC: NASA, 1971, p. 211–223. (Spec. NASA Rep. SP-269)
25. **Snell PG, Martin WH, Buckey JC, and Blomqvist CG.** Maximal vascular leg conductance in trained and untrained men. *J Appl Physiol* 62: 606–610, 1987.
26. **Taylor JA, Joyner MJ, Chase PB, and Seals DR.** Differential control of forearm and calf vascular resistance during one-leg exercise. *J Appl Physiol* 67: 1791–1800, 1989.
27. **Thornton WE, Moore TP, and Pool SL.** Fluid shifts in weightlessness. *Aviat Space Environ Med* 58: A86–A90, 1987.
28. **Vissing SF, Scherrer U, and Victor RG.** Relation between sympathetic outflow and vascular resistance in the calf during perturbations in central venous pressure. Evidence for cardiopulmonary afferent regulation of calf vascular resistance in humans. *Circ Res* 65: 1710–1717, 1989.
29. **Wallin BG, Victor RG, and Mark AL.** Sympathetic outflow to resting muscles during static handgrip and postcontraction muscle ischemia. *Am J Physiol Heart Circ Physiol* 256: H105–H110, 1989.
30. **Wilkerson MK, Muller-Delp J, Colleran PN, and Delp MD.** Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology. *J Appl Physiol* 87: 2115–2121, 1999.

