

## Increased vasomotor sympathetic nerve activity and decreased plasma nitric oxide release after head-down bed rest in humans: disappearance of correlation between vasoconstrictor and vasodilator

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### Abstract

We hypothesized that the relationship between resting levels of sympathetic vasoconstrictor nerve traffic and dilator substance nitric oxide (NO) release is altered after exposure to microgravity, resulting in abnormal peripheral resistance. To examine the hypothesis, we assessed muscle sympathetic nerve activity (MSNA) (microneurography), an indicator of NO release (plasma nitrite/nitrate concentrations) and leg vascular resistance (venous occlusion plethysmography) in 20 healthy male volunteers before and after 14 days of 6° head-down bed rest (HDBR), the ground-based analogue of microgravity. MSNA increased, while plasma nitrite/nitrate concentrations decreased after HDBR. A significant positive correlation observed between MSNA and plasma nitrite/nitrate concentrations before HDBR disappeared after HDBR. Leg vascular resistance increased after HDBR. In conclusion, an imbalance between sympathetic vasoconstrictor traffic and NO release might contribute to elevated peripheral vascular resistance following HDBR. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Bed rest; Microneurography; Muscle sympathetic nerve activity; Vascular resistance; Vasoconstrictor; Vasodilator

Changes in regulation of peripheral vasculature are observed after spaceflight [2,4,19] and its ground-based simulation model, 6° head-down bed rest (HDBR) [3,4]. Impaired regulations of peripheral vasculature during upright posture may relate to orthostatic intolerance after spaceflight and HDBR [2,4,20]. However, considerable controversy exists as to the effect of real and simulated microgravity on resting peripheral vascular resistance, with studies demonstrating decreased [1] and increased [3] vascular resistance. Moreover, the mechanisms responsible for altered resting peripheral resistance following microgravity are still unknown.

Resting level of limb vascular resistance is determined by not only vasoconstrictor but also vasodilator systems. The strength of sympathetic vasoconstrictor outflow positively

correlates with the release of the vasodilator substance, nitric oxide (NO) [16]. The vasoconstrictor effect of sympathetic traffic is counteracted by the vasodilator effect of NO coupled to the strength of sympathetic activity [16]. This coupling may contribute to the maintenance of normal resting arterial tone. Against this background, we hypothesized that the relationship between resting levels of sympathetic vasoconstrictor traffic and NO release is altered after exposure to microgravity, resulting in abnormal peripheral resistance at rest.

It remains unclear how spaceflight and HDBR influence sympathetic vasoconstrictor outflow and vasodilator substance NO. Our preliminary reports showed increases in muscle sympathetic nerve activity (MSNA) after HDBR [10–12]. Changes in the dilating substance NO following microgravity are absolutely unclear in humans, although only one rat study [5] suggested a downregulation of the NO-dependent vasodilatory mechanism after hindlimb unweighting, allowing greater myogenic tone in

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cerebral arteries. Moreover, the relationship between resting vasoconstrictor nerve activity and NO release after exposure to microgravity has not been examined.

To examine the hypothesis above, we performed 14 days of HDBR, and measured the vasomotor sympathetic outflow to the leg skeletal muscle as muscle sympathetic nerve activity (MSNA) with a microneurographic technique, the plasma nitrite/nitrate concentrations ( $[\text{NO}_x]$ ) as an indicator of NO release, and the leg vascular resistance with a venous occlusion plethysmography, in the resting supine position before and after 14 days of HDBR.

Twenty healthy male volunteers with a mean age of  $22 \pm 1$  (SE) years (range, 19–36 years), mean height of  $169.1 \pm 1.0$ , and mean weight of  $60.9 \pm 1.4$  kg were studied. All subjects were evaluated as having normal physical fitness by detailed medical history, physical examination, resting electrocardiogram, a panel of blood chemistry analyses and psychological testing. No subject had a habit of smoking, experience with recreational drug, or chronic medical problem. All subjects gave informed consent to participate in the study, which was approved by the Ethical Committee of the National Space Development Agency of Japan (NASDA) and the Committee of Human Research, Research Institute of Environmental Medicine, Nagoya University.

During 14 days of  $6^\circ$  HDBR, the subjects were continuously monitored by staff nurses to ensure that they remained head-down without interruption and that no physical exercise was performed. Dietary intake was restricted between 2000 and 2100 kcal per day (55% carbohydrate, 25% fat, 20% protein) and fluid intake from daily drinks was ad libitum; the average was  $1241 \pm 31$  ml per day.

All variables were measured 1–2 weeks before and immediately after HDBR. Each subject lay in bed in a supine position for at least 30 min. Measurements of MSNA, heart rate, blood pressure, and leg blood flow were performed for 10 min, after which blood sample were collected from the antecubital vein. MSNA and leg blood flow data were continuously recorded and stored on a DAT recorder (PC-216Ax, Sony Precision Technology, Tokyo, Japan) for further analysis.

MSNA was recorded by microneurography from an unilateral tibial nerve by microneurographic technique using a tungsten microelectrode with a shaft diameter of  $120 \mu\text{m}$  and an electrode impedance of 2–5  $\text{M}\Omega$  (model: 26-05-1, Federick Haer and Co., Bowdoinham, ME) without anesthesia. Nerve signals were fed into a preamplifier (Kohno Instruments, Nagoya; input impedance: 100  $\text{M}\Omega$ ; gain:  $\times 40\,000$ ), with two active band pass filters set between 500 and 5000 Hz, and were monitored with a loudspeaker. MSNA was identified similarly to the previous studies [7,10–12,16,]. MSNA was full-wave rectified and fed through a resistance-capacitance integrating circuit with a time constant of 0.1 s to obtain the integrated MSNA. MSNA was expressed as MSNA burst rate, i.e. the mean number of sympathetic bursts per minute.

The electrocardiogram with chest lead II was monitored. Blood pressure was measured every minute by an automated upper arm sphyngomanometer (BP203MII, Nippon Colin Co., Komaki-City, Japan). Mean blood pressure was calculated as the sum of the diastolic blood pressure plus one-third of the pulse pressure.

Leg blood flow was measured by venous occlusion and mercury-in-Silastic strain gauge plethysmography [6]. While the subject was in the supine position, the calf was elevated 10–15 cm above the right atrium to collapse the veins. Occlusion cuffs were placed around the thigh just above the knee and around the ankle. Care was taken to place the strain gauge in the same place in the pre- and post-HDBR trials. With the ankle cuff inflated to 250 mmHg to arrest circulation to the foot, leg blood flow was measured by inflating the thigh cuff to 50 mmHg for 7 s each time. Leg blood flow determinations were performed at 1-min intervals. Leg vascular resistance was calculated by dividing mean blood pressure by leg blood flow.

During 48 h before blood sampling for plasma nitrite and nitrate concentrations (plasma  $[\text{NO}_x]$ ), each subject was requested to maintain a nitrite/nitrate-restricted diet and to refrain from heavy physical exercise, to ensure that plasma  $[\text{NO}_x]$  levels reflected endogenous NO formation [9]. All subjects completed the diet.

Blood samples were collected into glass tubes, and were centrifuged at  $6000 \times g$  for 50 min to obtain plasma samples. Then, collected plasma samples were ultrafiltered through a 10 kDa molecular weight cut-off filter at  $6000 \times g$  for 50 min. The final products of NO in vivo are nitrite and nitrate and their relative proportions are variable and cannot be predicted with certainty. Therefore, we measured the sum of both nitrite and nitrate concentrations as the index of total NO production with a commercially available assay kit (Nitrate/Nitrite ColoRiMEtRiC Assay Kit, Cayman Chemical CO), which uses the Griess reaction as previously described [17,18]. Filtered samples were incubated for three hours with nitrate reductase and its co-factor to convert nitrate to nitrite. They were incubated for another 20 min with Griess reagents, which included sulfanilamide and *N*-(1-Naphthyl) ethylenediamine to convert nitrite to a deep purple azo compound. The absorbances were measured at 540 nm using a microplate reader and converted to  $\text{NO}_x$  concentrations by a nitrate standard curve. Recovery of nitrate was  $>95\%$ , and the assay results were not significantly affected by the presence of plasma proteins.

Data are expressed as means  $\pm$  SE. A Wilcoxon signed-rank test was performed to compare MSNA, leg blood flow, leg vascular resistance, plasma  $[\text{NO}_x]$ , heart rate and blood pressure after HDBR to those before HDBR. The relationships between MSNA or plasma  $[\text{NO}_x]$  versus blood pressure or leg vascular resistance were assessed by regression analysis. Significance was set at  $P < 0.05$ .

Table 1 summarizes the comparison of variables between before and after HDBR. MSNA significantly increased by

Table 1

MSNA, plasma [NO<sub>x</sub>], leg blood flow, leg vascular resistance, heart rate, and systolic, diastolic and mean blood pressure in resting supine position before and after 14 days of HDBR<sup>a</sup>

	Before HDBR	After HDBR
MSNA (bursts/min)	14 ± 2	19 ± 2*
Plasma [NO <sub>x</sub> ] (μM)	29 ± 3	16 ± 2*
Leg blood flow (ml 100 ml <sup>-1</sup> min <sup>-1</sup> )	3.4 ± 0.2	2.5 ± 0.2*
Leg vascular resistance (units)	25.1 ± 1.5	38.1 ± 2.5*
Heart rate (beats/min)	65 ± 2	72 ± 2*
Systolic blood pressure (mmHg)	116 ± 1	119 ± 1*
Diastolic blood pressure (mmHg)	59 ± 1	65 ± 2*
Mean blood pressure (mmHg)	78 ± 1	83 ± 2*

<sup>a</sup> Values are mean ± SE. \**P* < 0.05 vs. before HDBR.

38% from 14 ± 2 bursts/min before HDBR to 19 ± 2 bursts/min after HDBR. Plasma [NO<sub>x</sub>] significantly decreased by 46% from 29 ± 3 μM before HDBR to 16 ± 2 μM after HDBR. Leg blood flow significantly decreased by 26%, while leg vascular resistance significantly increased by 52% after HDBR compared with their pre-HDBR levels. Heart rate and blood pressure (systolic, diastolic and mean arterial pressure) was significantly higher after than before HDBR.

Fig. 1 shows the relationships between MSNA and plasma [NO<sub>x</sub>] prior to and following HDBR. There was a significantly positive linear correlation between them before HDBR (*r* = 0.60, *P* < 0.005), however, no linear correlation after HDBR.

In contrast, there were no correlation between blood pressure (systolic, diastolic and mean arterial pressure) and MSNA or plasma [NO<sub>x</sub>], in pre- and post-HDBR conditions. No correlation was also found between leg vascular resistance and MSNA or plasma [NO<sub>x</sub>] before and after HDBR.

The new finding of the present study is that the positive linear correlation between MSNA and plasma [NO<sub>x</sub>] was observed before HDBR, but disappeared after HDBR, with increased MSNA and decreased plasma [NO<sub>x</sub>]. The altered relationship between sympathetic vasoconstrictor outflow and vasodilator substance NO release may contribute to increased leg vascular resistance following HDBR.

Before HDBR, the resting sympathetic vasoconstrictor nerve activity was indeed positively correlated to the release of the dilating substance NO at rest. In addition, only one MSNA or plasma [NO<sub>x</sub>] did not correlate with the resting levels of blood pressure and leg vascular resistance. These findings are consistent with the previous study [16] which reported that the vasoconstrictor effect of vasomotor sympathetic outflow may be counteracted by the dilator effect of NO [13,16], and contribute to the lack of relationship between resting levels of MSNA and blood pressure.

Thus, the relationship between sympathetic vasoconstrictor outflow and vasodilator NO release rather than simple effect of each of them may be a contributing factor to resting arterial tone.

The increase in MSNA after 14 days of HDBR reported in the present study is consistent with previous studies showing increased MSNA after relatively short (3 and 6 days) [7,11] and prolonged (60 and 120 days) [10] duration of simulated microgravity. The present study is the first report of a decrease in plasma NO release following HDBR. It is known that NO is an important vasodilator substance and plays a role in the control of arterial tone throughout many vascular beds in humans [8,13,18]. This finding may thus provide new information for cardiovascular deconditioning during and after exposure to microgravity.

We suppose that the opposite directional changes in resting vasoconstrictor outflow and tonic NO release (increase in MSNA and decrease in plasma [NO<sub>x</sub>]) following HDBR may partly contribute to augmented peripheral vascular resistance. Moreover, the linear relationship between MSNA and plasma [NO<sub>x</sub>] did not shift right-downward keeping a positive correlation, but disappeared after HDBR. The imbalance between vasoconstrictor and dilator factors after HDBR may suggest a new (somewhat unstable) condition in the control of peripheral vasculatures. However, further investigations are needed to interpret the physiological nature of this altered relationship after microgravity.

One possible explanation for the lack of positive linear correlation between sympathetic vasoconstrictor outflow and dilator NO release after HDBR is as follows. Resting MSNA has a strong positive correlation to the plasma nore-

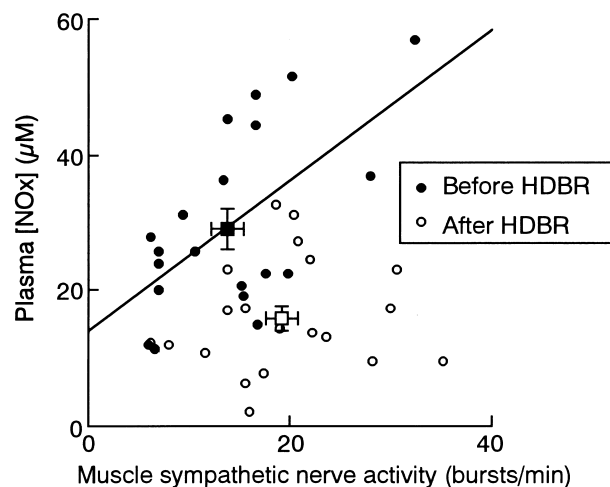


Fig. 1. Circles show individual levels of resting MSNA and plasma [NO<sub>x</sub>] before (closed circle) and after (open circle) HDBR in 20 subjects. Squares indicate mean levels before (closed square) and after (open square) HDBR. Regression line shows positive linear correlation between MSNA and plasma [NO<sub>x</sub>] before HDBR (*r* = 0.60, *P* < 0.005). However, open circles show the lack of relationship between MSNA and plasma [NO<sub>x</sub>] after HDBR (*r* = 0.12, *P* < 0.62).

pinephrine concentration [16]. Because endothelial cells have adrenergic receptors which respond to circulating norepinephrine and stimulate NO release [16], resting MSNA may have an apparent positive correlation to basal NO release. There are several possibilities of the reduction of endothelial NO release after HDBR. First, an inactivity (no physical exercise) during HDBR might attenuate NO release at rest, because regular physical exercise is linked to an upregulated expression of the endothelial NO synthase gene in vascular [15], and to elevated resting levels of NO release [16]. Second, HDBR-induced hypovolemia (12% of estimated plasma volume loss in this study) could decrease a shear stress in vessels, thus reducing a shear stress-induced vascular formation of NO [8], while this hypovolemia might stimulate cardiopulmonary baroreceptors to cause a reflex increase in MSNA. In addition, the reduction in basal NO release itself could also be involved in the increase in MSNA at rest after HDBR, because systemic inhibition of NO synthase by drug treatment has an excitatory effect on resting MSNA in humans [14]. These possible reductions in endothelial NO release may contribute to the lack of correlation between resting levels of MSNA and NO release.

In conclusion, 14 days of HDBR provided opposite directional changes, with increased sympathetic vasoconstrictor traffic and decreased vasodilator NO release at rest. Thus, the positive linear correlation between resting sympathetic vasoconstrictor outflow and tonic NO release disappeared after HDBR. These alterations might contribute to the elevated peripheral vascular resistance following HDBR.

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