

## Muscle sympathetic nerve activity during handgrip and post-handgrip muscle ischemia after exposure to simulated microgravity in humans

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### Abstract

To examine the effect of 6° head-down bed rest (HDBR) on vasomotor sympathetic responses to isometric forearm exercise, 16 healthy male subjects aged 20–36 years performed voluntary isometric handgrip (HG) at 30% of maximal voluntary contraction until fatigue, followed by 2 min of post-handgrip muscle ischemia (PHGMI) with 250 mmHg of cuff inflation, before and after 14 days of HDBR. Time to fatigue and maximal voluntary HG force were not affected by HDBR. Pre-exercise baseline muscle sympathetic nerve activity (MSNA, measured by microneurography), heart rate (measured by electrocardiogram) and mean blood pressure (measured by Portapres) increased after HDBR. Increases in MSNA were similar during HG but significantly lower during PHGMI ( $P < 0.01$ ) after HDBR. Responses of heart rate and mean blood pressure during HG and PHGMI were not affected by HDBR. These results suggest that the magnitude of muscle metaboreflex during isometric forearm exercise might be attenuated after 14 days of simulated microgravity. © 2000 Published by Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Autonomic nervous system; Bed rest; Exercise; Microneurography; Muscle metaboreflex; Spaceflight

Spaceflight alters the autonomic nervous system [5,6,15] and the neuromuscular system [4]. Severe muscle atrophy reduces the ability of the astronaut to exercise and work. If neural regulation of cardiovascular function during exercise is impaired in microgravity, the astronaut's ability to exercise would be reduced. The maintenance of exercise ability will become more important for astronauts in prolonged exposure, i.e. space station. However, the effects of microgravity on autonomic neural control of cardiovascular function during exercise, in particular isometric forearm exercise, still remains unclear.

During isometric exercise, the autonomic nervous system plays a crucial role in the integrative control of arterial blood pressure and blood flow to active muscle in humans [9,13]. Increased sympathetic and decreased parasympathetic outflow to the heart cause increases in heart rate and ventricular contractility, resulting in elevations of cardiac output and systemic oxygen transport [13]. Increased vasomotor sympathetic outflow to resistance

vessels in the non-active skeletal muscles and the viscera mediates peripheral vasoconstrictions, which have an essential role in redirection of the elevated oxygen transport to the contracting skeletal muscles [13]. Previous studies reported that real or stimulated microgravity alters autonomic control of the heart, with decreased resting vagal tone [6,15] and reduced carotid–cardiac baroreflex function [2,6,15], and changes neural and integrative control of peripheral vasoconstriction, with increased resting arterial tone [3] and impaired peripheral vasoconstriction under orthostatic stress [1,7]. We raise a possibility that exposure to microgravity alters neural control of cardiovascular function during isometric exercise. It will provide advantageous information for astronauts to examine how microgravity alters neural control of cardiovascular function during forearm isometric exercise, because forearms are usually used in their activity in space.

The purpose of the present study was to clarify the effects of microgravity on autonomic neural control of cardiovascular function during forearm isometric exercise, in particular on vasomotor sympathetic response to isometric forearm exercise. We performed handgrip (HG) exercises

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followed by circulatory arrest (post-handgrip muscle ischemia (PHGMI)) with an inflated cuff on upper arm, before and after 14 days of 6° head-down bed rest (HDBR), a well established ground-based analog of microgravitational environment, and compared responses of muscle sympathetic nerve activity (MSNA), heart rate and arterial blood pressure between prior to and following HDBR.

The subjects were 16 healthy male volunteers with a mean age of  $23 \pm 1$  (SE) years, mean height of  $168.2 \pm 1.1$  cm, and mean weight of  $63.1 \pm 2.1$  kg. All subjects were evaluated as physically healthy by the detailed medical history, physical examination, complete blood count, resting electrocardiogram, a panel of blood chemistry analyses and psychological testing. No subject was a smoker, used recreational drugs, or had chronic medical problems. Each subject signed informed consent after a complete explanation of the testing procedures. 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.

Each subject was exposed to 14 days of strict adherence to 6° HDBR. During 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 2000–2100 kcal/day (55% carbohydrate, 25% fat, 20% protein) and fluid intake from daily drinks was ad libitum; the average was  $1141 \pm 57$  ml/day. Each subject underwent HG tests 7–12 days before the start of HDBR and immediately after the end of 14 days of HDBR.

The subjects were required to take a supine position. Each subject performed two brief (<5 s) maximal contractions to determine his maximal voluntary contraction (MVC) by using a handgrip dynamometer. The average of these two contractions was determined as the MVC. After the subjects remained at rest quietly in the supine position for over 30 min, pre-exercise baseline recordings of sympathetic and cardiovascular variables were performed for 5 min. HG tests were challenged at 30% of MVC on each day until fatigue by dominant arm followed by a 2 min period of post-handgrip muscle ischemia (PHGMI). Fatigue was defined as the point when the subject could not maintain the predefined forces (30% of MVC). PHGMI was performed as follows: 5 s before the release of isometric HG, the upper arm cuff was inflated to suprasystolic blood pressure (>230 mmHg) and the subjects then released their grip. A two-min recovery period followed PHGMI. Variables were continuously recorded during pre-HG rest, HG, PHGMI and recovery, and stored on a DAT recorder (PC-216Ax, Sony Magnescale, Japan) for further analysis except intermittent upper arm pressure measurements.

The muscle sympathetic nerve activity (MSNA) was recorded by microneurography from unilateral tibial nerve using a tungsten microelectrode with a shaft diameter of 120

$\mu\text{m}$  and electrode impedance of 2–5 M $\Omega$  (model: 26-05-1, Federick Haer and Co., Bowdoinham, ME) without anesthesia. Nerve signals were fed into a high input-impedance preamplifier (Kohno Instruments, Nagoya, input impedance: 100 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 [8,11]. 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 min.

The electrocardiogram from chest lead II, and beat-to-beat blood pressure in the peripheral artery with a pneumatic finger cuff (Portapres™, TNO Institute of Applied Physics Biomedical Instrumentation TPD) [16] were monitored. Blood pressure values were confirmed every minute by an automated upper arm sphyngomanometer (BP203MII, Nippon Colin, Komaki, Japan). The finger cuff of the Portapres was non-invasively attached to two digits of the left hand at the height of the heart level and inflated alternately to prevent the pain due to continuous air pressure load. Mean blood pressure was calculated as the sum of the diastolic blood pressure plus one-third of the pulse pressure.

Data are expressed as means  $\pm$  SE MSNA, heart rate and mean blood pressure were determined for 5 min of pre-HG baseline periods, for each 30 s of the initial 90 s of HG tests, for last 15 s of HG tests (defined as ‘at fatigue’), and for 2 min of PHGMI. A two-way repeated measures analysis of variance [condition (before and after HDBR) and time (pre-HG rest, HG and PHGMI)] was performed. Tests for simple effects were done with a Bonferroni–Dunn comparison procedure when the interaction term was found to be significant. A Wilcoxon signed-rank test was performed to compare baseline levels of MSNA, heart rate and mean blood pressure during pre-HG rest between before and after HDBR. Statistical significance was set at  $P < 0.05$ .

The strength of maximal voluntary HG after HDBR ( $46.6 \pm 1.7$  kgw) was not different from that before HDBR ( $42.6 \pm 1.5$  kgw). Time to fatigue in HG test after HDBR ( $109 \pm 13$  s) was similar to that before HDBR ( $107 \pm 13$  s).

Pre-HG baseline MSNA significantly increased from  $14 \pm 2$  before HDBR to  $19 \pm 2$  bursts/min after HDBR. Baseline heart rate ( $70 \pm 2$  beats/min) after HDBR was higher than before ( $65 \pm 2$  beats/min). Baseline mean blood pressure also increased from  $78 \pm 1$  before HDBR to  $82 \pm 2$  mmHg after HDBR.

Responses of variables ( $\Delta$  increases from pre-HG baseline levels) to the HG test followed by PHGMI are shown in Fig. 1. MSNA gradually increased during HG, and decreased but remained elevated above baseline levels during PHGMI before and after HDBR. There was a significant difference in these MSNA responses between before and after HDBR (time  $\times$  condition interaction;  $P < 0.01$ ).

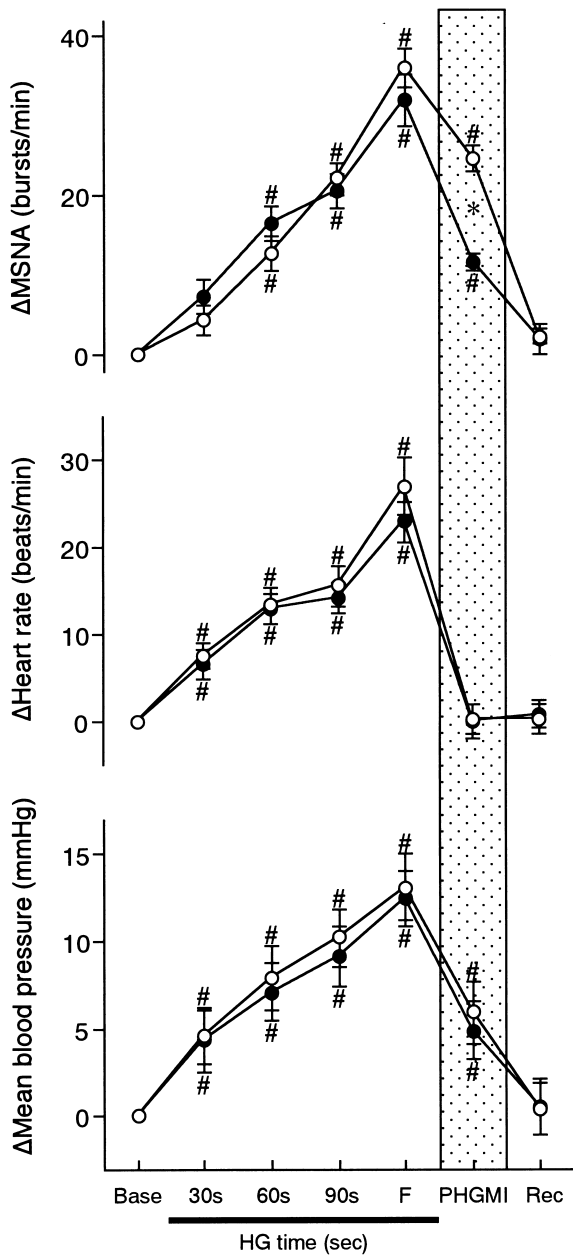


Fig. 1. Increases in MSNA, heart rate and mean blood pressure during HG (each 30 s of the first 90 s of HG, and the last 15 s at fatigue in HG), PHGMI (2 min) and recovery (2 min) before (open circle) and after (closed circle) 14 days of HDBR. Base, baseline; HG, handgrip; F, at fatigue; PHGMI, post-handgrip muscle ischemia; Rec, recovery. Values are means  $\pm$  SE, \* $P < 0.05$  vs. before HDBR. # $P < 0.05$  vs. corresponding pre-HG baseline levels.

Increases in MSNA during the initial 90 s and at fatigue in HG test were similar before and after HDBR. However, an increase in MSNA during PHGMI was significantly lower after HDBR ( $P < 0.01$ ).

Heart rate progressively increased during HG, but returned to baseline levels during PHGMI prior to and following HDBR. Mean blood pressure also progressively increased during HG, and decreased but remained at higher

levels above the pre-HG baseline. These responses of heart rate and mean blood pressure did not differ between before and after HDBR.

The major new finding of the present study was that increases in MSNA were not affected during HG but attenuated during PHGMI in HG tests at 30% of MVC after HDBR compared with before it. In contrast, the time course and magnitude of heart rate responses during HG and PHGMI were similar before and after HDBR, as in the previous study [14]. These results suggest that 14 days of HDBR might affect the vasomotor sympathetic response, but not the cardiac response, to isometric HG followed by PHGMI.

The muscle metaboreflex is the primary mechanism for an increase in MSNA during isometric exercise in humans [10,13]. The strongest evidence for this concept is the finding that during PHGMI, MSNA remained elevated or further increased compared with the level during the last minute of HG [8,10,13]. Metabolites (i.e. lactic acid and hydrogen ions) accumulated in the contracting skeletal muscles during HG are entrapped in the muscles by PHGMI, and activate the muscle metaboreflex, while there is no central command and no muscle mechanoreflexes during PHGMI [10,13]. The reduced increases in MSNA during PHGMI after HDBR thus suggest a decreased magnitude in the muscle metaboreflex after HDBR. Several possible explanations may be given. First, HDBR might change the muscle metabolism, and impair accumulations of metabolites in contracting skeletal muscles. However, this explanation seems unlikely, because if HDBR could impair accumulations of metabolites (i.e. intramuscular hydrogen ions) during HG, the fatigue time in HG would be prolonged after HDBR, since a fall in muscle pH impairs a force development. But, our results showed that time to fatigue remained unchanged after HDBR, with an absolute HG intensity similar to before. Second, sensitivity of receptors (free nerve endings) of chemically sensitive group III and IV muscle afferents [10] might be impaired after HDBR. Third, central modulation of metaboreceptor inputs could be altered after HDBR, resulting in reduced vasomotor sympathoexcitation despite the pre-HDBR level of activation of chemically sensitive muscle afferents.

In contrast to the reduced MSNA responses during PHGMI, increases in MSNA during HG remained unchanged after HDBR. These findings suggest several possible mechanisms, which compensate for the reduced magnitude of muscle metaboreflex and serve to cause the pre-HDBR level of MSNA activation during HG after HDBR. First, the arterial baroreflex-mediated inhibition of MSNA in response to blood pressure elevation during isometric HG [12] might be reduced after HDBR, because the vascular arm of arterial baroreflex may be compromised after 1–2 weeks of microgravity. [1,15]. Second, the stimulation of mechanically sensitive muscle afferents might be augmented during an inactivity of HDBR. Third, central modulation of parallel activation of somatomotor and vaso-

motor sympathetic pathways during static exercise [13] might be altered after HDBR. However, central command plays only a minor role in the activation of MSNA [13].

Muscle metaboreflex-mediated peripheral vasoconstrictions in the non-active skeletal muscles and the viscera play an important role in redirection of the elevated oxygen transport to the contracting skeletal muscles [13]. The present study raises one possibility that prolonged exposure to microgravity (i.e. space station) may impair muscle metaboreflex, and could induce early fatigue and reduce the ability to work with the forearm despite no atrophy in the contracting forearm muscles.

In conclusion, after 14 days of HDBR, the increases in MSNA during muscle ischemia after isometric forearm exercise until fatigue were attenuated, suggesting a reduced magnitude of muscle metaboreflex after HDBR.

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