FINAL REPORT

Microcirculatory Adjustments to Simulated Microgravity Exposure in Humans

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INTRODUCTION

Exposure of an individual to microgravity activates a series of compensatory alterations. The two problems addressed in this grant are likely related to these changes. Reduced tolerance to orthostatic stress is a primary concern when the individual returns to a 1-G environment, whereas reduced thermoregulatory capability could affect performance in space as well as following the flight. To this end we performed a series of experiments using an approach which addressed both issues by assessing the effects of simulated microgravity on the control of the cutaneous vasculature at the level of the microcirculation. These studies were performed using non- and minimally invasive techniques to assess SkBF simultaneously with administration of vasoactive substances primarily via intradermal microdialysis. With this information dose response relationships were constructed. It is recognized that the cutaneous circulation may not appreciably be involved in the maintenance of blood pressure during orthostasis in normothermia. To this regard two important points should be made, a) information regarding the effects of simulated microgravity on the cutaneous microcirculation may be valuable in identifying mechanisms associated with altered control of the microvasculature in regions more important to the regulation of blood pressure, but less easily studied. b) In hyperthermia 50-60% of cardiac output is directed towards the skin (25). In this environment the skin represents the largest fraction of total vascular conductance, and its control is, therefore, critical in the maintenance of blood pressure. This final point is of particular concern since the astronauts encounter hyperthermic conditions prior to landing (24). The following five hypotheses were tested. In each case microgravity exposure was simulated via 14 days of 6° head-down tilt (HDT) bed rest, and a subset of these subjects exercised throughout HDT exposure to see if impaired responses could be improved by aerobic exercise training.

1) Prolonged HDT exposure decreases cutaneous adrenergic vasoconstrictor responsiveness.
2) Prolonged HDT exposure reduces cutaneous vasoconstriction associated with the venoarteriolar response.
3) Prolonged HDT exposure reduces maximal cutaneous vasodilator responsiveness
4) Prolonged HDT exposure impairs sweat gland function
5) Impaired thermoregulatory responses due to HDT bed rest will be preserved in subjects exercising through bed rest exposure.

METHODS

Twenty-two subjects (19 male and 3 female) were exposed to 14 days of strict 6° HDT bed rest. Fourteen of these subjects were randomly assigned to an exercise group (including 2 females), while eight subjects served as control subjects and thus did not exercise during HDT bed rest. This study was a component of an 18 day HDT bed rest study. However, the reported data were obtained from subjects after 14 days of HDT bed rest. On the final day of HDT bed rest (i.e. day 18) subjects in the exercise group were further subdivided to investigate questions unrelated to the present findings. Thus, for the reported data there are an unequal number of subjects in the control and exercise groups. The subjects' average age was 34 ± 2 years and all were of normal height (180 ± 2 cm), weight, (82 ± 4 kg), and health. A written informed consent from each subject was obtained prior to participating in the institutionally approved study.

Bed Rest & Exercise

Strict bed rest was maintained in the 6° HDT position for 14 days. Subjects remained in this position at all times, except they were allowed to elevate on one elbow for meals and were horizontal for exercise, transport, and bathing. Subjects were housed in a hospital based General Clinical Research Center and given a controlled diet, while fluids were allowed *ad libitum*. Pre-bed rest data were obtained within a week of the onset of bed rest. Post-bed rest data were collected on day 14 of bed rest. For the exercise group, exercise was performed at 75% of pre-bed rest heart rate maximum for 90 minutes per day using a supine cycle ergometer (Collins). Subjects chose to exercise for this duration either in 2 bouts of 45 minutes or 3 bouts of 30 minutes per day.

Hypothesis #1: Two intradermal microdialysis membranes were placed in dorsal forearm skin. This technique involved placing a small (200 µm outer diameter, 10 mm length) sterile, semi-permeable membrane intradermally using a 25-gauge needle. Construction and insertion of microdialysis probe and assembly system are reported elsewhere (6, 18). The microdialysis membranes were perfused with Ringer's solution at a rate of 2 $\mu l \cdot min^{-1}$ via an infusion pump (Harvard). The protocol commenced once skin blood flow (SkBF) returned to normal levels after needle insertion trauma (~60-120 minutes). Local temperature was controlled at 34° C via a local heating element (Perimed) that housed a laser-Doppler flow probe centered directly above the microdialysis membranes. Once SkBF stabilized, eight doses of NE were delivered (in 10 fold increments) at a flow rate of 2 $\mu l \cdot min^{-1}$ for 5 minutes per dose. Data are presented from doses of $1 \cdot 10^{-9}$ to $1 \cdot 10^{-2}$ M, which represent both threshold and saturation for the subjects. Microdialysis membranes were placed at similar regions of the forearm between pre- and post-HDT bed rest trials. However, it is recognized that slight variations in probe placement likely occurred between these periods. For each period (i.e. pre- and post-HDBT), responses between the two sites for each dose were averaged.

Hypothesis #2: With the subject in the supine position and the arms and legs at heart level, the forearm venoarteriolar response was assessed by inflating a cuff placed around the upper arm to 45 mmHg. The same procedure was performed to assess the venoarteriolar response of the lower leg except the cuff was placed around the thigh. Previously, it has been reported that venous stasis needs to be above 25 mmHg to elicit the venoarteriolar response (15). The cuff remained inflated at this pressure for a minimum of two minutes. SkBF, assessed via laser-Doppler flowmetry (Perimed), was measured at two sites on the dorsal forearm for the forearm assessment. For the lower leg assessment, laser-Doppler measures were obtained from non-glabrous skin over the tibialis anterior 39 ± 1 cm from the bottom of the foot. When SkBF from two sites were measured, the laser-Doppler signals were averaged between those sites. Anatomical measurements were obtained to place the laser-Doppler flow probes in the same region between pre- and post-bed rest trials. However, as indicated above, given the small sampling area of the laser-Doppler flow probes, it is doubtful that the probes were placed at the exact location between trials.

Hypothesis #3: Assessment of maximal cutaneous vasodilation was accomplished by exposing the left subject's forearm to 42° C for 45 min using a cylindrical water spray device that encircled the subject's forearm (2, 21). A thermocouple was attached to forearm skin within the cylindrical water spray device to verify skin temperature was held at 42° C. Prior to and

following the 45 min heating protocol, the average of six forearm blood flow measurements was obtained using venous occlusion plethysmography (28). Arterial blood pressure was obtained under both conditions via R-wave triggered auscultation of the brachial artery (SunTech Medical Instruments, Raleigh, NC), and forearm vascular conductance (FVC) was calculated from the ratio of forearm blood flow and mean arterial blood pressure. The units for FVC are ml/100ml tissue/min/100 mmHg, however for clarity these units will be reported as FVC units. Since local heating of the forearm does not alter muscle blood flow (1), elevations in total forearm blood flow and FVC are due solely to elevations in forearm skin blood flow and forearm cutaneous vascular conductance, respectively.

Hypothesis #4: Two intradermal microdialysis membranes were placed in dorsal forearm skin. Probes were placed a minimum of 3 cm apart and at the same location for both pre- and post-HDT trials. This technique involved placing a small (200 µm outer diameter, 10 mm length) sterile, semi-permeable membrane intradermally using a 25-gauge needle. Construction and insertion of the microdialysis probe and assembly system are reported elsewhere (6, 18). The depth of probe placement was not identified, although Kellogg et al. (18), who used the same procedure, reported these probes were placed at a depth of 0.3 - 1.0 mm. Both microdialysis membranes were perfused with Ringer's solution at a rate of 2 $\mu l \cdot min^{-1}$ via an infusion pump (Harvard Apparatus, Holliston, MA). The protocol commenced once skin blood flow returned to normal levels after needle insertion trauma (~60-120 minutes). Chambers having a small window (10 x 5 mm, i.e. surface area of 0.5 cm^2) were positioned over each membrane to measure sweat rate by the ventilated capsule method using compressed nitrogen as the perfusion gas. The gas was delivered at a rate of 150 ml/min. Location of capsule placement was aided through the use of markings on the tubing that indicated the center of the membrane portion of the microdialysis probe. Humidity of the effluent gas was measured via a humidity/temperature probe (Vaisala, Woburn, MA) that was positioned 1 meter from the capsule on the skin. The humidity/temperature probe connected to a humidity data processor (Vaisala, Woburn, MA) that calculated absolute humidity from the measurement of relative humidity and temperature. Local skin temperature around the microdialysis membranes was maintained at 40° C through the use of a lamp and a thermocouple. Sweat gland function was assessed by perfusing nine doses ($1x10^{-6}$ to 2 M) of acetylcholine through the microdialysis membranes. Each dose of acetylcholine was administered for 5 min, while sweat rate over the microdialysis membranes was continuously recorded. Sweat rate during the final minute of each 5 min period was averaged and used for the statistical analysis. Sweat rates from both sites averaged and are reported as a single value for each dose of acetylcholine. Previously we reported the validity and reproducibility of this technique in assessing sweat gland function in humans (27). Following the acetylcholine challenge, with the sites continuing to be perfused with the highest dose of acetylcholine, the number of activated sweat glands was assessed in triplicate over the microdialysis membranes using the starch-iodine technique (19).

Hypothesis #5: For this component 10 subjects (9 male and 1 female) participated in the study. Throughout HDT bed rest subjects exercised at 75% of pre-bed rest heart rate maximum for 90 minutes per day on a supine cycle ergometer (Collins). Heart rate was monitored (Polar) during these exercise bouts. Immediately prior to beginning HDT bed rest and 13th day of bed rest thermoregulatory responses were assessed throughout a 25 min of supine cycle ergometry exercise tested in a 28°C room. The same absolute workload was performed during both the pre-

and post-HDT testing. Upon entering the laboratory, each subject was instrumented for the measurement of mean skin temperature (Tsk) from the weighted average of six thermocouples placed on the skin. Internal temperature (Tcore) was measured by thermister or telemetry pill. Thermister probe measured esophageal temperature and was inserted through the nose to a distance equal to one-quarter of the subject's heights. Telemetry pill was used in a subject who refused to insert esophageal temperature probe and was swallowed 2 hours before thermoregulatory response test. Heart rate (HR) was monitored using the ECG. Arterial blood pressure was obtained under both conditions via R-wave triggered auscultation of the brachial artery (SunTech Medical Instruments, Raleigh, NC). Mean arterial pressure (MAP) was calculated as one-third of pulse pressure plus diastolic pressure. Skin blood flow (SkBF) on the chest and forearm was measured by laser-Doppler flowmetry (Perimed), and sweat rate on the forearm (SR) by the ventilated capsule method using compressed nitrogen as the perfusion gas delivered at a rate of 500 ml/min. Cutaneous vascular conductance (CVC) was indexed from the ratio of SkBF to MAP. CVC was normalized relative to maximum CVC obtained via local heating of the skin to 42°C for 30 min. Humidity of the effluent gas was measured via a humidity-temperature probe (Vaisala, Woburn, MA) that was positioned 1 meter from the capsule on the skin. The absolute humidity was calculated from the measurement of relative humidity and room temperature.

RESULTS

Hypothesis #1

The mean goodness of fit (R^2) of the dose-response model was high both pre- (exercise: 0.94 ± 0.02 and non-exercise: 0.95 ± 0.03) and post- (exercise: 0.93 ± 0.02 and non-exercise: 0.95 ± 0.03) HDT bed rest. Neither HDT bed rest alone nor exercise during HDT bed rest had an effect on the concentration of NE that caused 50% of maximal cutaneous vasoconstriction (i.e. EC_{50}, HDT bed rest: $P > 0.30$; exercise during HDT bed rest: $P > 0.15$); see Figure 1. Similarly, maximal and minimal responses from the dose-response model were not altered by HDT bed rest (maximal: $P > 0.30$ and minimal: $P > 0.40$) or exercise during HDT bed rest (maximal: $P > 0.50$ and minimal: $P > 0.15$). Finally no interactions were observed between main factors of the ANOVA for any of the dose-response variables.

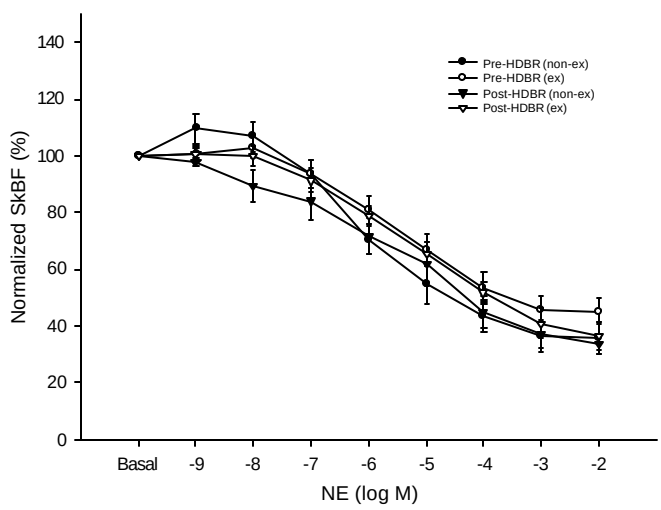

Figure 1: Effects of HDT bed rest with and without exercise during bed rest on cutaneous vasoconstrictor responses to exogenous norepinephrine.

Hypothesis #2

The magnitude of forearm vasoconstriction associated with the venoarteriolar response was not affected by HDT bed rest regardless of the group (pre- HDT bed rest non-exercise: 53.1 ± 4.2 %; post- HDT bed rest non-exercise: 52.2 ± 4.5%; pre- HDT bed rest exercise: 56.6 ± 2.1%; post- HDT bed rest exercise: 52.7 ± 3.0%); see Figure 2A. Probability values from the ANOVA were P > 0.30 for HDT bed rest, P > 0.70 for exercise during HDT bed rest, and P > 0.50 for the interaction found between pre/post- HDT bed rest and the exercise/non-exercise main factors. In contrast, cutaneous vasoconstriction of the lower leg due to the venoarteriolar response was significantly attenuated following HDT bed rest in both groups (pre- HDT bed rest non-exercise: 51.0 ± 5.0%; post- HDT bed rest non-exercise: 43.0 ± 6.6%; pre- HDT bed rest exercise: 48.4 ± 7.4%; post- HDT bed rest exercise: 36.4 ± 8.5%); see Figure 2B. Probability values from the ANOVA were P < 0.03 for HDT bed rest, P > 0.50 for exercise during HDT bed rest, and P > 0.60 for the interaction between pre/post- HDT bed rest and the exercise/non-exercise main factors.

Hypothesis #3

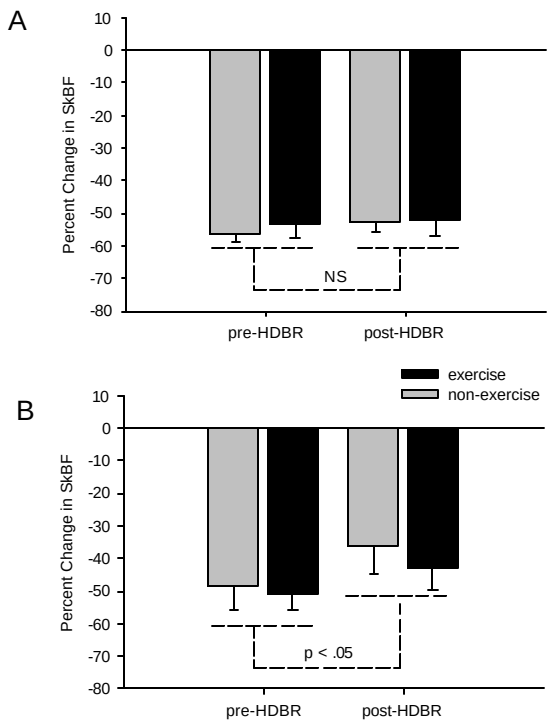

Figure 2: Effects of HDT bed rest with and without exercise during bed rest on cutaneous vasoconstrictor responses due to the venoarteriolar response in the arm (panel A) and leg (panel B). Cutaneous vasoconstriction due to this response was significantly attenuated in the leg in both the control and exercise groups.

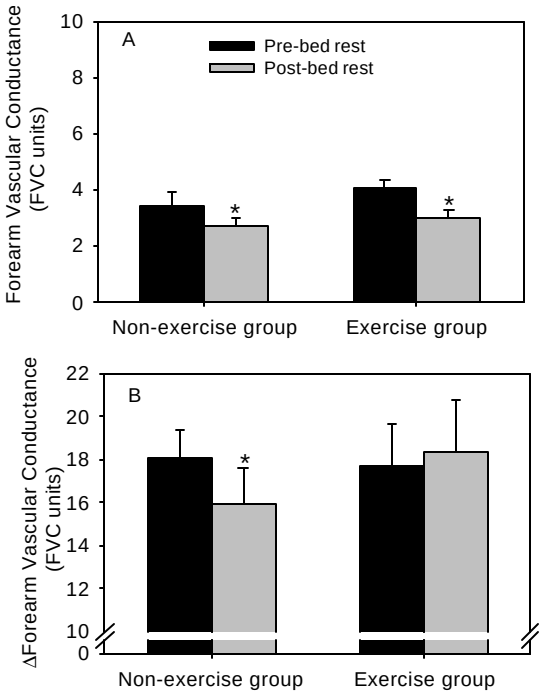

Figure 3: Effects of HDT bed rest with and without exercise during bed rest on basal forearm vascular conductance (panel A) and maximal forearm cutaneous vascular conductance (panel B). Maximal forearm cutaneous vascular conductance was significantly attenuated by HDT bed rest. However this response was preserved in subjects who exercised during HDT bed rest. * Significantly different from pre-bed rest levels.

In the non-exercise group both maximal FVC (21.5±1.3 to 18.7±1.7 FVC units; P=0.01) and the change in FVC with local heating (i.e. ΔFVC; 18.1±1.2 to 15.9±1.7; P=0.01) were significantly attenuated following HDT bed rest (Figure 3). In contrast, in the exercise group when reported either as maximal FVC (21.8±2.0 to 21.4±2.5 FVC units; P=0.39) or as the

increase in FVC due to local heating (17.7±1.9 to 18.4±2.4; P=0.32), these responses were not significantly different between pre- and post-HDT trials.

Hypothesis #4

In both the exercise and non-exercise groups the EC_{50} of the sweating response was unaffected by HDT bed rest (Figure 4). However, maximal stimulated sweat rate for the non-exercise group was significantly attenuated following HDT bed rest (0.63±0.06 to 0.42±0.03 mg/cm^2/min; P=0.001), while no significant difference was observed for the exercise group (0.81±0.15 to 0.77±0.10; P=0.31). Regardless of the group, HDT bed rest did not significantly change the number of activated sweat glands during administration of the highest dose of acetylcholine.

Hypothesis #5

Exercise during HDT exposure preserved VO_{2max}, blood volume, and plasma volume relative to pre-HDT bed rest levels. Internal temperature prior to exercise and at the end of exercise was not different between pre- and post-HDT trial. The onset of sweating and maximal sweat rate during exercise were

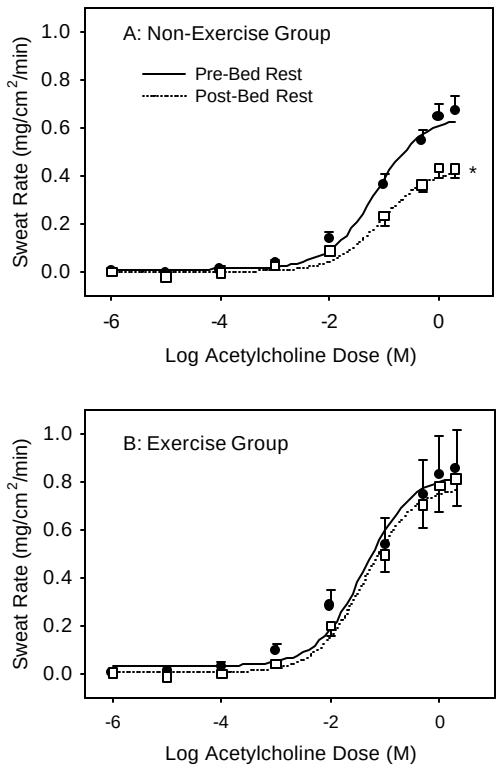

Figure 4: Effects of HDT bed rest with and without exercise during bed rest on sweat gland function. Maximal sweat gland function was attenuated by HDT bed rest. However this response was preserved in subjects who exercised during HDT bed rest.

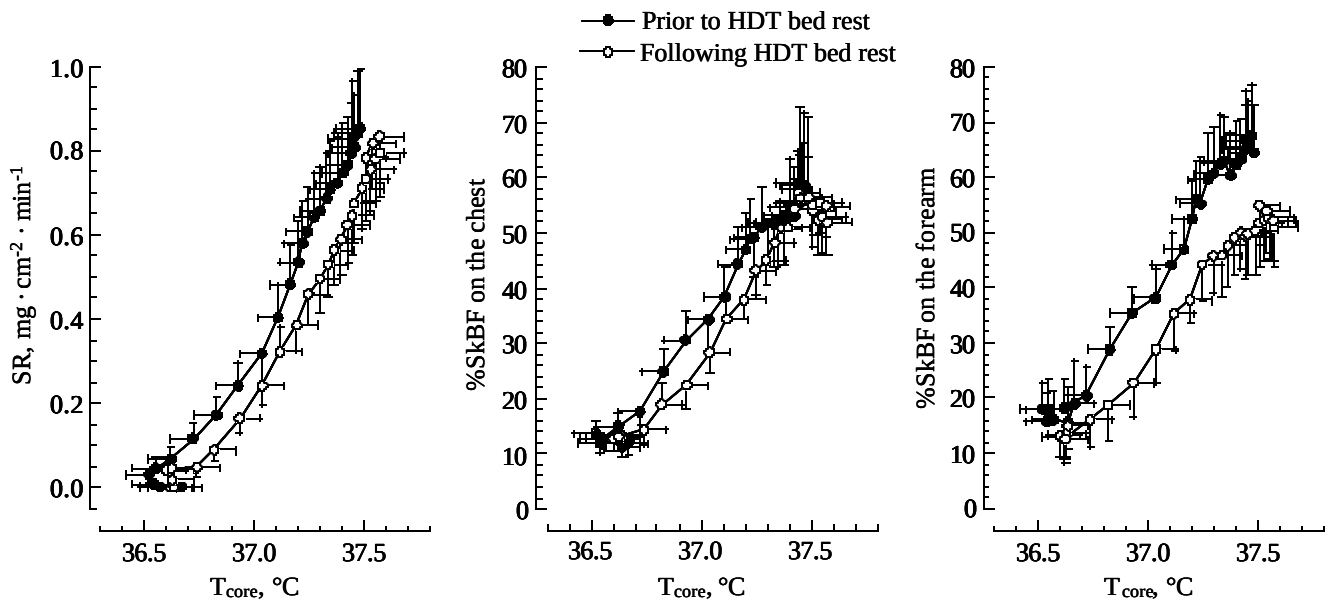

Figure 5: Effects of a combination of HDT bed rest with 90 min of exercise during bed rest on thermoregulatory responses. The elevation in sweat rate, skin blood flow on the chest, and skin blood flow on the forearm were preserved by exercise during HDT bed rest. These findings are in contrast to impaired thermoregulatory responses during HDT bed rest in subjects that do not exercise during bed rest.

no different between pre- and post- HDT trials (Figure 5). Moreover, cutaneous vascular responses were also preserved by exercise throughout HDT (Figure 5). Taken together, these data indicate that 90 min of exercise throughout HDT bed rest preserves thermoregulatory responses.

DISCUSSION

These data were obtained to identify the effects of 14 days HDT bed rest on the control of the microcirculation and sweating. Moreover, the affects of aerobic exercise training during bed rest on the hypothesized impaired responses, as well as on thermoregulatory responses during exercise, were assessed. The primary findings are a) HDT bed rest does not alter cutaneous post-synaptic vasoconstrictor responsiveness; b) vasoconstriction associated with the venoarteriolar response is impaired following HDT bed rest regardless of whether the subject exercises during bed rest; c) maximal cutaneous vasodilator and sweating responses are impaired following HDT bed rest, however these responses are preserved if the subject exercises during bed rest; and d) aerobic exercise during bed rest preserves thermoregulatory responses previously shown to be attenuated after HDT bed rest.

Hypothesis 1: No differences were observed in the $EC_{50,}$ maximum, or minimum SkBFs during exogenous norepinephrine administration between pre- and post- HDT bed rest, regardless of the group (i.e., exercise or control group). These data suggest HDT bed rest does not affect cutaneous post-synaptic vasoconstrictor responsiveness even when norepinephrine is infused via the interstitial space (i.e. ablumen). A lack of an effect of bed rest on vasoconstrictor responsiveness confirms prior work in humans in which the elevation of blood pressure due to systemic infusion (i.e. via the lumen of the vascular) of NE (3) and phenylephrine (4) were similar between pre- and post- HDT bed rest conditions. However, these findings are in contrast to animal studies which suggest space flight and hindlimb suspension (simulated microgravity exposure) reduces vasoconstrictor responsiveness in isolated-perfused preparations (9, 22, 23, 26). Taken together, these data suggest that in humans HDT bed rest does not alter post-synaptic vasoconstrictor responsiveness, and therefore this mechanism may not contribute to post-space flight orthostatic intolerance. In contrast, post-synaptic vasoconstrictor responsiveness seems to be impaired during in animals following actual and simulated space flight.

Hypothesis 2: In addition to adrenergic-mediated vasoconstriction, vasculature resistance can be increased by other means such as the venoarteriolar response (15-17). Although the exact mechanism causing vasoconstriction due to the venoarteriolar responses is unknown, vasoconstriction occurs via a non-adrenergic local mechanism (8) secondary to venous congestion (15-17); perhaps through a local neural network or a myogenic response (8). During orthostasis it has been reported that as much as 45% of the increase in systemic vascular resistance is due to vasoconstriction of cutaneous, subcutaneous, and muscle vascular beds as a result of this response (14, 15). In the present study cutaneous vasoconstriction of the leg due to the venoarteriolar response was significantly attenuated following HDT bed rest regardless of whether the subjects exercised during bed rest. In contrast, HDT bed rest did not change vasoconstrictor responsiveness in the arm due to the venoarteriolar response. It remains a matter of speculation whether impaired venoarteriolar responses in the lower limbs following HDT bed rest or space flight contributes to reduced orthostatic tolerance.

Hypothesis 3 and 4: A number of studies report greater increases in internal temperature during exercise following bed rest deconditioning relative to pre-bed rest conditions (10, 12, 13). Possible mechanisms leading to this response are reduced cutaneous vasodilation (7, 20) and/or reduced sweating responsiveness (12, 13). In support of these mechanisms, Fortney et al. (11) identified pronounced reductions in sweat rate and cutaneous vasodilation during exercise in two astronauts following 115 days in space. Thus, there is strong evidence to support the hypothesis that thermoregulation is altered following space flight and its bed rest analogs. Less clear are the mechanisms leading to the aforementioned diminished thermoregulatory responses. In the present study we observed that 14 days HDT bed rest reduced maximal cutaneous vasodilation and maximal acetylcholine-induced sweat rate. Moreover, prevention of detraining associated with HDT via chronic aerobic exercise during HDT preserved both of these responses. In contrast, sweat gland sensitivity (defined as the EC_{50} of the sweating response) and the number of activated sweat glands during exogenous administration of acetylcholine were unaffected by HDT bed rest. These data indicate that HDT bed rest alters post-synaptic responses associated with the cutaneous vasculature and sweating, and that these responses are normalized when subjects perform aerobic exercise training throughout bed rest. Attenuation of cutaneous vasodilator capacity and sweat gland function following HDT bed rest provides some mechanistic possibilities as to why thermoregulatory responses are impaired following these exposures. Moreover, data from the present study raise the possibility that thermoregulatory responses to an internal (i.e. exercise) or external (i.e elevated environmental temperature) heat stress may be preserved in subjects who exercise throughout simulated or actual microgravity exposure (see hypothesis 5 below).

Hypothesis 5: Previously we (5) and others (20) suggested that ground-based analogs of microgravity impair sweating and cutaneous vascular responses during passive heat stress or exercise. Moreover, thermoregulatory responses are impaired following actual space flight (11). In present challenge, aerobic exercise training during HDT bed rest preserved thermoregulatory responses during exercise. These findings are supported by the observation that the internal temperature threshold for the onset of sweating and cutaneous vasodilation was not difference between prior to and post HDT bed rest. Moreover, the slopes expressing the relationship between internal temperature and heat dissipating mechanisms (sweat rate, skin blood flow, and CVC) were also not different between pre- and post-HDT trials. These data suggest that exercise during HDT bed rest or space flight is beneficial in preserving thermoregulatory responses in humans. It remains unclear whether 90 min of aerobic exercise training per day is required to preserve thermoregulatory responses during HDT or whether similar effects can be observed following shorter duration of exercise.

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<u>Complete listing of all presentations this grant supported</u>:

1) Pharmacological assessment of the venoarteriolar response. Experimental Biology Meeting, April 1999.
2) Assessment of sweat gland function using intradermal microdialysis and capacitance hygrometry: A methodological investigation. American College of Sport Medicine Meeting. June, 1999.
3) Potential mechanism leading to impaired thermoregulation following microgravity." Space Biomedical Investigators' Workshop. NASA, January, 1999.
4) Evidence for metaboreceptor stimulation of sweating in normothermic and heat-stressed humans. American College of Sport Medicine Meeting. June, 2000.
5) Fourteen days head-down tilt does not alter maximal forearm cutaneous vascular conductance" Bioastronautics Investigators' Workshop, NASA, January 2001
6) Effects of prolonged head-down tilt (HDT) bed rest on sweat gland function and maximal forearm cutaneous vascular conductance. American College of Sport Medicine Meeting. June, 2001.
7) Effects of local warming on human cutaneous alpha-adrenergic vasoconstrictor. responsiveness. American College of Sport Medicine Meeting. June, 2001.
8) Effects of whole-body heating on cutaneous α-adrenergic vasoconstrictor responsiveness. Experimental Biology Meeting, April 2001.
9) Acetylcholine released from sudomotor nerves is capable of contributing to cutaneous vasodilation during heating in humans. IUPS Thermal Physiology Satellite Meeting. August 2001.
10) Exercise during simulated microgravity exposure preserves thermoregulatory responses. Experimental Biology Meeting, April 2002.
11) Skin-surface cooling maintains cerebral blood flow velocity and improves orthostatic tolerance during tilting in the heated human. Experimental Biology Meeting, April 2002.

12) Effect of head-down tilt bed rest on arm and leg cutaneous venoarteriolar responses in humans. Experimental Biology Meeting, April 2002.
13) Effect of head-down tilt bed rest on human cutaneous vasoconstrictor responses to exogenous norepinephrine. American College of Sport Medicine Meeting. June, 2002.
14) Phenylephrine-induced elevations in blood pressure is attenuated in heat stressed humans. American College of Sport Medicine Meeting. June, 2002.

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