ALTERATIONS IN HUMAN BARORECEPTOR REFLEX REGULATION OF BLOOD PRESSURE FOLLOWING 15 DAYS OF SIMULATED MICROGRAVITY EXPOSURE

DISSERTATION

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

by

Craig G. Crandall, B.S., M.S.
Denton, Texas
August 1993

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Prolonged exposure to microgravity is known to invoke physiological changes which predispose individuals to orthostatic intolerance upon re-adaptation to the earth's gravitational field. Attenuated baroreflex responsiveness has been implicated in contributing to this inability to withstand orthostatic stress. To test this hypothesis, eight individuals were exposed to 15 days of simulated microgravity exposure using the 6° head-down bed rest model. Prior to, and after the simulated microgravity exposure, the following were assessed: a) aortic baroreflex function; b) carotid baroreflex function; c) cardiopulmonary baroreflex function; and d) the degree of interaction between the cardiopulmonary and carotid baroreflexes. Fifteen days simulated microgravity exposure increased the gain of the aortic-cardiac baroreflex, expressed as the change in heart rate for a given change in blood pressure (0.45±0.07 to 0.84±0.18 Δbpm/ΔmmHg; $p<0.05$). Similarly, the gain of the cardiopulmonary baroreflex increased post-head down bed rest (1.5±0.4 to 2.4±0.6 PRU/mmHg $p<0.05$) when assessed by comparing the changes in forearm vascular resistance to the changes in central venous pressure. In contrast, the carotid-cardiac baroreflex and the interaction between the cardiopulmonary and carotid baroreflexes were not affected by simulated microgravity exposure ($p>0.05$), whereas the gain of the carotid-vascular

baroreflex was significantly attenuated following the exposure (0.17±0.02 to 0.14±0.2 mmHg/mmHg). These results suggest that global baroreflex desensitization is not the mechanism causing post-microgravity exposure orthostatic intolerance; rather the increases in the aortic and cardiopulmonary baroreflex gains will serve to attenuate, not accentuate, such an occurrence. Therefore, other mechanisms are likely involved in causing the observed orthostatic intolerance, possibly related to the maintenance of stroke volume during the orthostatic stress.

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Invited Presentation: "Baroreflex control of blood pressure following 15 days simulated microgravity exposure." NASA, Ames Research Center, Life Science Division. December 18, 1992.

Invited Presentation: "Validation of the Cosmed K2 telemetric oxygen uptake analyzer." NASA, Johnson Space Center, Houston, Texas; October 13, 1990.

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LIST OF ABBREVIATIONS

A_1	range of response (HR, RRI or MAP)
A_2	gain coefficient
A_3	estimated CSP for equal pressor and depressor responses
A_4	minimal HR, MAP or maximal RRI response
ALD	aldosterone
ANP	atrial natriuretic peptide
ATPS	atmospheric temperature and pressure saturated
AVP	arginine vasopressin
bpm	beats per minute
BV	blood volume
CSP	carotid sinus pressure
CSP_{sat}	saturation pressure
CSP_{thr}	threshold pressure
CSDP	carotid sinus distending pressure
C/T	cardiothoracic ratio
CVP	central venous pressure
ECG	electrocardiogram
EDRF	endothelium derived relaxing factor
FBF	forearm blood flow
FVR	forearm vascular resistance
g	gravitational force
GXT	graded exercise test
HDT	head-down tilt
HR	heart rate
$\Delta HR/\Delta MAP$	ratio of the changes in HR to the changes in MAP
LBF	leg blood flow
LBNP	lower body negative pressure
LVR	leg vascular resistance
MAP	mean arterial pressure

mmHg	millimeters of mercury
NASA	national aeronautic and space administration
NP	neck pressure
NTS	nucleus of the tractus solitarus
$PaCO_2$	arterial partial pressure of carbon dioxide
$PetCO_2$	end-tidal partial pressure of carbon dioxide
$P\bar{v}CO_2$	mixed venous partial pressure of carbon dioxide
PE	phenylephrine
PRA	plasma renin activity
PV	plasma volume
PVP	peripheral venous pressure
$\mathring{Q}$	cardiac output
RBC	red blood cell
RRI	interbeat interval
SEM	standard error of the mean
SV	stroke volume
STPD	standard temperature and pressure dry
TPR	total peripheral resistance
$\mathring{V}CO_2$	rate of carbon dioxide production
$\mathring{V}O_{2max}$	maximal rate of oxygen uptake
$\mathring{V}O_{2peak}$	peak rate of oxygen uptake
$\mathring{V}i$	inspiratory ventilatory rate

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CHAPTER I

INTRODUCTION

> The aspiration to travel beyond the atmosphere is like the desire to study the ocean floor, the interior of the Earth's crust, to invent a submarine, to fly through the air, improve life, treat disease, and explore the heavens.
>
> Konstantin Tsiolkovsky, 1857-1935
> A Russian School Teacher

The desire of individuals to live, work and play under a variety of environmental conditions, whether it be changes in pressure, temperature or gravitational states, requires the physiological systems to adapt to these environments. The challenge to the environmental physiologist is to understand these stresses, with a goal of providing insight which may be beneficial in allowing the individual to better adapt to the new environment.

Over the past three decades, numerous individuals have left the earth's atmosphere and its accompanying gravitational force to explore and gain a greater understanding of the vast regions of space around us. Such exposure to microgravity has been demonstrated to induce a multitude of physiological responses affecting cardiopulmonary, musculoskeletal, neurohormonal and neurovestibular systems (187), which have resulted in a large number of investigations and subsequent reports detailing the scientific findings concerning human sojourns in space. With the introduction of human

ground-based microgravity analogs, such as water immersion, supine bed rest, head-down tilt bed rest (HDT), as well as animal microgravity analogs of body casting and tail suspension, the database of physiological findings is continually growing.

Orthostatic hypotension, defined as the inability to maintain blood pressure during orthostatic stress, and subsequent syncope or syncopal symptoms, has been well documented following space flight (19, 29, 36, 84). Prior to the Space Shuttle era, less attention was placed on this problem since the astronauts re-entered the earth's atmosphere on their backs in a descending capsule, which does not predispose the individuals to syncope since orthostatic stress is absent in this position. Conversely, Shuttle astronauts now re-enter the earth's gravitational field in the sitting position, and therefore are susceptible to the orthostatic stress.

The mechanisms leading to post-space flight orthostatic intolerance are poorly understood. Blömqvist and Stone (29) suggested that the reduction in tolerance to orthostatic stress following microgravity exposure likely includes inadequate cardiac filling volume due to reduced blood volume, depressed baroreflex mediated responses to a fall in arterial pressure or a combination of these mechanisms. Additionally others have demonstrated that increased venous compliance of the lower extremities (55, 122) and decreased left ventricular end-diastolic volume resulting in a lowering of stroke volume and cardiac output, were observed following actual or simulated microgravity exposure (108) and therefore may be primary contributors to post-space flight orthostatic intolerance.

Nixon et al. (192) demonstrated that 6° HDT produced a central fluid shift, orthostatic intolerance and reduced exercise capacity. The decreased orthostatic tolerance and reduced exercise capacity were suggested to be a result of diuresis induced reduction in blood volume. However in a similar 24 hour HDT study, Blömqvist et al. (28) reinfused saline sufficient to bring central venous pressure (CVP) back to pre-HDT values into half of the subjects, and compared these subjects' orthostatic tolerance to that of the control subjects. The reinfusion of saline was not sufficient to improve orthostatic tolerance, and the authors concluded that decreases in blood volume alone were not responsible for the decreased tolerance. Furthermore, Greenleaf et al. (108) assessed the effects of exercise training during 30 days of HDT on orthostatic hypotension. Cardiovascular responses and tolerance time to 6° degree head-down tilt were measured in three groups of subjects who had undergone no exercise, endurance exercise or resistive exercise two times a day, five days a week during the HDT period. The endurance exercised subjects maintained their plasma volume (PV) at pre-HDT levels while the other two groups were found to have significant reductions in PV. However, tilt tolerance times were significantly reduced in all groups following HDT with no differences between the groups. Since the blood volume was maintained in the endurance exercised group, these data provide further evidence that physiological mechanisms other than intravascular blood volume must be important for blood pressure control following long term microgravity exposure.

Since hypovolemia can contribute to, but is not a sufficient explanation of the reported orthostatic hypotension and syncope following simulated

microgravity exposure, the possibility that inadequate reflex control of blood pressure may be associated with orthostatically-induced syncope was considered. Of the three baroreceptor populations, only the effects of microgravity on the carotid baroreflex has been investigated, and more specifically, only the cardiac (i.e. heart rate) efferent limb of that reflex. Such work was conducted by Convertino et al. (54) in which the changes in the carotid-cardiac baroreflex was assessed during 30 days of simulated weightlessness. The maximal slope (i.e. gain) of the relationship between estimated carotid sinus pressure and the change in interbeat interval (RRI) was reduced by HDT, indicating that the sensitivity of the vagally mediated carotid-cardiac baroreflex was attenuated. Plasma volume for all subjects decreased 15% following the procedure, however no significant correlation was found between the changes in plasma volume and the changes in the sensitivity of the carotid-cardiac baroreflex. In a separate study, the same group assessed the carotid-cardiac baroreflex of eight subjects at three different blood volemic states: hypovolemia; normovolemia; and hypervolemia (246). The results demonstrated that neither expanded nor depleted plasma volume altered the response of the carotid-cardiac baroreflex. Therefore, Convertino and associate's results indicate that a) simulated microgravity exposure decreases the response of the carotid-cardiac baroreflex; and b) the alterations observed in the carotid-cardiac baroreflex are independent of blood volume status.

Using a non-human primate model, Billman et al. (22) placed whole-body casts on rhesus monkeys and placed them in the horizontal position for 28 days. At control and days 7, 14 and 28 of the procedure, they observed the

changes in the RRI due to a 30 mmHg increase in arterial pressure induced by a bolus injection of phenylephrine. A reduced chronotropic response to the rise in blood pressure occurred as early as 7 days of horizontal body casting, and was observed to persist for the 28 day duration. This suggests that passive detraining could be associated with a decreased baroreflex responsiveness.

Only one study has assessed autonomic blood pressure regulatory alterations due to actual microgravity exposure (83). Carotid-cardiac baroreflex data were collected before and following 4-5 days of space exposure using the protocol previously described. The results obtained following the flight indicated that the baseline RRI was reduced, demonstrating tachycardia at rest. Additionally, the slope of the estimated carotid sinus pressure to RRI tended to decrease after landing (5.0±1.0 vs 3.4±0.5 msec/mmHg), but not significantly. This lack of statistical significance was attributed to a large variation of response in the relatively small number of subjects. However, the trend was thought to be consistent enough to conclude that the carotid-cardiac baroreflex function may be impaired following as little as 4-5 days of microgravity exposure.

Due to the ease of measurement, only the carotid-cardiac baroreflex has been investigated following simulated or actual microgravity in humans, therefore, little is known about the effects of microgravity on the cardiopulmonary baroreflex, aortic baroreflex, or the interaction between the cardiopulmonary and carotid baroreflexes. Without information regarding the effects of microgravity exposure on baroreceptor populations other than the carotid-cardiac baroreflex, erroneous conclusions may be made as to baroreflex function during and following space flight. Moreover, in light of

the data from Mancia et al. (176), Ferguson et al. (77) and Sanders et al. (221) demonstrating a predominance of the aortic baroreflex over the carotid baroreflex in controlling heart rate and peripheral resistance, it is vital to gain a greater understanding of microgravity-induced alterations of this baroreflex.

Statement of the Problem

The effects of microgravity exposure on baroreflex functions is not well known. The purpose of these series of experiments was to systematically evaluate the effects of simulated microgravity exposure on the cardiopulmonary baroreflex, aortic baroreflex, carotid baroreflex and the interaction between the cardiopulmonary and carotid baroreflexes.

Hypotheses

Although this study was not specifically designed to draw a direct correlation between tolerance to orthostatic stress following simulated microgravity exposure and baroreflex function, it was designed to investigate some of the mechanisms which occur as a result of simulated microgravity exposure that may explain why orthostatic intolerance is so prevalent following space flight. Therefore, the following hypotheses were tested in this investigation:

i. The gain of the aortic-cardiac baroreflex is attenuated following simulated microgravity exposure when compared to pre-HDT control.

ii. Simulated microgravity exposure results in a decreased cardiopulmonary baroreflex gain when evaluated against pre-HDT values.

iii. The same amount of cardiopulmonary baroreceptor unloading will result in less of an augmentation of the carotid-cardiac and carotid-vascular baroreflexes following simulated microgravity exposure.

iv. The increase in heart rate following HDT can be attributed to microgravity-induced reduction in basal vagal neural activity.

Delimitations of the Investigation

Due to methodological concerns, the following delimitations of this study were recognized.

i. The individuals who participated in the study were all Caucasian men with ages ranging from 26 to 45 years, therefore these findings may not be representative of the general population.

ii. The blood pressure values were obtained indirectly from the middle finger. Since direct intra-arterial measurements were not obtained, inherent experimental errors may have occurred regarding the absolute blood pressures obtained due to the placement of the cuff on the middle finger. These errors were minimized by verifying the pressures via auscultation of the brachial artery. However, the changes in blood pressure occurring during the various perturbations are assumed and expected to be accurate.

iii. Central venous pressure was estimated from peripheral venous pressure using the technique of Gauer and Sieker (91). Such a technique has been reported to accurately track central venous pressure under a variety of conditions (90).

iv. The change in carotid sinus distending pressure induced by neck pressure or suction is not well known. Only one study has investigated

the changes in carotid sinus transmural pressure during neck suction and pressure in humans (169), demonstrating that 64% of the external suction was transmitted to the region of the carotid sinus while 86% of external pressure was transmitted. Since no work has been conducted regarding the effects of the drug used during the aortic isolation procedure (phenylephrine) on the transmission characteristics of the pressure delivered to the carotid sinus region, we assumed 70% transmission of the pressure to ensure the carotid sinus distending pressure had returned to control tensions.

v. Low levels (<20 torr) of lower body negative pressure was expected to only perturb the cardiopulmonary baroreceptors (without affecting the arterial baroreceptors) because it has been found that pressures <20 torr do not result in changes in mean aortic pressure, arterial pulse pressure, maximal rate of aortic pressure rise or heart rate (130).

vi. The technique used to determine the vagal neural activity (i.e. heart rate variability) was assumed to be accurate as described by Pomeranz et al. (205) since muscarinic blockade eliminated the response while beta block did not alter the response.

vii. The model used to simulate microgravity exposure (6° HDT) has been repeatedly used, and has been demonstrated to be a valid analog of actual microgravity exposure when observing physiological systems (28, 52, 108, 223). However, no work has been conducted to determine if such a model is applicable when elucidating the effects of such exposure on the baroreceptors. We, therefore, assumed that the 6° HDT model is a valid analog of actual microgravity exposure, thus

enabling us to conduct this experiment without actually putting the subjects into space.

viii. This study presumed that central venous pressure was an index of cardiopulmonary baroreceptor loading. Repeated studies have verified the negative linear relationship between central venous pressure and forearm vascular resistance (130, 172), however, it is not known if central venous pressure is a true index of cardiopulmonary baroreceptor loading or unloading.

ix. Unlike the aortic baroreflex procedures, during the carotid baroreflex assessment, the transmission of the neck pressure/suction is assumed to be 100%. Applying this assumption to the data will not affect the results since the same assumption is made with both the pre- and post-HDT data.

CHAPTER II

REVIEW OF RELATED LITERATURE

Physiology is not merely the study of natural processes of the body, rather it encompasses the homeostatic adaptations which occur when the body is exposed to a variety of environmental conditions. Microgravity exposure is such a condition in which the body adapts to a new environment resulting in a new homeostatic condition which is conducive to the perturbation placed upon it. Although the body can function very well when exposed to microgravity conditions, the physiological changes which occur during space flight are known to be detrimental to the individual between the time when the individual has returned to the earth's gravitational field, and when a new 1-g homeostatic state has been attained. The purpose of this chapter is to: a) provide a brief summary of the U.S. and Soviet space programs; b) review the ground-based analogs of weightlessness; c) detail the physiological adaptations which occur during microgravity exposure; d) review baroreflex physiology, and its relevance to microgravity exposure; e) discuss post-flight orthostatic intolerance and exercise capacity.

Prior to beginning the subsequent sections, the reader should be advised that, although the Soviet Union has been conducting space related research for a similar time period as the U.S., due to political reasons, much of the results of these projects have not been made available. With the recent demise of the metaphorical "Iron Curtain", more data are becoming accessible

to the scientific community. Therefore, where pertinent Soviet data are available, the results will be presented, but the reader should be cautioned that they are not all inclusive.

Review of the U.S. Space Program

In 1958 the National Aeronautic and Space Administration (NASA) was formed and given the charge to launch a man into space at the earliest possible date and provide an environment for him in which he could perform effectively in space and return safely to the earth (190). On May 5, 1961, Alan Shepard was launched into space and experienced suborbital flight for 15 minutes. This flight initiated the Mercury space project. For the next two years, six Mercury flights and six astronauts were flown having a maximal duration of 34 hours and 20 minutes. Physiological data was obtained using a pre/post design with many of these flights. The primary physiological findings of the Mercury project were a reduced body weight presumed to be due to dehydration, as well as evidence for an impairment of the cardiovascular system (190). Furthermore, Dietlien (67) demonstrated orthostatic intolerance and hemoconcentration following the longest of these flights. However, the most important finding of this project was the knowledge that man could survive in the space environment.

Following the Mercury project, the Gemini project was initiated. Contrary to the Mercury capsule which could hold only one individual, the Gemini capsule was able to hold two individuals and provided life support for more than 14 days, thus enabling the physiologist to investigate the effects of extended microgravity exposure. Of the ten Gemini missions, three (Gemini 4, 5, and 7 lasting 4, 8 and 14 days respectively) were of particular

interest to physiologists since these missions included numerous in-flight physiological experiments. Such in-flight experiments were unique since the Mercury missions did not have the capabilities of in-flight experimentation. In addition to other questions, a primary question investigated with the Gemini project was whether the cardiovascular alterations observed in the Mercury missions were self limiting adjustments (190). Table 1 lists some of the significant perturbations observed during the Gemini project.

Table 1
Significant cardiovascular perturbations observed during the Gemini program

Loss of red blood cell mass (ranging 5-20% from baseline)
Post-flight orthostatic intolerance in all crew members
Loss of exercise capacity compared with pre-flight baselines
Higher than predicted metabolic cost of an extravehicular activity

Table adapted from Nicogossian (190).

As a result of President John F. Kennedy's directorate of putting a man on the moon, the Apollo program was instigated to attain this goal. Since the purpose of these projects were not directly related to biomedical research, less time was provided to investigate the physiological effects of microgravity. The majority of the physiological experiments were conducted prior to launch and after landing. Table 2 illustrates the major biomedical findings of these flights. Upon comparison of these changes with those in table 1 and the findings from the Mercury program, it is obvious that little new physiological information was obtained from the Apollo project.

Table 2
Significant biomedical findings in the Apollo program

Vestibular disturbances
Post-flight dehydration and weight loss (recovery within one week)
Decreased orthostatic tolerance (tilt and LBNP tests)
Reduced post-flight exercise tolerance (first 3 days)
Decreased red blood cell mass (2-10%) and plasma volume (4-9%)

Adapted from Nicogossian (190), LBNP: Lower body negative pressure.

Once the goal of sending individuals to the moon had been realized, NASA introduced the Skylab program. The Skylab was a space habitat and in-flight laboratory capable of containing three astronauts for durations of up to three months. The maximal stay in the structure was 84 days, enabling the researchers to establish a time course of the physiological processes which occurred during extended microgravity exposure. The large structure housed exercise equipment and a lower body negative pressure (LBNP) box for in-flight testing of exercise capacity and orthostatic tolerance, respectively. Echocardiographic analysis was obtained with an onboard echocardiograph (see table 3 for summary of the findings). The primary insight of the Skylab experiments was the ability to differentiate the microgravity-induced physiological alterations which were self limiting from those which would continue throughout the exposure (67).

Between November, 1973 and the first Shuttle flight in April, 1981, no manned U.S. launched mission was attempted. However, from this date to present (except for a period of 33 months following the Challenger accident) numerous successful Shuttle flights have been performed.

Table 3
Cardiovascular changes identified during the Skylab Missions

Cardiovascular deconditioning observed during flight appeared to be adaptive and tended to stabilize after 4-6 weeks.
Cardiovascular changes did not impair crew health or ability to function effectively in flight.
In-flight LBNP tests provided a fairly reliable predictive index of post-flight cardiovascular status.
In-flight cardiac electrical activity was not altered.
Decreased cardiac output noted in crewmen post-flight; thought to be related to reduced blood volume.
No significant decrement in work capacity or physiologic responses to exercise during flight.
Decreased work capacity and altered physiological responses to exercise after flight.
Increased exercise by the Skylab crew was thought to be a factor in improving recovery rate.

Table adapted from Levy and Talbot (162).

The convenience of the Shuttle is its capability to hold a Spacelab module in its bay. Such a module could be equipped with numerous pieces of equipment depending on the experimentation planned. Thus far, three fully dedicated biomedical research flights have been realized, with two more planned in the future. With the use of these Spacelab modules, more intricate and invasive experimentation are being conducted enabling more mechanistic rather than descriptive research. An additional advantage of the Shuttle is the greater capability, when compared to its predecessor vehicles, to control and reproduce the environmental conditions (i.e. barometric

pressure, temperature, inspired gas concentrations, etc.) which the experiments are conducted.

Prior to using the Shuttle, the problem of orthostatic intolerance was acknowledged, but was not of great concern because it posed no real threat to the astronauts since they re-entered the earth's gravitational field in a Gx force vector (see figure 1) which does not predispose the astronauts to an orthostatic challenge. However, since re-entry in the Shuttle results in a Gz force vector greater than 1-g for approximately 17 minutes, concentrated efforts have been placed upon this problem as well as potential countermeasures.

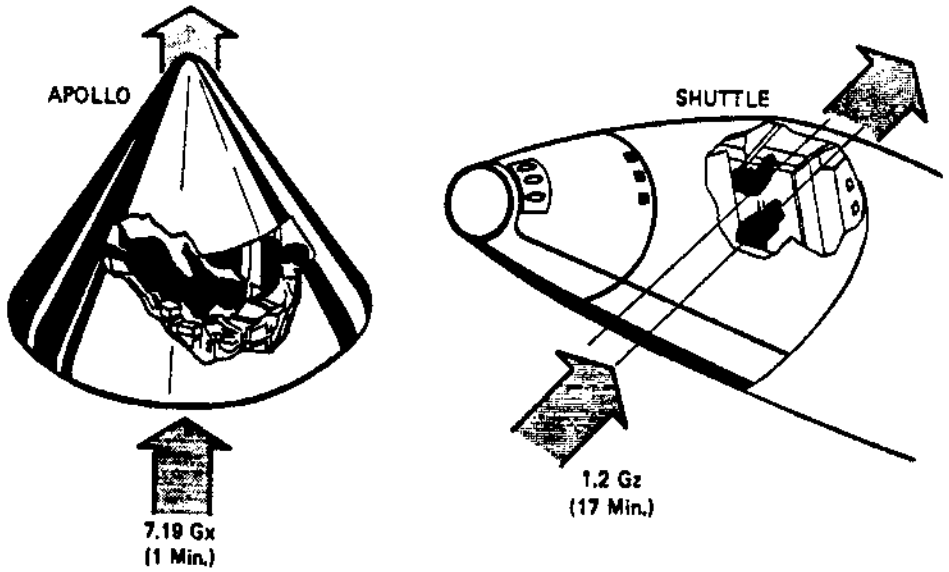

Figure 1: Comparison of the gravitational forces experience by astronauts in a capsule and Space Shuttle.

Soviet Space Program

The Soviet Space Agency, prior to the removal of the "Iron Curtain", was very active in space exploration and related research having placed over 140 astronauts in space for durations of up to 367 days over the past three decades (190). The Soviets have used capsules (similar to the Apollo capsules) with a connecting work compartment (Soyuz program), Salyut space stations, as well as the multi-modular Mir space station to conduct physiological research. As previously stated, traditionally the findings of this research have not been made available; only recently an increase in Soviet space related data is being presented to the scientific community. Results of some of the better known Soviet missions will be discussed in the subsequent sections.

Ground-Based Analogs of Microgravity Exposure

The cost of sending individuals and equipment into space is astronomical, this coupled with the limited number of subjects being sent on missions, necessitated the use of ground-based analogs of microgravity exposure. When considering the cardiovascular changes associated with weightlessness in humans, four analogs have traditionally been employed: a) bed rest, b) head-down tilt bed rest (HDT), c) water immersion, and d) hyperbolic aircraft flights.

Bed rest studies have been conducted since the mid-1800s resulting in volumes of data (for a comprehensive review see refs (81, 107, 170, 191)). However, it wasn't until the beginning of the U.S. and Soviet space programs that bed rest was used as an analog of microgravity exposure; primarily because the cardiovascular deconditioning observed during space flight was similar to that which occurred during bed rest (81).

More recently HDT, typically -4° to -6°, was introduced as a more effective means of inducing the cardiovascular changes associated with space flight (135). Since that study, numerous American and Soviet studies have further verified this technique (28, 52, 53, 192, 232, 252). The results of HDT are similar to bed rest, however, the changes are accelerated. Contrary to bed rest, HDT results in more pronounced fluid shifts along with the related symptoms of facial puffiness, nasal congestion, fullness of the head, and sensory realignments similar to that experienced by astronauts (189). These results were further verified by a joint 7-day bed rest study conducted by Soviets and Americans in which the effects of horizontal and 6° HDT were compared (94). In addition to the previously mentioned variables, this study suggested that 6° HDT resulted in a more pronounced cardiovascular deconditioning, as measured by post-bed rest LBNP and exercise tolerance, than bed rest alone.

Water and "dry" immersion have been used to observe the renal and circulatory changes which may occur during space flight (86). Unlike bed rest which removes the hydrostatic gradient, immersion mimics the microgravity-induced vascular changes by increasing the pressure around the vessels thereby translocating fluid thoracically. This invokes the same series of neurohumoral responses believed to occur during space flight, ultimately resulting in similar cardiovascular deconditioning but more rapidly. The primary problem with water immersion is the potential for skin maceration, making this technique impractical for durations of more than 24 hours (189). The Soviets have employed a technique called "dry immersion" in which the subjects are immersed, however, their skin is isolated from the water by a

thin plastic sheet, thus enabling long-term immersion studies. Results have demonstrated that this is an effective alternative to HDT (102).

Contrary to the previously described procedure, actual microgravity is possible with hyperbolic aircraft flights in which the investigators are repeatedly exposed to brief periods of weightlessness for durations of 30-40 seconds. Although this analog is the only "ground-based" analog which exposes the individual to 0-g, this technique is not useful to describe the physiological alterations due to long-term microgravity exposure.

Cardiovascular Alterations Occurring During Actual or Simulated Microgravity Exposure

Although microgravity exposure induces changes in many physiological systems such as muscle, bone, neurovestibular and cardiovascular, the primary focus of the research being presented is the cardiovascular system. Therefore the remainder of this chapter will be specific to the effects of simulated microgravity exposure on the cardiovascular system.

Fluid Shifts

Humans spend approximately 2/3 of their existence in the upright position. In the upright position the heart is 1.2 to 1.5 meters above the feet. Due to the vascular hydrostatic forces and the compliant nature of the veins, approximately 70 to 75 percent of the total blood volume is residing in veins below the level of the heart (216). However, upon initiation of either microgravity or an analog, the hydrostatic gradient is removed (or opposed in the case of immersion) resulting in more equal distribution of blood as well as intravascular pressures throughout the general circulation. An estimated

700-1500 ml of blood from the lower extremities and pelvic region is transferred to the thoracic circulation increasing cardiopulmonary blood volume (168) as well as slightly increasing blood volume to the vasculature of the arms and head (9, 35). This translocation of volume is reflected in an elevated central venous (28, 85, 192), pulmonary arterial and capillary-wedge pressures (165) and end-diastolic volumes (38). Evidence of this shift has been pictorially documented in astronauts (pre-flight versus in-flight) showing signs of facial edema, periorbital puffiness, thickening of the eyelids and distention of the jugular and facial veins (247). Multiple circumferential measurements of the legs and arms obtained pre-flight, in-flight and post-flight and converted to volumetric estimates were obtained. In-flight leg volumes decreased within the first 24 hours in space and continued to decrease through day 57 with the post-flight leg volume being lower than pre-flight, while arm volume changed very little (247). In support of these findings, acute HDT data have demonstrated changes in the Starling forces promoting filtration in the lower lip, suggesting an increased vascular hydrostatic component greater than the increased interstitial hydrostatic pressure (196). These data support the concept that microgravity exposure rapidly induces fluid shifts from the lower extremities to the upper regions of the body.

Plasma Volume

When interpreting the data of the immediate changes in plasma volume (PV) during simulated microgravity exposure one needs to be cautious of the position the individual is in during the control measurements. If the controlled condition is upright (usually seated) an

initial 6-7% increase in PV is observed within the first few hours of HDT (38, 99) or horizontal bed rest (255). In contrast, if the controlled condition is the supine position, no initial hemodilution occurs, rather hemoconcentration is observed (192, 230). The difference in the results of the two techniques is likely due to the customary thirty minute supine period prior to the evaluation of blood volume (113); during this time the hemodilution has occurred such that the controlled supine condition is conceivably at a higher PV than the controlled upright position. The initial hemodilution, when going from the upright to supine of HDT positions, is a result of decreased hydrostatic forces in the legs. For example, upon the initiation of HDT, capillary pressure in the toe goes from 90 mmHg (standing) to 20 mmHg (HDT) (81). Such a decreased capillary pressure will alter the Starling forces in the vasculature, resulting in a net reabsorption into the vascular space, promoting hemodilution (114). Further evidence is provided in a study by Hargens et al. (115) in which interstitial fluid loss in the legs was hypothesized to cause the observed reduction in interstitial fluid pressure. While it is clear that the Starling forces in the lower extremities result in a net reabsorption, the opposite effect occurs in the upper region of the body favoring net filtration. However, the amount of intravascular fluid gained by reabsorption in the lower regions is not offset by filtration in the upper regions probably due to the greater compliant nature of the vessels in the upper regions of the body.

As the duration of the exposure continues, a gradual reduction in PV is observed as a result of a reduction in thirst and a concomitant diuresis (78, 131, 156). The mechanisms causing this diuresis is unclear; initially it was

thought to be a result of the Gauer-Henry reflex. Gauer (88, 89) hypothesized that the normal operating condition for humans is the upright posture rather than the supine posture, such that any perturbation resulting in cardiovascular changes altering the normal operating condition would invoke a series of reflexes to return the condition similar to that which occurs while the individual is upright. As central blood volume is elevated, this hypothesis states that the low pressure mechanoreceptors (presumably the cardiopulmonary baroreceptors) are stimulated resulting in an increased vagal afferent traffic from these receptors. Such stimuli will decrease hypothalamic vasopressin release (64, 87), inhibit the renin-angiotensin system (167, 175, 220), reduce sympathetic nerve activity (173, 175), and decrease thirst (79, 240), all of which importantly affect fluid homeostasis.

However, more recent data has not verified the Gauer-Henry reflex to be the primary mechanism resulting in the loss of PV during simulated microgravity exposure, due to the lack of change or relatively little decrease in plasma vasopressin concentration (AVP), plasma renin activity (PRA), and plasma aldosterone concentration (ALD) during simulated microgravity experiments (6, 111, 192, 230). Further evidence of the lack of influence from the Gauer-Henry reflex was shown by Gilmore et al. (101) demonstrating that primates who underwent vagal and sino-aortic denervation failed to prevent diuresis in response to a 15% PV expansion. In addition, cardiac denervated patient's hormonal response to 6° HDT were similar to normal healthy counterparts (58).

Following actual microgravity exposure, these volume regulating hormones have not decreased, and in some cases even increased (125).

However, it should be noted that the blood samples for these hormones were not typically collected immediately after entering the microgravity environment, but are likely delayed.

More recent thinking regarding the mechanisms for the reduction in PV during space flight invoke the role of atrial natriuretic peptide (ANP). The stretch of the atria by the thoracic translocation of blood stimulates the release of ANP into the blood resulting in a natriuretic and diuretic effect (95). Various reports have demonstrated a transient increase (up to two-fold) in plasma ANP concentration during HDT (6, 98). However others have suggested that even these increases in plasma ANP concentration may not be sufficient to elicit the observed decreases in PV (3).

Other proposed hypotheses for the diuretic response are: a) a change in the adequacy of the responses of the kidney to the fluid-regulating hormones (185) resulting in a dissociation between plasma AVP concentration and free water clearance, and b) a decrease in total plasma protein content due to a reduced return of protein from the lymphatic system (249).

Red Blood Cells

As early as the Gemini flights, 5-20% decrease in red blood cell (RBC) mass was recorded. Skylab data indicate that RBC mass will continue to decrease until plateauing around 60 days in space (125). Similar reductions in RBC count have also been observed during actual space flight (156, 158) and may be a result of reduced erythropoietin which in one mission was reported to decrease 50% within 24 hours after launch (155). It should be noted that these changes in RBC count and erythropoietin occurred in a 100% oxygen environment, however, reductions in RBC mass have been observed in the

absence of hyperoxia (156). Moreover, in bed rest studies of durations greater than two weeks, significant reductions in RBC mass have been documented (37, 143). The likely mechanisms for the reduction in RBC mass may be due to reduced cell synthesis subsequent to reduced erythropoietin, increased destruction of RBC evidenced from increased ferritin (155), production of inhibitors of RBC production (243), splenic trapping (131) or changes in bone marrow sensitivity to erythropoietin (243).

Cardiac Function

Cardiac Size. Pre and post flight data using standard chest X-rays of Skylab crew members demonstrated "modest" decreases in cardiothoracic (C/T) ratios. These data combined with data from flights onboard Mercury, Gemini and Apollo space crafts demonstrated a C/T ratio reduction of 0.018, approximately 5%, (186) indicating that heart size had decreased during flight (121). However, it has been suggested that the decrement in the C/T ratio was a result of decreased ventricular volumes and not due to a "loss in thickness of the cardiac muscle" (19). With the use of more modern echocardiography equipment, an estimated 11% reduction in left ventricular mass (pre- versus post-flight) has been estimated (35). Direct estimations of rat cardiac mass during a form of simulated microgravity (immobilization) have resulted in a 24% reduction in left ventricular mass and a 26% reduction in right ventricular mass following 20 days of exposure (151, 224).

Using in-flight echocardiographic data from four crew members of a Shuttle flight, ventricular volume data as early as four hours into the flight were obtained (35). Left ventricular diastolic volume index as well as stroke volume index were elevated on flight day one, then decreased to 15% of pre-

flight levels. Right ventricular dimensions were also decreased in-flight by 30%. Similar changes in ventricular volumes have been obtained during simulated microgravity exposure, that is increased volumes initially (8, 28, 192), followed by decreased volumes with extended exposure (8, 14, 152, 219). Such changes were suggested to be associated with the changes in intravascular volume rather than morphological factors, since end-diastolic diameters rapidly returned to normal following exposure (14).

Cardiac Output. With simulated microgravity, there is an initial gradual increase in cardiac filling pressure and stroke volume (SV) (85, 166, 168). Cardiac output ($\dot{Q}$) has been shown to increase (38, 85, 166, 168) or stay the same (28, 165, 192) depending on the degree of vasodilation and bradycardia which may occur as a result of the increases in thoracic filling, or depending upon which position (i.e. upright or supine) was defined as the control position (81).

As the bed rest or HDT duration continues, the intravascular volume decreases resulting in decreased SV and a concomitant reduction in $\dot{Q}$, even though heart rate was elevated post-exposure (7, 8, 15, 207), suggesting greater relative reductions in SV. Interestingly, in-flight $\dot{Q}$s aboard a French-Soviet space flight (Aragatz) of one astronaut via echocardiography indicated a reduction in SV of 10%, however, HR increased approximately 25% resulting in little change or a slight increase in $\dot{Q}$ (8) depending upon the day the measurement was obtained. The discrepancies between this and the comparative 28 day HDT study were not discussed in the paper. Further in-flight studies (7 day flights) of three astronauts similarly showed a decreased SV and an increased HR in all astronauts, however, $\dot{Q}$ was elevated

in one astronaut and decreased in the other astronauts (207). It appears, therefore, that the resultant change in $\dot{Q}$ is a function of whether the HR can increase sufficiently to counter the volume-induced decrease in SV during flight.

Cardiac Contractility. Acute simulated microgravity exposure has not demonstrated any significant reduction in indices of cardiac contractility measured with echocardiography (28, 29, 168, 192). Nixon et al. (192). found small increases in left ventricular function with no change in heart rate, arterial pressure or the velocity of circumferential fiber shortening within 24 hours of HDT. These data are compatible with the Frank-Starling mechanism as a result of the increased pre-load, without changes in ventricular contractility.

As the duration of actual or simulated microgravity exposure continues, conclusions regarding myocardial contractility become less clear. Following ten days of bed rest, Goldwater et al. (104) found no significant alterations in the cardiac function curve obtained with echocardiography and LBNP. These data are supported by Georgizyevskiy et al. (96) who also saw no differences in myocardial contractility in individuals following 62 days of bed rest. Contrary findings demonstrating a decreased contractility, have been observed by Hung et al. (124), Baisch et al. (14) and Katkov et al. (136) following bed rest of ten days, or HDT of seven and five days, respectively. However in a subsequent study of Katkov et al. (137), no change in contractile state was found after 5-6 days of 15° HDT.

No direct arterial catheterization in humans has occurred during actual space flight with the intent to determine indices of myocardial contractile

function. However, studies primarily constructing ventricular function curves with echocardiography and LBNP, have indicated little change in contractile function (93, 121).

Therefore, actual or simulated microgravity either does not change, or slightly decreases myocardial contractile function.

Cardiovascular Autonomic Regulation

Heart Rate. Subjects of extended bed rest studies frequently demonstrate an increase in HR as the duration of the exposure increases (8, 16, 24, 27, 96, 127, 183, 204). An average 0.5 bpm/day increase in HR has been observed with durations of bed rest of 30 days (244), while a 62-day study demonstrated a 25 bpm increase in HR by the end of the study (224). Conversely, studies have reported no change in HR as a result of HDT exposure (17).

Increases in HR have been observed following, as well as during space flight (8, 35, 83, 123), however others have reported HRs "practically within preflight limits" (110) during flight and HR being accelerated upon re-adaptation to the 1-g environment (182).

The mechanisms for the cardio-acceleration observed during simulated and actual microgravity exposure are still unknown, but are likely related to autonomic adjustments occurring during the exposure (81). Goldberger et al. (103) hypothesized that the accelerated HRs were due to decreased parasympathetic nervous system activation since 7-10 days of HDT induced significant reductions in heart rate variability (an index of vagal activation) in subjects given atropine. However, two confounding influences cloud these data: a) the heart rate variability without atropine (i.e. controlled condition)

was not affected by the HDT exposure, and b) the data which were retrospectively analyzed, were obtained during LBNP exposure, which is known to increase sympathetic and decrease parasympathetic nervous system activation because of arterial baroreceptors unloading (133, 248, 250). This finding is in contrast to the work of Beregovkin et al. (17) who observed a three-fold increase in respiratory arrhythmia during bed rest suggesting increased parasympathetic nervous system function. Not surprisingly, these investigators did not demonstrate an increased HR in the three subjects who underwent 30 days of 4° HDT. Further support for an increase in parasympathetic nervous system function was presented by Davydova et al. (61), when they recorded elevated resting whole blood acetylcholine concentrations during 120 days of 4.5° HDT.

Sullivan et al. (241) suggested that post-HDT cardio-acceleration is not a result of depressed cardiac muscarinic receptor mechanisms because the change in HR due to an infusion of atropine sulfate was not altered following three weeks of HDT.

Blood Pressure. Blood pressure has been reported to increase (42, 96, 132, 134) or not change (54, 100, 105) following simulated or actual microgravity exposure. Assuming blood pressure was elevated to some extent following such exposure, the mechanisms for this change are likely to be related to increases in total peripheral resistance (TPR) as $\dot{Q}$ remains the same or decreases slightly following microgravity exposure (see above). Factors resulting in this increased TPR are likely related to increased concentrations of vasoactive hormones, stress-relaxation properties or decreased concentrations of endothelial derived relaxing factor.

Repeated bed rest studies have demonstrated either no change (44, 54, 202) or a decrease (59, 61, 100, 226) in blood and urinary catecholamine concentrations. Similar findings have been observed during space flight (125, 154). Although the concentration of vasopressin (AVP) as well as plasma renin activity (PRA) are known to remain the same or slightly decrease during HDT exposure (6, 98, 111), during space flight their concentrations have been shown to slightly increase (125) or decrease (157). No work has been reported regarding the effects of actual or simulated microgravity exposure on circulatory vasodilator concentrations. Therefore, the changes in TPR as a result of HDT exposure are not thought to be a result of increased circulating concentrations of catecholamines or other vasoactive hormones. However, the effects of the changes in the plasma concentration of these vasoactive hormones on TPR in-flight is unknown.

Autonomic receptor function has traditionally been determined by measuring cardiovascular responses to selective infusions of receptor agonists and antagonists. Graded doses of both norepinephrine and angiotensin II were administered to individuals following 2-3 weeks of bed rest (44). Interestingly, the infusion required to elicit a given increase in blood pressure was not significantly altered, indicating no change in post-junctional receptor function. When similar infusions of tyramine were administered after twelve days of bed rest, vasoconstrictor responses were attenuated (226). Since tyramine has no significant direct action on vessels, and its effect is produced solely by releasing norepinephrine from the nerve endings (82), this finding suggests a decreased synthesis, storage or an increased uptake of norepinephrine in the pre-junctional nerve terminals following bed rest.

However, these findings are not supportive of the increased TPR observed in studies in which blood pressure was elevated and $\dot{Q}$ reduced.

Baroreceptor Function

As the primary focus of this paper is the investigation of baroreflex function following simulated microgravity exposure, a more comprehensive review of the baroreceptors and their function will be provided. Baroreceptors participate in the reflex control of blood pressure by being a component part of the baroreflex arc. A reflex arc includes a sensory component (the afferent receptor), afferent nerves (which in the case of baroreceptors travel to the nucleus tractus solitarius of the medulla), central integration, efferent nerve fibers of the sympathetic and parasympathetic nervous system, and efferent organ(s) (which, in the case of the arterial baroreceptors, is the heart and the smooth muscle of the vasculature).

Arterial Baroreceptors

This section will focus primarily on the arterial baroreceptors, or in other words, the baroreceptors on the arterial side of the circulation, namely the carotid and aortic baroreceptors.

Carotid Baroreceptors. The carotid baroreceptors are located primarily on the internal carotid artery just distal to the bifurcation of the common carotid arteries. However, in humans, receptors may also be located at the bifurcation and at the external carotid arteries (23). Due to their accessibility relative to the aortic baroreflexes, much more research has been focused on these receptors in humans. The region of the carotid baroreceptors is innervated by a branch of the glossopharyngeal nerve (cranial nerve IX) called the Hering nerve (69). These small myelinated fibers (2-4 mm) innervate the

thin walls of the sinus and terminate adjacent to the media (97). The smooth muscle content of these areas is known to be reduced (13, 209), this coupled with an increased elastin (12), results in a more compliant region when compared to the surrounding vasculature.

These baroreceptors are actually stretch receptors that are stimulated not only by the absolute deformation (mean pressure), but also the change in deformation (pulsatile pressure) of the adventitial tissue in which the nerve endings reside (41, 69, 257). These findings were eloquently demonstrated by Hauss et al. (118) who showed that when the carotid sinus was "imprisoned" in plaster of Paris, so that it could not deform due to the pressure within the sinus, the reflex was not elicited. Therefore, anything that will distend the carotid sinus, such as increased intravascular pressure or decreased tissue pressure surrounding the carotid sinus, will invoke the reflex.

Aortic baroreceptors. The aortic baroreceptors have been located in the region of the aortic arch and the roots of major arterial branches from the aorta in the dog, whereas additional receptors have been located in the common carotid artery in the cat and rabbit (11). The aortic baroreceptors are innervated with thick myelinated fibers of the aortic depressor branch of the vagus nerve. These fibers similarly terminate in the neurofibril end plates of the medial-adventitial interface (2). The aortic baroreceptors also function as stretch receptors, however, in contrast to the carotid baroreceptors, there is no reduction in smooth muscle around the aortic baroreceptors (69).

Physiology of the arterial baroreceptors. The carotid and aortic baroreceptors, being located on the arterial side of the circulation, are identified as arterial baroreceptors. The afferent fibers from these receptors

synapse within the nucleus tractus solitarius (NTS) in the medulla (60, 146, 210). The efferent limb of these reflex arcs include sympathetic pre-ganglionic neurons in the intermediolateral cell columns of the thoracic spinal cord, and vagal neurons, in the nucleus ambiguus and dorsal motor nucleus of the vagus (237).

In 1931 Koch (145) demonstrated that mean arterial pressure exhibited an inverse sigmoidal relationship with intrasinus (carotid) pressure when intrasinus pressure was changed in a stepwise manner. These findings were further substantiated by Bronk and Stella (33) who found that the impulse frequency in the Hering nerve exhibited a positive sigmoidal relationship to changes in static sinus pressure. Thus, these and other findings have established three distinguishing features of baroreceptor discharge characteristics (150): a) a threshold pressure at which baroreceptors begin to discharge; b) a pressure range for which the discharge rate increases with a rise in the mean arterial blood pressure; and c) an asymptotic pressure beyond which there is little increase in baroreceptor discharge.

When comparing the functional differences between the carotid and aortic baroreceptors, the threshold for stimulation of the aortic baroreceptors has been determined to be substantially higher than that of the carotid baroreceptors. Pelletier et al. (201) has determined that the threshold pressure sufficient to change activity in the aortic depressor nerve was 95 mmHg, whereas the pressure for the carotid sinus nerve (Hering nerve) was 62 mmHg in the dog. These authors suggested that the aortic baroreceptors primarily buffer hypertensive pressures and are less effective in buffering a reduction in systemic blood pressure below normal levels (200).

No work has been conducted to determine if the aforementioned mentioned characteristics also apply to humans. However, studies have been conducted to determine the relative contribution of the aortic and carotid baroreflexes to systemic blood pressure regulation. The first of which was presented by Mancia et al. (174) who concluded that the extra-carotid baroreceptors play a more important role than the carotid baroreceptors in the control of heart rate in man. These findings have been substantiated by Ferguson et al. (77) and Shi et al. (229) using a technique which employs a combination of an alpha agonist, LBNP, and carotid sinus neck pressure to isolate the aortic baroreflex. Similarly, Sanders et al. (221) determined that in the human, the aortic baroreflex also predominated in blood pressure regulation over the carotid baroreflex, using muscle sympathetic nerve activity as an index of vasomotion.

The arterial baroreceptors are known to have the capacity to reset due to chronic and acute perturbations. Chronic resetting was first observed by McCubbin et al. (180) who observed changes in carotid sinus nerve activity to changes in intrasinus pressures of 60, 120 and 240 mmHg in normotensive and chronic-renal hypertensive dogs. The intrasinus pressure of 60 mmHg demonstrated intermittent bursts in the control dogs without any change in nerve activity of the hypertensive dogs. At 120 mmHg, both the hypertensive and control dogs demonstrated intermittent nerve activity. Interestingly, at 240 mmHg, the control dogs demonstrated continuous nerve activity, whereas the hypertensive dogs were still displaying intermittent nerve activity. The authors proposed three mechanisms to account for the resetting of the baroreceptors in hypertension: a) alteration in the natural distensibility

of the carotid sinus; b) inactivation of some of the fibers having lower threshold pressures; and c) adaptation of the baroreceptors to increased pressure (being a phenomenon which may be related to the change in the threshold pressure and sensitivity of the receptors). Similar shifts in aortic baroreflex threshold and sensitivity have been found in one-kidney Page hypertensive rabbits (1) and in spontaneously hypertensive rats (4). Such resetting has also been observed following acute hypertension preconditioning for 5-20 minutes (47, 68, 120). These acute resetting investigations focused specifically on afferent receptor resetting. It should be emphasized that resetting of a complete baroreflex loop could occur as a result of changes in the afferent sensory receptors, central integration, efferent neurons, end-organ responses, or a combination of these mechanisms.

As previously mentioned, the aortic and carotid baroreceptors exert their influences through sympathetic and parasympathetic nerve fibers. However the responses of the parasympathetic and sympathetic systems do not have the same speed of transmission through central pathways. The latency between electrical stimulation of the carotid sinus or aortic nerve and activation of vagal cardiac branches was 30-90 ms (129, 153). This is in contrast to a 180-240 ms lag time between carotid sinus nerve stimulation and the onset of inhibition of sympathetic nerve activity (211). Therefore, the response to an increased blood pressure will result in an increase in cardiac vagal activity much faster than the corresponding sympathetically mediated reduction in blood pressure.

The arterial baroreceptors act to maintain blood pressure constant around an operating point (225). When blood pressure varies around this

operating point, the reflexes are invoked to return blood pressure to the operating point. Changes in blood pressure are detected at the carotid sinus and aortic baroreceptors. The nerve impulses from these structures travel to the NTS of the medulla. Sympathetic efferent nerve activity innervating the sino-atrial (SA) and atrial-ventricular (AV) nodes, myocardium, vascular smooth muscle and the adrenal medulla result in an increase in HR, myocardial contractility, TPR and epinephrine release. Moreover, baroreflex induced sympathetic stimulation has been demonstrated to affect splanchnic (214), cutaneous (141) and venous circulations (231).

Vagal efferent nerves primarily innervate the SA and AV nodes resulting in a decreased HR with increased nerve activity. The HR responses to vagal stimulation are very rapid initiating in less than a second, whereas changes in HR invoked by sympathetic stimulation take five seconds or more (161).

A gain of a controlled system can be expressed as the ratio of a change in output to a change in input (218). When identifying the baroreflex gains, both open loop and closed loop gains must be understood. An open loop gain is determined when the input is changed (i.e. pressure in an isolated carotid sinus) and the output (resultant blood pressure) is measured. This is in contrast to a closed looped gain in which the input and output are connected in an intact organism (225). These definitions will be helpful in understanding the methods used to determine baroreflex sensitivity in the subsequent section.

Methods of assessing baroreflex sensitivity in humans (selective).

Carotid baroreflex assessment will vary depending upon the invasiveness of the study. Perturbations such as direct electrical nerve stimulation (253) or temporary denervation with anesthesia (112) or permanently with surgical ablation (5) have been performed on humans. All of these procedures had potential problems in addition to the degree of invasiveness (198).

Clinically, carotid massage has been used to treat occurrences such as superventricular tachycardia (32). A variation of this technique was used by Roddie and Shepherd (213) who instructed subjects to compress their carotid arteries while observing the resultant changes in blood pressure. However, this method does not afford one the ability to quantify the changes which occur at the carotid sinus, therefore is of little use as a research tool.

Neck pressure/suction has been used to transmit pressure or suction through the tissue of the neck and thereby artificially produce changes in carotid distending pressure (76, 238). More recently, Eckberg et al. (71) developed a system in which rapid changes in neck pressure and neck suction are delivered to the anterior 2/3 of the subject's neck. This process allowed quantification of the carotid baroreflex using 10-15 seconds of data collection by assessing the resultant changes in RRI (or blood pressure) and plotting these changes against estimated carotid sinus pressure (see the methods for a more complete explanation of the procedure) to completely characterize the stimulus-response curve (236). Since this procedure results in a rapid beat-by-beat change in the RRI, it has been used as an index of an opened looped carotid baroreflex response.

The primary limitation of this technique is the lack of quantification of transmission of the pressure/suction through the tissue of the neck. Ludbrook et al. (169) inserted catheters in a jugular vein beneath the collar and another catheter percutaneously adjacent to the intra-jugular catheter in order to determine the transmural pressure across the carotid sinus. They determined that 86% of the applied pressure, and 64% of the applied suction was transmitted to the carotid sinus. However, it is likely that individuals differ in transmission characteristics, furthermore, the collar used by Ludbrook et al. (169) during these procedures is quite different than the current collars used in most research laboratories.

Method of assessing aortic baroreflex sensitivity in humans. The isolated aortic baroreflex can be assessed using the techniques developed in Mark's laboratory (77, 221) in which they produced a sustained increase in arterial pressure using a steady-state phenylephrine infusion. Subsequently, carotid sinus pressure was maintained at control levels by the application of neck pressure at levels equal to the elevation in blood pressure (after correcting for transmission characteristics), while the increases in CVP were counteracted with LBNP. During this period of aortic baroreceptor isolation (i.e. when neck pressure and LBNP were applied during steady state infusion of phenylephrine) the ratio of the change in HR to the change in MAP, or the ratio of the change in muscle sympathetic nerve activity to the change in MAP can be used as an index of aortic baroreflex sensitivity. No additional methods have been devised to assess the aortic baroreflex in the human.

Methods of assessing baroreflex sensitivity in humans (non-selective). A variety of non-selective perturbations have been used to assess global

baroreflex function. However the primary drawback with these procedures is that all baroreceptor populations are likely perturbed, therefore, conclusions cannot be made regarding a single baroreceptor population. Furthermore, since these methods use steady state conditions, they can only be interpreted as closed looped responses.

Probably the most common technique to assess baroreflex function is the use of vasoactive drugs. This method involves infusing a vasoactive (pressor or depressor) substance into an individual while observing the resultant changes in HR and blood pressure. Pressor substances such as norepinephrine, angiotensin II and phenylephrine have traditionally been used, while depressor agents such as trinitroglycerin, amyl nitrate or sodium nitroprusside have been employed (198). The primary drawback of these methods is that since vasoactive drugs are administered, only baroreflex control of HR and not blood pressure can be assessed. However, techniques such as muscle sympathetic nerve activity are being used as an index of baroreflex mediated changes in vascular resistance (221).

Passive perturbations such as LBNP (234) and head-up tilting (233) have been used to assess baroreflex function. Typically these procedures involve the assessment of the change in heart rate to the change in blood pressure induced by the LBNP or head-up tilt, with the slope of this relationship being indicative of baroreflex function. However, Smith et al. (235) demonstrated that blood pressure increases were augmented greater than that which could be accounted for by venous return when the subjects were asked to perform low-level isometric exercise. These findings may

confound the use of tests such as the stand test as a test of baroreflex function when postural muscle are contracted (184).

Cardiopulmonary Baroreceptors

In contrast to the relatively discrete locations of the arterial baroreceptors, the cardiopulmonary baroreceptors are much more diffuse. These receptors are comprised primarily of vagal C-fibers which innervate the great veins of the thorax, atria, heart and pulmonary vasculature (177). These afferent nerve fibers travel via the vagus nerve to the NTS of the medulla and tonically inhibit vasomotor centers (203). Activation of these receptors by volume expansion (212) or a balloon distending the atria (208) result in reflex reduction in renal sympathetic nerve activity and subsequent diuresis. Conversely, reductions in blood volume result in stimulation of sympathetic nerve activity (245). Therefore, these receptors are thought to monitor central venous pressure to ensure adequate blood is available to be pumped through the heart into the arterial circulation, for this reason they have been called the low pressure baroreceptors.

In humans, arguably the best known cardiopulmonary baroreflex response is the vasoconstrictor effect of non-hypotensive reductions in central blood volume and cardiac filling pressure. Importantly, the decreased central blood volume and cardiac filling pressure must not be sufficient to change arterial blood pressure, thus it is termed a non-hypotensive reduction. This limitation was investigated by Johnson et al. (130) using a slow continuous ramp of LBNP at a rate of 1 torr·minute^{-1} to invoke central hypovolemia, while simultaneously monitoring heart rate, right atrial pressure, aortic pressure, pulse pressure and the maximal rate of rise of aortic

pressure. They demonstrated that from 0 to -20 torr LBNP no detectable change in these parameters were observed, suggesting that these levels of exposure do not change the stimulus to the arterial baroreceptors. However, forearm blood flow decreased progressively with the LBNP. Since the arterial baroreceptors were not perturbed, the reduction in forearm blood flow was a result of the cardiopulmonary baroreflex.

The sensitivity of the cardiopulmonary baroreflex has traditionally been assessed by comparing the changes in central venous pressure with the increases in forearm vascular resistance, with the resultant slope of this relationship being an index of baroreflex sensitivity (172).

Interaction of the Cardiopulmonary and Carotid Baroreflexes

Animal investigations have found that cardiopulmonary afferents affect carotid baroreflex function. For example, vagal deafferentation in dogs augmented the vasoconstriction invoked by carotid sinus hypotension (147). Moreover, the blood pressure responses to carotid occlusion was less than normal during hypervolemia; the opposite was also the case (39). Further evidence is provided by Shepherd (228) in which the response of the carotid baroreflex was assessed with vagal cooling (reducing cardiopulmonary input) with the aortic nerve cut. This investigator concluded that a maximal response to the vagal cooling occurred when the carotid sinus pressure was lowest, and an elevated carotid sinus pressure combined with the vagal cooling led to a less pronounced response due to the divergence of the signals. However, Chen et al. (43) found that cardiopulmonary baroreceptor vagal nerve activity did not affect the carotid sinus baroreflex gain between carotid

sinus pressures of 100 and 150 mmHg, but reduced its gain below 100 mmHg. Thus, the findings of Shepherd (228) may not be due to the divergence of the signals, rather the reduction in carotid sinus pressure.

In humans, Victor and Mark demonstrated that neck pressure potentiated the forearm vasoconstrictor response to low level LBNP (251). Pawelczyk and Raven (199) conclusively demonstrated the interaction of the cardiopulmonary and carotid baroreflexes in man. Using LBNP, they reduced central venous pressure at six stages to -50 torr while assessing the sensitivity of the carotid-cardiac and carotid-vascular baroreflexes. They determined that the reduced central venous pressure and/or reduced blood volume invoked by LBNP augmented both HR and blood pressure carotid baroreflex responses by reducing the tonic inhibitory influence (203) from the cardiopulmonary baroreflex.

Baroreflex Function and Simulated or Actual Microgravity Exposure

Multiple animal studies and three human studies have investigated the effects of simulated and actual microgravity exposure on baroreflex function. Care must be taken in interpreting these data since the animal studies have classically observed the closed loop global baroreflex function by administering a vasoactive drug, whereas the human studies have investigated an open loop carotid-cardiac baroreflex function.

Animal studies. The most commonly used animal model for microgravity research is the hindquarters suspension model in rats (194), because the hemodynamic and blood volume responses were comparable to that of a human exposed to simulated microgravity (29, 222). This model

involves suspending the hindquarters of the rat in a harness resulting in a 20-30° head-down position. Such a procedure was performed for 24 hours on rats while baroreceptor sensitivity was assessed by calculating the change in HR to the change in blood pressure relationship to graded doses of phenylephrine and sodium nitroprusside (179). These investigators (179) demonstrated a reduction in this relationship, suggesting that the baroreflex sensitivity was attenuated. Such findings are not universal. Using similar techniques, for durations of 9, 90, and 120 days, a slight increase (164) or no change (194) in the baroreflex sensitivities were observed. Baroreflex function was assessed on rhesus monkeys prior to, and following, 28 days of horizontal casting from the axilla to the ankles (a method of simulated microgravity) (66). These investigators (21) determined that the gain of the baroreflex was 50% depressed post-casting.

Human studies. Both Convertino et al. (54) and Eckberg et al. (72) have determined the open loop carotid-cardiac baroreflex gain of humans using the neck pressure/suction technique previously described. Eckberg et al. (72) determined that the RRI response and maximal slope of the carotid baroreflex response was not affected by 10 days of head-down tilt bed rest. Interestingly, when Convertino et al. (54) repeated the study, however exposing the subjects to 30 days head-down tilt bed rest, they demonstrated significant reductions in the maximal slope of the carotid-cardiac baroreflex at day 12, with the decrement persisting until day 30. Furthermore, the two subjects with the greatest reduction in maximal slope of this response also had the greatest post-bed rest orthostatic intolerance. These findings corroborate recent findings of Shuttle astronauts prior to and following 4-5 days flight, resulting

in a reduction of the maximal carotid-cardiac baroreflex gain from 5.0±1.0 pre-flight to 3.4±0.5 ms/mmHg post-flight (83).

In summary, the animal data, investigating global closed looped baroreflexes demonstrate mixed results with the longer duration models suggesting no change or a slight increase in baroreceptor function. These data are in contrast to the open looped data of the carotid baroreflex in humans, which suggest diminished carotid baroreflex function following actual or simulated microgravity.

Orthostatic Intolerance

A primary concern of many within the United States space program is the reoccurring orthostatic intolerance frequently observed following simulated and actual microgravity exposure. The concern stems from two issues: a) will orthostatic intolerance affect the pilot's ability to safely land the Shuttle; and b) if there was a problem in which an emergency egress from the Shuttle is required, would the astronauts be able to exit the Shuttle without assistance from emergency personnel. This problem was first recognized following the 34 hour Mercury flight of David Simmons in 1957, and since has been the focus of many research projects both on the ground and in space (17, 26, 54, 108, 116, 128, 135).

In a recent review article, Nicogossian et al. (188) cited 30 to 50 percent of the astronauts studied following missions of eight days or less exhibited orthostatic intolerance manifested by increased heart rates, decreased blood pressure and clinical symptoms of syncope or near syncope. However, following a 9 day mission, Gaffney et al. (84) reported significant decreases in orthostatic intolerance in all seven crew members.

Of particular concern is the lack of a concrete functional definition of orthostatic intolerance (81). After two weeks of bed rest, during a stand test, the increase in HR and decrease in SV were almost double the respective changes pre-bed rest (117). However, higher HRs and lower SVs have not been proven to be indicative of orthostatic hypotension. Miller et al. (183) demonstrated that 100% of the subjects exhibited an elevated HR during a 30 minute 90° head-up tilt test following 4 weeks of bed rest exposure, however, only 42% actually exhibited presyncopal symptoms. Fortney et al. (80) measured LBNP tolerance following 13 days of 6° HDT; although all ten subjects had significantly elevated HRs during the LBNP test post-HDT, only five of the subjects decreased the LBNP level tolerated. It is clear that the elevated HR is a compensatory response during orthostatic stress following bed rest, it may not be appropriate to ascribe the same response as being indicative of orthostatic intolerance. Therefore, when reviewing studies in which orthostatic tolerance is assessed, one must be aware that the definition of orthostatic tolerance may vary between laboratories.

In discussing orthostatic intolerance, one needs to recall the basic physiological equation defining blood pressure regulation:

$$MAP = HR * SV * TPR$$

where MAP is mean arterial pressure, HR is heart rate, SV is stroke volume and TPR is total peripheral resistance. Thus, changes in the physiological state which affect one of the variables controlling blood pressure during orthostatic stress, without an appropriate compensation from the others, will predispose the individual to orthostatic intolerance. Factors influencing orthostatic tolerance, and therefore, the variables in the preceding equation,

have been suggested to include decreased blood volume, increased compliance of the vessels in the lower body, decreased baroreceptor function and altered autonomic function (29).

Initially, the primary focus was placed upon the changes in blood volume which occur during space flight (126, 128), however, in-flight countermeasures which should maintain blood volume have not consistently maintained orthostatic tolerance (80, 108, 239, 241). Moreover, when saline was infused sufficient to return central venous pressure to pre-HDT pressure, orthostatic tolerance was not affected (28). Conversely, investigators have reported improved orthostatic tolerance when PV was returned to pre-HDT conditions (31, 109, 128).

More recently, greater emphasis has been placed upon potential decrements in baroreceptor function (54, 83), compliance of the vessels of the lower limbs (55, 181) and cardiac dynamics (159) in an attempt to explain the decrements in orthostatic tolerance following microgravity exposure.

Exercise Capacity

Exercise capacity, defined as the level of maximal oxygen uptake ($\dot{V}O_{2max}$) attained during dynamic exercise, is known to be reduced following simulated or actual microgravity exposure (for a comprehensive review, see ref. (51)). The most pronounced reduction in $\dot{V}O_{2max}$, 26.4% and 34.6% have been reported following 20 and 30 days of bed rest respectively (139, 219). Similar reductions, however not of the same magnitude, have been reported following actual microgravity exposure (217). Interestingly, with the longer duration Skylab flights in which the astronauts performed regular exercise, decrements in $\dot{V}O_{2max}$ were not observed in-flight or post-flight (217), and

actually increased in some astronauts. Also of interest, those astronauts and cosmonauts who performed regular aerobic exercise in space, required less time post-landing for their cardiovascular parameters to return to normal (35).

Factors adversely affecting $\dot{V}O_{2max}$ following real or simulated microgravity exposure may include reduced blood volume, ranging from 4-16% (78, 131, 156); impaired maximal SV and $\dot{Q}$, possibly a result of the reduced blood volume and changes in cardiac hemodynamics (34, 50, 182); and cellular mechanisms at the skeletal muscle evidenced by greater acidosis for a given workload (56, 138, 182). Changes in pulmonary function is not likely implicated in the reduction in $\dot{V}O_{2max}$, since gas exchange during exercise in space or on the ground is not altered (135, 182).

Summary and Conclusion

Since the inception of the U.S. and Soviet space era in the early 1960's, an extraordinary amount of resources have been used in an effort to identifying the effects and associated mechanisms of microgravity exposure. Due to the apparent self-limiting nature of the effects of microgravity exposure on the cardiovascular system, prolonged space flight is not expected to adversely affect the cardiovascular system while journeying to locations such as Mars. For these reasons, it has been suggested that the astronaut's problem is not the adaptation to space flight, rather the effects of space flight on the re-adaptation to the earth's gravitational field.

Therefore, of paramount importance to space agencies such as NASA, is the development of countermeasures to be used by the astronauts throughout the microgravity exposure to minimize the physiological changes

that occur during space flight. Before such countermeasures can be developed, greater knowledge needs to be obtained to clarify as well as identify the effects of microgravity exposure on the human physiological systems.

CHAPTER III

PROCEDURES AND METHODS

The aim of this project was to investigate the effects of simulated microgravity exposure on aortic, carotid and cardiopulmonary baroreflex function, and to determine if such exposure influences cardiopulmonary-carotid baroreflex interaction. The duration of the simulated microgravity procedure was chosen to equal the maximal amount of time an individual could remain in space using an extended duration orbitor mission from the current orbitor fleet. Eight men with ages similar to the current astronaut corps (28-48 years), were selected to participate in the study. All but one of these subjects had participated in at least two previous HDT studies of durations greater than seven days. Prior to the initiation of the HDT position, descriptive metabolic and cardiovascular data were obtained on each subject, and additionally, each subject was familiarized with the procedures used in the study by experiencing a "dry run" exposure to the techniques.

Subjects

Volunteers were recruited from the subject pool at NASA's Ames Research Center as well as the San Francisco Bay Area. Prospective subjects were excluded if they did not conform to the descriptive criteria previously set (men, aged 25-50 years), or exhibited abnormal responses during either the physical exam, echocardiographic screening or exercise stress test. Two prospective subjects were excluded due to various complications which were

diagnosed during the initial screening procedures.

Each subject was thoroughly informed of the techniques to be used, as well as the purpose of these techniques, and each signed an informed consent. All procedures were approved by the Internal Review Board For Use of Human Subjects at both NASA-Kennedy Space Center and NASA-Ames Research Center. In addition, all measurement techniques performed by the principle investigator were approved by the Institutional Review Board of the Texas College of Osteopathic Medicine. Each subject completed a medical history and underwent a physical exam conducted by a clinic not associated with NASA-Ames. This exam included a resting 12-lead electrocardiogram (ECG) and a graded exercise test. Furthermore, a resting echocardiogram was performed, and another graded exercise test was administered to volitional fatigue to determine peak oxygen uptake ($\dot{V}O_{2peak}$).

Of the eight subjects chosen, only two participated in regular aerobic exercise. Their exercise regimens were comprised primarily of running at least four times a week, whereas the other six subjects did not perform any regular aerobic exercise.

Procedures

Test Days

Within the two weeks prior to the head-down tilt (HDT) exposure, the subjects reported to the laboratory to be oriented and familiarized with the techniques and procedures used during the HDT. The actual experimental protocol for the study consisted of three days ambulatory control, sixteen days bed rest in the 6° HDT position, and two days of ambulatory recovery. HDT exposure days and their corresponding tests are listed in Table 4.

Table 4
General Protocol

Day	Procedure
Control day 2	α_1 sensitivity Cardiac output
Control day 3	Aortic baroreflex Cardiopulmonary baroreflex Carotid baroreflex Cardiopulmonary-carotid baroreflex interaction
Control day 4	Plasma volume Estimation of cardiac autonomic function
Bed rest day 1	6° HDT position
Bed rest day 15	Aortic baroreflex Carotid baroreflex Cardiopulmonary baroreflex Cardiopulmonary-carotid baroreflex interaction Estimation of cardiac autonomic function
Bed rest day 16	Plasma volume α_1 sensitivity Peak oxygen uptake

Protocols

Graded Exercise Test

Each subjects' peak oxygen uptake ($\dot{V}O_{2peak}$) was determined during a graded maximal exercise test (GXT) in the supine position on an electronically-braked cycle ergometer (Quinton #845) on a control day prior to the beginning of HDT. The exercise consisted of incrementally graded

intensities at a pedaling frequency ranging from 60-70 revolutions per minute. The work rate for the initial 4 minutes was 200 kpm/min, after which the work rate was increased each minute by 100 kpm/min until the subject reached volitional fatigue. In an attempt to motivate the subject to provide a maximal effort, the subject was verbally encouraged to continue working until a minimum of 60 rpm was no longer able to be maintained.

Prior to the beginning of the test, pre-jelled silver-silver chloride electrodes were affixed to the subject's chest. An anterior (lead II) and lateral (V5) leads were monitored continuously throughout the test (Lifepack-6). At each stage of the GXT, systolic and diastolic blood pressure via auscultation and heart rate from the electrocardiogram (ECG) monitor's strip chart were obtained.

Peak oxygen uptake was obtained via open circuit spirometry. The subject breathed through a wide bore (2.2 cm internal diameter) mouthpiece connected to a flow-meter (Pneumotach, S-301) for the determination of minute ventilation ($\dot{V}e$). The expired air was collected in a mixing chamber from which gas samples were continuously drawn and analyzed for oxygen (O_2) and carbon dioxide (CO_2) percentages (Beckman, LB2). The gas and ventilation signals underwent analog-to-digital conversion (Vacumed) and subsequently were analyzed for determination of $\dot{V}O_2$, rate of carbon dioxide production ($\dot{V}CO_2$) and $\dot{V}e$. Calculations were performed on-line using a personal computer and a software package (Vacumed) employing standard algorithms (49).

Alpha Receptor Mediated Mechanisms

On control day 2 and bed rest day 16 cardiovascular responses to graded steady-state infusions of an α_1-adrenoceptor agonist [phenylephrine (PE)] was recorded. The subject was placed in a quiet environment while baseline measurements of heart rate (ECG), blood pressure (Finapres), cardiac output (CO_2 re-breathe) and leg blood flow (occlusion plethysmography) were obtained.

Alpha Adrenoreceptor Sensitivity: PE was infused at three steady-state rates of 0.25 ug/kg/min, 0.50 ug/kg/min and 1.00 ug/kg/min. Each infusion interval continued for nine minutes during which the previously mentioned variables were obtained. The test was ended with the completion of the last infusion, or if systolic blood pressure increased greater than 20 mmHg above resting pre-infusion control. The sensitivity of the alpha receptor mechanisms were evaluated by comparing the relationship between the PE dose and the increases in leg vascular resistance (see techniques section for method of analysis).

Aortic Baroreflex Sensitivity

On control day 3 and on bed rest day 15 the subjects were instrumented for the measurement of heart rate (HR), arterial pressures and peripheral venous pressure (PVP) [using the "dependent arm" method of central venous pressure (CVP) determination (91)]. The subjects were placed in the lateral decubitus position in a lower-body negative pressure (LBNP) device and sealed at the iliac crest.

A schematic of the experimental protocol is illustrated in figure 2. Following instrumentation, the subjects rested quietly in the LBNP box for fifteen minutes, after which, three minutes of resting data were obtained.

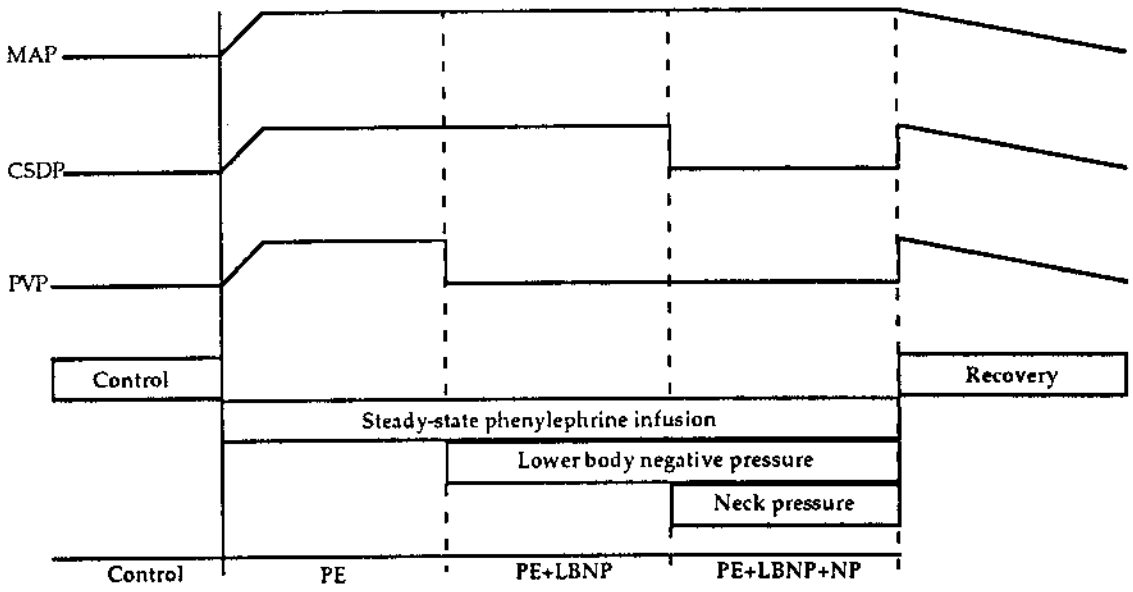

Figure 2: A schematic of the experimental protocol used to isolate the aortic baroreflex. PE increased MAP, CSDP and PVP. The addition of LBNP returned PVP to baseline, while subsequent NP returned CSDP values to baseline, resulting in only the aortic baroreceptors being loaded. PE: steady-state phenylephrine infusion; LBNP: lower body negative pressure; NP: pressure applied to the anterior 2/3 of the subject's neck; MAP: mean arterial pressure, CSDP: carotid sinus distending pressure; PVP: peripheral venous pressure.

Subsequently, a steady-state infusion of phenylephrine (PE) was begun with the goal of increasing mean arterial pressure (MAP) by 15 mmHg. Beginning at 30 mg/min, every two to three minutes the infusion rate was increased by 15 mg/min until the desired rise in MAP was obtained (PE stage). This infusion rate was maintained constant throughout the remaining procedures. After three minutes of data collection following the desired increase in MAP, LBNP was applied until the individual's index of CVP was returned to pre-PE infusion levels. Returning CVP back to baseline removes the PE-induced loading of the cardiopulmonary baroreceptors (PE+LBNP stage). Two

minutes of data were recorded once CVP had returned to baseline. Neck pressure (NP) was then applied to the anterior 2/3 of the subject's neck (31) to the amount of 1.4 times the increase MAP, with the intention of returning carotid sinus distending pressure (CSDP) to pre-PE infusion values (PE+LBNP+NP stage). This amount of NP was chosen to ensure complete transmission of pressure through the tissue of the neck (22). In the subsequent recovery period, PE infusion was terminated while the subject was monitored during the return of blood pressure to baseline.

Data analyses were conducted on the hemodynamic variables during the last minute of the control, PE and PE+LBNP stages. To reduce the possibility of carotid baroreceptor resetting when NP was applied during the PE+LBNP+NP stage, data were analyzed from the first 30 seconds of this period. This time frame was chosen following an evaluation of the HRs for the cumulative period encompassing 0-10 seconds, 0-20 seconds and 0-30 seconds after the application of NP. The mean HRs for each of these periods were not significantly different from each other either pre- or post-HDT (pre: 49.6±2.9, 49.2±2.6, 48.8±2.5 bpm; post: 52.0±3.2, 52.4±3.3, 52.2±3.1 bpm for 0-10, 0-20 and 0-30 cumulative second periods, respectively). Therefore, we were confident that the mean HRs of the 0-30 second period would: a) be representative of the HRs as a result of CSDP being returned to pre-PE values, and b) avoid any possible resetting of the carotid baroreceptor population.

The sensitivity of the baroreflexes was calculated as the ratio of the differences in HR to MAP ($\Delta HR/\Delta MAP$) between baseline and experimental stages. The PE stage was indicative of global baroreceptor loading, and therefore provided an index of total baroreflex-cardiac response. The

PE+LBNP condition was designed to eliminate the cardiopulmonary baroreceptor influence leaving only the arterial baroreceptors loaded. The PE+LBNP+NP stage was used to eliminate the contribution from the carotid sinus baroreceptors isolating the influence of the aortic baroreceptors.

The resultant decrease in HR due to the PE induced elevations in MAP was presumed to be a result of enhanced parasympathetic neural activity to the heart, since at rest, sympathetic neural activity to this organ is known to be minimal (10).

Cardiopulmonary Baroreflex Sensitivity

The sensitivity of the cardiopulmonary baroreflex was assessed on control day three and bed rest day fifteen. The subject was placed in the lateral decubitus position with his lower body sealed at the iliac crest in the LBNP device. By applying progressive vacuum to the box, the pressure within the box was reduced resulting in the pooling of blood in the lower limbs at the expense of blood in the thoracic region, thus unloading the cardiopulmonary baroreceptors. Lower body negative pressure (LBNP) was applied at 5 torr increments through 20 torr in the following order: 0 torr for 5 min; 5 torr for 3 min; 10 torr for 4 min; 15 torr for 15 min; 20 torr for 3 min. Blood pressure, HR and PVP were monitored beat-by-beat continuously throughout the procedure, while forearm blood flow was obtained during each stage. Pressures no greater that 20 torr were employed since such pressures have been demonstrated to not alter HR, pulse pressure, mean aortic pressure or the maximal rate of rise of aortic pressure (130), and, therefore, presumably do not unload the arterial baroreceptors. The sensitivity of the cardiopulmonary

baroreflex was assessed by comparing the relationship between peripheral venous pressure and forearm vascular resistance.

Carotid Baroreflex Sensitivity

The carotid baroreflex was assessed on control day three and bed rest day fifteen by applying pressure and suction to the anterior regions of the subject's neck to mimic carotid hypotension and hypertension, respectively. A silicon-lined collar was placed around the anterior 2/3 of the subject's neck. At end-expiration, the subject held his breath and a pressure of 40 torr was delivered to the chamber and held for five consecutive R-waves of the subject's ECG. Then, with each successive R-wave, the pressure was sequentially stepped to 25, 10, -5, -20, -35, -50, -65 torr. Each pressure step was triggered 50 ms after the R-wave in order to superimpose the pressure and suction stimuli upon the naturally-occurring carotid pulses (236). A minimum of five complete trains were obtained for each subject, in which the average HR and MAP responses were plotted against the carotid sinus distending pressure.

Carotid baroreflex responsiveness was determined using the mathematical modeling technique of baroreflex function as described by Kent et al. (142), employing the following equation:

$$\text{HR or MAP} = A_1 \cdot \{1+e^{[A_2(\text{neck chamber pressure}-A_3)]}\}^{-1}+A_4$$

where: A_1 = range of the response (maximum-minimum)

A_2 = a gain coefficient (a function of neck chamber pressure)

A_3 = neck chamber pressure required to elicit equal pressor and depressor responses (centering point)

A_4 = minimum HR or MAP response

This model permits the calculation of carotid sinus threshold and saturation pressures (the difference being the operating range), while the sensitivity (i.e. slope) of the baroreflex can be determined from the first derivative of the logistic function model (see figure 3).

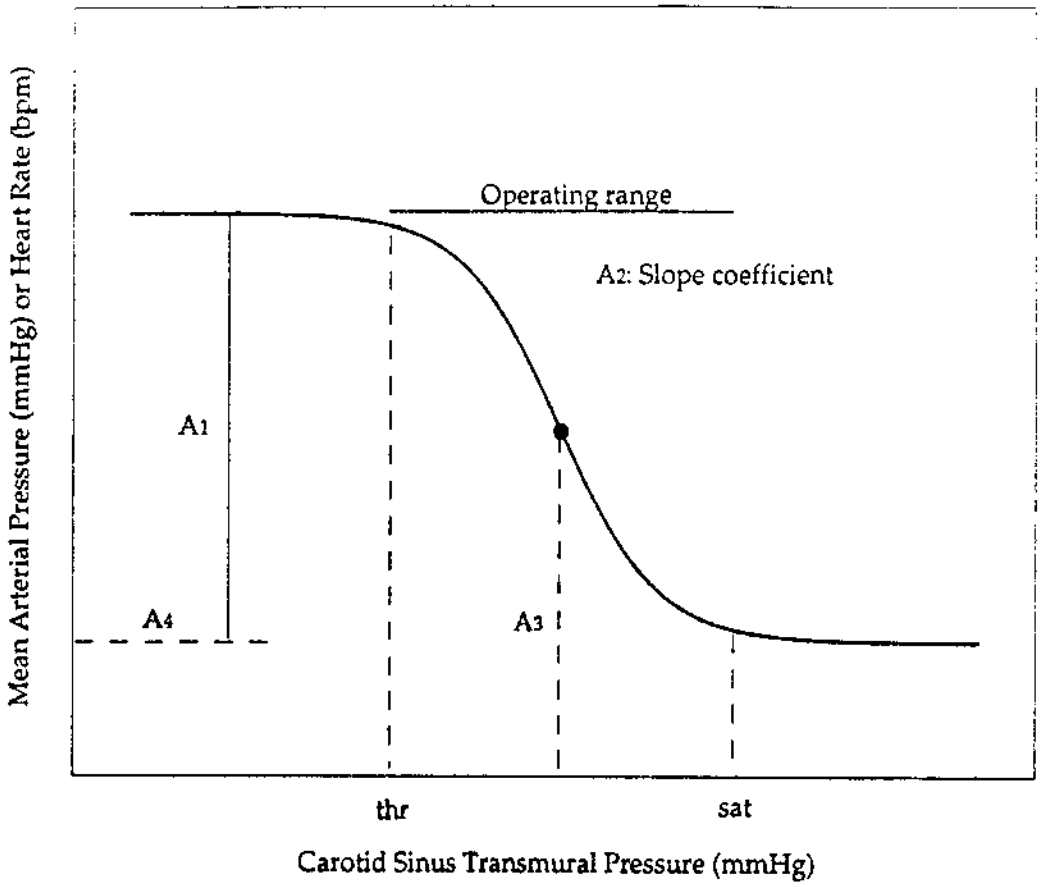

Figure 3: An illustration of the logistic model of the carotid sinus stimulus-response relationship. A_1 represents the difference between the maximum and minimum responses of HR or MAP. A_2 is the slope coefficient. A_3 is the centering point of the carotid sinus pressure which equal pressor and depressor responses can be observed, and is therefore the point of maximum gain. A_4 represents the minimum HR or MAP. Thr. and Sat. denote the threshold and saturation pressures (respectively) of the carotid sinus stimulus-response curve, with the distance between these points being the operating range.

Cardiopulmonary-Carotid Baroreflex Interaction

To determine the degree of interaction between the cardiopulmonary baroreceptors and the carotid baroreflex , the aforementioned carotid sinus distending pressure-HR reflex and carotid sinus distending pressure-blood pressure reflex stimulus-response relationships were obtained at 0, 15 and 30 torr LBNP. During these trains, the subjects lie in the lateral decubitus position such that estimates of CVP could be obtained from PVP. The degree of interaction between these baroreflexes were quantified by evaluating the relationship between the maximal gain of the carotid-cardiac (HR) and carotid-vascular (MAP) responses to the reduction in PVP induced by LBNP. Fifteen torr LBNP was chosen since such a level of LBNP would decrease cardiopulmonary baroreceptor loading without affecting the arterial baroreceptors (130). However the 30 torr LBNP would decrease MAP, and this level provided an important third level of cardiopulmonary baroreceptor unloading from which to examine carotid baroreflex function. Greater than 30 torr LBNP was not utilized in this procedure, since such levels of LBNP could induce syncopal symptoms particularly following the HDT exposure.

Estimation of Cardiac Autonomic Neural Activity

On control day four and bed rest day fifteen, cardiac autonomic activity was assessed as follows. The subjects quietly rested for fifteen minutes in the supine position. This was followed by five minutes of heart rate data collection in which respiratory rate was held at fifteen breaths per minute via a metronome. Shortly thereafter, another five minutes of heart rate data were collected, however, during this time the subjects were allowed to breathe at their own spontaneous voluntary respiratory rate. The order of the

five minute heart rate data collection periods (i.e. controlled or spontaneous breathing) was randomly chosen pre-bed rest, with this order being reversed post-bed rest.

The ECG signal was recorded on 8 mm FM tape (Teac, MR-40) and later digitized at a sampling rate of 1Khz , while the sequential RRIs were timed to the nearest millisecond. The controlled breathing data were analyzed using spectral analysis techniques, while the spontaneous breathing data were analyzed with time-series signal processing.

Spectral analysis of the non-spontaneous breathing RRIs data was performed using fast Fourier transform techniques (193, 195). The power spectral areas were analyzed around the frequency range of 0.23-0.27 Hz representing the high frequency range, and 0.08-0.12 representing the low frequency range. The high frequency area is known to be indicative of basal vagal neural activity whereas the ratio of the low to high frequencies is used as an index of sympathetic neural activity (119, 193).

A time-series analysis technique was utilized to remove non-periodic baseline fluctuations in RRI with a third-order 21 point polynomial function (63). The frequency band of 0.14 to 0.4 Hz (corresponds to respiratory rates of 8 to 24 breaths per minute) was used to analyze the respiratory frequency induced heart rate variability. Following this filtering, the natural logarithm of the variance of the RRIs were calculated. These data were calculated from the spontaneous breathing data set.

A third technique to estimate cardiac vagal neural activity, the standard deviation of the RRIs, was used on the spontaneous breathing data set. Although this technique is more "crude" than the previously mentioned

techniques, it has been show to be a valid index of the change in cardiac vagal neural activity (119). Therefore, the standard deviation of the heart rate period was also obtained pre- and post-HDT during both the controlled and spontaneous breathing to serve as an additional quantitative estimate of cardiac vagal neural activity.

Techniques

6° Head-Down Bed Rest

Six degree HDT was maintained during the bed rest period, and monitored closely for compliance. The subjects were not permitted to change their HDT position, except during their bathing period (15 minutes, once per day), in which they were supine. Furthermore, physical activity was not permitted, other than that required to daily functions such as eating.

The subjects were kept on a strict diet of 2500 to 2800 kcals per day (45% carbohydrate, 38% fat, 17% protein), with dietary sodium and potassium being held constant at approximately 120 and 70 mEq per day, respectively. All subjects abstained from autonomic nervous system stimulants (i.e. caffeinated and alcoholic drinks). Fluid intake was *ad libitum*, however, it was restricted to 2000 ml per day. The photo-period was sixteen hours light to eight hour dark with lights on at 7:00 AM.

Cardiac Output

Cardiac Output ($\dot{Q}$) was determined pre- and post-HDT utilizing carbon dioxide (CO_2) re-breathing techniques which are based upon the Fick principle. CO_2 partial pressures were measured with a CO_2 infra-red analyzer (Beckman, LB-2), and the CO_2 signal was interfaced with a multi-pen chart recorder (Soltec 1286) for subsequent analysis. The CO_2 analyzer and chart

recorder tracings were calibrated before each experiment using calibrated gases of known CO_2 concentrations.

The volume of CO_2 produced ($\dot{V}CO_2$) was determined by having the subject breath through a mouthpiece which was connected to a respiratory valve (Koegel). On the inspiratory side of the respiratory valve, a turbine flow meter was attached (Pneumoscan), while the expiratory side of the valve was attached to a five-liter mixing chamber via tubing having an internal diameter of 3.2 cm. The flow meter's signal was interfaced with a multi-pen chart recorder (Soltec 1286) for future analysis of minute inspiratory ventilation ($\dot{V}_i$); this ventilatory system was calibrated prior to each subject's experimentation with a three-liter syringe. The mixed expired air was collected from the mixing chamber by the CO_2 analyzer at a flow rate of 500 ml/min. The subject breathed through this system for a minimum of three minutes prior to data collection to ensure adequate washout of air previously in the mixing chamber and accompanying tubing. Appropriate conversion of the ventilatory volumes from ATPS to STPD were accomplished prior to $\dot{V}CO_2$ determination.

Following $\dot{V}CO_2$ determination, end-tidal CO_2 samples were obtained for approximately 30 seconds, of which the last five breaths were averaged for subsequent estimation of arterial CO_2 partial pressure (178). Following these 30 seconds of end-tidal monitoring, the subject signaled the investigator at the end of a normal expiration, and the investigator connected the subject to a 5 liter latex re-breathing bag containing a gas mixture of 4% CO_2 and 96% O_2 via a sliding 3-way valve (Hans Rudolph, 2770). The subject re-breathed from the re-breathe bag for ten breaths at a respiratory rate of approximately 30 breaths

per minute. The exponential rise in the partial pressure of end-tidal CO_2 ($PetCO_2$) was use to calculate mixed venous CO_2 partial pressure ($P\bar{v}CO_2$) employing the extrapolation method of Defares (62).

The three variables obtained with this procedure ($\mathring{V}CO_2$, $PaCO_2$ and $P\bar{v}CO_2$) were then used to calculate cardiac output following the appropriate conversion of partial pressures to blood content using the standard dissociation equation (45). Mean HR was obtained during the re-breathing procedure such that SV could be calculated from the product of $\mathring{Q} \cdot HR^{-1}$, while TPR was calculated from the product of $MAP \cdot \mathring{Q}^{-1}$ with the blood pressures obtained prior to the re-breathe procedure.

Heart Rate and Blood Pressure

Heart rate data was continuously monitored from the ECG signal (Quinton) via a typical five lead electrode placement on the subject's chest. The electrode sites were shaved (if required) and the skin abraded to remove dirt and loose epidermal tissue. Silver-silver chloride electrodes were than placed on the chest and electrode leads attached. The ECG signal was processed to determine the frequency of the RRI which was digitally displayed on the ECG monitor. Furthermore, when beat-by-beat analysis was required, the ECG signal was recorded on 8 mm tape (Teac, MR-40) and subjected to analog to digital conversion at a frequency of 1 KHz with subsequent RRI and HRs collected and displayed by a customized software package accurate to ±1 msec.

Beat-by-beat systolic, diastolic and mean arterial pressures were obtained with a Finapres (Ohmeda 2300) blood pressure monitor. The Finapres monitor measures blood pressure using a small finger cuff that

contains a photoplethysmographic volume transducer and an inflatable air bladder. The cuff is connected to a fast-response servo control system that instantaneously regulates the pressure applied to the finger through the bladder and, thus, the pressure applied to the walls of the arteries. As blood pressure increases, the arterial wall expands, increasing the volume of the finger. This volume differential is measured by the plethysmographic transducer. The Finapres monitor responds to the increasing volume by increasing cuff pressure until the original arterial size and blood volume are again reached. The external pressure continuously adjusted by the cuff closely follows the intra-arterial pressure within the finger, allowing measurements of the external pressure as a function of the arterial blood pressure (30). Prior to data collection, the arterial pressure obtained from the Finapres was verified by auscultation. The beat-by-beat output from the Finapres was stored on 8 mm tape as well as a computer hard drive in an ASCII format.

Peripheral Venous Pressure

Central venous pressure (CVP) was estimated from peripheral venous pressure (PVP) using the dependent arm technique of Gauer and Sieker (91). The subject lies in the LBNP chamber in the right lateral decubitus position (see figure 4) with his arm extended downward through a cut-out in the table. A 20-gauge Teflon over needle catheter (Angioset) was inserted into an antecubital vein and connected to a sterile tubing and disposable pressure transducer (Baxter, Uniflow™), of which the output signal was interfaced with a pressure monitor (Hewlett Packard 78342A). The transducer was then centered at the level of the subject's mid-sternum. While in this position,

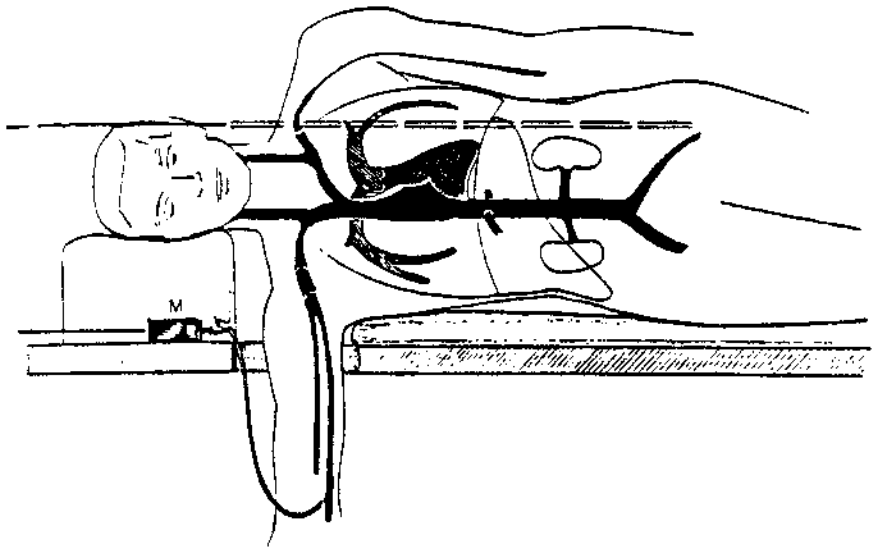

Figure 4: Schematic description of the technique used to estimate central venous pressure from peripheral venous pressure.

with the right arm extended downward, the venous valves become incompetent such that the PVP measured in the antecubital vein is reflective of CVP. The output from the pressure monitor was directed to a chart recorder and 8 mm tape for ensuing beat-by-beat analysis.

Blood and Plasma Volumes

Plasma volumes were determined using a modified Evans blue dye dilution technique (106). Shortly after the subjects woke in the morning, a control baseline blood sample was drawn and an intravenous injection of 11.5 mg of dye pre-diluted with isotonic saline solution (2.5 ml) was administered. A 5 ml aliquot of the control baseline blood sample was used as the pre-dye concentration. The dye from a 10 minute post-injection blood sample recovered from the plasma with a wood cellulose powder chromatographic column (Solka-Floc SW40A), was compared with a standard dye solution at

615 nm with a spectrophotometer. Plasma volume was obtained using the standard dilution equation:

$$PV = M1 \cdot (C2-C1)^{-1}$$

Where: PV = Plasma volume (ml)

M1 = Content of dye injected (mg)

C1 = Concentration of dye in the plasma prior to the dye injection (should be zero) (mg/ml).

C2 = Concentration of dye in the plasma following 10 minutes of mixing (mg/ml).

Total blood volume was calculated from plasma volume and peripheral venous hematocrit (Hct) values following the corrections for trapped plasma and plasma skimming in the determination of Hct (106).

Forearm and Leg Blood Flow

Forearm and leg blood flows (FBF and LBF, respectively) were determined using venous occlusion plethysmography, employing a dual-loop mercury in silastic strain gauge as described by Whitney (254). Prior to, and following all experimentation in which FBF or LBF was obtained, the strain gauge was calibrated. The output of the strain gauge circuitry was directed to a multi-pen chart recorder (Grass 7D). During each measurement, a wrist (ankle for the leg blood flow) occlusion cuff was inflated to 270 torr to stop blood flow to the hand (or leg) for approximately one minute. A cuff placed around the upper arm (thigh for leg blood flow) was inflated and deflated to 40 torr at 10 second increments for two minutes. The strain gauge, placed at the maximum circumference of the forearm or calf, was stretched as the efflux of blood was inhibited with the venous occlusion cuff. The volume of

the forearm/calf was calculated and treated as a cylinder with a length of 1 cm., such that the linear change in circumference during the venous occlusion was used to calculate FBF (or leg blood flow) as follows:

$$V = [(2 \cdot C_2) \cdot C_1^{-1}] \cdot 100$$

$$FBF = V \cdot T^{-1}$$

Where: C_1 = initial forearm or calf circumference (cm)

C_2 = change in forearm or calf circumference (cm)

V = change in forearm or calf volume (ml · 100 ml tissue^{-1})

T = time for measurement (min)

The mean of the six determinations were representative of the FBF response for that perturbation. Forearm vascular resistance (FVR) or leg vascular resistance (LVR) was calculated from the product of MAP·FBF^{-1} (or MAP·LBF^{-1}).

Lower Body Negative Pressure

LBNP was applied to the lower portions of the subject's body by placing the subject in a LBNP device and sealing the device around the subject's iliac crest while in the lateral decubitus position. The negative pressures generated caused a pooling of blood in the legs, resulting in a decrease in thoracic blood volume with a concomitant decrease in CVP. Such CVP changes were required when determining both aortic and cardiopulmonary baroreflex sensitivity, as well as the interaction between the cardiopulmonary and carotid baroreflexes.

The pressures within the box were decreased using a vacuum source (Shop-vac) and a customized computer controller which adjusted the size of an aperture attached to the device, thereby controlling the amount of air allowed to leak into the device. Negative pressures were verified with a

digital pressure monitor (Bio-Tek). With such a system, negative pressures within the device could be controlled with an accuracy of ±1 torr.

Statistics

Pre- and post-HDT baseline hemodynamic variables, maximum gains of the carotid and cardiopulmonary baroreflexes, as well as the slope of the relationship between CVP (cardiopulmonary baroreflex) and the maximal gain of the carotid baroreflex, were compared using a paired t-test analysis when the appropriate parametric assumptions were attained. For tests in which parametric assumptions were not met, a Wilcoxon Matched Pairs-Signed Rank Analysis was conducted. The slopes of the cardiopulmonary baroreflex (CVP versus FVR) and the interaction of the cardiopulmonary-carotid baroreflex (CVP versus maximal gain of the carotid baroreflex) were attained using least-squares linear regression techniques.

The aortic baroreflex HR, MAP and PVP data were evaluated using a (2X4) 2-factor repeated measures ANOVA (see figure 5) with factors of time (pre- and post-HDT) and experimental stage (control; PE; PE+LBNP; PE+LBNP+NP). Post hoc comparisons were accomplished with a Student-Newman-Kuels test when significant group effects were observed. A paired t-test was used to evaluate the aortic baroreflex and the calculated carotid-cardiac baroreflex gains pre- and post-HDT.

The alpha level for all statistical analysis was set at $p=0.05$. All values are presented as mean±standard error of the mean (SEM). Statistical analysis was accomplished using Statistic Analysis Systems (SAS Institute Inc.).

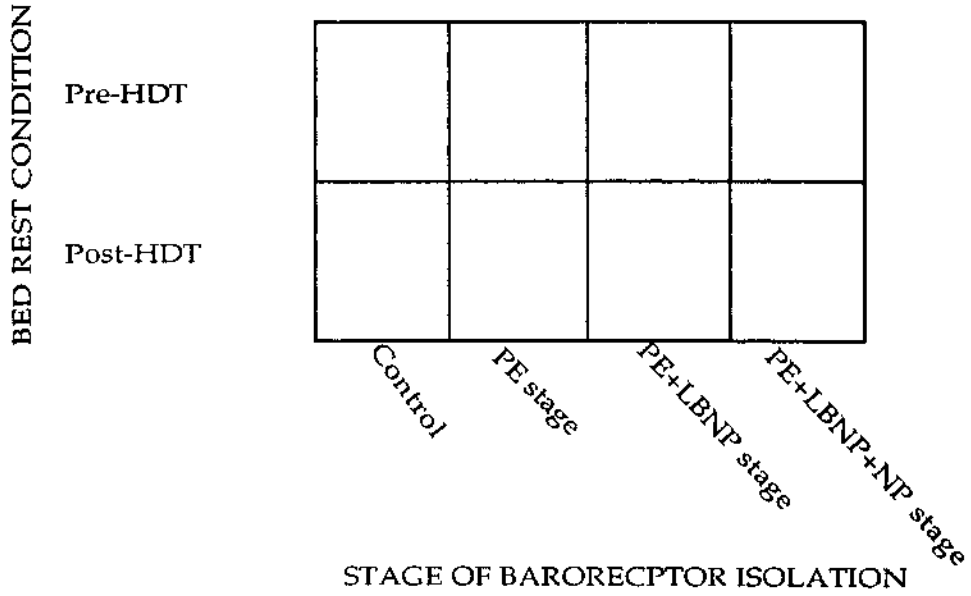

Figure 5: A statistical model used to analyze the hemodynamic variables observed as a result of a combination of bed rest and baroreceptor isolation stage. See figure 2 for an explanation of the aortic baroreceptor isolation stages.

CHAPTER IV

RESULTS

The primary objective of this study was to investigate the effects of simulated microgravity on aortic, carotid and cardiopulmonary baroreflex function. Additionally, the potential alterations of the interaction between the cardiopulmonary and carotid baroreflexes were also characterized. In the subsequent chapter the mean values and statistical analysis of the observed effects are reported. Additionally, graphic depictions of the effects of simulated microgravity exposure on baroreflex function are presented.

Descriptive Comparisons

A summary of the effects of 6° head-down tilt (HDT) on physiological and anthropometric variables are illustrated in table 5. Simulated microgravity exposure significantly decreased peak oxygen uptake ($\dot{V}O_{2peak}$), and resting cardiac output ($\dot{Q}$), while heart rate (HR) increased resulting in a significant stroke volume (SV) reduction. Even though $\dot{Q}$ was reduced, mean arterial pressure (MAP) was significantly elevated due to an increased total peripheral resistance (TPR) post-HDT. Both blood volume (BV) and plasma volume (PV) were significantly reduced as a result of the HDT exposure, and were the likely cause of the reduced body weight.

The low coefficient of variation of the pre-HDT variables (less than 14%, with a mean of 6.4%) demonstrate that the physiological and anthropometric variables of the subjects were similar. Furthermore, a statistical test of

normality (Shapiro-Wilk test) of the subject's age, height, weight, $\dot{V}O_{2peak}$ and blood volume (BV) indicated that the subject population was not significantly different from a normal distribution ($p>0.05$), and therefore it was concluded that the subjects represented a homogeneous population.

Table 5
Summary of the changes in physiological and anthropometric variables

Variable	Pre-HDT	Post-HDT
Age (yrs)	38.4±1.9	-
Height (cm)	182.9±1.9	-
Body Weight (kg)	80.5±3.6	79.5±3.5*
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	36.3±1.1	31.4±1.4*
HR (bpm)	56.0±3.8	61.8±4.5*
$\dot{Q}$ ($l \cdot min^{-1}$)	5.2±0.7	4.7±0.6*
SV (ml)	91.0±11.0	80.0±10.5*
MAP (mmHg)	86.3±3.8	89.8±3.2*
TPR ($mmHg \cdot l^{-1} \cdot min^{-1}$)	18.6±2.3	21.8±3.2*
PVP (mmHg)	8.1±0.6	6.0±0.4*
BV ($ml \cdot kg^{-1}$)	84.1±4.4	72.7±4.8*
PV ($ml \cdot kg^{-1}$)	47.1±2.0	40.3±2.2*

* Signifies significant differences between pre- and post-HDT conditions. PVP is peripheral venous pressure which is used as an index of central venous pressure.

Aortic Baroreflex Function

Aortic-cardiac baroreflex function was isolated using the technique of Ferguson et al. (77) in which a combination of phenylephrine (PE), LBNP and neck pressure (NP) were employed with the resultant change in HR to the change in MAP being recorded at each stage (see figure 2 in the previous chapter). Due to technical difficulties in performing this procedure on one of

the subjects, the subject was excluded from the data analysis and resulted in a subject sample size of seven.

By design, PE elevated ($p<0.05$) MAP from baseline during all stages in which the drug was administered (table 6). This elevation in blood pressure brought about a baroreflex mediated reduction in HR ($p<0.05$) during all experimental stages in which PE was administered. The PE infusion caused PVP to rise (7.2±0.4 to 10.0±0.7 mmHg; $p<0.05$), while LBNP returned PVP to control values. The return of PVP to pressures not statistically different than control verifies that the appropriate amount of LBNP was applied during the procedure to return cardiopulmonary baroreceptor distending pressures to pre-PE infusion values.

Table 6
Cardiovascular measurements pre- and post-HDT

	Control	PE	PE+LBNP	PE+LBNP+NP
HR (beats/min)				
Pre-HDT	56.0±3.8	48.8±2.8 *	48.4±2.5 *	49.8±2.7 *
Post-HDT	61.8±4.5	51.3±3.7	51.7±3.4	52.4±3.0
MAP (mmHg)				
Pre-HDT	95.7±3.1	106.4±3.6 *	107.2±3.6 *	109.8±3.3 *
Post-HDT	103.8±2.8	115.4±3.3	114.0±2.8	115.3±3.2
PVP (mmHg)				
Pre-HDT	8.1±0.6 * *	11.0±0.7	8.4±0.6 * *	8.7±0.4 * *
Post-HDT	6.0±0.4	8.7±0.6	6.9±0.2	6.6±0.4

*: significantly different from the control stage (collapsed across pre/post HDT); **: significantly different from PE stage (collapsed across pre/post HDT). PE: steady-state phenylephrine infusion (arterial and cardiopulmonary baroreflexes); PE+LBNP: phenylephrine infusion plus lower-body negative pressure (arterial baroreflexes); PE+LBNP+NP: phenylephrine infusion plus lower-body negative pressure and neck pressure (aortic isolated condition).

The average PE-induced increase in MAP, along with the associated NPs, are illustrated in table 7. Prior to HDT, the PE infusion increased MAP an average of 13.1±1.8 mmHg, while post-HDT, MAP was increased an average of 11.9±1.5 mmHg post-HDT, resulting in no significant difference between these values. Similarly, no significant difference was found between the NPs required to return CSDP to baseline (pre-HDT: 18.5±1.6 torr and post-HDT: 16.9±1.4 torr).

Table 7
Phenylephrine induced increases in blood pressure with the corresponding applied neck pressure

	Pre-HDT		Post-HDT	
Subject	ΔMAP (mmHg)	NP (torr)	ΔMAP (mmHg)	NP (torr)
1	15.2	18.2	12.7	17.0
2	17.7	21.0	6.1	12.0
3	15.1	25.0	16.3	22.0
4	9.1	14.0	14.0	18.0
5	18.7	22.0	16.2	21.0
6	10.7	15.0	9.2	14.0
7	5.5	7.7	9.0	14.0
Means±SEM	13.1±1.8	18.5±1.6	11.9±1.5	16.9±1.4

No significant difference was observed between pre- and post-HDT ΔMAPs, nor pre- and post-HDT NPs. ΔMAP: Increased mean arterial blood pressure due to the phenylephrine infusion. NP: The amount of neck pressure applied to counter this increase in MAP.

To verify that the appropriate amount of NP was applied, the NP applied was divided by 1.4 [the correction value for incomplete transmission of the NP through the neck tissue (169)], and this value was compared with the elevations in MAP for each subject. The results are illustrated in figure 6. No significant differences were found between the adjusted NP and ΔMAP

values either pre- or post-HDT, thus confirming that the correct amount of NP was applied to decrease CSDP back to pre-PE infusion values.

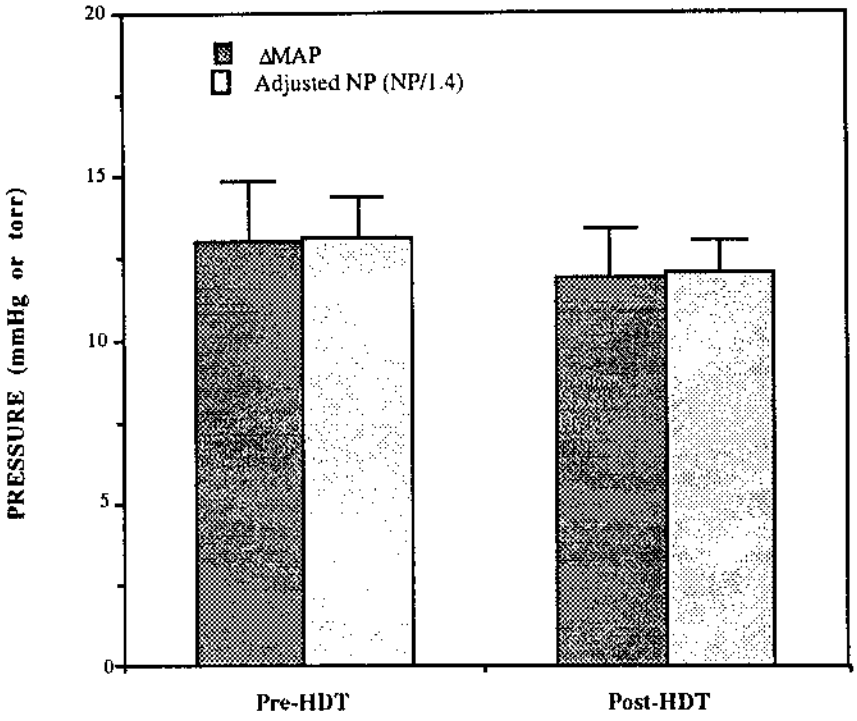

Figure 6: The PE-induced change in MAP (ΔMAP) is compared with the adjusted NP both pre- and post-HDT. The adjusted NP was calculated by dividing the NP applied on each subject by 1.4 (see methods for explanation). This figure illustrates that the appropriate amount of NP was applied to return CSDP to baseline pressures.

The calculated baroreceptor-cardiac gains (ΔHR/ΔMAP) for each experimental stage are illustrated in table 8. The sensitivity of the aortic-cardiac baroreflex (PE+LBNP+NP) was significantly increased (0.45±0.07 to 0.84±0.18 bpm/mmHg) following the HDT exposure. Interestingly, the gain of the global baroreflex (PE stage) did not significantly change following HDT (0.69±0.08 to 0.86±0.10 ms/mmHg), nor did the gain of the arterial-cardiac reflex (aortic and carotid-cardiac; PE+LBNP stage) significantly change following HDT (0.72±0.16 to 0.98±0.21 ms/mmHg).

Table 8
Baroreflex gains (ΔHR/ΔMAP)

	Pre-HDT			Post-HDT		
		Arterial	Aortic		Arterial	Aortic
	PE	PE+LBNP	PE+LBNP+NP	PE	PE+LBNP	PE+LBNP+NP
Means	0.69	0.72	0.45	0.86	0.98	0.84*
±SEM	±0.08	±0.16	±0.07	±0.10	±0.21	±0.18

*: Indicates significant differences between the pre- and post-HDT conditions for the aortic isolated (PE+LBNP+NP) stage. Arterial and Aortic represent the activated baroreflex with the arterial being a combination of the aortic and carotid baroreflexes.

Individual subject gains for the aortic isolated condition are illustrated in figure 7. All but one of the seven subjects demonstrated an increased aortic-cardiac baroreflex gain following HDT exposure.

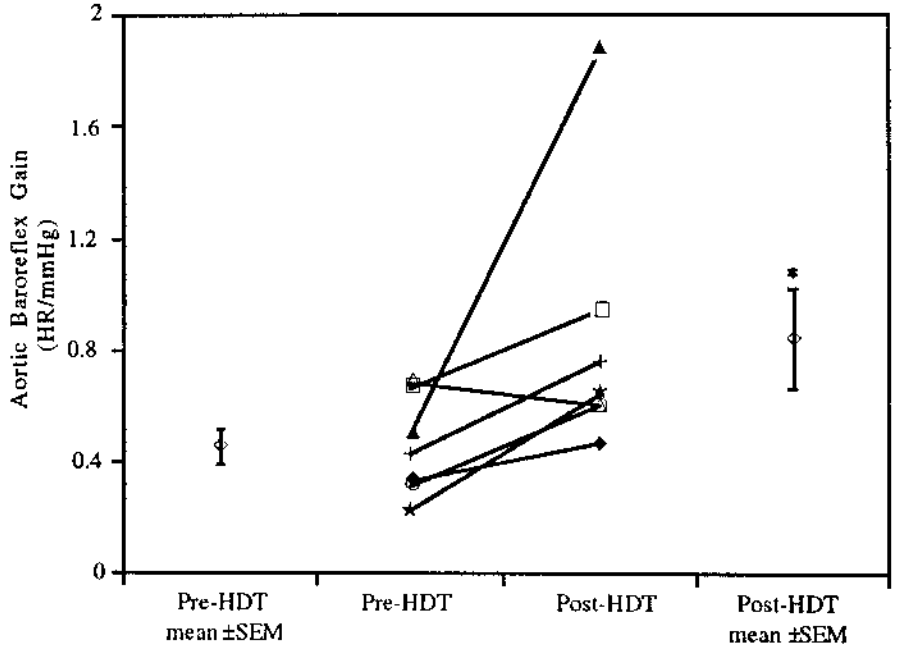

Figure 7: Individual and mean±SEM aortic-cardiac baroreflex gains. Six of the seven subjects increased their aortic-cardiac baroreflex gain following 15 days of HDT exposure, resulting in a significant increase in the mean aortic-cardiac baroreflex gain (*: $p<0.05$).

By subtracting the aortic-cardiac baroreflex (PE+LBNP+NP) gain from the arterial-cardiac baroreflex (PE+LBNP) gain, the calculated carotid-cardiac baroreflex gain was obtained. Figure 8 illustrates that the calculated carotid-cardiac gain tends to decrease (0.26±0.11 to 0.14±0.05 bpm/mmHg) following HDT (p=0.21). An increased aortic-cardiac gain, accompanied with a non-significant decreased carotid-cardiac gain, accounted for a moderately increased arterial-cardiac baroreflex gain (p=0.19) post-HDT. Additionally, there tended to be an increased relative contribution of the aortic-cardiac baroreflex gain to the total arterial-cardiac baroreflex gain (p=0.12) following HDT.

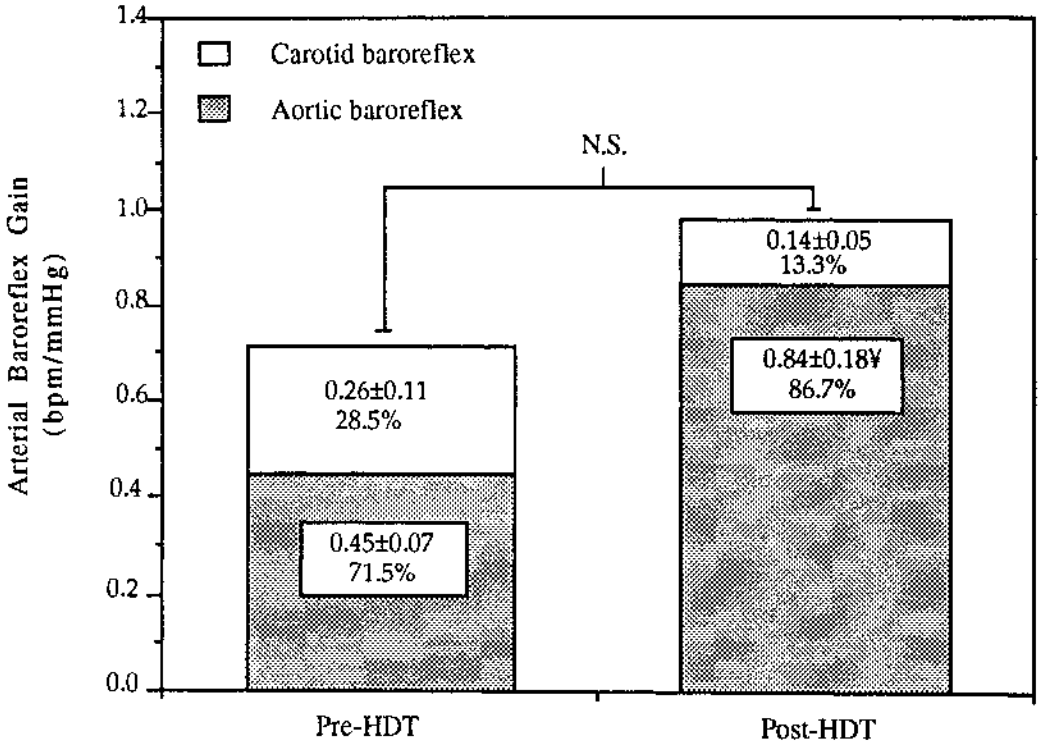

Figure 8: Relative and absolute contribution of the aortic, carotid and arterial-cardiac baroreflexes pre- and post-HDT. The total height of the column represents the arterial-cardiac baroreflex gain; the aortic-cardiac baroreflex gain was obtained as described in the text. The carotid-cardiac gain was calculated as the difference between the arterial and aortic-cardiac baroreflex gains. The aortic-cardiac baroreflex gain significantly increased due to HDT exposure (¥: $p<0.05$).

Carotid Baroreflex Function

The carotid-cardiac and carotid-vascular baroreflexes were assessed using the neck pressure/neck suction technique described by Sprenkle (236). These techniques involved measuring the resultant HR (or inter-cardiac beat interval [RRI]) due to the changes in carotid sinus distending pressure (CSDP) for the carotid-cardiac baroreflex, or, for the carotid-vascular reflex, measuring the resultant changes in MAP due to the changes in CSDP. The changes in heart rate with carotid stimulation occur within a few hundred milliseconds. The vascular response to carotid stimuli is not as rapid as the cardiac response, therefore, the resultant blood pressures for the particular carotid stimuli were "offset" to account for this latency (199).

The stimulus-response characteristics of both the carotid-cardiac and carotid-vascular baroreflexes follow a sigmoidal shaped curve. This permits the utilization of logistic modeling techniques (142), from which parameters characterizing the curve can be derived and analyzed statistically.

Carotid-cardiac baroreflex responses

Regardless of whether HR or RRI were analyzed, HDT induced statistically significant changes in the same parameters (see tables 9 and 10). The carotid sinus pressure (CSP) at the point of maximal gain (A_3) was significantly increased post-HDT, resulting in a rightward shift of the stimulus-response relationship for the HR and RRI response to the changes in CSDP (see the arrow on figure 9 on page 77 and figure 10 on page 78).

Table 9
Effects of HDT on the carotid-cardiac baroreflex
expressed as heart rate

Parameter	Pre-HDT	Post-HDT	p value
A_1 (bpm)	9.73±2.3	9.51±2.1	0.44
A_2 (units)	0.087±0.03	0.087±0.03	0.49
A_3 (mmHg)	98.0±5.2	112.4±2.5	0.015
A_4 (bpm)	52.1±3.2	57.9±3.4	0.01
CSP_{sat} (mmHg)	122.9±4.3	138.0±4.2	0.001
CSP_{thr} (mmHg)	73.1±7.0	86.71±4.7	0.11
Operational range (mmHg)	49.8±5.0	51.3±7.3	0.45
Maximal gain	-0.204±0.04	-0.211±0.06	0.46

Significant differences where $p<0.05$. A_1: physiological range of the dependent variable; A_2: gain coefficient; A_3: carotid sinus pressure at maximal gain; A_4: maximum RRI during the train (representative of the minimal HR); CSP_{sat}: carotid sinus pressure where saturation occurs; CSP_{thr}: carotid sinus pressure where threshold occurs; Operational range: the difference between CSP_{sat} and CSP_{thr}; Maximal gain: the maximal gain of the stimulus-response relationship.

Table 10
Effects of HDT on the carotid-cardiac baroreflex
expressed as inter-cardiac beat interval

Parameter	Pre-HDT	Post-HDT	p value
A_1 (ms)	185.7±38.4	147.6±28	0.12
A_2 (units)	0.086±0.01	0.088±0.03	0.45
A_3 (mmHg)	100.0±5.0	114.4±2.4	0.015
A_4 (ms)	1183.1±73	1159.3±61	0.02
CSP_{sat} (mmHg)	125.7±4.5	139.8±4.3	0.005
CSP_{thr} (mmHg)	74.4±6.7	88.9±11.6	0.085
Operational range (mmHg)	51.3±5.6	50.93±7.2	0.48
Maximal gain	3.84±0.8	3.35±0.9	0.28

See table 9 for a description of the parameters.

Since the HDT procedure resulted in an elevated HR, the minimal HR (maximal RRI) of the relationship was significantly altered, as was the centering point. Figure 9 shows that the rightward shift of the stimulus response relationship, exemplified by the increase in CSP at maximal gain, in combination with the upward shift due to the tachycardia, resulted in a right-upward shift of the stimulus response relationship (right-downward shift for the RRI data, figure 10) post-HDT; such a shift has been termed "resetting" (215). Consistent with such a shift of the stimulus response curve, the CSP_{sat} was elevated post-HDT and the CSP_{thr} tended to increase, resulting in no significant change in the operational range of the response. All other parameters did not statistically change.

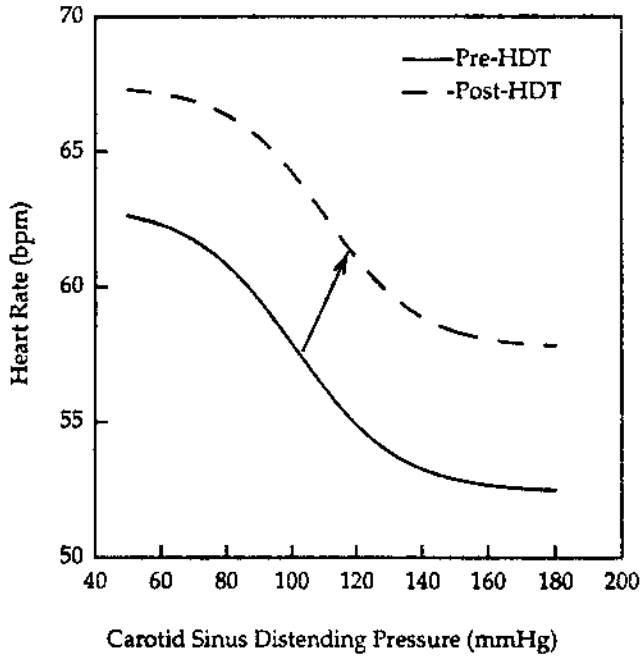

Figure 9: Data obtained from modeled carotid cardiac baroreflex (HR) responses pre- and post-HDT. Simulated microgravity shifts the stimulus response relationship upwards and to the right indicating a re-setting the carotid-cardiac baroreflex.

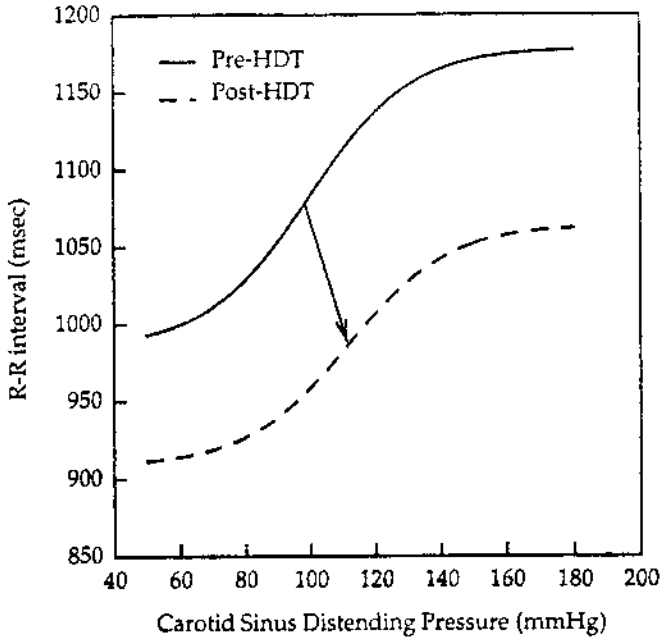

Figure 10: Data obtained from modeled carotid-cardiac baroreflex (RRI) responses pre- and post-HDT. Simulated microgravity shifts the stimulus response relationship downwards and to the right indicating a re-setting the carotid-cardiac baroreflex.

Carotid-vascular baroreflex responses

Unlike the carotid-cardiac baroreflex, simulated microgravity significantly attenuated the maximal gain of the carotid-vascular response (see table 11 and figure 11) suggesting a decreased sensitivity of this baroreflex. As expected with an elevated blood pressure post-HDT, minimal MAP (A_4) was elevated post-HDT. This increased MAP coupled with no change in the CSP at maximal gain (A_3) resulted in an upward shift of the stimulus response relationship (figure 11) without the corresponding rightward shift observed in the carotid-cardiac baroreflex.

Table 11
Effects of HDT on the carotid-vascular baroreflex

Parameter	Pre-HDT	Post-HDT	p value
A_1 (mmHg)	10.23±1.9	8.49±1.4	0.13
A_2 (units)	0.074±0.01	0.064±0.02	0.18
A_3 (mmHg)	108.0±4.8	112.2±3.8	0.30
A_4 (mmHg)	86.2±4.0	96.2±7.9	0.024
CSP_{sat} (mmHg)	139.3±8.6	146.1±5.6	0.23
CSP_{thr} (mmHg)	77.6±3.8	78.3±5.8	0.47
Operational range (mmHg)	61.7±9.4	67.8±8.4	0.28
Maximal gain	-0.167±0.02	-0.136±0.2	0.05

See table 9 for the description of the parameters.

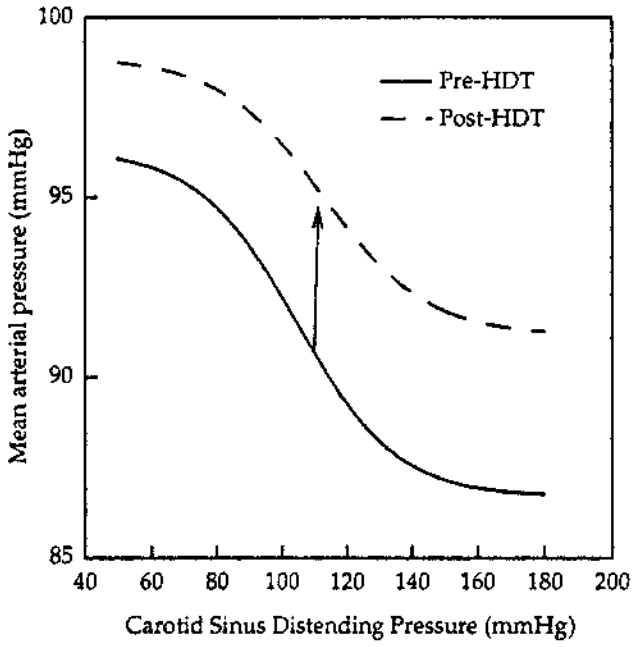

Figure 11: Data obtained from modeled carotid-vascular baroreflex responses pre- and post-HDT. Since HDT did not change the carotid distending pressure at maximal gain, HDT induced an upward shift in this baroreflex with an accompanying depression of the maximal gain.

In summary, these data demonstrate that both the carotid-cardiac and the carotid-vascular baroreflexes have altered stimulus-response characteristics following the simulated microgravity exposure. HDT exposure significantly attenuated the gain of the carotid-vascular baroreflex and shifted the function curve upward, while the gain of the carotid-cardiac baroreflex was unchanged, and the function curve exhibited a parallel right-upward resetting.

Cardiopulmonary Baroreflex Function

To assess the cardiopulmonary baroreflex responsiveness pre- and post-HDT, the subjects were exposed to graded LBNP to unload the cardiopulmonary baroreceptors while the resultant changes in FVR were monitored.

LBNP significantly reduced PVP below control levels, while the slope of the relationship between LBNP and PVP (see figure 12, page 81) was attenuated post-HDT ($p=0.003$), indicating that for the same amount of negative pressure applied, there was less reduction in PVP post-HDT. The baseline PVP (0 mmHg LBNP) was significantly reduced post-HDT. As previously mentioned, this reduction was likely a result of the HDT-induced decreases in blood volume.

When comparing the individual slopes (i.e. gains) of the PVP-FVR response pre- to post-HDT (figure 13, page 82), seven of the eight subjects exhibited an increased slope post-HDT resulting in an overall significant increase in this response ($p=0.023$). The mean FVRs at each level of cardiopulmonary unloading (estimated CVP), are illustrated in figure 14 on page 83. Such a response indicates that, for the same degree of

cardiopulmonary baroreceptor unloading, a greater increase in FVR will result post-HDT.

An increased cardiopulmonary baroreflex gain, accompanied with a decreased CVP unloading for the same amount of LBNP leads one to hypothesize that the mechanism causing the increased baroreflex sensitivity is multi-factorial including possible changes in end-organ responses (i.e. a or b receptors), central integration and/or efferent mechanisms.

Of these potential mechanisms, the α_1 receptor component of the efferent limb was investigated by administering graded doses of the α_1 receptor agonist, phenylephrine (PE), while simultaneously obtaining leg vascular resistance (LVR). The results are summarized in figure 15, page 83.

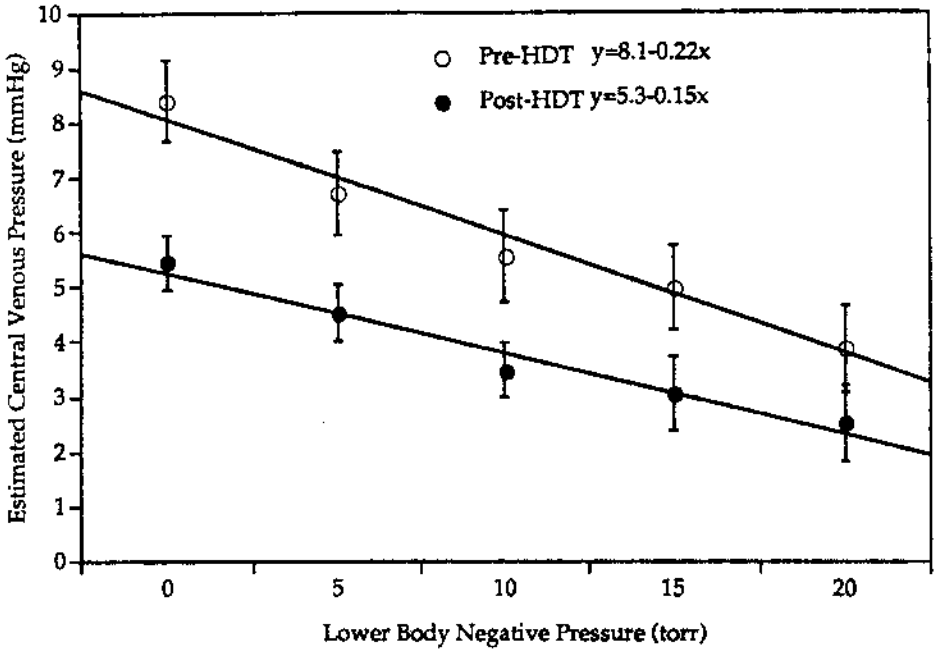

Figure 12: Estimated central venous pressures responses (PVP) to lower body negative pressure (LBNP). The slope of the relationship between LBNP and PVP was attenuated post-HDT (p=0.003), suggesting that for the same amount of negative pressure, there was less of a reduction in PVP post-HDT.

Consistent with the finding of this study, simulated microgravity increased vascular resistance demonstrated here by an increased LVR, however, the slope of the relationship between the PE dose and LVR was not altered by HDT exposure. No change in the slope of this relationship indicates that an increased α_1 receptor mediated mechanisms is not a mechanism resulting in the heightened cardiopulmonary baroreflex gain post-HDT. The potential mechanisms resulting in an increased LVR in the subjects post-HDT (i.e. the intercept of the relationship), are reviewed in the discussion.

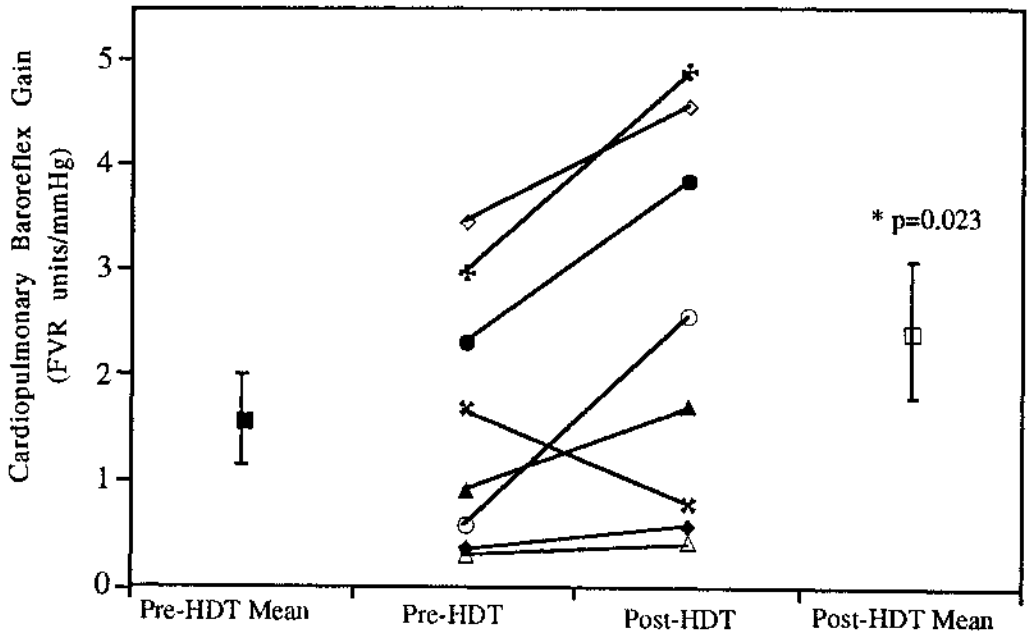

Figure 13: Average and individual cardiopulmonary baroreflex gains. Seven of the eight subjects tested exhibited an increased cardiopulmonary baroreflex gain resulting in a significantly increased overall gain post-HDT.

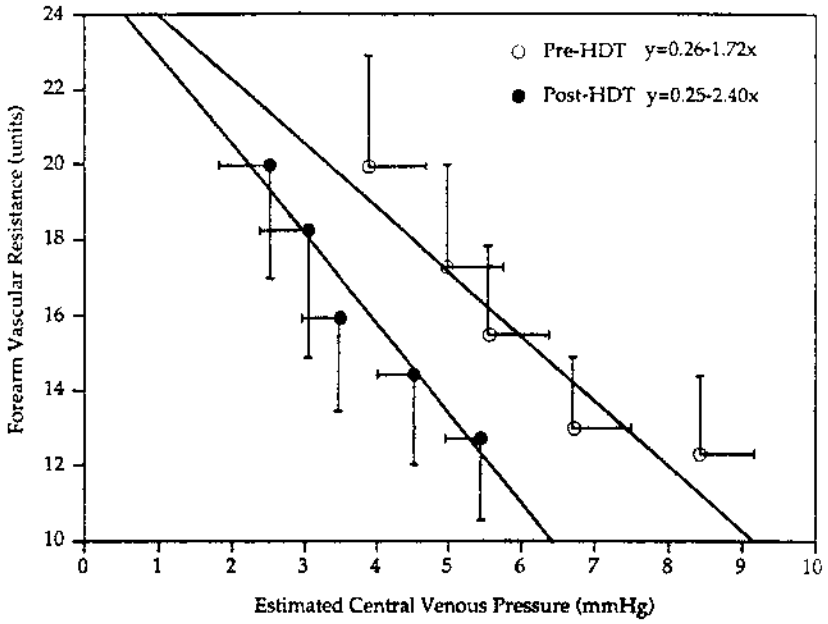

Figure 14: Mean cardiopulmonary baroreflex response pre- and post-HDT. Simulated microgravity exposure significantly increased the slope of the relationship between CVP and FVR suggesting that the cardiopulmonary baroreflex responsiveness was augmented post-HDT.

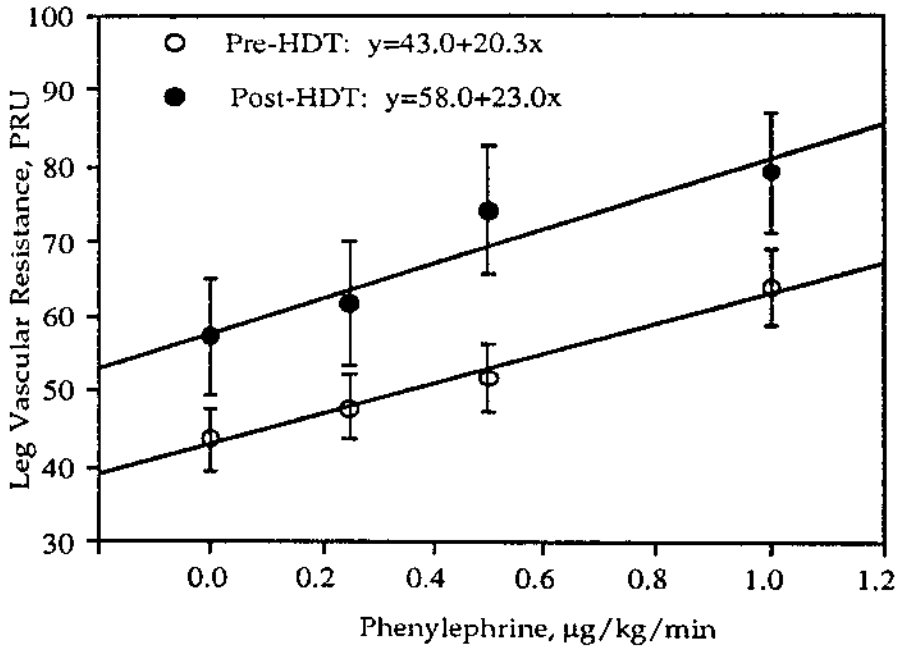

Figure 15: Dose response relationship between phenylephrine (PE) dose and the resultant change in leg vascular resistance (LVR). Consistent with the data, vascular resistance was significantly elevated, however the slope of the PE-LVR relationship was not altered by HDT (p=0.24).

Interaction Between Cardiopulmonary and Carotid Baroreflexes

To determine the degree of interaction between cardiopulmonary and carotid baroreflexes, the carotid baroreflex sensitivity was assessed as described in the methods, at three levels of cardiopulmonary baroreceptor unloading. The slope of the relationship between cardiopulmonary baroreceptor unloading (expressed as PVP) and the gain of the carotid-cardiac (using HR and RRI) and the carotid-vascular are graphically depicted in figures 16, 17 and 18 respectively, while individual subject data are listed in appendix A.

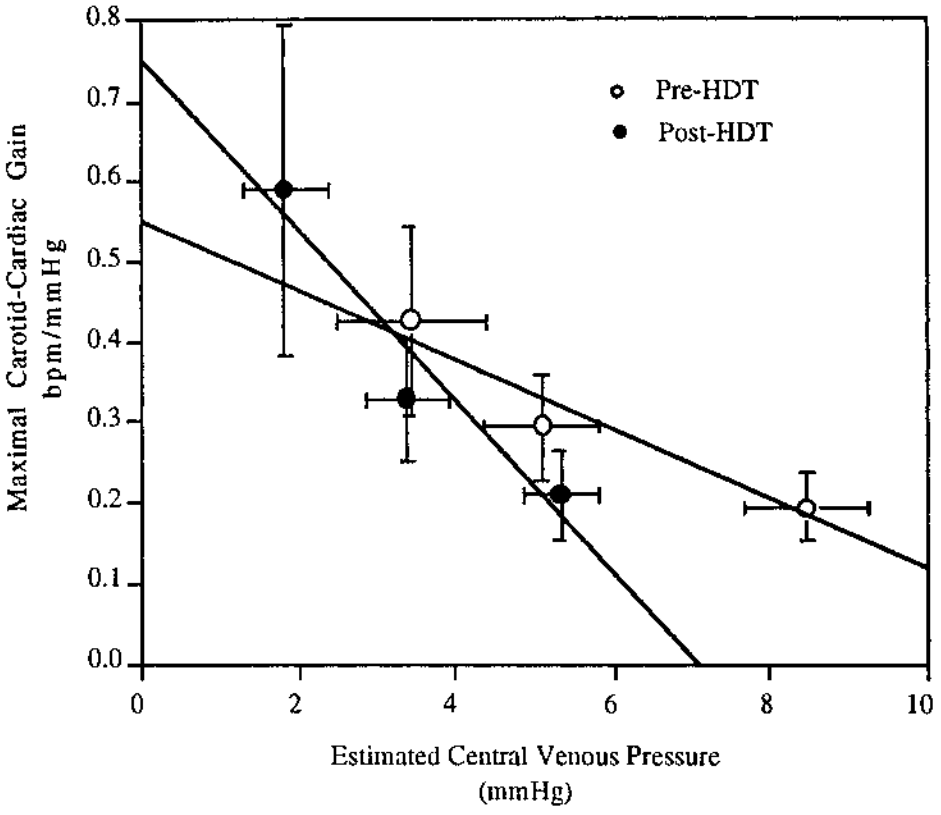

Figure 16: Mean responses of the maximal gain of the carotid-cardiac baroreflex expressed as HR during graded reductions in estimated CVP pre- and post-HDT. Following the HDT exposure, the degree of interaction expressed as the slope of the relationship between estimated CVP and the maximal gain of the carotid-cardiac baroreflex tended to be heightened, however not significantly (p=0.10).

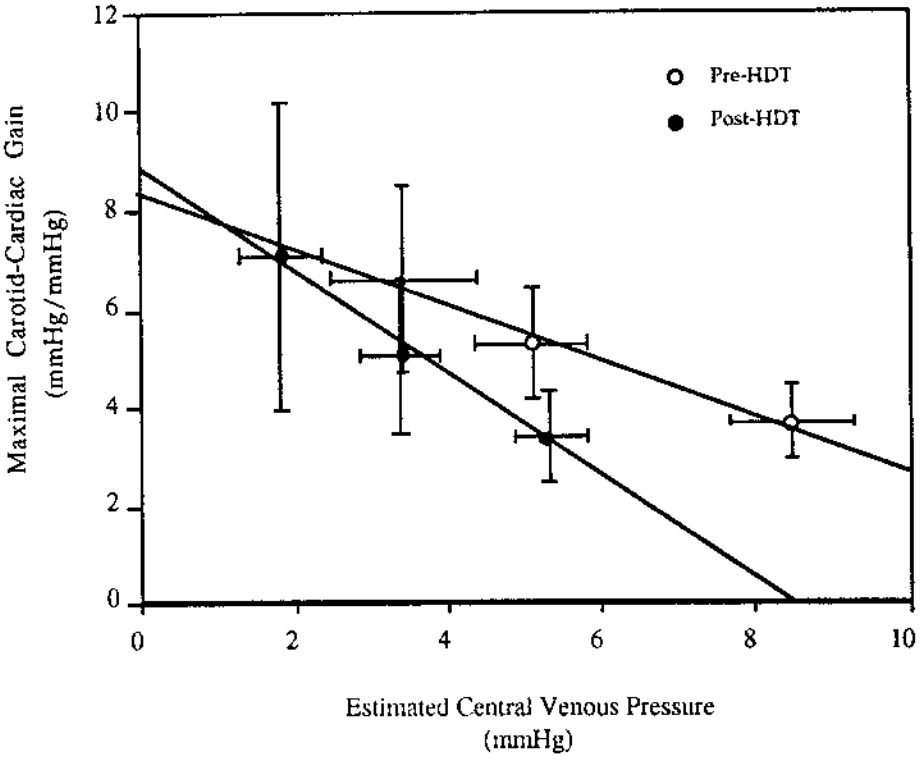

Figure 17: Mean responses of the maximal gain of the carotid-cardiac baroreflex expressed as RRI during graded reductions in estimated CVP pre- and post-HDT. Following the HDT exposure, the degree of interaction expressed as the slope of the relationship between estimated CVP and the maximal gain of the carotid-cardiac baroreflex tended to be heightened, however not significantly (p=0.18).

Simulated microgravity exposure resulted in a slight tendency of the slope of the relationship between the estimated CVP and the maximal gain of the carotid-cardiac baroreflex to be heightened when expressed as HR (p=0.10) or RRI (p=0.18). Although the slope of these relationships were almost doubled post-HDT, statistical significance was not obtained due to the high degree of variability inherent to the technique. Conversely, the degree of interaction between the cardiopulmonary and carotid-vascular baroreflex was not affected by simulated microgravity exposure (p=0.44). However,

consistent with this study's previously reported findings, the maximal gain of the carotid-vascular baroreflex was depressed post-HDT across all levels of LBNP (determined using a 2-way ANOVA with main effects of LBNP stage and time [pre/post-HDT], p=0.05).

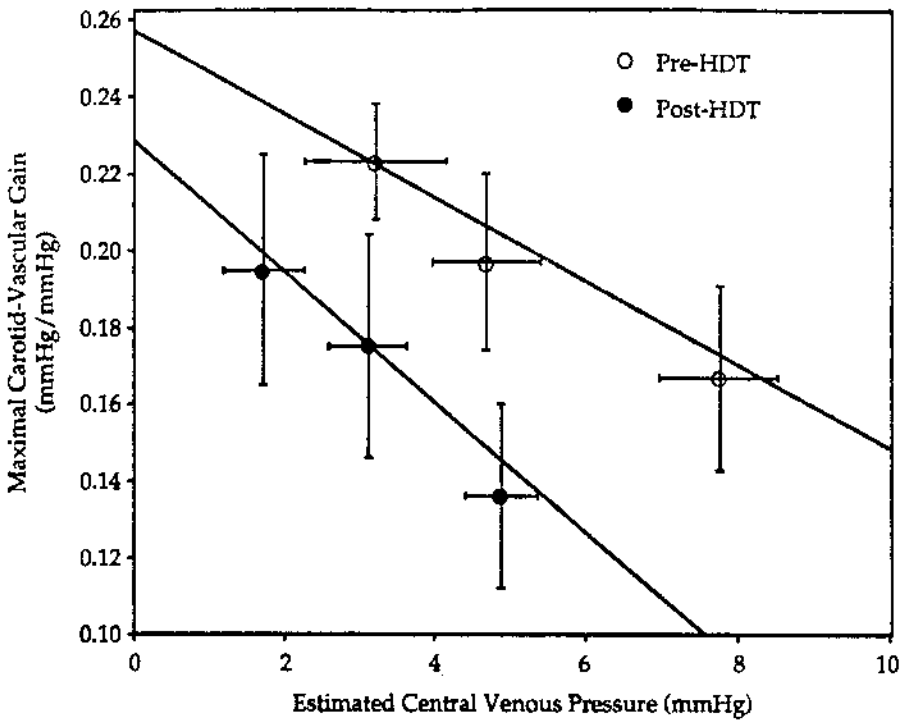

Figure 18: Mean responses of the maximal gain of the carotid-vascular baroreflex during graded reductions in estimated CVP pre- and post-HDT. Simulated microgravity exposure did not alter the degree of interaction between the cardiopulmonary and carotid-vascular baroreflexes (p=0.44). However, of interest, is the depressed carotid-vascular baroreflex maximal gain at any estimated CVP post-HDT.

Autonomic Neural Activity Analysis

Spectral Analysis

The simulated microgravity exposure significantly increased both the low and high frequency components from the spectral analysis (table 12). The high frequency component is representative of vagal neural activity and was significantly decreased due to the HDT exposure, thus suggesting that vagal neural activity was reduced. In contrast, the sympathetic neural activity, represented by the ratio of the low to high frequencies, was not significantly altered by the simulated microgravity exposure.

Table 12
Quantification of autonomic activity determined from power spectral analysis of RRI

	Low frequency (0.08-0.12 Hz eq)	High frequency (0.23-0.27 Hz eq)	Low/High ratio
Pre-HDT	60.0±27.8	95.3±28.5	1.18±0.72
Post-HDT	31.1±13.8 *	48.2±17.4 *	0.69±0.39
P-value	0.012	0.017	0.49

The values under the low and high frequency categories represent the normalized power spectral densities ($msec^2$) for the corresponding frequencies. The low frequency is understood to be a result of a combination of parasympathetic and sympathetic influences, whereas the high frequency domain is believed to represent solely parasympathetic influences. The low/high ratio represent the ratio of the low to high power spectral densities and is indicative of sympathetic neural activity.

Time Based Analysis

Both methods of time based analysis (standard deviation and time series analysis) showed similar changes as that seen with the spectral analysis (Table 13). That is, simulated microgravity exposure significantly reduced

these indices of vagal neural activity, thus suggesting that vagal neural activity was reduced post-HDT.

Table 13
Quantification of autonomic activity determined from time based analysis of RRI

	SD (msec)	Band Variance (time series analysis)
Pre-HDT	42.2±4.8	7.44±0.25
Post-HDT	33.3±3.3	7.08±0.24
P-value	0.02	0.01

SD: mean standard deviation of the heart periods; Band Variance: the natural logarithm of the heart period variance within the specified frequencies of 0.12 to 0.40 Hz.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Four hypotheses were presented at the beginning of this project, which are presented here in the null form: a) the gain of the aortic-cardiac baroreflex is not attenuated following simulated microgravity exposure when compared to pre-HDT control; b) simulated microgravity exposure does not decrease the cardiopulmonary baroreflex gain when evaluated against pre-HDT values; c) the same amount of cardiopulmonary baroreceptor unloading will not decrease the degree of augmentation of the carotid-cardiac and carotid-vascular baroreflexes following simulated microgravity exposure; and d) simulated microgravity will not decrease basal vagal neural activity. The first three hypotheses were not rejected, whereas the final hypothesis was rejected.

Throughout the course of the experiment, it became quite obvious that the overall hypothesis posed in which simulated microgravity exposure will reduce baroreflex function, was untenable. Our findings indicate that HDT exposure increased the gain of the aortic and cardiopulmonary baroreflexes, decreased the gain of the carotid-vascular baroreflex and did not change the gain of the carotid-cardiac or the interaction between the cardiopulmonary and carotid baroreflexes. In this chapter, the perturbation of simulated microgravity exposure on the following will be discussed: a) basic physiological and anthropometric variables; b) aortic baroreflex function; c) carotid baroreflex function; d) cardiopulmonary baroreflex function;

e) interaction between carotid and cardiopulmonary baroreflexes; and f) summary and directions for future research.

Basic Physiological and Anthropometric Variables

The primary goal for subject selection was to choose subjects whose physiological and anthropometrical variables would be similar to those of the current astronaut corps. Table 14 illustrate these variables compared to astronauts selected in 1984 and 1985. As this table depicts, age and height were similar between the groups, however the subject pool chosen did not reflect the same degree of maximal oxygen uptake as the astronaut corps. This lower "fitness" was also reflected in the increased average weight of the subjects.

Table 14
Comparison of physiological and anthropometric variables between the subjects and selected astronauts

Variable	Subject	Astronaut
Age (years)	38.4±1.9	39.0±3.3
Height (inches)	70.5±2.3	69.8±1.7
Weight (lbs)	177.3±10.4	166.5±14.5
$\dot{V}O_{2peak}$	36.3±1.1	48.6±5.5†

Astronaut data compiled from ref. (206), †: these data are $\dot{V}O_{2max}$ and not $\dot{V}O_{2peak}$, therefore they are expected to be approximately 7% higher than $\dot{V}O_{2peak}$.(10)

As reflected in table 5 on page 69 multiple physiological and anthropometrical variables were affected by the HDT exposure. Similar to previous HDT as well as actual space flight studies (6, 78, 80, 131, 158, 167, 255), there was a significant reduction in blood and plasma volume. The reduction of these variables likely explains the reduction in estimated central venous

pressure (PVP), stoke volume (SV), cardiac output ($\dot{Q}$), body weight and peak oxygen uptake ($\dot{V}O_{2peak}$).

Mean Arterial Pressure and Total Peripheral Resistance

Of interest, and more difficult to explain, was the post-HDT increase in mean arterial blood pressure (MAP) and total peripheral resistance (TPR) measured at rest. These increases in MAP and TPR are not unique to this study, and are reported repeatedly in other bed rest studies (42, 96, 132, 134). It was clear that the increased MAP post-HDT was strictly a result of increased TPR, since, cardiac output $\dot{Q}$ decreased during the exposure. However, less clear was the mechanism causing the increased TPR. The only data obtained which shed light on this question is illustrated in figure 15 on page 83. When graded doses of phenylephrine were administered pre- and post-HDT, the slope of the relationship between phenylephrine dose and leg vascular resistance was not altered, however the intercept was significantly elevated. No difference in the slope of this relationship suggests that α_1 receptor mechanism responsiveness leading to vascular smooth muscle contraction apparently was not altered. This, coupled with a decreased norepinephrine and no change in plasma renin activity or vasopressin concentrations in the blood, suggest the possibility of an increased intrinsic tone of the vasculature. The mechanism causing such an increased vascular tone is not clear. This effect is not likely a result of myogenic activity since such a phenomenon would predict decreases in TPR with the decreased flow reflected in the decreased $\dot{Q}$. One unsubstantiated mechanism could be flow induced reductions in EDRF (20, 163).

Heart Rate

It is clear from the results that regardless of the method used to quantify vagal neural activity to the heart, vagal neural activity was reduced following simulated microgravity exposure. This reduced neural activity was not likely a factor of changes in parasympathetic receptor function on the heart since the heart rate response to atropine sulfate is not altered after three weeks of bed rest (241). Conversely, the index of sympathetic neural activity, indicated that HDT did not alter the sympathetic influence to the heart. From these data, it was clear that the increased HR was a result of a decreased basal vagal neural activity, without any changes in sympathetic neural activity. Figure 19 illustrates a conceptual model of the possible changes in vagal neural activity.

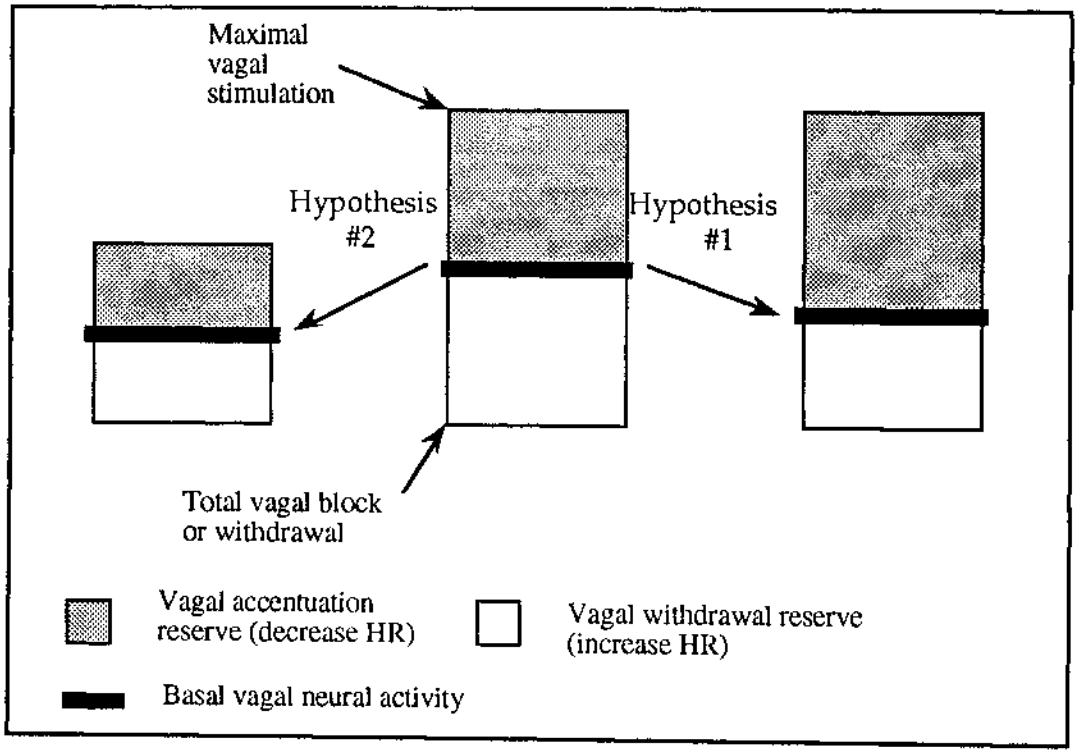

Figure 19: Conceptual model of the effects of vagal neural activity on the heart due to simulated microgravity exposure. See text for explanation.

The total box (encompassing both shaded and non-shaded areas) represents the range of vagal neural activity to the heart from maximal stimulation to total absence. Therefore, the shaded areas represent the vagal neural activity accentuation reserve (the capacity to decrease HR) and the non-shaded areas represent the vagal neural activity withdrawal reserve (the capacity to increase HR). The corresponding arrows depict the possible changes as a result of microgravity exposure. This model allows for two hypotheses to be considered:

Hypothesis #1: This hypothesis predicts a decrease in vagal neural activity due to a reduction of basal vagal neural activity without changing the maximal vagal range (i.e. reflects the difference between maximal withdrawal and maximal stimulation). As the schematic illustrates, such a shift will result in an increased reserve to decrease heart rate by increasing the range of vagal neural stimulation. This hypothesis also predicts a decreased reserve to increase heart rate.

Hypothesis #2: This hypothesis states that the total range of vagal activity is reduced and the relative location of the basal vagal neural activity has not changed (i.e. the vagus nerve will still have equal relative capacity to increase or decrease HR). With such a hypothesis the ability to either increase and decrease HR by changing vagal input has been attenuated.

Current knowledge of the effects of simulated microgravity exposure precludes concrete conclusions regarding which of these proposed hypotheses are correct. Further research would need to be conducted, likely using pharmacological intervention, to answer these questions. However, this

information is of great value, particularly when investigating the effects of microgravity on vagally mediated baroreflex function.

Such a reduction in parasympathetic neural activity, associated with microgravity-induced cardiovascular "detraining", reinforces the well-established link between increased vagal neural activity and the corresponding bradycardia in individuals who undergo aerobic exercise training (46, 75). We propose that post-HDT reduction of vagal neural activity may be a factor of chronic hypovolemia, since, in contrast to these subjects, aerobically trained individuals have larger blood volumes and increased vagal neural activity (144).

Aortic Baroreflex Function

We found, contrary to our initial hypothesis, that simulated microgravity exposure increased the sensitivity of the aortic-cardiac baroreflex. Additionally, HDT exposure tended to increase the relative and absolute contribution of the aortic-cardiac baroreflex gain to the total arterial-cardiac baroreflex gain (figure 8, page 74). Similar work conducted by Convertino et al. (54), as well as Fritsch et al. (83), concluded that the carotid-cardiac baroreflex gain was attenuated following HDT or actual microgravity exposure. The tendency for a depressed closed loop calculated carotid-cardiac gain in our data are in agreement with data from Convertino et al. (54) and Fritsch et al. (83). However, a reduced carotid-cardiac gain, accompanied with an increased sensitivity of the aortic-cardiac baroreflex, are puzzling in light of data demonstrating significant reductions in orthostatic tolerance following actual or simulated microgravity exposure (31, 36, 54, 108, 128, 181, 256). These data, coupled with data verifying a predominance of the aortic-cardiac and

aortic-vascular baroreflexes over the corresponding carotid-cardiac and carotid-vascular baroreflexes in humans (77, 174, 221), would lead one to conclude that the reduced arterial baroreceptor sensitivity is not a primary contributor to the reduction in orthostatic tolerance frequently observed following microgravity exposure. Conversely, an increased aortic-cardiac baroreflex gain may reduce the amount of orthostatic intolerance experienced by maintaining cardiac output during an orthostatic challenge.

An increased aortic-cardiac gain may provide insight to explaining the augmented HR response observed during stand tests or lower body negative pressure (LBNP) following simulated microgravity when little or no change in MAP occurred (26, 116, 126, 192). Convertino et al. (54) evaluated non-syncopal and syncopal subjects during a stand test following 30 days of HDT. The non-syncopal subjects exhibited a significant elevation in HR during the stand test post-HDT, even though systolic blood pressure was not significantly reduced during the stand test. Moreover, this occurred in individuals having a relatively small yet an apparent decrease in their carotid-cardiac baroreflex gain from 3.1 to 2.7 ms/mmHg. Prior to the work reported in the present study, the mechanisms causing this tachycardiac response were unclear. However, in light of our findings, it is probable that a primary mechanism causing the increased HR during the post-HDT orthostatic challenge tests may be attributed to an augmented aortic-cardiac baroreflex gain accompanied with a decreased vagal neural activity.

Possible explanations for an increased aortic-cardiac baroreflex gain could be attributed to: a) mechanisms associated with HDT-induced reductions in $\dot{V}O_{2max}$; b) a decreased cardiopulmonary inhibitory influence

on the aortic-cardiac baroreflex; c) changes in basal vagal neural activity and d) an up-regulation of the aortic baroreceptors.

Using a similar protocol to isolate the aortic-cardiac baroreflex response, Shi et al. (229) evaluated the sensitivity of the aortic-cardiac baroreflex and its contribution to the total arterial-cardiac baroreflex on average fit (mean $\dot{V}O_{2max}$: 42.9+1.0 ml/kg/min) and highly fit (mean $\dot{V}O_{2max}$: 62.3+1.8 ml/kg/min) individuals. The average fit individuals had a significantly increased aortic-cardiac baroreflex sensitivity and contribution to the total arterial-cardiac baroreflex when compared to the high fit individuals. Since the subjects in the present study experienced a 13% reduction in $\dot{V}O_{2max}$, their increased aortic-cardiac baroreflex gain may be a consequence of HDT-induced detraining. However, contrary to this hypothesis, Shi et al. (229) reported a tendency for the average fit individuals to have an elevated calculated carotid-cardiac baroreflex gain (average fit: 0.35±0.09 and highly fit: 0.19±0.02; p=0.10) , whereas the present investigation demonstrated the calculated closed loop carotid-cardiac baroreflex gain tended to be depressed (p=0.12) following HDT (figure 8, page 74). The reduced calculated carotid-cardiac baroreflex gains are in agreement with the findings of previous studies (54, 83). These data suggest that the augmented aortic-cardiac baroreflex gains may be selectively coupled with mechanisms resulting in decreased aerobic fitness, while the reductions in the carotid-cardiac baroreflex gains may be a result of the perturbation of microgravity exposure.

Such a paradoxical change in the aortic and carotid baroreceptor gains due to simulated or actual microgravity exposure, may be related to differences in the receptor's anatomical location relative to the arterial

hydrostatic indifference point. Both mean arterial and pulse pressures at the location of the carotid baroreceptors have been demonstrated to be lower in erect humans when compared to the supine posture (92). Conversely, since the aortic baroreceptors are located closer to the hydrostatic indifference point relative to the carotid baroreceptors, substantially smaller shifts in relative hydrostatic pressure will occur during posture changes at the aortic baroreceptors. When an individual is placed in a microgravity environment (or an analog), the carotid baroreceptors will be exposed to, and therefore detect, greater increases in pressure relative to an erect position (due to the hydrostatic gradient) when compared with similar postural-induced changes in pressures at the aortic baroreceptors. Such an increased arterial pressure at the carotid sinus, purely due to the decreased hydrostatic gradient for the duration of the exposure, may provide the stimulus to down regulate the baroreceptors in this region (40) without similarly affecting the aortic baroreceptors. Although this hypothesis provides a plausible explanation for the tendency of the calculated carotid-cardiac baroreflex gain to decrease following HDT, it is not suitable in explaining an increased aortic-cardiac baroreflex gain, since such a hypothesis would predict little change in the aortic-baroreflex gain.

Altered integration with the cardiopulmonary baroreceptors could lead to an augmentation of the aortic-cardiac baroreflex gain. Pawelczyk and Raven (199) demonstrated that unloading of the cardiopulmonary baroreceptors resulted in an increased sensitivity of the carotid-cardiac baroreceptors. Similar interaction between the cardiopulmonary baroreceptors and the aortic baroreceptors have been identified in rabbits (25).

Furthermore, DiCarlo and Bishop (65) concluded that decreased arterial baroreflex function in trained rabbits was a result of enhanced inhibitory influence from cardiac afferents, possibly due to the training-induced increases in blood volume (BV) or changes in the compliance of the cardiac tissues. Since Convertino et al. (57) reported that the compliance of the venous system did not change following 10 weeks of endurance training, it was likely that the changes DiCarlo and Bishop (65) observed were due to changes in volume rather than compliance. In contrast to the increases in BV, HDT exposure decreased BV (table 5 page 69). Therefore, the decreased blood volume may be implicated in the reduction of our index of CVP (8.1±0.6 to 6.0±0.4 mmHg) post-HDT. Such a reduction in CVP would decrease the stretch placed upon the cardiopulmonary baroreceptor region, thereby reducing the cardiopulmonary-mediated tonic inhibitory integration with the aortic baroreflex within the ventrolateral medulla resulting in an accentuation of the aortic-cardiac baroreflex gain. However, one would expect this reduced cardiopulmonary inhibitory influence to likewise augment the carotid-cardiac baroreflex gain, which was not the case. Furthermore, it could be hypothesized that a sustained decrease in CVP throughout the HDT may result in a resetting of the cardiopulmonary baroreceptor's accentuation of the arterial baroreceptors. Under these circumstances, a reduced cardiopulmonary inhibitory tone would not be expected.

Changes in basal vagal neural activity may contribute to the augmented aortic-cardiac baroreflex. The method used to isolate the aortic baroreflex involved infusing a vasoactive drug, phenylephrine, to increase blood pressure, and observe the concomitant relationship between the

increases in blood pressure and decreases in heart rate. As it is known that basal neural activity to the heart is predominantly parasympathetic with minimal sympathetic neural influence at rest (18, 160, 161), a baroreflex mediated decrease in HR will likely be the result of increased vagal neural activity rather than withdrawal of sympathetic neural activity. Referring to hypothesis #1 of the conceptual model of the effects of HDT on vagal neural activity (figure 19, page 92), one can see that this hypothesis allows a greater reserve for vagal stimulation. If such occurred in this study, the increased aortic-cardiac gain may be related to a greater capability to slow the heart rate post-HDT due to this greater reserve of vagal neural activity .

An additional mechanism by which the aortic-cardiac baroreflex sensitivity could increase, might include an up-regulation of the aortic baroreceptors. As an increased pulsatile stretch of the aortic baroreceptors has been demonstrated to down-regulate these receptors (40), one might conclude that a reduced pulsatile stretch may consequently lead to an up-regulation of this baroreceptor group. Cardiac output was reduced while heart rate was elevated after HDT and resulted in a reduced stroke volume (see table 5 page 69). Assuming the compliant nature of the aorta had not changed due to HDT, a reduced stroke volume would decrease the pulsatile deformation at the region of the aortic baroreceptors, thereby providing a stimulus for baroreceptor up-regulation. What is not known is the mechanisms causing such a change. Suggestions have included changes in the distensibility of the baroreceptor region and/or an increased sensitivity of the baroreceptor's low threshold afferent neurons (150).

Carotid Baroreflex Function

Carotid-Cardiac Baroreflex

Much debate has occurred regarding the units used when expressing the cardiac response to the carotid stimuli, whether it be HR or inter-cardiac interval (RRI). The primary issue is due to the non-linearity of these units, which is graphically depicted in figure 20. As one can see from this figure, if one changes heart rate from 80 to 60 bpm, the change in RRI will be smaller than the corresponding change in RRI from 60 to 40 bpm. Therefore, since the carotid baroreflex "trains" decrease heart rate during the simulated hypotensive phase (with a range of 9.7 bpm as seen with this study's data), the corresponding change in RRI will be greater pre-bed rest than post-bed rest since the resting HR is significantly elevated post-bed rest. If reported as HR, such a non-linear relationship results in less of a change in HR to the same carotid sinus stimulation post-bed rest than if the response was reported as RRI. Arguments supporting the RRI method are based on the linear relationship between cardiac-vagal nerve traffic and RRI, whereas the relationship between cardiac-vagal nerve activity and HR is curvilinear (140, 197). However to avoid criticism from both schools of thought, both HR and RRI data were analyzed and reported.

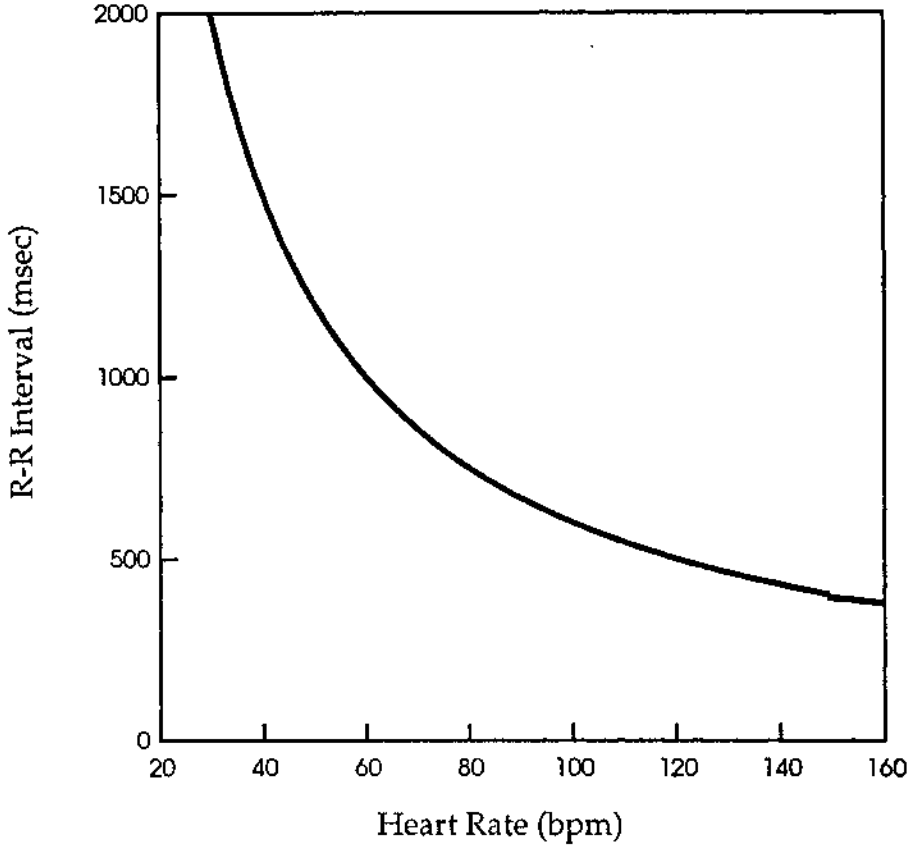

Figure 20: Relationship between heart rate and RRI across different heart rates.

Fifteen days simulated microgravity did not significantly affect the maximal gain of the carotid-cardiac response, regardless of the method of analysis. However, the average maximal gain was non-statistically increased post-HDT when expressed as HR, whereas the average maximal gain when expressed as RRI decreased (also non-significantly) post-HDT. The opposing directions of these non-statistically significant changes were due to this non-linear relationship between HR and RRI. These data are in contrast to both Convertino et al. (54) and Fritsch et al. (83) who demonstrated significant decreases in the carotid-cardiac baroreflex post-HDT and space flight

respectively, but are consistent with Eckberg et al. (72) who did not demonstrate such a change. The apparent difference of these findings and those of Convertino et al.'s (54) and Fritsch et al.'s (83) is the lack of a significant change in responding range in our data (responding range is the range of RRI response during the carotid sinus perturbations: A_1; only the RRI data were compared, as Convertino et al. and Fritsch et al. only reported RRI data). Our data indicate a trend for a decreased responding range (pre: 185.7±38.4 ms to post:147.6±28 ms; $p=0.12$), whereas Convertino et al. (54) reported a significantly decreased responding range (pre: 165±20 to post: 122±20 $p \leq 0.05$); interestingly the differences in the relative reductions between these means were similar.

As illustrated in figures 16 and 17, there is a right-upward parallel shift of the HR curve and a right-downward parallel shift of the RRI curve. The upward shift (downward for the RRI graph) is simply the effects of HDT on heart rate, resulting in a basal cardio-acceleratory state compared to pre-HDT. Thus the stimulus response relationship start at a higher HR (lower RRI). The rightward shift of these curves are more difficult to explain. Both curves had a significantly increased carotid sinus pressure at the point of maximal gain (A_3). This point represents the pressure around which symmetrical responses in heart rate are produced (74). Thus, the blood pressure in which equal cardio-acceleration and cardio-deceleration can be attained, was shifted to a higher blood pressure. Such a shift represents a relocation of the stimulus response curve so it may continue to effectively buffer acute changes in blood pressure at the higher basal blood pressure.

Also of interest, and likely related to the increased blood pressure, is the tendency for an increased carotid sinus pressure threshold (CSP_{thr}), and a significantly increased carotid sinus pressure saturation (CSP_{sat}). These shifts indicate that the pressure at which the carotid-cardiac baroreflex begins to functionally change heart rate (CSP_{thr}) and the point at which it can no longer change heart rate (CSP_{sat}) has been shifted to higher pressures. Such changes would be consistent with the "resetting" of the carotid-cardiac baroreflex to higher arterial pressures.

The mechanism for this type of resetting is not known, but may be a result of the chronically increased pressure at the carotid-sinus. The effects of chronic hypertension on the carotid baroreflex supports this hypothesis (150). Animal models, either spontaneously hypertensive or which were surgically or pharmacologically made hypertensive, have reported similar shifts in the pressure at which carotid-sinus nerve activity increased (i.e. threshold) and carotid-sinus nerve activity no longer increased (i.e. saturation) (180). The authors concluded that this resetting tended to maintain, rather than to prevent, the elevation of blood pressure. Factors such as changes in the distensibility of the carotid sinus or inactivation of some of the fibers having lower thresholds, were implicated in causing this shift (180). However, other variables may also be implicated in this type of a shift of the baroreflex function curve, which might be related to central nervous system mediated mechanisms (149, 227).

Carotid-Vascular Baroreflex

Unlike the carotid-cardiac baroreflex, there was a significant decrease in the maximal gain of the carotid-vascular baroreflex. The difference between

these two limbs of the carotid baroreflex (i.e. the cardiac and vascular limbs) may be a result of the efferent pathways utilized for the particular limb. The techniques used to assess the carotid-cardiac baroreflex are exclusively vagally mediated mechanisms, since atropine sulfate abolished the effects of this reflex while beta blockade did not affect it (70, 73). In contrast to the carotid-cardiac reflex, the efferent limb of the carotid-vascular baroreflex are sympathetically mediated events. In our experience, changes in blood pressure due to ramped changes in carotid sinus transmural pressure, were offset by three to four beats and out of phase with the observed changes in heart rate (198). This delayed response is thought to be due to the slower response characteristics of the sympathetic neural activity when compared to parasympathetic neural activity (148, 227). The fact that the blood pressure response was out of phase with the heart rate response, suggest that the changes in blood pressure were not associated with heart rate mediated changes in cardiac output, but were a result of sympathetic nervous system mediated changes in vascular resistance. Therefore, the differences in response between the maximal gain of the carotid-cardiac and carotid-vascular baroreflexes may be a result of differences in central integration of afferent information, efferent neural activity and specific end-organ adaptations.

Another divergent response with respect to the effects of simulated microgravity on the carotid-cardiac and carotid-vascular baroreflexes was the change in the characteristics of the stimulus-response curve. In contrast to the carotid-cardiac baroreflex, the carotid-vascular baroreflex did not exhibit a change in the carotid sinus pressure at maximal gain (A_3), rather there was a

vertical displacement of the response at the same carotid sinus transmural pressure (see figure 18) without the accompanying rightward shift observed for the carotid-cardiac baroreflex. An upward displacement of this type is not an uncommon observance. Korner et al. (149) demonstrated such a shift when measuring the carotid-cardiac baroreflex responses in dogs exposed to hypoxia resulting in arterial PO_2 of 30 mmHg. The authors suggested the resultant shift was due to centrally mediated changes associated with an increased chemoreceptor and lung inflation receptor activity. Clearly, these mechanisms were not the cause of the displacement seen with the carotid-vascular baroreflex of the present investigation, however they do demonstrate that centrally mediated events can induce a similar displacement of the baroreflex function curve.

An additional mechanism for this upward displacement of the carotid-vascular baroreflex function curve, unrelated to central nervous system mediated events, may be associated with the increased tone of the vasculature. We have demonstrated that the vascular tone is increased post-HDT, therefore, the changed vessel tone related to carotid sinus perturbation would be additive to the heightened vascular tone. Thus, the complete carotid-vascular baroreflex function curve is displaced to a heightened MAP for the same CSDP without any change in the location of the carotid sinus pressure at maximal gain (A_3).

A consequence of the HDT-induced upward displacement of the stimulus response curve (without an accompanying rightward shift) would diminish the baroreflex's reserve to buffer increases in pressure, while increasing the reserve capability of the reflex to buffer decreases in blood

pressure. This type of a displacement would be advantageous in controlling blood pressure during an orthostatic challenge when blood pressure was reduced, however, this advantage would be nullified due to the decreased carotid-vascular baroreflex gain that resulted from simulated microgravity exposure.

Cardiopulmonary Baroreflex Function

Unloading of the cardiopulmonary baroreceptors results in a sustained increase of sympathetic discharge to the arms and legs (251), culminating in increased peripheral vascular resistance that is correlated to the reduction in central venous pressure (CVP). Furthermore, Mack et al. (172) and Takeshita et al. (242) demonstrated that the slope of the relationship between CVP and forearm vascular resistance (FVR) was indicative of cardiopulmonary baroreflex sensitivity. Thus, this technique was utilized to determine the sensitivity of this reflex following 15 days of simulated microgravity exposure. The findings of our investigation indicate that the cardiopulmonary baroreflex was significantly accentuated following the HDT exposure. Changes in the cardiopulmonary baroreflex gains are not without precedent. Mack et al. (172) assessed the cardiopulmonary baroreflex in individuals with varying degrees of aerobic fitness and determined that the higher fit individuals had significantly reduced cardiopulmonary baroreflex gains. They hypothesized that the elevated blood volume may have resulted in a greater loading of the cardiopulmonary baroreceptors causing a resetting of these receptors. This was subsequently confirmed when these investigators compared the cardiopulmonary baroreflex gain of the same individuals following volume expansion, and demonstrated that when volume

expanded, the gain of the cardiopulmonary baroreflex was significantly attenuated (171). Since both volume expansion and chronic aerobic training increase central venous pressure (57, 229), the mechanism thought to be responsible for the decreased cardiopulmonary baroreflex gain was the increased loading of the cardiopulmonary baroreceptors, reflected by the elevated CVP. Furthermore, decreases in blood volume, induced by the diuretic furosemide, increased the sensitivity of the cardiopulmonary baroreflex (246).

The results of this investigation demonstrated a significant increase in the gain of the cardiopulmonary baroreflex. Since our index of CVP was reduced post-HDT, the increased cardiopulmonary baroreflex gain was likely associated with the chronically reduced CVP.

The exact mechanism causing the increased cardiopulmonary baroreflex gain, whether it be afferent, central or efferent mechanisms, is not known. However to determine if the response was a result of accentuated α_1 mediated events, graded doses of the α_1 agonist, phenylephrine, was administered while the changes in leg vascular resistance (LVR) were determined. As illustrated in figure 15, the slope of this relationship was not significantly different pre- to post-HDT. Even though baseline LVR was significantly elevated post-HDT the slope of the relationship between the dose and LVR was not changed. Such findings suggest that an accentuation of α_1 mediated events was not the mechanism resulting in an increase cardiopulmonary baroreflex gain. These data, coupled with data demonstrating a diminished synthesis or storage of norepinephrine in the pre-junctional nerve terminals following bed rest (82), suggests that the

increased cardiopulmonary baroreceptor sensitivity following HDT was a result of altered afferent receptor or central integrative mechanisms. These mechanisms were not directly, or indirectly, assessed in this study, so conclusions regarding which, or both, of these components of the baroreflex loop contributed to the increased cardiopulmonary baroreflex gain were not determined.

Interaction of Cardiopulmonary and Carotid Baroreflexes

Presumably, of greater importance than the cardiopulmonary baroreflex itself in short term blood pressure regulation is its role in modulating the arterial baroreflexes (203). To the author's knowledge, no investigator has analyzed the cause and effect relationship of any perturbance (such as HDT) on the degree of interaction between the cardiopulmonary baroreflex and carotid-cardiac baroreceptors (degree of interaction being quantified as the slope of the relationship between maximal gain of the carotid baroreflex and CVP).

This study demonstrated that the degree of interaction between the carotid-cardiac or carotid-vascular and cardiopulmonary baroreflexes were not significantly altered by HDT exposure. Although the average difference in the slope of this relationship was apparently doubled post-HDT for the carotid-cardiac baroreflex, due to the high degree of variability and the small sample size, statistical significance was not attained. Post-hoc power analysis (48) of this data, using the observed differences in the means, demonstrated that a subject sample size of eleven would have been required to attain the needed statistical significance. Because of the high cost and enormous amount of resources used to conduct such a study, limited number of beds for the

subjects and the fact that the authors chose the aortic baroreflex to be the primary focus of the study (which required a subject sample size of six), such a subject sample size was not obtained.

Conversely, the apparent trend demonstrated with the interaction between the carotid-cardiac and cardiopulmonary baroreflex was not apparent when investigating the effects HDT on the interaction between the carotid-vascular and cardiopulmonary baroreflexes. The slope of these relationships were almost equal (see figure 18).

Summary and Directions for Future Research

This investigation is the first of its kind in which the effects of simulated microgravity exposure on baroreflexes other than the carotid-cardiac reflex were studied. The results demonstrated that this exposure significantly increased the aortic-cardiac baroreflex gain and the cardiopulmonary baroreflex gain, decreased the carotid-vascular baroreflex gain, and did not change the carotid-cardiac baroreflex or the relationship between the carotid and cardiopulmonary baroreflex. Consistent with other bed rest studies, body weight, estimated CVP, BV, PV, $\dot{Q}$ and $\dot{V}O_{2peak}$ were reduced, while HR, MAP and TPR were elevated. However, unique to this study, is the finding that the elevated HR seen post-HDT is likely a result of the reduced vagal neural activity observed following this exposure.

With this information, greater understanding of the effects of microgravity on the ability of the individual to regulate blood pressure can be reviewed. Previously, the factors affecting basic blood pressure regulation,

and thus post-flight orthostatic hypotension were summarized using the following equation:

$$MAP = HR * SV * TPR$$

Therefore, any changes associated these variable without compensation from the other variables, would result in post-flight orthostatic hypotension. By applying the findings of this study to this basic equation, the results are depicted in figure 21.

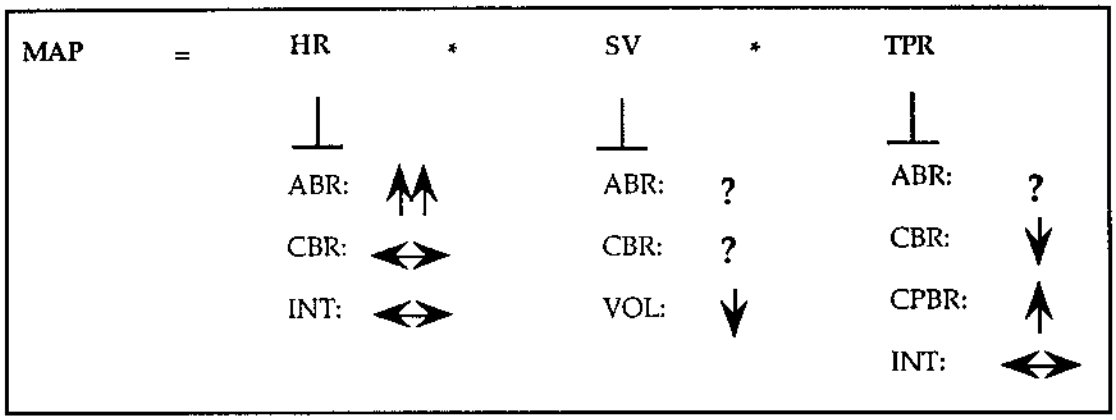

Figure 21: Baroreflex modulation of factors controlling blood pressure. ABR: aortic baroreflex; CBR: carotid baroreflex; CPBR: cardiopulmonary baroreflex; INT: interaction between the carotid and the cardiopulmonary baroreflex and VOL: blood volume. The arrows indicate the direction of change of these parameters following simulated microgravity exposure.

Beginning with the effects of HDT on the baroreflexes controlling HR, the most pronounced finding of this study is the increased aortic baroreflex gain that occurred as a result of the exposure. Unlike the reports from others (54, 83), fifteen days simulated microgravity exposure did not significantly change the carotid-cardiac baroreflex. Although there was a tendency for an increased interaction between the carotid-cardiac and cardiopulmonary baroreceptors, statistical significance was not attained.

The effects of these baroreflexes on the vascular responses were identified under the TPR parameter. Due to the experimental procedure in which an α_1 agonist was administered, the direct effects of the aortic-vascular baroreflexes was not obtainable. Although the carotid-vascular baroreflex gain decreased, the cardiopulmonary baroreflex increased and the interaction between the carotid-vascular and cardiopulmonary baroreflexes did not change.

The effects of the arterial baroreflexes on variables affecting SV, primarily being cardiac contractility, have been well established (225). However, how space flight or any of its analogs affect this relationship is still a matter of conjecture. Although, this study did conclude that SV was depressed following the HDT exposure, and this reduction was likely attributed to a combination of reduced blood volume and an increased HR.

It should be obvious that the question marks in the preceding equation could become the foci of future research projects. With the advancement of microneurography techniques, greater understanding can be obtained from the stimulus response characteristics using muscle sympathetic nerve activity as the dependent variable. Such a technique will enable quantification of the influence HDT exposure has on aortic baroreflex control of vascular resistance. Similarly, using beat-by-beat echocardiographic analysis during baroreflex perturbation, changes in the baroreflex response characteristics affecting stroke volume could also be quantified.

Recently Levine et al. (159), has suggested non-baroreflex mechanisms contributing to orthostatic intolerance. They implicated cardiac compliance and Frank-Starling mechanisms as a potential cause of orthostatic

intolerance. Pulmonary capillary wedge pressure, SV and left ventricular end-diastolic volumes were obtained in athletes and non-athletes. The Starling curves (figure 22) of these groups indicate that for the same decreases in pulmonary capillary wedge pressure, greater decreases in SV occurred in the athletes. When pulmonary capillary wedge pressure was plotted against end-diastolic volume (figure 23), providing an index of left ventricular compliance, the athletes were predisposed to greater reductions in filling for the same reductions in pulmonary capillary wedge pressure, indicating the athletes' left ventricle was more compliant than the non-athlete. This increased left ventricular compliance would be facilitative in increasing the athlete's SV during aerobic activity, however it would be detrimental when exposed to orthostatic stress.

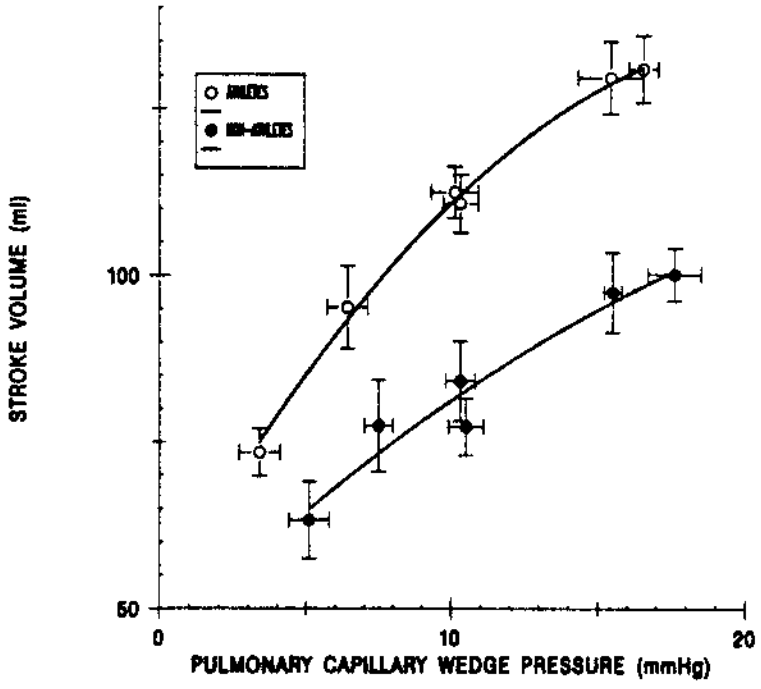

Figure 22: Comparison of Frank-Starling relationships between athletes and non-athletes. Figure from ref. (159).

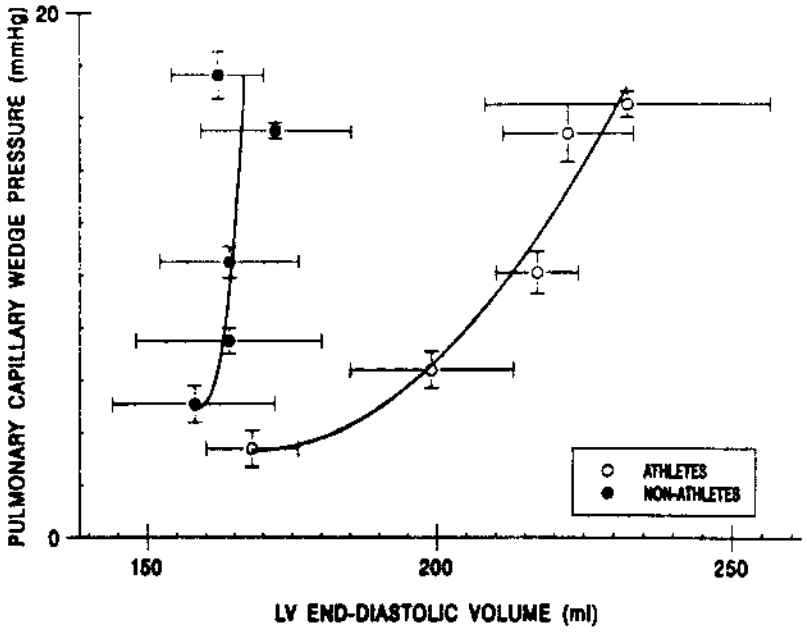

Figure 23: Comparison of pressure/volume relationships between athletes and non-athletes. Figure from ref. (159).

Relating this information to space flight, these authors hypothesized two potential mechanisms being responsible for post-flight orthostatic intolerance (159).

a) A change in dynamic compliance caused by altering ventricular volume and shifting the operational position on the same pressure/volume curve (i.e. shifting from the plateau to the steep portion of the same compliance curve).

b) A change in global cardiac chamber compliance that alters pressure/volume relations over the entire range of filling pressures (i.e. shift to a different compliance curve).

Both conditions have the potential to predispose the individual to greater reductions in end-diastolic volume for the same reduction in left ventricular

filling pressure. These authors suggest that regardless of how effective the baroreceptors are in controlling HR and TPR, maximal baroreflex activation will not prevent orthostatic hypotension if cardiac filling cannot be adequately maintained (159).

Thus, the summation of this data demonstrate that simulated microgravity exposure does not reduce the sensitivity of global baroreflex function as had been previously concluded (54, 83), rather changes associated with this exposure result in an overall increase in the sensitivity of many of these baroreflexes. Such an increase would attenuate, not accentuate, post-space flight orthostatic hypotension. Therefore, other mechanisms are likely involved in causing this phenomenon, possibly being related to the maintenance of SV during orthostatic stress.

The use of additional techniques such as direct measurements of sympathetic nerve activity, intra-cardiac pressures and volumes during orthostatic stress following space flight, would be of great benefit in determining the mechanisms causing microgravity-induced orthostatic hypotension.

APPENDIX

INDIVIDUAL SLOPES OF THE INTERACTION BETWEEN THE CARDIOPULMONARY AND CAROTID BAROREFLEXES

Individual slopes of the relationship between the cardiopulmonary and carotid-cardiac (HR) baroreflex

Subject Number	Pre-HDT	Post-HDT	
1	$-3.4e^{-2}$	$-2.6e^{-2}$	
2	$-1.4e^{-2}$	$-2.1e^{-2}$	
3	$-9.0e^{-2}$	$-2.6e^{-2}$	
4	$-1.6e^{-2}$	$-7.8e^{-2}$	
5	$-10.9e^{-2}$	$-35.6e^{-2}$	
6	$-2.7e^{-2}$	$-8.9e^{-2}$	
7	$-1.6e^{-2}$	$-7.9e^{-2}$	
mean±	$-4.4e^{-2}$	$-9.6e^{-2}$	
SEM	$1.5e^{-2}$	$4.5e^{-2}$	p=0.10

Individual slopes of the relationship between the cardiopulmonary and carotid-cardiac (RRI) baroreflex

Subject Number	Pre-HDT	Post-HDT	
1	-0.32	-0.13	
2	-0.34	-0.11	
3	-0.96	0.10	
4	-0.28	-0.88	
5	-1.72	-4.27	
6	-0.23	-1.11	
7	-0.10	-0.48	
mean±	-0.56	-0.98	
SEM	0.22	0.57	p=0.18

Individual slopes of the relationship between the cardiopulmonary and carotid-vascular (MAP) baroreflex

Subject Number	Pre-HDT	Post-HDT	
1	$-4.0e^{-2}$	$-2.1e^{-2}$	
2	$-1.6e^{-2}$	$-4.8e^{-2}$	
3	$-2.0e^{-2}$	$1.7e^{-2}$	
4	$-1.3e^{-2}$	$-2.5e^{-2}$	
5	$-1.3e^{-2}$	$-2.8e^{-2}$	
6	$3.4e^{-2}$	$-8.0e^{-2}$	
7	$-6.8e^{-2}$	$-1.4e^{-2}$	
mean±	$-1.3e^{-2}$	$-1.2e^{-2}$	
SEM	$0.5e^{-2}$	$0.6e^{-2}$	p=0.44

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