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HUMAN CARDIOVASCULAR RESPONSES TO SIMULATED PARTIAL GRAVITY AND A SHORT HYPERGRAVITY EXPOSURE

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HUMAN CARDIOVASCULAR RESPONSES TO SIMULATED PARTIAL GRAVITY AND A SHORT HYPERGRAVITY EXPOSURE

DISSERTATION

A dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the
College of Engineering at the University of Kentucky

By

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ABSTRACT OF DISSERTATION

HUMAN CARDIOVASCULAR RESPONSES TO SIMULATED PARTIAL GRAVITY AND A SHORT HYPERGRAVITY EXPOSURE

Orthostatic intolerance (OI), i.e., the inability to maintain stable arterial pressure during upright posture, is a major problem for astronauts after spaceflight. Therefore, one important goal of spaceflight-related research is the development of countermeasures to prevent post flight OI. Given the rarity and expense of spaceflight, countermeasure development requires ground-based simulations of partial gravity to induce appropriate orthostatic effects on the human body, and to test the efficacy of potential countermeasures.

To test the efficacy of upright lower body positive pressure (LBPP) as a model for simulating cardiovascular responses to lunar and Martian gravities on Earth, cardiovascular responses to upright LBPP were compared with those of head-up tilt (HUT), a well-accepted simulation of partial gravity, in both ambulatory and cardiovascularly deconditioned subjects. Results indicate that upright LBPP and HUT induced similar changes in cardiovascular regulation, supporting the use of upright LBPP as a potential model for simulating cardiovascular responses to standing and moving in lunar and Martian gravities.

To test the efficacy of a short exposure to artificial gravity (AG) as a countermeasure to spaceflight-induced OI, orthostatic tolerance limits (OTL) and cardiovascular responses to orthostatic stress were tested in cardiovascularly deconditioned subjects, using combined 70º head-up tilt and progressively increased lower body negative pressure, once following 90 minutes AG exposure and once following 90 minutes of -6º head-down bed rest (HDBR). Results indicate that a short AG exposure increased OTL of cardiovascularly deconditioned subjects, with increased baroreflex and sympathetic responsiveness, compared to those measured after HDBR exposure.

To gain more insight into mechanisms of causal connectivity in cardiovascular and cardiorespiratory oscillations during orthostatic challenge in both ambulatory and cardiovascularly deconditioned subjects, couplings among R-R intervals (RRI),

systolic blood pressure (SBP) and respiratory oscillations in response to graded HUT and dehydration were studied using a phase synchronization approach. Results indicate that increasing orthostatic stress disassociated interactions among RRI, SBP and respiration, and that dehydration exacerbated the disconnection. The loss of causality from SBP to RRI following dehydration suggests that dehydration also reduced involvement of baroreflex regulation, which may contribute to the increased occurrence of OI.

KEYWORDS: Cardiovascular Regulation, Lower Body Positive Pressure, Artificial Gravity, Orthostatic Stress, Phase Synchronization.

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March 24, 2015

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March 24, 2015

DEDICATION

I dedicate this dissertation to my family, especially,

to my parents, for supporting me each step of the way;

to my wife Linyuan, for her understanding and support.

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CHAPTER 1 INTRODUCTION

Missions of astronauts to Mars are proposed in the future. It is probable that crewmembers will be exposed to a reduced gravitational environment for about two years or more. Specifically, a mission to Mars will expose crewmembers to about six months of microgravity during transition from Earth to Mars, followed by a stay on Mars for about one year in 3/8 g (g is the strength of a gravitational field) and then the transition back to Earth through another six months of microgravity.

Physiological malfunctions following exposure to microgravity are well documented. Microgravity exposure can cause muscle atrophy [1-3], bone demineralization[4, 5], immune function [6-8] decrement, neurovestibular [9] defects and impaired cardiovascular function (see reference [10] for review), e.g., reduced plasma volume (PV) [11] and red cell count, decreased autonomic function [12], cardiac arrhythmia (see reference [13] for review) and decreased orthostatic tolerance [14-18] upon reentry to higher gravitational environment. Orthostatic intolerance (OI), the inability to maintain blood pressure (BP) upon assumed upright posture, is one crucial microgravity-induced malfunction [16]. Approximately 28% to 65% of astronauts experience symptoms of OI during post-flight standing test or head-up tilt (HUT) test [14, 16, 18], which is a great risk to the safety and performance of astronauts. Thus, development of effective countermeasures to protect against OI is an important area of interest. In the meantime, since physiological data is limited or lacking for real space exploration, countermeasure development requires ground-based simulations of partial gravity to induce appropriate gravitational effects on the human body, and to test the efficacy of potential

countermeasures.

Therefore, the objective of this dissertation was to explore future Mars exploration-related cardiovascular regulation, in terms of testing new simulation models of partial gravitational environments of the moon and Mars, designing and testing countermeasures to spaceflight induced OI, and understanding mechanisms of spaceflight induced OI. The specific aims of the dissertation are as follows:

1.1 Aim 1: Test the Efficacy of Upright Lower Body Positive Pressure to Simulate Cardiovascular Responses to Lunar and Martian Gravities.

To assess cardiovascular regulation in partial gravity, ground-based simulations of cardiovascular responses to activities in lunar and Martian gravities are necessary, since actual physiological data are limited for moon activity and are lacking for Mars activity. The harness suspension system [19, 20] currently used by the National Aeronautics and Space Administration (NASA) provides a good simulation of the dynamic aspects of exercise in reduced gravity environments. However, this system does not alter the hydrostatic pressure gradient along the body axis and, therefore, imposes only a minimal impact on the cardiovascular system. In contrast to the harness suspension system, HUT is a well-accepted model to simulate cardiovascular regulation in response to lunar and Martian gravities [21]. Nevertheless, the motion restriction of the HUT model limits its application in simulating exercise in partial gravities. With the advantage of freeing subjects to exercise, applying lower body positive pressure during standing [i.e., upright lower body positive pressure (LBPP)] can also change the hydrostatic pressure gradients along the body axis via the alteration of pressure differentials [22, 23].

However, no previous study, except our previous study [22], has directly compared these two models, i.e., HUT and upright LBPP, regarding the cardiovascular responses of standing in lunar (1/6 g) and Martian (3/8 g) gravities. This study [22] indicated that upright LBPP is comparable to HUT in terms of simulating cardiovascular responses to standing in partial gravity in ambulatory subjects. Nevertheless, spaceflight is accompanied by cardiovascular deconditioning, whether upright LBPP is comparable to HUT in simulating cardiovascular responses to partial gravity following cardiovascular deconditioning is unknown.

To answer this question, in this study, cardiovascular responses to standing in lunar and Martian gravities, simulated by upright LBPP, were compared to those simulated by HUT. It was hypothesized that upright LBPP would provide a model comparable to HUT for simulating cardiovascular responses to standing in lunar and Martian gravities in both ambulatory and cardiovascularly deconditioned subjects.

1.2 Aim 2: Test the Efficacy of a Short Artificial Gravity Exposure in Postponing the Occurrence of Orthostatic Intolerance Symptoms.

Among different countermeasures proposed to prevent OI, artificial gravity (AG) provided by an onboard short-arm centrifuge has been suggested as a gravity-based countermeasure for future spaceflight (see reference [24] for review). To obtain optimal effects, different AG protocols have been tested. Although no data are currently available concerning effects of AG on the cardiovascular system during spaceflight, results from ground-based studies using intermittent artificial gravity (IAG) provided by a short-arm centrifuge are promising [25-27]. Nevertheless, IAG protocols require substantial time to complete, therefore, the development of an effective countermeasure that could be

applied over a relatively short time period would be of particular importance. So far, there is only one study investigating effects of a short AG exposure in postponing OI symptoms in ambulatory men [28]. However, effects of a short AG exposure in preventing OI are unknown in cardiovascularly deconditioned men and women.

To bridge the gap, cardiovascular responses to orthostatic stress in cardiovascularly deconditioned men and women were assessed, once following 90 minutes exposure of AG and once following 90 minutes exposure of -6º head-down bed rest (HDBR). The hypothesis of this study was that a short AG exposure would be capable to postpone the OI symptoms in cardiovascularly deconditioned men and women.

1.3 Aim 3: Explore Changes in Cardiovascular and Cardiorespiratory Couplings in Response to Orthostatic Stress and Dehydration.

The cardiovascular system is influenced by several feedback and feed-forward mechanisms regulating cardiovascular homeostasis [29]. Different signal processing methods, including time domain and frequency domain methods [30], have been widely applied to assess coupling strengths among cardiovascular subsystems. For a more detailed understanding of regulation of cardiovascular system, it is essential not only to detect interactions but also to identify causal relationships [31, 32]. Since cardiovascular (sub)systems very likely interact with each other in a nonlinear way, it is more appropriate to analyze the interactions using nonlinear approaches (see reference [33] for review) in addition to conventional linear methods [34, 35].

In this study, a phase synchronization approach was applied to investigate effects of different gravitational environments (moon, the Mars and the Earth) and reduced blood

volume on cardiovascular and cardiorespiratory couplings in a ground-based simulation of space exploration to obtain more information concerning spaceflight induced OI.

The remainder of the dissertation is organized as follows: CHAPTER 2 provides background of physiological mechanisms regulating cardiovascular hemostasis as well as details of spaceflight related cardiovascular adaptations; CHAPTER 3 describes the study exploring the use of upright LBPP to simulate cardiovascular responses to standing in lunar and Martian gravities; CHAPTER 4 demonstrates the efficacy of delaying the occurrence of OI symptoms using a short AG exposure; CHAPTER 5 describes alterations of coupling strength and coupling direction in the cardiovascular system in response to orthostatic stress and dehydration. CHAPTER 6 summarizes the major findings of the dissertation and points out several directions of future work.

CHAPTER 2 BACKGROUND

The purpose of this chapter is to review some important topics that are discussed within this dissertation. These topics include effects of orthostatic stress on cardiovascular homeostasis (section 2.1), microgravity induced cardiovascular adaptations (section 2.2) and alterations of cardiovascular regulation to higher gravitational environments following spaceflight (section 2.3). This chapter also introduces current ground-based studies in terms of countermeasures to spaceflight induced cardiovascular deconditioning (section 2.4), and simulating microgravity and partial gravity effects on cardiovascular regulation (section 2.5).

2.1 Cardiovascular Responses to Standing in Earth's Gravity

The cardiovascular system is constantly subjected to gravitational stress on the surface of the Earth. Standing up from a supine position in a gravity field imposes a substantial challenge to the human cardiovascular system, i.e., there is a significant fluid redistribution to the lower body due to the increase in hydrostatic pressure gradient along the body axis (head-to-foot) [36]. The fluid redistribution induces a drop in venous return to the heart and a consequent reduction in BP, which is a great risk to the maintenance of cerebral perfusion. Throughout the evolution of humankind, the cardiovascular system has been adapted to combat these gravitational effects. To optimize the distribution of blood volume and to maintain BP, several reflexes [36, 37], which are mediated by both nerves and hormones, are activated.

Instantaneous changes in autonomic cardiovascular control are modulated by the baroreflex mechanism. Pressure-sensitive receptors located in the walls of

cardiopulmonary veins and the right atrium, and within the carotid artery and aorta monitor venous and arterial pressure, respectively. Blood pressure-induced wall distention stimulates these receptors and sends information to the brainstem. Information from the venous baroreceptors and from the aortic arch baroreceptors are carried centrally via the vagus nerve. Carotid sinus baroreceptor nerve activity is relayed centrally by passage through the carotid sinus nerve and then through the glossopharyngeal nerve before it arrives at the brainstem. The first synapse of afferent fibers from these baroreceptors is in the nucleus tractus solitarius (NTS) of the medulla oblongata [38]. In one reflex circuit, these inputs activate NTS neurons, and in turn, excite cardiac parasympathetic preganglionic neurons in the nucleus ambiguous (NA, location of vagal motor neurons). In contrast, the NTS projects inhibitory pathways to the caudal ventrolateral medulla (CVLM) and from the CVLM to the rostral ventrolateral medulla (RVLM), where neurons that originate sympathetic tone are located, via the inhibitory neurotransmitter γ-aminobutyric acid (GABA) [39]. These cell bodies send their efferent projections through the intermediolateral gray column of the spinal cord.

These efferent neural signals innervate end-organs, such as heart and blood vessels, to adjust heart rate (HR) and vascular resistance, respectively [37]. For the heart, the vagus nerve synapses at the sinoatrial node (primarily right vagus) and atrioventricular node (primarily left vagus). Acetylcholine, the parasympathetic neurotransmitter, binds muscarinic receptors, and slows HR through an inhibitory G-protein system and a stimulus G-protein system. In addition, post-ganglionic sympathetic neurons innervate the whole heart. Norepinephrine, the sympathetic neuron transmitter, binds to β1 receptors in the heart and increases HR by increasing sodium influx and increasing

atrioventricular conductivity. For blood vessels, the post-ganglionic sympathetic neurotransmitter, norepinephrine, binds to α-adrenoreceptors on the surface of vascular smooth muscle cells and causes muscle contraction. In addition, epinephrine, binds to β2 receptors as well as α-adrenoreceptors in the vasculature. In skeletal muscle, which has high β2 density, physiological concentration of epinephrine causes vasodilation.

Baroreflex function can be simplified as follows: an increase in arterial pressure is detected by arterial baroreceptors, which increase their input into the NTS; activation of the NTS causes a greater inhibitory output to the RVLM; inhibition of cells in the RVLM results in a compensatory reduction in sympathetic tone. An increase in arterial pressure also leads to activation of the NTS and of the NA, with increased parasympathetic activity. These modulations result in a reduction in vascular resistance and HR. In converse, a decrease in arterial pressure (i.e., the case of standing in gravity field) results in decreased firing in the NTS, withdrawal of the inhibitory influence of this nuclei on the RVLM, and a compensatory increase in sympathetic tone. In addition, a decrease in arterial pressure leads to inhibition of the NTS and of the NA, with reduced parasympathetic activity. These modulations increase vascular resistance and HR.

Concurrently, endocrine response is also triggered [37]. As described above, sympathetic outflow releases epinephrine and norepinephrine from the adrenal medulla gland. Renal sympathetic nerve activity stimulates the release of renin which converts angiotensinogen to angiotensin I, and eventually to angiotensin II. Angiotensin II is an important factor in cardiovascular control: on the one hand, it is a vasoconstrictor; on the other, it stimulates aldosterone secretion, which stimulates sodium and water retention in the kidneys.

As a result, the reflex response to standing in a gravitational environment is increasing HR and cardiac contractility, vascular resistance and blood volume, all in an attempt to maintain BP and cerebral perfusion. However, when exposed to microgravity, the absence of gravity pulling effects induces several cardiovascular adaptations.

2.2 Cardiovascular Adaptation to Microgravity

When exposed to microgravity, the absence of gravity eliminates the gravity-induced pressure gradient, i.e., reduced pressure in the lower body and increased pressure at the heart and brain level, and results in cephalic fluid shift. The head-ward fluid shift initializes some cardiovascular responses to microgravity exposure. This fluid shift distends the central vasculature and this is detected by volume sensors (located in the walls of vena cava, of the pulmonary vasculature and of the atria) as a fluid-volume overload. The cardiovascular system responds to this by an immediate reduction of vascular resistance, together with a reduction of HR. In the meantime, the kidney is informed about the fluid volume overload. This fluid shift can elicit a variety of hormonal responses that lead to increased diuresis and fluid loss, including elevated secretion of atrial natriuretic peptide, decreased secretion of vasopressin, and decreased activation of the renin–angiotensin system [40]. In addition, many astronauts experience space motion sickness, which can also contribute to a contraction of the blood volume [41, 42]. It has been well documented that spaceflight induces 10~20% reduction of PV [11, 16, 40].

Numerous studies have also confirmed that microgravity exposure induces important changes in baroreflex function [43]. Most of the data have been obtained by evaluating baroreflex function during the immediate post-spaceflight time [16, 44-46]. Fritsch *et al.* [44] found that the slope, range and position of operational points on the carotid

transmural pressure-sinus node response relation were all reduced at rest on landing day after 4-5 days missions, relative to preflight. Fritsch-Yelle *et al.* [45] also found reduction in the slope, range and position of operational points of the carotid baroreceptor reflex response following 8-14 days missions. The reduction in baroreflex sensitivity was also reported using a time-domain baroreflex computation method following 10-11 days spaceflight [46]. In addition, direct assessments of baroreflex function during spaceflight were performed [47-50]. It has been shown that baroreflex sensitivity was reduced at the late stage of spaceflight [47-49]. Di Rienzo *et al.* [50] showed that rest baroreflex sensitivity increased at the early stage of spaceflight and tended to return to baseline in the subsequent days during a 16 days spaceflight.

As the stay in microgravity environment increases, the final effect of the above cardiovascular adaptations is the establishment of a new set point of the cardiovascular system, different from that on the Earth. In other words, the cardiovascular system is now "deconditioned" with respect to the Earth's gravitational field.

2.3 Post-spaceflight Orthostatic Intolerance

Upon reentering to the Earth's gravity after spaceflight, astronauts are affected by several different symptoms, such as dizziness, an inability to maintain assumed standing position, presyncopal feelings and reduced exercise capacity. These symptoms can be summarized using the term "cardiovascular deconditioning". The cardiovascular deconditioning is caused by the ineffective control of BP due to resetting of baroreceptors, to the PV reduction, and to the consequent mismatch of the beat-to-beat adjustment of cardiac output (CO) to venous return.

The occurrence of OI is a critical component of cardiovascular deconditioning, and it depends on the duration of microgravity exposure and the type of orthostatic test used. The occurrence of OI after spaceflight is well documented [14-18]. Buckey *et al.* [16] reported that 9 out of 14 crew members are unable to tolerate a 10 minutes stand test following 9-14 days in space. Meck *et al.* [15] indicated that five out of six astronauts become presyncopal after long term (120-190 days) spaceflight, while only one of six astronauts become presyncopal following a short duration spaceflight, during two 10 minutes stand tests. Meck *et al.* [14] also reported that 10 out of 23 astronauts cannot tolerate 10 minutes 80º HUT test after 5-18 days spaceflight.

2.4 Countermeasures to Microgravity-induced Cardiovascular Deconditioning

Several countermeasures to spaceflight-induced cardiovascular deconditioning have been tested with different levels of success in preventing post-flight cardiovascular deconditioning (see reference [10] for review).

2.4.1 Currently Used Countermeasures

To help restore some of the spaceflight-induced reduction in PV, the standard fluid loading protocol (~ one liter of isotonic fluid) before landing was adopted by the NASA. Results of orthostatic tests in astronauts participating in spaceflight demonstrated that this protocol is beneficial for crewmembers with flights lasting less than a week [51]. For longer flights of 10-15 days, the benefits of this protocol were reduced [52]. To protect against OI during re-entry and immediately after landing, NASA also routinely requires that astronauts wear an anti-gravity suit [53] to provide mechanical counter-pressure over the legs and the abdominal compartment. However, these protective effects are lost when the garments are disconnected from the pressure supply provided by the space shuttle.

Midodrine, an α1 adrenergic agonist, has also been shown to be effective in preventing OI after simulated [54] and real [55] short-duration spaceflight. However, its efficacy during re-entry, as well as following long-duration flights, is unknown. In addition, in-flight exercise has also shown some beneficial effects on post-flight cardiovascular responses to standing as well as exercise responses [56].

2.4.2 Potential Countermeasure: Artificial Gravity

Since cardiovascular deconditioning is virtually all attributed to the loss or reduction of gravity, a logical way to prevent cardiovascular deconditioning in a microgravity or partial gravity environment is to provide an artificial source of gravity (see reference [24] for review). Artificial gravity provides a way to simulate natural 1 g environment (i.e., Earth-like), and to challenge all physiological systems. As a consequence, several spaceflight related physiological problems, including bone loss, cardiovascular deconditioning, and muscle atrophy, can be dealt with well. For these reasons, AG can be treated as a countermeasure for multiple physiological systems.

Theoretically, AG can be generated by accelerating or decelerating a spacecraft with a constant linear acceleration. However, generating a constant linear acceleration cannot be achieved by today's state of spacecraft propulsion. To assess the physiological effects of AG generated by spacecraft rotation, a prototype, i.e., long-arm centrifuge (Figure 2.1, *left*) has been designed and tested. A more realistic way to generate AG is transporting a short-arm centrifuge (Figure 2.1, *right*) onboard. Although current space transports are restricted to relatively small payloads, transporting a short-arm centrifuge is feasible and could provide an AG source for use during spaceflight. Therefore, current ground-based AG studies have focused on the application of short-arm centrifuge.

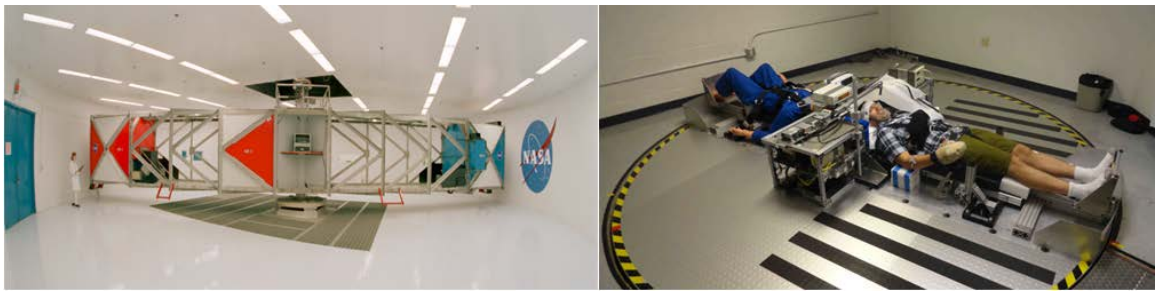

Figure 2.1 Long-arm centrifuge (*left*) and short-arm centrifuge (*right*) at the NASA Ames Research Center.

On a short-arm centrifuge, the generated artificial gravity (g) is determined by the radial distance from the center of rotation (r) and the square of the rotation angular velocity (ω), i.e., $g \propto r\omega^2$ (Figure 2.2). On a short-arm centrifuge, the subject is generally lying on his/her back on radius, with the head towards the center and the feet outwards, and the body perpendicular to the rotation axis. The generated gravitational force is parallel to the body axis and directed head-to-foot, and we call this force as +Gz. The +Gz level at the head is close to zero, while the +Gz level at the feet can reach 5 g maximum.

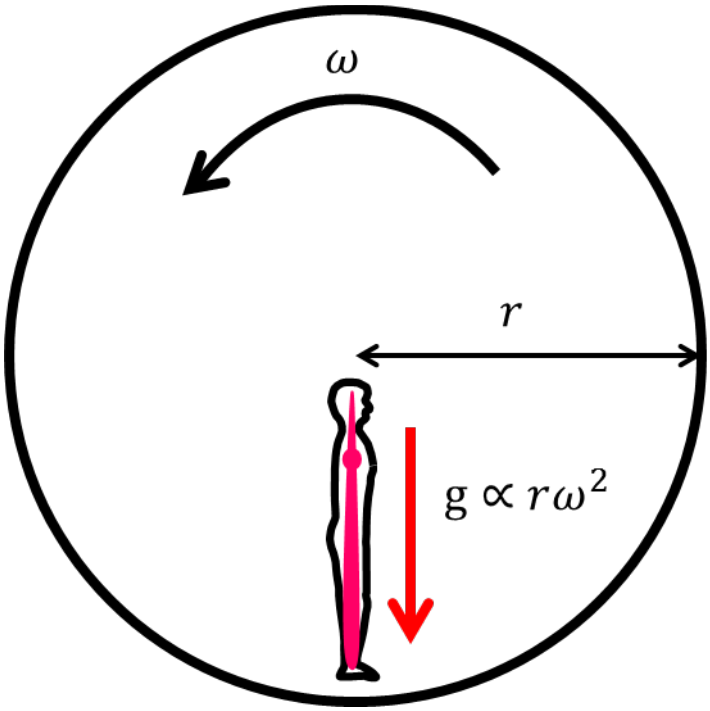

Figure 2.2 An illustration of centripetal force generated during rotation of a short-arm centrifuge.
g, centripetal force from head-to-foot; r, distance from the center of the rotation; ω, rotation angular velocity.

Although AG provides a way to simulate natural 1 g gravity, it is important to notice that AG is not equal to Earth's gravity. As shown in Figure 2.3, the +Gz profile (*red numbers*) of centrifugation induced AG is different from that of the real gravity, which is constant (i.e., 1 g) along the body axis. In addition, the AG-induced hydrostatic pressure gradient (*green numbers*) is also different from those induced by Earth's gravity. Therefore, with the aim of optimizing positive physiological effects of AG exposure, different AG protocols should be designed and tested to answer the following critical questions, i.e., 1) how much AG is needed? 2) how often should AG be applied? and 3) how long should AG be applied?

Researches of the AG effects on cardiovascular regulation are still in the initial stage;

nevertheless, results from previous studies have shown that AG training could prevent several symptoms of cardiovascular deconditioning, such as OI [25-27, 57] and reduced aerobic capacity [26].

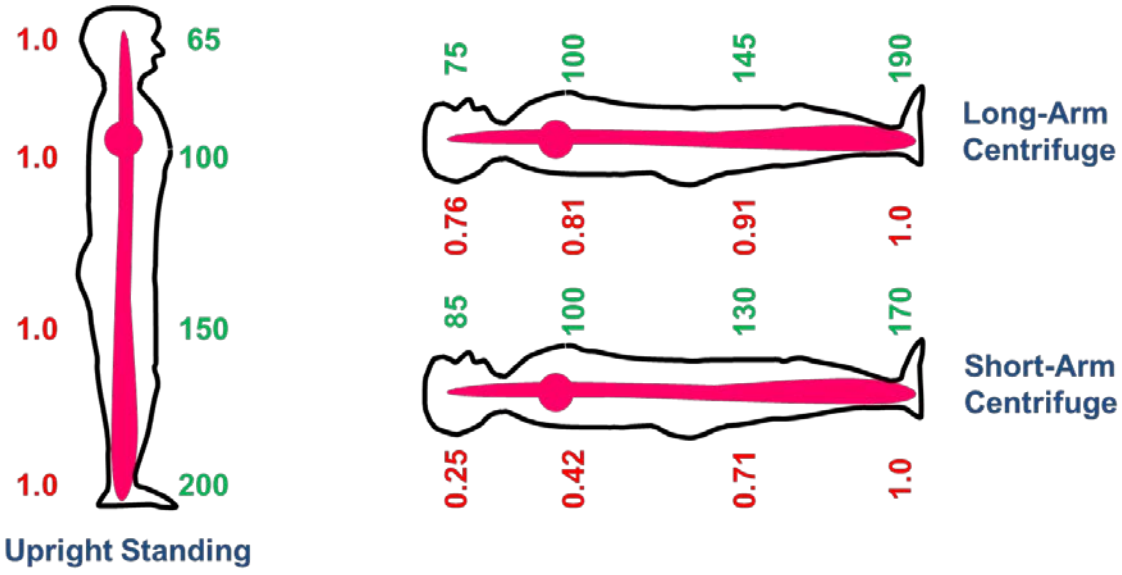

Figure 2.3 Comparison of typical +Gz profiles (g, *red numbers*) and hydrostatic pressure (*mmHg, green numbers*) gradients induced by Earth's gravity exposure (*left*) and artificial gravity exposure provided by long (*top right*) and short (*bottom right*) arm centrifuge. This figure was generated based on data in [58].

2.5 Ground-based Simulations of Spaceflight Effects on Cardiovascular Control

Since physiological data from actual spaceflight are limited, Earth-based simulations are required to better understand mechanisms of spaceflight-induced cardiovascular deconditioning and to assess prospective countermeasures. To date, several methods have been proposed and tested in simulating cardiovascular effects of microgravity, as well as partial gravity.

2.5.1 Simulations of Microgravity Effects on Cardiovascular Regulation

Head-down Bed Rest. Head-down bed rest (HDBR) is a well-accepted model to simulate most physiological effects of spaceflight (see reference [59] for review). Head-down bed

rest can induce a fluid shift from the lower to the upper compartment of the body. This fluid shift results in a transient increase of "sensed" PV, as more fluid moves into the vascular compartment from the lower body than that is filtered out of capillaries into the upper body. As in spaceflight, this head-ward fluid shift together with PV expansion stimulate central volume carotid, aortic and cardiac receptors inducing an increase in diuresis and a reduction in PV.

Acute Dehydration. As mentioned in section 2.2, a major effect of spaceflight induced cardiovascular deconditioning is PV reduction, therefore, it might be possible to reproduce the spaceflight induced changes in cardiovascular regulation by reproducing the spaceflight induced dehydration. Iwasaki *et al.* [60] compared cardiovascular responses to furosemide induced dehydration with those of HDBR, and found that the changes in reflex control are comparable. These results suggest that PV reduction may be largely responsible for the observed changes after HDBR.

2.5.2 Simulations of Partial Gravity Effects on Cardiovascular Regulation

Several simulation methods have been proposed and tested to advance Earth-based simulations of partial gravity environments. Head-up tilt is a well-accepted model to simulate partial gravity effects on the cardiovascular system. Hydrostatic pressure (P) gradient along the head-to-foot body axis at a distance h from the hydrostatic indifferent level can be altered by changing tilt angles, according to the following expression,

$$P = \rho g h[\sin(\theta)], \qquad \textbf{(2.1)}$$

where ρ is blood density, g is the acceleration of gravity, θ is the tilt table angle from horizontal. Pavy-Le Traon *et al.* [21] recommended that a 10º HUT model can be used to

simulate the acute effects of 1/6 g (i.e., lunar gravity) on the cardiovascular system.

One limitation of the HUT model is the inability to assess cardiovascular responses to activities in simulated partial gravity. In addition, as addressed before (see section 2.4), exercise alone, or in combination with other procedures are potential effective countermeasures to cardiovascular deconditioning. Therefore, in order to understand the cardiovascular control and to test the efficacy of potential countermeasures containing exercise, it is necessary to have subjects performing exercise within a simulated partial gravity environment. Currently, there are three methods available to simulate exercise in partial gravity environments, that is, the partial gravity simulator (POGO) [19], supine lower body negative pressure (LBNP) [61-63], and upright LBPP [63, 64]. The POGO is a harness suspension system developed by NASA to simulate the dynamic aspects of exercise in partial gravity environments. The POGO system can unload the musculoskeletal system, but cannot alter the hydrostatic pressure gradient along the body axis. Therefore, it has a minimal impact on the cardiovascular system. By altering the pressure differentials of inside the chamber and atmosphere pressure, supine LBNP and upright LBPP can change the hydrostatic pressure gradient along the body axis by loading and unloading to the corresponding body weight in partial gravity, respectively. Although there are previous studies investigating biomechanical and cardiovascular responses to supine LBNP [62, 63] and upright LBPP [23, 63-66], there are only a few studies assessing the validity of supine LBNP and upright LBPP [22] in simulating cardiovascular effects of standing and exercising in lunar and Martian gravities.

CHAPTER 3 CARDIOVASCULAR RESPONSES TO STANDING IN SIMULATED LUNAR AND MARTIAN GRAVITIES

The purpose of this work was to test the efficacy of upright LBPP as a model simulating cardiovascular responses to standing in lunar and Martian gravities. In an effort to understand this, cardiovascular responses to upright LBPP were compared to those of HUT, a currently well-accepted model of simulating cardiovascular responses to standing in partial gravity. This chapter is adapted from a previously published journal article [67].

3.1 Introduction

Reduced PV and altered cardiovascular regulation result from exposure to microgravity [11]. Diminished blood pressure regulation following spaceflight has been attributed to reduced central PV and inadequate vasoconstriction in response to the orthostatic stress imposed by return to gravity [11]. To maintain adequate BP and cerebral perfusion during orthostatic stress, reflex regulation is evoked by increased HR and vasoconstriction to compensate for reduced preload and stroke volume (SV).

Head-up tilt is a widely accepted maneuver used to induce passive orthostatic stress by decreasing venous return and central blood volume accompanied by a consequent unloading of cardiopulmonary and arterial baroreceptors. However, motion restriction during HUT limits further investigation of activities in space missions. Motion restriction is not a significant problem in chambers that use LBPP to reduce weight bearing during upright activity [23, 64]. In this setting, cardiovascular responses to graded LBPP have been studied in standing men and women [23, 68, 69] with results indicating that body compartment fluid redistribution in response to upright posture combined with LBPP

could also change preload, thereby loading both high- and low-pressure baroreceptors to regulate BP. Thus, combining upright LBPP with treadmill activity provides a way to simulate activity in reduced gravity environments with the advantage of producing a realistic environment in which subjects can perform tasks (i.e., walking, running, hill climbing or performing directed activities) under relevant physiological conditions [23, 63, 64]. In addition, cardiovascular deconditioning associated with spaceflight can be simulated by pharmacologically-induced acute dehydration [60].

The combination of orthostatic stress in euhydrated (normal blood volume) and dehydrated (reduced blood volume) men and women makes this study unique, since most studies [21, 64-66, 68, 70], including previous studies of our group [22, 23], using upright LBPP or HUT simulated activity in euhydrated condition. Deconditioned physiologic responses [71-73] to orthostatic stress have been rarely studied, especially in simulating standing on the surface of Moon, the Mars and the Earth. The purpose of the present study was to compare cardiovascular responses between HUT and upright LBPP in both euhydrated and dehydrated conditions to assess the hypothesis that upright LBPP would provide a model comparable to HUT to simulate partial gravities with the advantage of freeing the subject to be active.

3.2 Materials and Methods

3.2.1 Subjects

Six men (24.2 ± 0.5 years in age, 171.8 ± 3.1 cm in height, and 74.2 ± 8.9 kg in weight) and six women (24.7 ± 0.5 years in age, 159.1 ± 1.5 cm in height, and 59.3 ± 2.1 kg in weight), who were non-smokers and normotensive, were recruited. None was a trained athlete. Each subject gave informed written consent to the experimental protocol,

approved by the University of Kentucky Institutional Review Board for the Protection of Human Subjects and the NASA Johnson Space Center Committee for Protection of Human Subjects. Selection of subjects was based on a screening evaluation that consisted of a medical history questionnaire, a 12-lead electrocardiogram, and BP measurement.

3.2.2 Experimental Protocol

Each subject reported to the lab on three separate visits. During the first visit, each subject was familiarized with the protocol and with instrumentation and data collection procedures. The two subsequent visits were separated by one week and both sessions occurred at the same time of day. Subjects were studied euhydrated and dehydrated. In the dehydrated session, intravenous furosemide (Lasix®, 0.5 mg per Kg body weight, 4 mg/min) was infused to reduce PV. Urine volume and BP were monitored for at least two hours after the furosemide infusion, and testing started after urine output and BP were stabilized. Prior to testing, weight, height, and distance between impedance leads, resting HR and BP of each subject were measured, and neoprene shorts (AlterG Inc., Fremont, CA) of appropriate size were donned. An antecubital vein catheter was placed for blood sample collection. Ambient temperature was maintained between 70 and 75 degrees Fahrenheit.

Head-up tilt protocol. As shown in Figure 3.1, the HUT protocol involved moving the subjects from supine to 10º, 20º and 80º HUT in a graded manner with an electric tilt table to simulate passive standing in lunar, Martian and Earth's gravity, respectively. The HUT trial began with subjects lying supine on the table while ultrasonic images of the heart (about 10 minutes) and non-invasive cardiovascular measurements (3 minutes) were made. The same procedure was repeated at each of the three tilt angles.

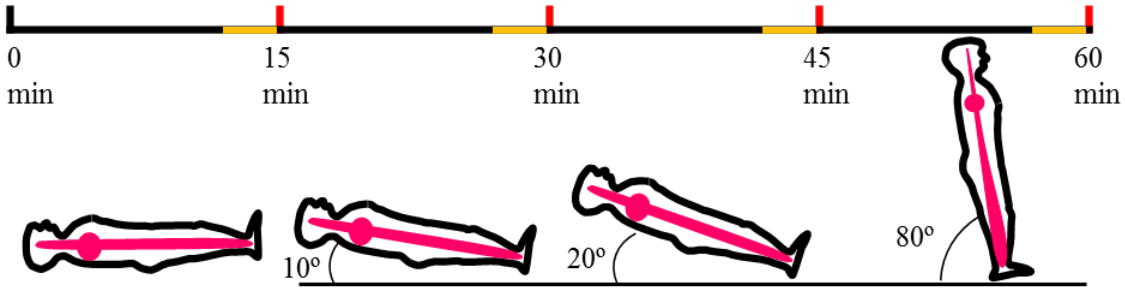

Figure 3.1 Schematic illustration of the head-up tilt protocol.

Upright lower body positive pressure protocol. Testing was conducted using a commercially available LBPP chamber with an enclosed treadmill (G Trainer, Alter G, Inc., Fermont, CA). As shown in Figure 3.2, the upright LBPP protocol included applications of positive pressure to subjects in the upright posture to reduce body weight (BW) to 20% and 40%, or remain at 100% BW (standing without a significant amount of LBPP) to simulate effects of lunar, Martian and Earth's gravity, respectively. The upright LBPP test began by standing upright with legs and hips sealed in the chamber. Ultrasound (about 10 minutes) and non-invasive cardiovascular measurements (3 minutes) were conducted at each BW.

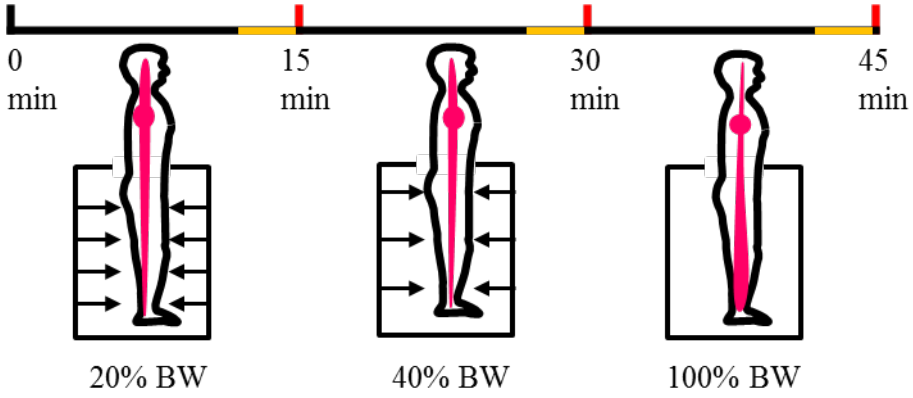

Figure 3.2 Schematic illustration of the upright lower body positive pressure protocol.

The order of HUT and upright LBPP testing was randomized among subjects, but the

same order of HUT and LBPP was used in both euhydrated and dehydrated tests for a given subject. If orthostatic hypotension symptoms developed (systolic blood pressure < 70 mmHg, HR drop > 20 beats per minute, lightheadness, dizziness or nausea), the HUT or upright LBPP test was terminated immediately. Subjects were de-instrumented and fed salty snacks and drinks after all tests were completed. The medical monitor was in charge of assessment of cardiovascular recovery and dismissal of subjects at the end of the study.

Blood samples. In both euhydrated and dehydrated conditions, a blood sample was collected from an intravenous antecubital catheter at the end of each stress (red marks in Figure 3.1 and Figure 3.2) for subsequent analysis of hematocrit (Hct) and hemoglobin (Hb). The percentage changes in plasma volume (%ΔPV) with furosemide administration and with orthostatic stresses were calculated using Hct and Hb [74] with the following equation

$$\%\Delta PV = 100 \times \left[\frac{Hb_B}{Hb_A} \times \frac{(1-Hct_A \times 10^{-2})}{(1-Hct_B \times 10^{-2})}\right] - 100, \qquad \textbf{(3.1)}$$

where A is after and B is before a time interval; Hb is in g/dL and Hct is in percent.

3.2.3 Instrumentation and Data Acquisition

Standard lead II electrocardiogram (Model 90623A, SpaceLabs Inc., Redmond, WA) was continuously monitored and collected. Continuous BP and HR were obtained at the finger using photoplethysmography (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands) with the hand positioned at heart level. Brachial artery BP was measured periodically using a manometer (UA-767, A&D Medical, San Jose, CA) placed around the upper arm for the calibration of continuous BP. Stroke volume was recorded with a

pulsed wave Doppler probe (Philips, Andover, MA). A tetra-polar high resolution impedance meter (UFI Model 2994D, Morro Bay, CA) was used to measure body segmental fluid shifts. The angle of the tilt table was recorded by an accelerometer (Crossbow, Jameco, CA), and pressure in the LBPP chamber was measured by an air pressure transducer (CyberSense CyQ 301, Nicholasville, KY). All data were collected by computer acquisition software (WinDAQ, DATAQ Instruments, Akron, OH) at 1000 Hz with subsequent analysis of mean, spectral power and baroreflex function using MATLAB (R2012b, Mathworks, Natick, MA).

3.2.4 Data Analysis

Mean values. Heart rate and R-R intervals (RRI) were computed by identifying R waves in the last three minutes of each data segment. Artifacts in the HR and BP signals were removed manually. Systolic (SBP) and diastolic (DBP) blood pressures were determined by computing the maximum and minimum values of BP for each heartbeat and were used to calculate mean arterial blood pressure (MAP, equals to two thirds DBP plus one third SBP). Estimates of total peripheral resistance (TPR) were calculated as MAP/CO. Mean values were computed for each three minutes time segment (orange segments shown in Figure 3.1 and Figure 3.2).

Spectral power. R-R intervals, SBP, and DBP were resampled at 4 Hz using a cubic spline method. Each data segment was then linearly detrended. Power spectral densities (PSD) of these variables were estimated using Welch's method of averaged periodograms (480-point Hamming window with 440-point overlap). Spectral powers in low (LF, 0.04 – 0.15 Hz) and high (HF, 0.15 – 0.40 Hz) frequency regions [75] were obtained using trapezoidal integration over the specified frequency ranges.

Baroreflex sequences. A sequence method [76] was used to provide information about the number of blood pressure ramps, the number of baroreflex sequences, baroreflex sensitivity and baroreflex effectiveness. Sequences of three or more consecutive heartbeats, in which progressively increasing or decreasing SBP (at least 1 mmHg) were followed by progressively lengthening or shortening of RRI (no less than 4 milliseconds), were identified. A sequence was accepted as a baroreflex sequence if the correlation coefficient of the regression line between SBP and RRI within the sequence was no less than 0.85 [76]. Spontaneous baroreflex sensitivity (BRS) was defined as the slope of the regression line for each sequence. The ratio between the number of baroreflex sequences and the total number of SBP ramps determined the baroreflex effectiveness index (BEI) [77]. For each subject, the numbers of SBP ramps and baroreflex sequences were normalized by the number of analyzed heartbeats in each data segment since both parameters depend on the number of analyzed heartbeats, which varied within and among subjects.

Statistics. Variables were compared using a mixed model analysis of variance (ANOVA) with Condition (euhydration vs. dehydration) × Stage (simulated gravitational environment of spaceflight, Moon, Mars and the Earth) × Technique (HUT vs. upright LBPP) for main effects and interactions. Significant interaction with Technique was considered most relevant for assessing the differential effect of HUT and upright LBPP. When significant effects were observed, Tukey's post-hoc analysis was performed to estimate pairwise comparisons. Significance was accepted at $p < 0.05$. Analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Results are presented as mean ± standard error of the mean (SEM).

3.3 Results

Complete data were collected from all euhydrated subjects. We collected no data from one man in the dehydrated condition since his BP was above 140/90 mmHg. The incidence of presyncope prevented collection of complete data segments from some dehydrated subjects. Specifically, we did not collect data from three additional dehydrated subjects at 100% BW and two at 80º HUT. Subjects experienced presyncope in three (all during HUT) of 24 tests in euhydrated conditions and in 13 (8 during HUT, and five during LBPP) of 20 tests in dehydrated conditions. The level of LBPP required to reduce subject's body weight was not different in euhydrated and dehydrated conditions (33.7 ± 1.7 vs. 31.6 ± 0.9 mmHg for 20% BW and 25.4 ± 1.3 vs. 24.0 ± 0.7 mmHg for 40% BW).

3.3.1 Steady State Hemodynamic Responses.

Table 3.1 shows average values of steady state hemodynamics at supine and in response to upright LBPP and HUT in both euhydrated and dehydrated conditions. Compared with the euhydrated condition, dehydration increased Hct (main effect of condition, $p < 0.0001$) and Hb (main effect of condition, $p < 0.0001$), resulting in a 9.5 ± 1.3% decrease of calculated resting PV (main effect of condition, $p < 0.0001$). This dehydration was accompanied by significant reductions in SV (main effect of condition, $p < 0.0001$) and CO (main effect of condition, $p < 0.0001$). Dehydration increased HR at simulated lunar ($p = 0.0008$), Martian ($p = 0.0002$) and Earth's ($p < 0.0001$) gravities, but had no significant effect on resting HR ($p = 0.4576$) or resting BP ($p = 0.3940$, 0.1909, 0.5120 for SBP, DBP and MAP, respectively).

Table 3.1 Steady state cardiovascular responses to supine rest and orthostatic stresses induced by head-up tilt and upright lower body positive pressure in euhydrated and dehydrated conditions.

	Baseline	Head-up tilt			Upright lower body positive pressure		
	Supine	HUT 10º	HUT 20º	HUT 80º	20% BW	40% BW	100% BW
				Euhydration (n=12)			
HR	64 ± 3	63 ± 3	64 ± 3	82 ± 3*	65 ± 3	68 ± 2	80 ± 2*
SBP	116 ± 4	113 ± 4	112 ± 4	104 ± 4*	118 ± 4	115 ± 4	114 ± 4‡
DBP	71 ± 2	70 ± 2	69 ± 3	70 ± 3	79 ± 2*‡	78 ± 3*‡	77 ± 3‡
MAP	86 ± 3	84 ± 3	84 ± 3	82 ± 3	92 ± 3‡	90 ± 3‡	90 ± 3‡
SV	65 ± 4	64 ± 4	61 ± 4*	47 ± 3*	61 ± 3	54 ± 2*	44 ± 2*
CO	3.85 ± 0.27	3.80 ± 0.27	3.67 ± 0.25	3.50 ± 0.21	3.86 ± 0.17	3.32 ± 0.15	3.23 ± 0.17
TPR	25.3 ± 1.4	23.2 ± 1.5	23.8 ± 1.6	24.1 ± 1.3	24.4 ± 1.3‡	27.6 ± 1.1‡	28.4 ± 1.5‡
Hb	13.6 ± 0.4	13.8 ± 0.4	13.8 ± 0.4	14.6 ± 0.4*	14.4 ± 0.4*‡	14.3 ± 0.4*‡	14.5 ± 0.4*
Hct	39.3 ± 1.1	39.7 ± 1.2	40.0 ± 1.2	41.9 ± 1.2*	41.6 ± 1.2*‡	41.2 ± 1.2*‡	41.8 ± 1.1*
%ΔPV	0	-1.5 ± 0.8	-2.5 ± 0.7	-10.5 ± 0.6*	-8.8 ± 1.0*‡	-7.7 ± 1.0*‡	-10.2 ± 0.8*
				Dehydration (n=11)			
HR	65 ± 2	67 ± 3†	70 ± 3†*	93 ± 5†*	71 ± 3†*	74 ± 3†*	93 ± 5†*
SBP	112 ± 4	109 ± 4	108 ± 3	99 ± 4*	112 ± 3	109 ± 2	120 ± 7‡
DBP	69 ± 2	70 ± 2	69 ± 3	69 ± 3	76 ± 2*	76 ± 2*	84 ± 2†*‡
MAP	83 ± 3	83 ± 2	82 ± 3	79 ± 3	88 ± 2	87 ± 2	96 ± 4†*‡
SV	50 ± 5†	49 ± 4†	44 ± 4†*	36 ± 3†*	49 ± 4†	42 ± 4†*	35 ± 3†*
CO	3.04 ± 0.20†	2.97 ± 0.18†	2.91 ± 0.16†	3.01 ± 0.23†	3.10 ± 0.22†	2.94 ± 0.21†	2.99 ± 0.21†
TPR	28.7 ± 2.4†	28.8 ± 1.8†	29.3 ± 2.3†	29.4 ± 2.5†	29.8 ± 2.2†‡	31.3 ± 2.9†‡	33.0 ± 2.7†‡
Hb	14.6 ± 0.4†	14.7 ± 0.4†	14.8 ± 0.4†	15.2 ± 0.4†*	15.1±0.4†*‡	15.1±0.4†*‡	15.1 ± 0.5†*
Hct	42.2 ± 1.1†	42.3 ± 1.1†	42.6 ± 1.1†	43.9 ± 1.1†*	43.8±1.0†*‡	43.5±1.1†*‡	43.5 ± 1.4†*
%ΔPV	-9.5 ± 1.3†	-9.9 ± 1.2†	-10.9 ± 1.2†	-16.3 ± 1.4†*	-15.9±1.1†*‡	-15.0±1.3†*‡	-16.3 ± 1.5†*

Values are mean ± SEM. HR, heart rate, beats per minute; SBP, systolic blood pressure, mmHg; DBP, diastolic blood pressure, mmHg; MAP, mean arterial pressure, mmHg; SV, stroke volume, ml; CO, cardiac output, L/min; TPR, total peripheral resistance, mmHg/(L/min); Hb, hemoglobin, g/dL; Hct, hematocrit, %; %ΔPV, plasma volume change compared with supine in the euhydrated

condition, %. † Significantly different from euhydration at the same stage, $p < 0.05$; * Significantly different from supine rest in the same condition using the same technique, $p < 0.05$; ‡ Significantly different from head up tilt response at matched level of orthostatic stress in the same condition, $p < 0.05$.

Compared with supine rest, increasing orthostatic stress, induced by HUT or upright LBPP, elevated HR (condition by stage interaction, $p = 0.0003$) and reduced SV (main effect of stage, $p < 0.0001$), with the result that CO was maintained. Hematocrit (technique by stage interaction, $p = 0.0004$) and Hb (technique by stage interaction, $p = 0.0006$) were increased, while PV (technique by stage interaction, $p < 0.0001$) was decreased by both HUT and upright LBPP. Compared with HUT, higher Hct and Hb, and greater PV loss, were observed at simulated lunar ($p < 0.0001$ for Hct, Hb and PV loss) and Martian ($p = 0.0309$ for Hct, $p = 0.0077$ for Hb and $p < 0.0001$ for PV loss) gravities during upright LBPP. Compared with supine rest, SBP (technique by stage interaction, $p < 0.0001$) was reduced by HUT and reached significance at 80^{o} ($p < 0.0001$), but DBP and MAP (Figure 3.3) were maintained during HUT in both conditions. During upright LBPP, SBP was maintained at supine values in both conditions; however, DBP was significantly increased at 20% ($p = 0.0027$) and 40% BW ($p = 0.0333$) in normovolemic condition and at 20% ($p = 0.0497$), 40% ($p = 0.0422$) and 100% BW ($p < 0.0001$) in hypovolemic condition. Mean arterial pressure was maintained at supine values in both conditions during upright LBPP, except at 100% BW in the hypovolemic condition ($p < 0.0001$), when it was significantly elevated. Hypovolemia resulted in increased DBP ($p = 0.0471$) and MAP ($p = 0.0111$) at 100% BW, but had no significant effect on BP responses at other stages. Significantly higher BP responses were observed during upright LBPP compared to HUT at Earth's gravity in both euhydrated ($p < 0.0001$ for SBP, $p = 0.0380$ for DBP, and $p = 0.0072$ for MAP) and dehydrated ($p < 0.0001$ for SBP, MAP and DBP) conditions. Compared with supine, TPR appeared to increase with upright LBPP and HUT (main effect of stage, $p = 0.0350$), but a post hoc analysis indicated the

increase was not significant even at simulated Earth's gravity (p = 0.0557). Higher TPR was observed during upright LBPP than during HUT (main effect of technique, p = 0.0069).

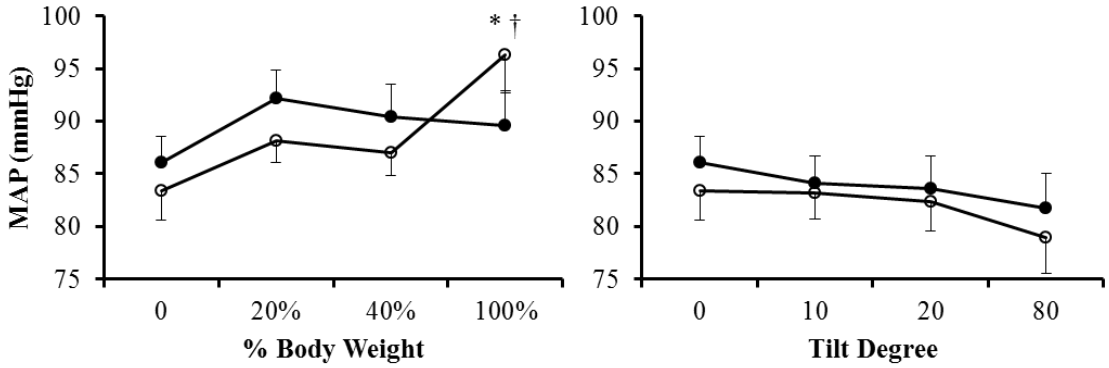

Figure 3.3 Mean arterial pressure (MAP) in euhydrated (*filled circle*) and dehydrated (*open circle*) conditions plotted as a function of increasing orthostatic stress evoked by upright LBPP (*left*) and HUT (*right*).
† Significantly different from normovolemia at the same stage, $p < 0.05$; * Significantly different from baseline in the same condition, $p < 0.05$.

Segmental impedance indicated that furosemide infusion induced significant central hypovolemia (main effect of condition, $p < 0.0001$) and both HUT and upright LBPP led to equivalent central fluid loss (Figure 3.4, main effect of technique, p = 0.5718), and equivalent fluid loss in the upper leg (not shown, main effect of technique, p = 0.1316). However, upright LBPP induced greater fluid pooling in the abdominal region (Figure 3.4, main effect of technique, p = 0.0002) and lower leg (not shown, main effect of technique, p = 0.0001) than did HUT.

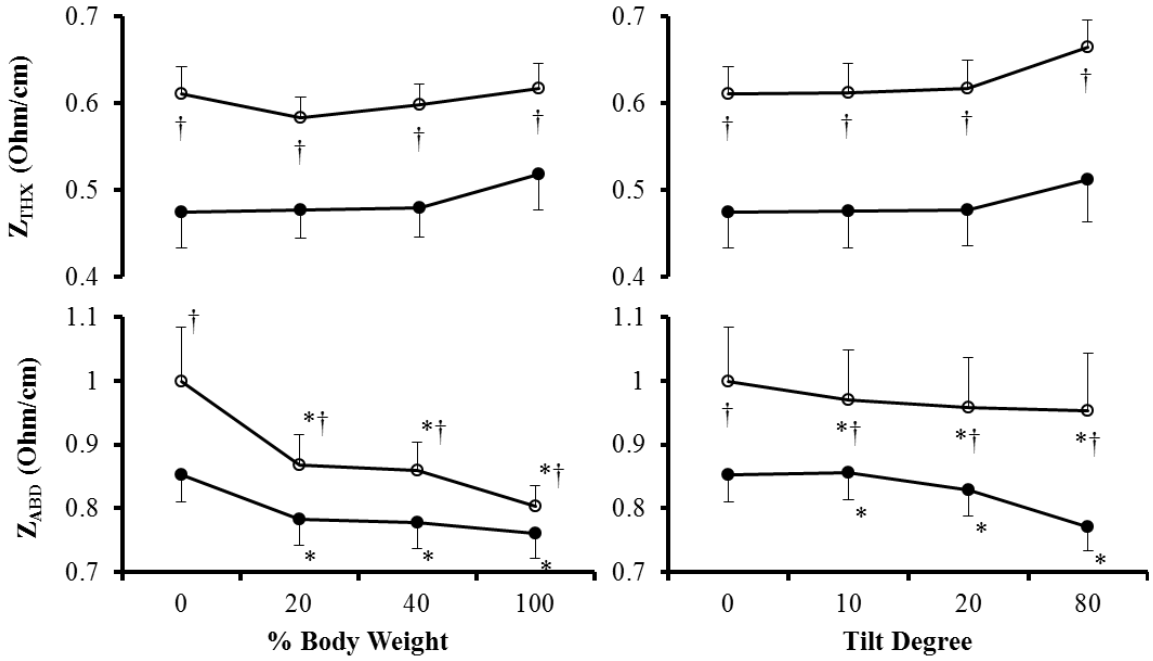

Figure 3.4 Normalized (by distance between electrodes) thoracic impedance (Z_{THX}) in euhydrated (*filled circle*) and dehydrated (*open circle*) conditions plotted as a function of increasing orthostatic stress evoked by upright LBPP (*top left*) and HUT (*top right*). Normalized (by distance between electrodes) abdominal impedance (Z_{ABD}) in euhydrated (*filled circle*) and dehydrated (*open circle*) conditions plotted as a function of increasing orthostatic stress evoked by upright LBPP (*bottom left*) and HUT (*bottom right*).
† Significantly different from normovolemia at the same stage, $p < 0.05$; * Significantly different from baseline in the same condition using the same technique, $p < 0.05$.

3.3.2 Neurally-mediated Cardiovascular Responses.

Table 3.2 shows regulatory cardiovascular responses to HUT and upright LBPP in euhydrated and dehydrated conditions. Compared with euhydration, dehydration resulted in increased low frequency spectral power of diastolic blood pressure oscillations (DBP_{LF}, main effect of condition, $p = 0.0178$), increased TPR (main effect of condition, $p < 0.0001$, Table 3.1), reduced BRS (main effect of condition, $p < 0.0001$) and increased BEI (main effect of condition, $p < 0.0001$), resulting from a significant increase in the number of baroreflex sequences in combination with no change in the number of BP ramps (not shown). The ratio of low to high frequency spectral power of R-R interval

oscillations ($RR_{LF/HF}$) was increased (main effect of condition, $p = 0.0184$) by dehydration. Normalized high frequency (by low and high frequency power) spectral power of R-R interval oscillations (RR_{HFnu}) was not different for euhydrated and dehydrated conditions at supine, but was reduced by dehydration at simulated lunar ($p = 0.0472$), Martian ($p = 0.0004$) and Earth's ($p = 0.0402$) gravities.

Table 3.2 Neurally-mediated regulatory responses to supine rest and orthostatic stresses induced by head-up tilt and upright lower body positive pressure in euhydrated and dehydrated conditions

	Baseline	Head-up tilt			Upright lower body positive pressure		
	Supine	HUT 10º	HUT 20º	HUT 80º	20% BW	40% BW	100% BW
				Euhydration (n=12)			
$RR_{LF/HF}$	1.5 ± 0.5	1.0 ± 0.2	1.4 ± 0.3	8.2 ± 2.2*	1.8 ± 0.4	1.6 ± 0.3	8.0 ± 2.5*
RR_{HFnu}	0.43 ± 0.05	0.53 ± 0.04	0.47 ± 0.04	0.16 ± 0.03*	0.43 ± 0.05	0.44 ± 0.04	0.21 ± 0.05*
DBP_{LF}	2.5 ± 0.5	2.6 ± 0.5	3.4 ± 0.8	5.4 ± 1.0*	3.5 ± 0.8	3.3 ± 0.5	5.4 ± 1.3*
BRS	26.9 ± 4.1	21.6 ± 3.5	27.8 ± 4.6	13.5 ± 2.3*	29.0 ± 5.7	22.9 ± 4.4	12.6 ± 2.0*
BEI	0.23 ± 0.05	0.28 ± 0.07	0.25 ± 0.05	0.49 ± 0.05*	0.21 ± 0.04	0.25 ± 0.04	0.43 ± 0.04*
				Dehydration (n=11)			
$RR_{LF/HF}$	1.4 ± 0.3†	1.8 ± 0.3†	2.2 ± 0.4†	13.8 ± 4.9$^{\dagger *}$	3.5 ± 1.3†	4.1 ± 1.3†	10.7 ± 1.9$^{\dagger *}$
RR_{HFnu}	0.47 ± 0.05	0.39 ± 0.04†	0.37±0.05$^{\dagger *}$	0.11±0.02$^{\dagger *}$	0.41 ± 0.08†	0.28±0.05$^{\dagger *}$	0.10±0.02$^{\dagger *}$
DBP_{LF}	3.2 ± 0.5†	3.0 ± 0.7†	4.1 ± 0.8†	7.2 ± 1.9$^{\dagger *}$	5.2 ± 1.6†	3.5 ± 1.0†	9.4 ± 2.5$^{\dagger *}$
BRS	23.5 ± 2.7†	20.6 ± 3.1†	21.6 ± 3.5†	7.2 ± 1.7$^{\dagger *}$	14.8 ± 1.7†	18.4 ± 2.6†	8.7 ± 1.5$^{\dagger *}$
BEI	0.29 ± 0.05†	0.41 ± 0.05†	0.42 ± 0.06†	0.51±0.06$^{\dagger *}$	0.30 ± 0.05†	0.39 ± 0.05†	0.52±0.07$^{\dagger *}$

Values are mean ± SEM. $RR_{LF/HF}$, ratio of low to high frequency spectral power of RR interval oscillations, a.u.; RR_{HFnu}, normalized high frequency spectral power of RR interval oscillations, a.u.; DBP_{LF}, low frequency spectral power of diastolic blood pressure, mmHg2; BRS, arterial baroreflex sensitivity, ms/mmHg; BEI, baroreflex effectiveness index, a.u.. † Significantly different from normovolemia at the same stage, $p < 0.05$; * Significantly different from supine rest in the same condition using the same technique, $p < 0.05$.

Compared with supine, Earth's gravity simulated by both upright LBPP and HUT resulted in significantly reduced BRS ($p < 0.0001$), and increased BEI ($p < 0.0001$), $RR_{LF/HF}$ ($p < 0.0001$) and DBP_{LF} ($p < 0.0001$) in both conditions. Significantly reduced supine RR_{HFnu} was observed at 80º HUT and 100% BW upright LBPP ($p < 0.0001$) in euhydrated condition. Significantly reduced supine RR_{HFnu} was also observed at Martian ($p = 0.0012$) and Earth's ($p < 0.0001$) gravities simulated by both HUT and upright LBPP in the dehydrated condition.

3.4 Discussion

Cardiovascular responses were compared between upright LBPP and HUT in euhydrated and dehydrated men and women. The primary findings were 1) there was no difference between upright LBPP and HUT with respect to changes of numerous cardiovascular indices, including HR, SV, CO, $RR_{LF/HF}$, RR_{HFnu}, BRS, BEI, TPR and DBP_{LF} in response to orthostatic stress and dehydration, 2) upright LBPP induced more PV loss at simulated lunar and Martian gravities compared to matched levels of HUT, and 3) although similar blood pressures were observed in response to orthostatic stress in the euhydrated condition, blood pressures increased during standing at 100% BW, but not during 80º HUT when dehydrated.

3.4.1 Similarities between Cardiovascular Responses to Upright Lower Body Positive Pressure and Head-up Tilt

Increased orthostatic stress is known to induce blood pooling in the lower body, leading to reduced central blood volume and venous return [36], as indicated by decreasing SV and increasing thoracic impedance in our study. Increasing tilt angle or decreasing LBPP unloads cardiopulmonary baroreceptors and reduces the inhibition of arterial

baroreceptors [23, 68] to increase HR to maintain CO near supine values. It is widely accepted that the HF power of R-R interval oscillations is mediated predominantly by changes in vagal activity, while LF power is determined by changes in both sympathetic and vagal activity [30, 78]. Therefore, increased $RR_{LF/HF}$ and decreased RR_{HFnu} associated with increased HR may indicate an enhancement of sympathetic activity and a shift of sympathovagal blance toward symapthetic control. Increased DBP_{LF} may indicate increased activation of reflex-mediated sympathetic pathways to peripheral vasculature, since LF spectral power of DBP oscillations has been shown to be related to vasomotor activity based on group mean [79]. In addition, the increase in BEI with increasing orthostatic stress indicated increased effectiveness of baroreceptors driving the sinus node, while reduced BRS suggested that the strength of the baroreflex was diminished [77]. Furosemide administration induced a PV loss comparable to the hypovolemia occurring after short term microgravity exposure [11]. As a result, a greater SV reduction was observed and a higher HR response was evoked at each stress following dehydration. The higher HR was associated with smaller RR_{HFnu} and greater $RR_{LF/HF}$, indicating inhibition of parasympathetic activity by the dehydration procedure. Dehydration also appeared to enhance sympathetic vasomotor activity [60, 71] and reduce baroreflex strength [60] since dehydration led to an increase in DBP_{LF} and a BRS reduction.

A diminished ability to maintain BP in response to orthostatic stress may result in OI. One factor in the development of OI may be the inability of subjects to adequately elevate their peripheral resistance [80]. The greater TPR during upright LBPP may have contributed to the reduced incidence of OI compared to HUT. It is unlikely that reflex-mediated vasomotion contributed to the difference of TPR since DBP_{LF} was comparable

between the two techniques, so local mechanisms need to be considered as those may intervene in baroreflex regulation [65]. Previous studies [65, 70] have suggested that an increase in intramuscular pressure can reflexly increase BP. The higher TPR during upright LBPP may result from mechanical compression of the vasculature in the lower body during upright LBPP, signaled via the intramuscular mechanoreflex [65, 68, 70]. Also, as indicated by greater blood pooling in the lower body, greater lower body transmural pressure existed during upright LBPP compared to HUT. Henriksen [81] observed that the venoarterial reflex was activated when transmural pressure exceeded 25 mmHg, and therefore might contribute to the higher TPR in upright LBPP due to greater venous distension. In addition, transmural pressure increased with increasing orthostatic stress, and was greater during upright LBPP compared to HUT at each stress level. So the myogenic response, in which vasoconstriction occurs when transmural pressure increases, might be greater in the arteries of the leg in upright LBPP than in HUT. After dehydration, although both DBP_{LF} and TPR were elevated, the reduction in PV appears to have been the primary reason for the increased incidence of OI.

3.4.2 Differences between Cardiovascular Responses to Upright Lower Body Positive Pressure and Head-up Tilt

In the present study, differential effects of upright LBPP and HUT were observed in Hct, Hb, PV change and BP responses. Comparable PV losses (Hct and Hb elevations) were observed at 80º HUT and 100% BW upright LBPP. Compared with standing, decreasing tilt angle preserved PV, while increasing LBPP had no significant effect to prevent PV loss, leading to greater PV losses at simulated lunar and Martian stresses in upright LBPP compared with HUT. Other studies have reported that 60 mmHg LBPP prevented PV

reduction during 60º HUT in men [66] and in standing men and women [73], respectively. However, in both studies [66, 73], the abdominal compartment was compressed by the antigravity suit that employed five bladders to provide positive pressure, the compression was applied either before tilting up [66] or for an extend time period (one hour) [73]. In our study, a maximum chamber pressure of less than 40 mmHg was applied for about 15 minutes, the abdominal compartment was not compressed, and LBPP was not applied before standing. It is speculated that increasing LBPP induced fluid pooling in the abdominal compartment, resulted in increased filtration at this vulnerable site in the present study. It is known that as hydrostatic pressure increases with upright posture, capillary filtration into the interstitial space increases, thereby reducing PV [82]. Reduced filtration with reducing tilt angle and increased filtration with increasing chamber pressure may contribute to the different effects of upright LBPP and HUT at intermediate stresses.

Dehydration changed BP responses to upright LBPP but not to HUT. In the euhydrated condition, application of LBPP resulted in non-significant increases of standing SBP, DBP and MAP, which are consistent with our previous studies [22, 23] and others [64]. However, Shi *et al.* [70] indicated that supine MAP increased by 3-6 mmHg at 20-30 mmHg LBPP and by 4-15mmHg at 40-50 mmHg LBPP. The relatively small BP responses to LBPP observed in the present study may be due to upright posture, as indicated by Nishiyasu and associates [68, 69] who determined that BP response to LBPP was dependent on body position. In the dehydrated condition, a significant elevation of DBP and MAP at 100% BW was observed. These BP findings are not without precedent. Kimmerly *et al.* [71] demonstrated augmented MAP at -40 mmHg lower body negative

pressure in hypovolemic subjects and attributed this to enhanced sympathetically mediated arterial baroreflex responses and an upward shift of cardiopulmonary baroreflex sensitivity. A normal rise in TPR and DBP_{LF} despite increased BP at 100% BW during dehydration suggests that sympathetic vasomotor responses to orthostatic stress may have been enhanced in this dehydrated condition during upright standing.

3.4.3 Limitations.

In the present study, different LBPPs were used for each subject to reduce BW to 20% and 40%, respectively, since the surface area of the waist seal of this commercially available device was constant. The technique that Boda *et al.* [62] used, radially changing the surface area of the waist seal to expose each subject to the same pressure, could eliminate this limitation. Also, our determination of sympathetic activity was indirect [79], but results from direct measurements of muscle sympathetic nerve activity (MSNA) in similar studies [65, 72] support our results.

3.5 Conclusions

The study documents cardiovascular responses to HUT and upright LBPP in terms of standing in lunar, Martian and Earth's gravities in euhydrated and dehydrated conditions. Cardiovascular responses were similar between HUT (10^{o} and 20^{o}) and upright LBPP (20% and 40% BW), which supports the use of upright LBPP as a potential model to simulate activity in fractional gravity. The normal rise in DBP_{LF} and TPR despite increased BP at 100% BW in the dehydrated condition indicates that dehydration may enhance the sympathetic vasomotor response to orthostatic stress.

CHAPTER 4 AUTONOMIC CARDIOVASCULAR RESPONSES TO ORTHOSTATIC STRESS FOLLOWING A SHORT ARTIFICIAL GRAVITY EXPOSURE

Although several studies have been conducted trying to maximize positive effects of AG, no study has been conducted testing the cardiovascular effects of a short AG exposure, especially in cardiovascularly deconditioned subjects. To bridge the gap, the effects of a short AG exposure on orthostatic tolerance limit (OTL) and autonomic cardiovascular control of cardiovascularly deconditioned subjects were evaluated in this study. This chapter is adapted from a manuscript [83] ready for submission.

4.1 Introduction

Microgravity [11] and its ground-based simulation, e.g., HDBR [59], result in the development of multiple cardiovascular dysfunctions that become apparent on returning to gravitational environments [10], one of which is the development of OI. Approximately 28% to 65% of astronauts experience symptoms of OI during post-flight stand/tilt tests [14, 16, 18], which is a great risk to the safety and performance of astronauts. Therefore, development of effective countermeasures to protect against OI is an important area of interest.

Among countermeasures [10] proposed to prevent OI, AG provided by a short-arm centrifuge has been suggested as a gravity-based countermeasure for future spaceflight [24]. Although no data are currently available concerning effects of AG on the cardiovascular system during spaceflight, results from ground-based studies using IAG provided by a short-arm centrifuge are promising [25-27, 57]. For example, three weeks

of IAG exposure has been shown to improve the OTL of ambulatory men [25, 27, 57], and to prevent OI of cardiovascularly deconditioned men [26]. Nevertheless, these IAG protocols [24-27, 57] required substantial time to complete, therefore, the development of an effective countermeasure that could be applied over a relatively short time period would be of particular importance. Schlegel *et al.* [28] determined that OTL and baroreflex responsiveness were improved by a single, sustained exposure to 30-min +3Gz AG in ambulatory men. Moreover, as PV loss is one factor contributing to post-flight OI [11, 59], and cardiovascular control of dehydrated subjects is more relevant to returning astronauts [67, 72], effects of a single AG exposure on cardiovascular responses during dehydration-induced deconditioning, which are unknown, need to be studied.

In addition, women have been reported to be more predisposed to OI than men [84]. However, most AG studies [24-26, 28], in particular, studies using a single AG exposure [28], have involved only male subjects. Convertino *et al.* [85] indicated that cardiovascular adaptations to IAG were different in men and women. Stenger *et al.* [27] reported passive IAG exposure did not significantly improve ambulatory women's OTL. Thus, whether a short AG exposure is effective to improve deconditioned women's OTL needs to be investigated.

To determine whether OTL would be improved following a short AG exposure, cardiovascular and cerebrovascular responses to orthostatic stress, once following 90 minutes AG exposure and once following 90 minutes HDBR exposure, were compared, in cardiovascularly deconditioned subjects. Mean values of the hemodynamic responses to orthostatic stress following AG or HDBR exposure have been reported elsewhere [86]. The purpose of the present analysis was, specifically, to determine effects of a short

duration AG exposure on autonomic cardiovascular function and baroreflex function when exposed to orthostatic stress, compared to those following HDBR exposure. It was hypothesized that a short AG exposure would improve subsequent sympathetic responses to orthostatic stress and baroreflex responsiveness compared to those following HDBR exposure.

4.2 Materials and Methods

4.2.1 Subjects

Nine men (38 ± 4 years in age, 175 ± 3 cm in height, and 81 ± 5 kg in weight) and seven women (30 ± 2 years in age, 168 ± 2 cm in height, and 71 ± 4 kg in weight), who were non-smokers and normotensive, were recruited. None was a trained athlete. Each subject gave informed written consent to the experimental protocol, approved by the NASA Ames Research Center and University of Kentucky Institutional Review Boards for the Protection of Human Subjects. Selection of subjects was based on a screening evaluation that consisted of a medical history questionnaire, a 12-lead electrocardiogram, and BP measurement.

4.2.2 Experimental Design and Protocol

Each subject attended two experimental sessions which were separated by 21 days and both sessions occurred at the same time of day. Both experimental sessions included each of the following: 1) dehydration protocol, 2) ~90 minutes HDBR exposure or ~90 minutes AG exposure, and 3) OTL test protocol. The order of treatment assignment (HDBR vs. AG) was randomized and counterbalanced.

Dehydration protocol. Subjects were given guidelines for sodium intake for 48 hours preceding each experimental session. On the day of each session, after a debriefing and a

check of the subject's potassium level, 20 mg furosemide was infused intravenously to reduce PV. Urine output and BP were monitored for up to two hours after the injection. Tests started after urine output and BP had stabilized.

Head-down bed rest protocol. Subjects were placed in -6º HDBR position for ~90 minutes before their OTL test.

Artificial gravity exposure protocol. The Human Performance Centrifuge (Figure 2.1, *right*) at the NASA Ames Research Center was used to provide AG. The subject lay on the centrifuge with his/her head toward the center in a fully extended body position. The acceleration and deceleration rates were 5 rpm/sec^2. The rotation speed was adjusted to obtain the desired +Gz level at the heart for each subject. After being instrumented and following five minutes supine baseline, each subject underwent an ~90 minutes "step up" AG protocol including a presyncopal limit test (PLT) followed by a training period. During PLT, men were taken to 0.6 g (at the heart level) and held there for five minutes, then incremented by 0.1 g at three minutes intervals until the development of presyncope (SBP < 90 mmHg, HR drop > 20 bpm or the subject experienced nausea, dizziness or light-headedness). The subject was then brought back to rest for 10 minutes. During the training period that immediately followed the 10 min supine rest, a step up protocol was used that went 0.2 g below the presyncopal limit. Women were brought to 0.4 g (at the heart level) at the beginning of PLT and training and then followed the same protocol as men since women were thought to be less tolerant of this stress than men. Following the training period, the subject rested for five minutes. Figure 4.1 shows a typical AG exposure protocol for a dehydrated male (*top,* height = 161.4 cm; distance between the heart and the center of centrifuge = 48.1 cm) and female (*bottom,* height = 161.5 cm;

distance between the heart and the center of centrifuge = 46.4 cm) subject.

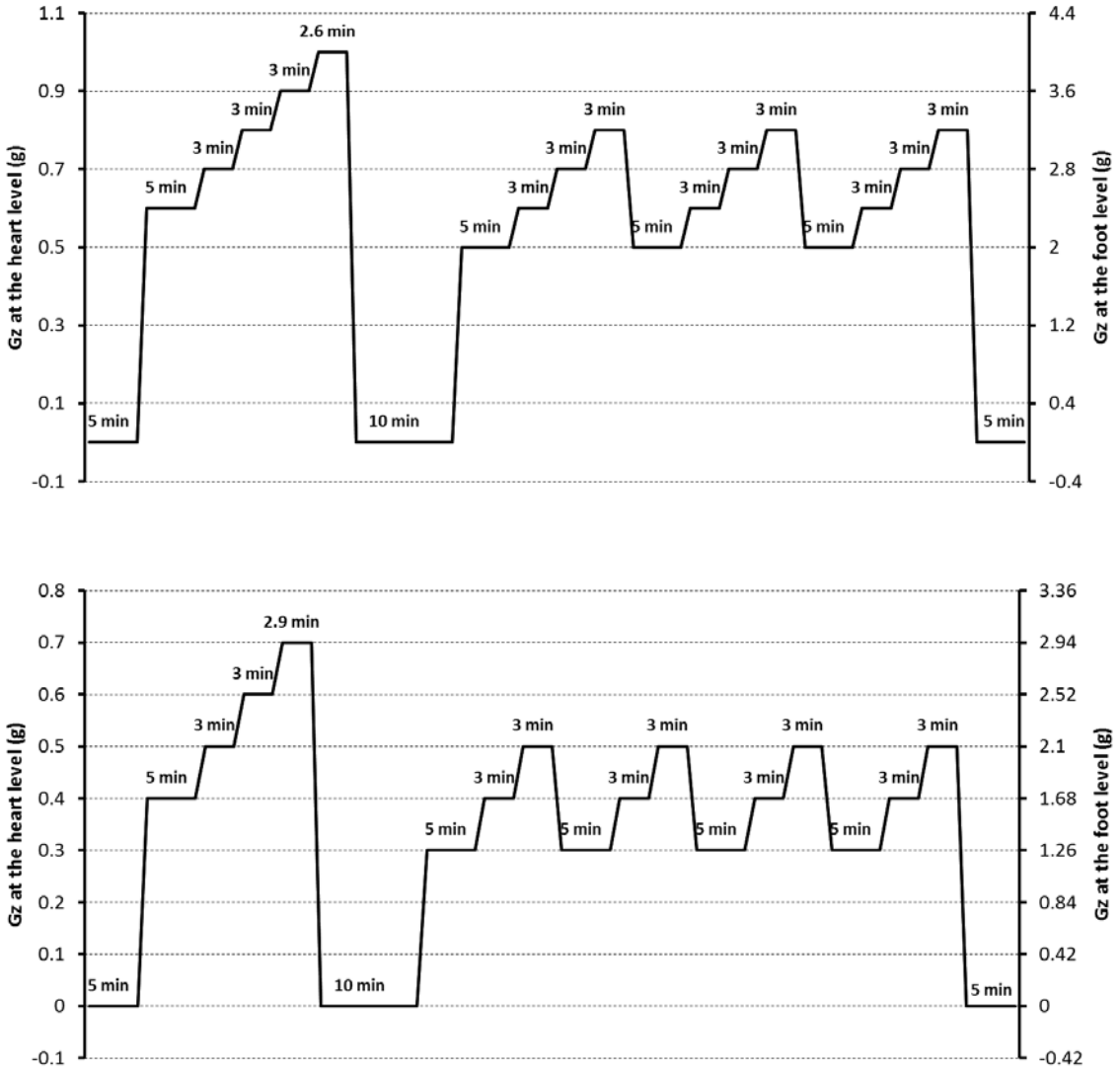

Figure 4.1 Artificial gravity exposure protocols for a cardiovascularly deconditioned male (*top*) and female (*bottom*) subject.

Orthostatic tolerance limits test protocol. Subjects lay supine for at least 15 minutes for instrumentation and equilibrium. Supine control data were taken for 10 minutes before HUT. The tilt table was then brought to 70º for 10 minutes, after which pressure inside the chamber was reduced 20 mmHg below atmospheric pressure for three minutes; subsequent 10 mmHg reductions in pressure were made at three minutes intervals until

the onset of presyncopal symptoms. At the onset of presyncope, the subject was placed in the Trendelenburg position (-6º) until BP and HR stabilized. Orthostatic tolerance limit was defined as the time elapsed between the start point of tilt up and the endpoint of presyncope. A orthostatic tolerance limit test protocol for a dehydrated male subject following a short AG exposure is shown in Figure 4.2.

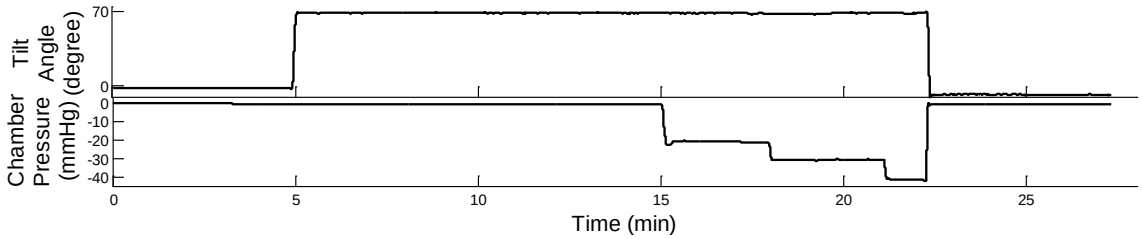

Figure 4.2 An illustration of orthostatic tolerance limit test protocol for a male subject following a short artificial gravity exposure.

4.2.3 Instrumentation and Data Acquisition

During the OTL test, standard lead II electrocardiogram (Model 90623A, SpaceLabs, Inc., Redmond, WA) was continuously monitored and recorded. Continuous BP and HR were obtained at the middle finger of the left hand using photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) with the hand positioned at heart level using a sling. The height correction feature of the Finometer was used to correct for hydrostatic difference between heart and finger sensor. Brachial artery BP was also measured periodically using a manometer (UA-767, A&D Medical, San Jose, CA) placed around the upper arm for the calibration of continuous BP. Changes in blood volumes of body segments were estimated with the use of a tetrapolar high-resolution impedance monitor four-channel digital impedance plethysmograph (UFI Model 2994D, Morro Bay, CA). Impedance was obtained for four anatomic segments, i.e., thorax (supraclavicular area to xyphoid process), abdomen (xyphoid process to iliac crest),

upper leg (iliac crest to knee) and lower leg (upper calf just below the knee to the ankle) [87]. Respiration was estimated using respiration-induced changes of thoracic impedance. All data were collected by computer acquisition software (WinDAQ, DATAQ Instruments, Akron, OH) at 1000 Hz with subsequent analysis using MATLAB (R2012b, Mathworks, Natick, MA). Figure 4.3 shows major physiological signals collected during OTL test for a cardiovascularly deconditioned male subject following a short AG exposure.

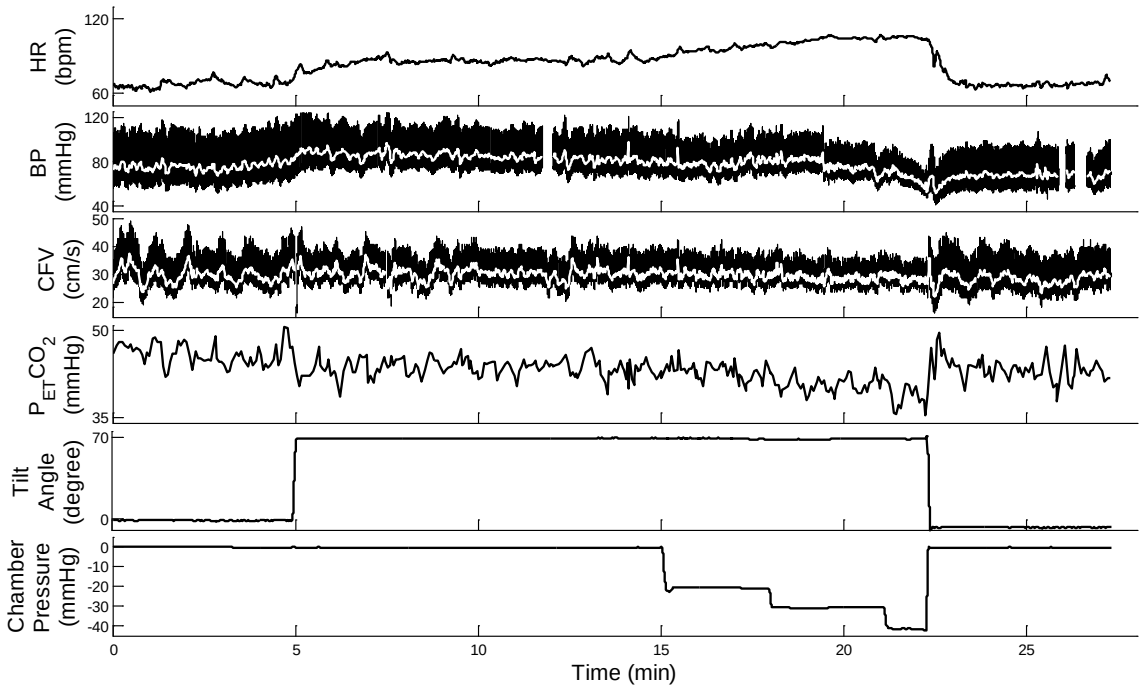

Figure 4.3 Major physiological signals collected during orthostatic tolerance limit test for a cardiovascularly deconditioned male subject following a short artificial gravity exposure.
HR, heart rate, beats per minute; BP, blood pressure, mmHg; CFV, cerebral flow velocity at the middle cerebral artery, cm/s; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide, mmHg. The white trace in BP waveform indicates mean arterial pressure, and the white trace in CFV waveform indicates mean cerebral flow velocity.

4.2.4 Data Analysis

Period selection. Data were summarized as two-minute averages. Segments including

two-minute before HUT, two-minute after tilt to 70º, two-minute before presyncope and two-minute following tilt back were chosen to determine cardiovascular regulation at supine control, the initial response to tilt (early tilt, ET), preceding presyncope (late tilt, LT) and recovery from OTL tests following AG compared to HDBR exposures, as shown in Figure 4.4 (*red segment*). Due to the inter-subject differences of OTL, late tilt period includes some levels of lower-body negative pressure for some subjects.

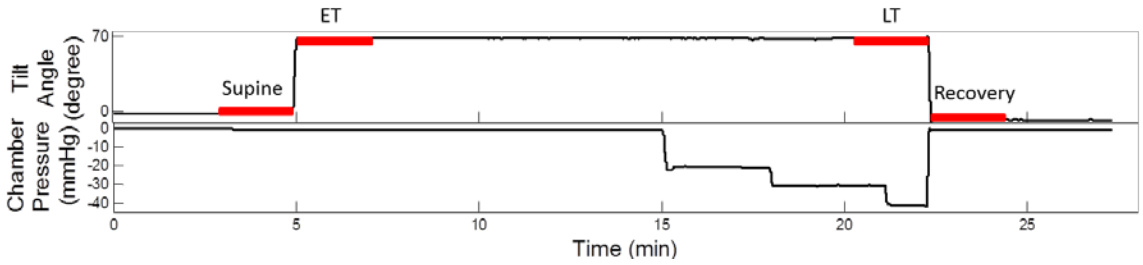

Figure 4.4 An illustration of data analysis period selection.
Each red segment indicates a two-minute segment used for data analysis. Supine, two-minute at supine position; ET, two-minute at 70º head-up tilt; LT, last two-minute before presyncope; Recovery, two-minute following tilt back.

Preprocessing. Locations of the R wave peak were identified in the ECG and R-R interval (RRI) time series were constructed. Local maxima and minima of BP within each heartbeat were identified and used to construct systolic (SBP) and diastolic blood pressure (DBP) time series, respectively. Respiratory rate (f_R) was obtained by identifying local minima of the respiratory waveform (i.e., the start of expiration). All artifacts were removed by visual inspection. Mean values of each two-min data were used to provide hemodynamic parameters. Each time series was then linearly interpolated, resampled at 4 Hz and linearly detrended for spectral and transfer function analyses.

Spectral power. Spectral power of RRI was calculated based on Welch's averaged periodogram method. Power spectral density estimates were made from 256-point (64

sec) windows with 32-point (8 sec) increments. This process resulted in eight segments of data for each recording. Mean values of spectral power in the low- (LF, 0.04-0.15 Hz) and high- (HF, 0.15-0.4 Hz) frequency ranges were calculated [30, 78]. The same methodology with SBP yielded SBP_{LF} and SBP_{HF}. The ratio of LF and HF spectral power of RRI ($RR_{LF/HF}$) and normalized HF spectral power of RRI (RR_{HFnu}, by the summation of LF and HF power) were also calculated to reflect sympathetic and vagal control of HR [30, 78], respectively.

Transfer function analysis. Coherence and transfer function gain and phase between spontaneous oscillations in SBP and RRI were determined using cross-spectral analysis in the LF range as this range is thought to be predominantly determined by the baroreflex [88]. To ensure robust gain and phase estimates within the LF band, we averaged only those gain and phase values where the corresponding coherence was greater than 0.5.

Baroreflex sequences. See section 3.2.4 for details.

Statistical analysis. A two-tailed, paired t-test was used to determine the significance of OTL following a short AG exposure compared with those following a short HDBR exposure. A three-way mixed model analysis of variance was used to determine the effects of gender, treatment (AG vs. HDBR) and time (supine, ET, LT and recovery) with two repeated factors (treatment and time). Least squares means method was used to assess pairwise comparisons. Logarithmic transformation was performed for parameters not normally distributed. Analysis was completed using SAS 9.3 (SAS Institute Inc., Cary, NC). Significance was accepted at $p < 0.05$, and p values less than 0.10 were noted throughout. Values are shown in mean ± standard error of the mean (SEM).

4.3 Results

4.3.1 Orthostatic Tolerance Limit.

As shown in Figure 4.5, a short AG exposure significantly increased OTL of male ($p = 0.0041$), but not female ($p = 0.5770$) subjects, compared with those following HDBR exposure.

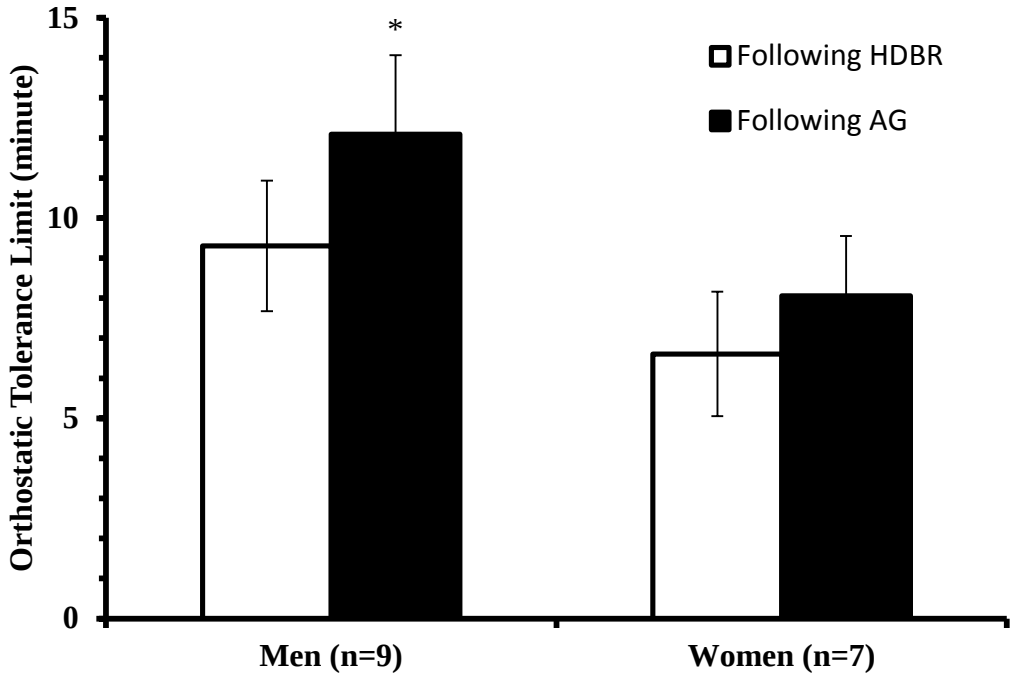

Figure 4.5 Group average of orthostatic tolerance limit following head-down bed rest (HDBR, *white bar*) and artificial gravity (AG, *black bar*) exposure in men and women.
* Significantly different compared to orthostatic tolerance limit following head-down bed rest exposure, $p < 0.05$.

4.3.2 Mean Values.

Table 4.1 shows group averages of hemodynamic parameters in response to orthostatic stress following AG compared with HDBR. Supine HR increased (Treatment × Time, $p = 0.0166$) with orthostatic stress. During recovery, HR following HDBR was lower than

supine ($p = 0.0008$) and lower compared with HR following AG ($p = 0.0472$). Overall, women had higher HR responses (Gender × Time, $p = 0.0005$), due primarily to ET response ($p = 0.0109$) after both AG and HDBR. Compared with HDBR, AG reduced men's ($p = 0.0192$), but did not change women's, SBP (Gender × Treatment, $p = 0.0442$). Compared to supine, SBP decreased during orthostatic stress and was not restored during recovery (Time, $p < 0.0001$). Resting DBP (Time, $p < 0.0001$) increased at ET ($p < 0.0001$) and decreased at recovery ($p < 0.0001$). Lower SBP ($p < 0.0001$) and DBP ($p < 0.0001$) were observed at LT compared with ET. Respiratory rate was not changed by AG compared with HDBR, and was not altered by orthostatic stress. Compared to supine, normalized thoracic impedance (Z_{THX}) increased during tilt and was restored during recovery (Time, $p < 0.0001$). Normalized abdominal impedance (Z_{ABD}, Gender × Time, $p = 0.0102$) decreased during tilt in both men and women. Recovery restored Z_{ABD} in men ($p = 0.0902$) but not in women ($p = 0.0006$). Compared with ET, Z_{THX} tended to increase ($p = 0.0696$) and Z_{ABD} decreased in both men ($p = 0.0002$) and women ($p = 0.0019$) during LT.

Table 4.1 Hemodynamic response to orthostatic stress after AG vs. HDBR in dehydrated men and women

	Following AG				Following HDBR			
	Supine	ET	LT	Recovery	Supine	ET	LT	Recovery
				Men (n=9)				
HR	68.9±4.9	84.9±4.1*	104.8±7.0*	70.3±4.5†	67.4±3.4	80.8±4.0*	94.4±5.1*§	64.0±3.6*
SBP	119.7±4.4†	122.3±5.1†	100.1±4.8*	107.8±3.3*	131.5±3.3	130.0±4.0	112.4±4.5*	113.7±3.8*
DBP	69.3±3.0	76.9±2.4*	68.6±3.1§	65.8±2.5*	75.8±2.2	79.6±2.5*	73.7±2.7§	67.8±2.5*
f_R	17.0±1.0	16.3±1.2	15.6±1.2	17.9±1.6	17.6±1.5	15.1±1.1	16.1±1.4	16.2±1.3
Z_{THX}	0.43±0.03	0.46±0.04*	0.47±0.04*	0.43±0.03	0.46±0.03	0.50±0.03*	0.50±0.03*	0.46±0.03
Z_{ABD}	0.85±0.03	0.80±0.03*	0.79±0.03*	0.84±0.03	0.88±0.04	0.83±0.04*	0.82±0.04*	0.88±0.04
				Women (n=7)				
HR	70.7±1.7	90.9±2.2*‡	101.3±4.3*	69.6±2.4†	71.2±2.1	94.4±2.5*‡	106.6±4.7*	64.4±2.4*
SBP	126.8±2.1	127.0±3.3	113.4±4.0*	109.6±3.0*	124.5±3.5	125.2±2.9	111.0±3.9*	107.0±5.7*
DBP	70.8±2.0	75.5±2.9*	71.3±3.0§	66.1±2.1*	71.2±1.7	77.4±2.5*	72.6±2.5§	64.2±3.4*
f_R	17.6±1.6	17.1±2.3	17.2±2.0	18.7±2.1	17.0±2.5	17.6±2.3	17.6±1.9	18.4±1.9
Z_{THX}	0.59±0.01‡	0.63±0.01*	0.64±0.01*	0.59±0.01‡	0.56±0.04‡	0.60±0.04*	0.60±0.04*	0.56±0.04‡
Z_{ABD}	1.00±0.02‡	0.93±0.03*	0.91±0.03*	0.99±0.02*	1.04±0.06‡	0.97±0.06*	0.96±0.06*	1.03±0.07*

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; HR, heart rate, beats/minute; SBP, systolic blood pressure, mmHg; DBP, diastolic blood pressure, DBP; f_R, respiratory rate, breaths/min; Z_{THX}, normalized (by distance between electrodes) thoracic impedance, Ohm/cm; Z_{ABD}, normalized (by distance between electrodes) abdominal impedance, Ohm/cm. * Significantly different from supine, $p < 0.05$; † significantly different from HDBR, $p < 0.05$; ‡ significantly different from male subjects, $p < 0.05$; § significant difference between ET and LT, $p < 0.05$.

4.3.3 Spectral Power.

Heart rate and blood pressure variability parameters are shown in Table 4.2. With respect to supine, $RR_{LF/HF}$ (Time, $p < 0.0001$) increased and RR_{HFnu} (Time, $p < 0.0001$) decreased during tilt. Lower $RR_{LF/HF}$ ($p = 0.0022$) and higher RR_{HFnu} ($p = 0.0015$) were observed at recovery compared with supine. Compared to HDBR, AG increased SBP_{LF} (Gender × Treatment × Time, $p = 0.0235$) in women ($p = 0.0441$) but reduced SBP_{LF} in men at supine ($p = 0.0524$) and at recovery ($p = 0.0271$). Women had greater SBP_{LF} at supine ($p = 0.0222$), ET ($p = 0.0219$) and recovery ($p = 0.0278$) than men following AG, and greater SBP_{LF} at ET ($p = 0.0302$) following HDBR. With respect to supine, SBP_{LF} increased at ET ($p = 0.0003$) in women and at ET ($p = 0.0023$) and LT ($p = 0.0035$) in men following AG, increased at ET ($p < 0.0001$) and LT ($p = 0.0077$) in women and tended to increase at ET ($p = 0.0803$) in men following HDBR. Compared to supine, SBP_{LF} decreased at recovery in men ($p = 0.0017$ and 0.0103) and women ($p = 0.0224$ and 0.0195) following AG and HDBR, respectively. With respect to supine, SBP_{HF} increased during tilt (Time, $p < 0.0001$) and was restored during recovery. Compared with ET, SBP_{HF} increased during LT ($p = 0.0422$).

Table 4.2 Heart rate variability and blood pressure variability responses to orthostatic stress following AG vs. HDBR in dehydrated men and women

	Following AG				Following HDBR			
	Supine	ET	LT	Recovery	Supine	ET	LT	Recovery
				Men (n=9)				
$RR_{LF/HF}$	7.0±2.2	11.5±2.3*	19.2±3.2*	4.1±1.4*	6.7±1.5	14.3±4.3*	18.4±4.5*	4.4±1.6*
RR_{HFnu}	0.24±0.07	0.11±0.03*	0.07±0.02*	0.30±0.05*	0.18±0.03	0.10±0.02*	0.10±0.04*	0.29±0.06*
SBP_{LF}	7.2±1.2	22.4±9.0*	22.6±5.8*	3.4±1.0*†	10.3±1.7	17.3±3.9	18.9±6.3	4.9±0.9*
SBP_{HF}	1.3±0.5	3.6±0.7*	6.0±1.2*§	1.0±0.1	1.1±0.2	2.5±0.6*	6.2±1.6*§	1.1±0.2
				Women (n=7)				
$RR_{LF/HF}$	5.8±1.6	16.1±4.8*	12.4±3.8*	3.0±0.6*	4.2±1.2	13.3±3.3*	14.4±3.6*	2.2±0.5*
RR_{HFnu}	0.19±0.04	0.09±0.02*	0.13±0.04*	0.30±0.05*	0.23±0.03	0.10±0.03*	0.10±0.03*	0.35±0.05*
SBP_{LF}	12.5±1.5†‡	36.0±6.3*‡	26.9±7.3	5.9±0.7*‡	9.4±2.4	34.5±8.3*‡	25.8±5.9*	4.2±1.4*
SBP_{HF}	1.2±0.4	5.4±2.1*	5.8±1.7*§	1.7±0.7	1.2±0.4	5.9±1.2*	7.4±2.3*§	0.8±0.4

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; $RR_{LF/HF}$, ratio of low frequency (0.04 – 0.15 Hz) power and high frequency (0.15 – 0.4 Hz) power of R-R intervals, normalized unit; RR_{HFnu}, normalized high frequency power of R-R intervals, normalized unit; SBP_{LF}, low frequency power of systolic blood pressure, $mmHg^2$; SBP_{HF}, high frequency power of systolic blood pressure, $mmHg^2$. * Significantly different from supine, $p < 0.05$; † significantly different from HDBR, $p < 0.05$; ‡ significantly different from male subjects, $p < 0.05$; § significant difference between ET and LT, $p < 0.05$.

4.3.4 Baroreflex Sequences.

Figure 4.6 shows baroreflex parameters calculated using the spontaneous sequence method. Compared to supine, the normalized number of SBP ramps (N_{ramps}) increased (Time, $p = 0.0002$) at ET ($p = 0.0745$) and LT ($p = 0.0072$) and decreased at recovery ($p = 0.0026$), the normalized number of baroreflex sequences (N_{seq}) decreased (Time, $p = 0.0011$) at LT ($p = 0.0011$) and at recovery ($p = 0.0285$). Compared with ET, N_{seq} decreased at LT ($p = 0.0002$). Women had greater N_{seq} (Gender × Treatment, $p = 0.0396$) than men following AG ($p = 0.0303$) but not HDBR. Compared to supine, BEI was reduced by orthostatic stress (Time, $p < 0.0001$), reaching significance at LT ($p < 0.0001$). Compared with ET, BEI was reduced at LT ($p < 0.0001$). Women had greater BEI than men (Gender, $p = 0.0390$). With respect to supine, BRS (Gender × Time, $p = 0.0125$) decreased at ET ($p = 0.0003$ and $p < 0.0001$) and LT ($p < 0.0001$ and $p < 0.0001$) in men and women, respectively. At recovery, increased BRS was observed in men ($p = 0.0092$), but not women, compared with supine, following both AG and HDBR. Compared with ET, BRS was reduced at LT in men ($p < 0.0001$). Compared to men, women had higher BRS at LT ($p = 0.0433$) and tended to have greater BRS at supine ($p = 0.0626$) following both AG and HDBR.

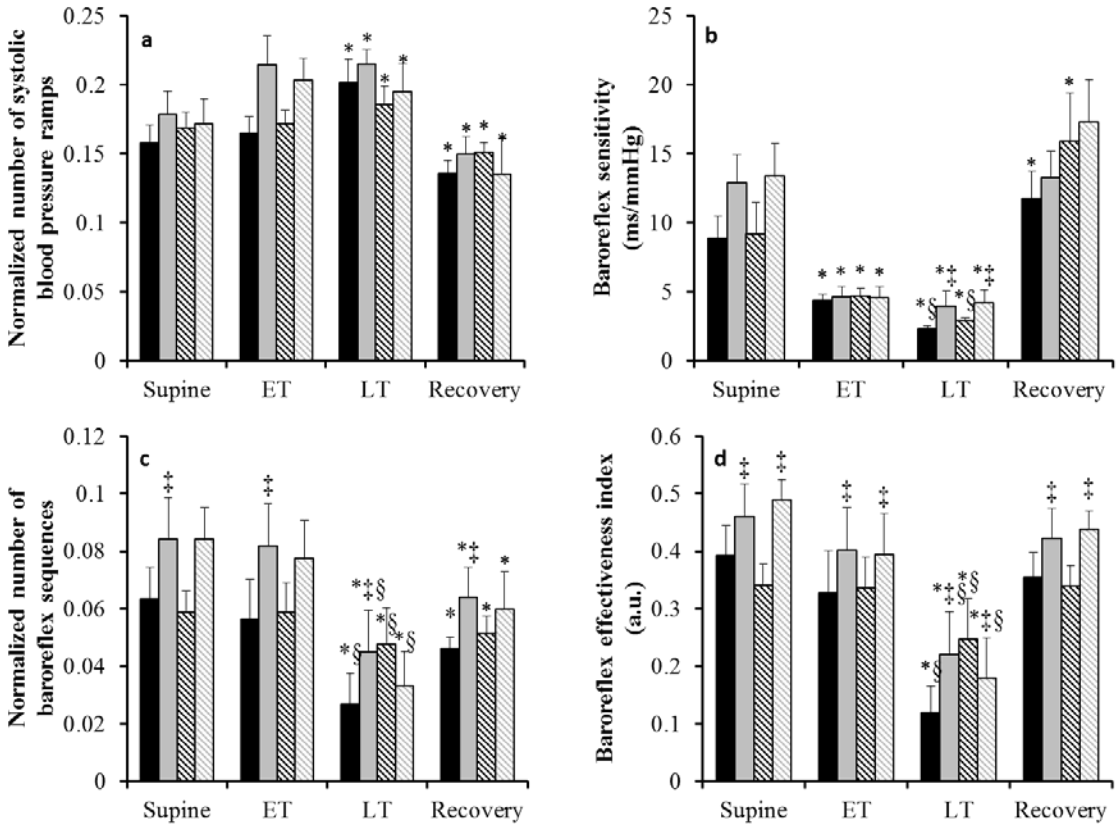

Figure 4.6 The normalized number of systolic blood pressure ramps (a), baroreflex sensitivity (b), the normalized number of baroreflex sequences (c) and baroreflex effectiveness index (d) responses to orthostatic stress after AG (*solid bars*) vs. HDBR (*hatched bars*) in dehydraed men (*black*) and women (*gray*).
ET, early tilt; LT, late tilt. * Significantly different from supine, $p < 0.05$; † significantly different from HDBR, $p < 0.05$; ‡ significantly different from male subjects, $p < 0.05$; § significant difference between ET and LT, $p < 0.05$.

4.3.5 Transfer Function Analysis.

Table 4.3 shows baroreflex parameters calculated using transfer function analysis. A significant three-way interaction was detected in COH_{LF} (Gender × Treatment × Time, $p = 0.0010$). Compared to results following HDBR, women had lower COH_{LF} at LT ($p = 0.0025$) and men had similar COH_{LF} across the OTL test ($p = 0.0729$ at supine) following AG. Compared to men, women had higher COH_{LF} at ET ($p = 0.0190$) and LT ($p = 0.0035$) following HDBR and at supine ($p = 0.0053$) and ET ($p = 0.0042$) following AG.

Compared with supine, women's COH_{LF} did not change during the OTL test following HDBR, but decreased at LT ($p = 0.0028$) and recovery ($p = 0.0068$) following AG. However, men's COH_{LF} was reduced at LT ($p = 0.0180$) following HDBR and did not change during the OTL test following AG. Compared with ET, women's COH_{LF} decreased at LT ($p < 0.0001$) following AG while men's COH_{LF} did not change following either AG or HDBR ($p = 0.0614$).

Orthostatic stress reduced $Gain_{LF}$ (Time, $p < 0.0001$) at ET ($p < 0.0001$) and LT ($p < 0.0001$) and increased $Gain_{LF}$ ($p < 0.0001$) at recovery, compared to supine. Compared with ET, $Gain_{LF}$ decreased at LT ($p < 0.0001$). Compared to supine, $Phase_{LF}$ (Treatment × Time, $p = 0.0271$) decreased at ET ($p = 0.0260$) following HDBR, while did not change in response to orthostatic stress following AG.

Table 4.3 Transfer function gain, phase and coherence between systolic blood pressure and R-R intervals in response to orthostatic stress after AG vs. HDBR in dehydrated men and women

	Following AG				Following HDBR			
	Supine	ET	LT	Recovery	Supine	ET	LT	Recovery
				Men (n=9)				
COH_{LF}	0.62±0.03	0.65±0.04	0.63±0.05	0.57±0.04	0.69±0.02	0.64±0.04	0.56±0.04*	0.63±0.06
$Gain_{LF}$	11.0±2.7	5.0±0.6*	2.2±0.7*§	16.9±1.7*	8.5±1.5	5.2±0.7*	2.8±0.5*§	19.9±4.0*
$Phase_{LF}$	-1.4±0.1	-1.3±0.2	-1.0±0.3	-1.2±0.2	-1.2±0.2	-1.5±0.2*	-1.5±0.1	-1.5±0.2
				Women (n=7)				
COH_{LF}	0.78±0.06‡	0.82±0.03‡	0.58±0.04*	0.59±0.02*	0.72±0.03	0.77±0.04‡	0.75±0.03‡	0.63±0.09
$Gain_{LF}$	9.8±1.6	4.7±0.5*	3.5±0.9*§	13.7±2.5*	11.1±1.6	5.5±0.9*	3.2±0.8*§	22.8±5.2*
$Phase_{LF}$	-1.3±0.1	-1.2±0.2	-1.4±0.2	-1.3±0.4	-1.2±0.2	-1.3±0.1*	-1.6±0.2	-1.4±0.3

Values are mean ± SEM. AG, artificial gravity; HDBR, head-down bed rest; ET, early tilt; LT, late tilt; COH_{LF}, coherence in low frequency range, a.u.; $Gain_{LF}$, transfer function gain in low frequency range, ms/mmHg; $Phase_{LF}$, transfer function phase in low frequency range, radians. * Significantly different from supine, $p < 0.05$; † significantly different from HDBR, $p < 0.05$; ‡ significantly different from male subjects, $p < 0.05$; § significant difference between ET and LT, $p < 0.05$.

4.4 Discussion

Reflex cardiovascular responses to orthostatic stress induced by combined HUT and progressive LBNP were tested in dehydrated men and women following a short exposure to AG and HDBR, respectively. Primary findings of this study are 1) a short AG exposure increased men's, but not women's, SBP_{LF} responses to orthostatic stress, relative to responses following HDBR exposure, and 2) in response to milder levels of orthostatic stress, the $Phase_{LF}$ between SBP and RRI was unchanged following AG but became more negative following HDBR exposure in both men and women, compared with the $Phase_{LF}$ at supine.

Upright posture is known to result in a caudal fluid translocation and central hypovolemia, as indicated by increased Z_{THX} and decreased Z_{ABD}. The furosemide-induced plasma volume reduction and postural fluid shift-induced central hypovolemia together challenged compensatory mechanisms to defend against BP reductions [67], similar to that encountered by astronauts returning to earth [11]. In order to maintain BP during orthostatic stress, the unloaded baroreceptors reduced vagal activity, as indicated by reduced RR_{HFnu}, and enhanced sympathetic activity, as indicated by increased $RR_{LF/HF}$ and SBP_{LF}, contributing to tachycardia and arterial vasoconstriction [78]. With increasing orthostatic stress, especially during LT, the decreased N_{seq} was consistent with loss of overall baroreflex activity; and the simultaneously reduced BEI and baroreflex sensitivity (i.e., BRS or $Gain_{LF}$) indicated that baroreceptors were less effective in driving the sinus atrial node and the effect of RRI to dampen SBP changes was reduced, which may lead to an unstable relationship between SBP and RRI; furthermore, the increase in N_{ramps} indicated increased BP variability, implying a maladaptive arterial baroreflex control

during orthostatic stress [89]. These parameters concerning baroreflex responsiveness indicated that malfunction of baroreflex regulation contributed to the occurrence of presyncope. During recovery, the relatively higher HR following AG compared with HDBR suggests that other mechanisms may have contributed to the regulation of the end-organ response (i.e., HR) following orthostatic stress since indices of autonomic regulation of HR were comparable. The increased baroreflex sensitivity may be due to the baroreflex overshoot following orthostatic stress [90]. The decreased $RR_{LF/HF}$ and SBP_{LF}, and increased RR_{HFnu} and baroreflex sensitivity, reflect an effort to increase venous return to restore decreased BP during recovery.

4.4.1 Mechanisms of Improved Orthostatic Tolerance Limit Following Artificial Gravity Compared With Head-down Bed Rest Exposure.

Figure 4.5 indicates that OTL was improved, following AG exposure compared with that following HDBR exposure, by ~30% in men and ~22% in women. This is accompanied by a significantly increased SV (~10%) in women and a significantly reduced TPR in men (~7%) and in women (~8%), as shown in another report of the present study [86]. It has been shown that IAG exposure increased venous return, enhanced venous constriction and enhanced sympathetic response to orthostatic stress in male subjects [25-27, 91]. In the present study, the AG-induced, significantly reduced TPR and SBP, as well as relatively greater SV and relatively lower SBP_{LF} at supine of men are consistent with our previous studies [25-27]. The post-AG reduction of TPR would likely result in reduced SBP and increased SV via reduced afterload and could also lead to a shift of blood to the venous circulation increasing SV via increased preload [25]. The tendency of reduced supine SBP_{LF} of men following AG may indicate the beneficial effect of AG,

since higher tolerance for upright posture has been observed in subjects with lower supine muscle sympathetic nerve activity (MSNA) [92]. Fu *et al.* [93] indicated that a subject may have a limited reserve for sympathetically medicated vasoconstriction, therefore, if resting sympathetic outflow is higher, then the sympathetic discharge upon upright posture would increase less. Fritsch-Yelle *et al.* [18] reported that astronauts who failed to complete their 10 minutes stand tests on landing day had significantly smaller increases in plasma norepinephrine levels with standing. In the present study, when exposed to orthostatic stress, male subjects had a non-significant increase of SBP_{LF} following HDBR, but a significantly elevated SBP_{LF} following AG, reflecting an increased sympathetic responsiveness to orthostatic stress in men after AG [25-27] compared to HDBR.

Cardiovascular responses of female subjects to orthostatic stress following ~90 minutes AG or HDBR exposure were somewhat different from those of men in the present study. In contrast to decreased supine SBP_{LF} in men, the increased supine SBP_{LF} following AG in women reflects elevated sympathetic vasomotor tone, which may be a reflex response to peripheral vasodilation, as indicated by reduced TPR, in an effort to regulate BP [94]. Indeed, we found no difference in women's BP, despite ~10% greater SV [86] following AG compared to HDBR. However, elevated supine sympathetic activity after AG may reduce sensitivity and responsiveness of the vasculature [93]. Nevertheless, this is not the case in the present study since orthostatic stress elicited comparable increases in SBP_{LF} and TPR [86] following AG relative to HDBR in women.

In addition to changes in hemodynamic parameters and autonomic control, increased baroreflex sensitivity [85, 91, 95, 96] and increased operational point (a measure of the

buffering capacity of the carotid baroreflex to a hypotensive stimulus) [28] have been reported following AG exposure. In the present study, we did not find elevated baroreflex sensitivity following AG, compared to that observed following HDBR, using either sequence (BRS) or transfer function analysis ($Gain_{LF}$) methods. Consistent with our previous IAG training study [57], these results indicate that the sensitivity of baroreceptors was not enhanced by a short AG exposure. However, not only gain response but also time delay response determines the efficiency of baroreceptors. Gulli *et al.* [97] have reported that subjects with different orthostatic tolerance have no differences in baroreflex sensitivity, while subjects with poor orthostatic tolerance have significantly longer phase delay between SBP and RRI, using cross-spectral analysis in the LF range. In a study investigating patients with a history of vasovagal syncope and healthy controls using spontaneous sequence analysis, Gulli *et al.* [98] indicated that most baroreflex responses occurred within 1 second in controls while taking longer than 2 seconds in patients. These results emphasize that in a closed-loop feedback system, a delayed response in the output signal (e.g., RRI) may lead to system instability [99]. Therefore, the increased LF phase delay in response to orthostatic stress following HDBR indicates a delayed response of HR to dampen BP oscillations, and the delayed effector response could generate an unstable state of regulation, which may lead to early onset of presyncope. Compared to the more negative phase following HDBR in response to 70º HUT, the sustained $Phase_{LF}$ between SBP and RRI following AG reflect enhanced baroreflex responsiveness to orthostatic stress. Thus, the finding of maintained $Phase_{LF}$, reflecting enhanced baroreflex responsiveness, is consistent with results from previous AG studies [25, 28, 85, 91, 95] although the pattern of this improvement is different.

Furthermore, it has been determined that the delay between SBP and RRI oscillations increased when vagal tone was low [100]. Westerhof *et al.* [90] reported that subjects who presented presyncopal symptoms during 70º and 90º HUT had extended phase delay during the first 2 minutes of 70º and 90º HUT, compared with those who did not, indicating sympathetic excitation. Gulli *et al.* [101] found a less negative phase in the HF range 2-3 minutes before and during presyncope in fainters, compared with non-fainters, indicating disengaged sympathetic activity. These results suggest that fainters seem to engage sympathetic activity earlier and also disengage earlier [90]. Therefore, in the present study, compared to supine, the increased $Phase_{LF}$ at ET indicated early sympathetic activation following HDBR exposure, while the well maintained $Phase_{LF}$ following AG may preserve the sympathetic adjustment and contribute to greater OTL than that observed following HDBR.

4.4.2 Gender Differences in Response to Orthostatic Stress Following Artificial Gravity Compared With Head-down Bed Rest Exposure.

In the present study, female subjects were more predisposed to OI, as evidenced by ~30% lower OTL following both AG and HDBR exposure. Several mechanisms, such as differences in hemodynamic responses [84], autonomic cardiovascular regulation [102], sympathetic neural responses [72] and baroreflex responses [103] to orthostatic stress, may contribute to poorer orthostatic tolerance in women. In the present study, the greater HR in women at ET indicated the presence of an important compensatory mechanism for relatively smaller SV [72], however, HR responses were similar when approaching syncope even though SV was still lower in women. The significantly greater SBP_{LF} at ET in women indicated that females achieved greater sympathetic excitation at milder levels

of orthostatic stress than males but might not have enough vasoconstrictor reserve to compensate for further central hypovolemia [93]. Indeed, we observed slightly reduced SBP_{LF} in women by ~10% during LT with respect to ET, reflecting reduced sympathetic outflow to the vasculature. In contrast to women, men maintained their SBP_{LF} levels during LT, compared with those during ET. The relatively greater COH_{LF} between SBP and RRI in women may be due to greater oscillations of the input signal [104], i.e., SBP_{LF}. In contrast to Laitinen *et al.* [103], we did not observe any difference of baroreflex sensitivity in men and women. The greater BEI in women was due to a greater N_{seq} for a comparable N_{ramps} following both AG and HDBR, which may reflect greater stimulation to baroreceptors and greater effectiveness of baroreceptors in driving the sinus node in women compared to men.

In this study, although OTL improved significantly for the group of men and women, the OTL improvement of women was not statistically significant as a separate group. This difference is not likely to be attributed to the relatively lower level of AG exposure since our previous studies [27, 57] indicated that three weeks of passive IAG exposure did not significantly improve women's OTL. However, IAG exposure with exercise training did increase women's OTL [27, 57]. Convertino *et al.* [85] reported that cardiovascular adaptations to hypergravity training was dependent of gender, and indicated that female had inherently limited capacity to improve their orthostatic performance. Therefore, efficient AG protocols or combination of AG and other countermeasures need to be further investigated to support optimal performance of both men and women during subsequent orthostatic stress.

4.4.3 Limitations.

We acknowledge three limitations of this study. First, we did not directly measure MSNA. The SBP_{LF}, although well correlated to MSNA, does not reflect the absolute levels of individual MSNA [79]. Second, we were unable to conduct AG and HDBR protocols at the same stage of women's menstrual cycle, which may affect the results due to hormone variations [105]. However, orthostatic tolerance and cardiovascular control have been shown not to be affected by phase of the menstrual cycle [106]. In our study, the menstrual cycle phase effects may have been counterbalanced since all the female subjects had their OTL tests during early follicular phase or late luteal phase, and the distribution of menstrual cycle phase was approximately equal on both AG and HDBR days. Finally, although this study was designed to study a passive AG countermeasure, subjects did a small amount of exercise during and following the AG exposure. During AG exposure, subjects were asked to bend their toes upward when development of presyncope was expected. After AG, to maintain gravitational exposure, subjects walked to the OTL station (~20 meters). After HDBR, to maintain the simulation of spaceflight, subjects were transported to the OTL station via gurney. These differences in activity may have some influences on the results.

4.5 Conclusions

We conclude that a short duration exposure to AG increased some aspects of baroreflex activity in men and women and sympathetic responsiveness in men to orthostatic stress, compared with exposure to 90 minutes of HDBR, in a pharmacologically induced dehydrated condition. Cardiovascular adaptions to AG may contribute to improved orthostatic tolerance when reentering a gravitational environment.

CHAPTER 5 CARDIOVASCULAR AND CARDIORESPIRATORY PHASE SYNCHRONIZATION IN EUHYDRATED AND DEHYDRATED HUMANS

In this chapter, a new data analysis procedure, the phase synchronization method, was applied to obtain more information about cardiovascular and cardiorespiratory interaction in nonlinear dynamics domain. This chapter is adapted from a previously published journal article [107].

5.1 Introduction

The cardiovascular system is influenced by several feedback and feed-forward mechanisms regulating cardiovascular homeostasis [29], as outlined in Figure 5.1. It is well known that HR and SBP interact with each other in a closed-loop via baroreflex feedback and mechanical feed-forward mechanisms [108-111]. Cardiovascular interaction is also perturbed by respiration (RESP) via mechanical effects on intrathoracic pressure and stroke volume [112], and effects on cardiac vagal motoneurons [113]. Different physiological [108, 111, 114-121] and pathological [31, 35, 109, 110, 122-126] states have been shown to alter cardiovascular coupling and/or cardiorespiratory coupling. Therefore, it is essential to assess cardiovascular and cardiorespiratory interactions using these output signals (e.g., HR, SBP and RESP) to provide important information concerning physiological mechanisms involved in regulation of cardiovascular and respiratory systems.

For a more detailed understanding of regulation of cardiovascular and respiratory systems, it is essential not only to detect interactions but also to identify causal relationships [31, 32]. Since cardiovascular and respiratory systems very likely interact

with each other in a nonlinear way, it is more appropriate to analyze the interactions using nonlinear approaches [33] in addition to conventional linear methods [34, 35]. Several nonlinear methods [33], such as higher-order statistics [122], nonlinear Granger causality [124], nonlinear prediction [31], entropy [109, 110], and phase synchronization approach [116-121, 123, 127-129], have been applied to analyze cardiovascular and cardiorespiratory couplings in health and disease conditions.

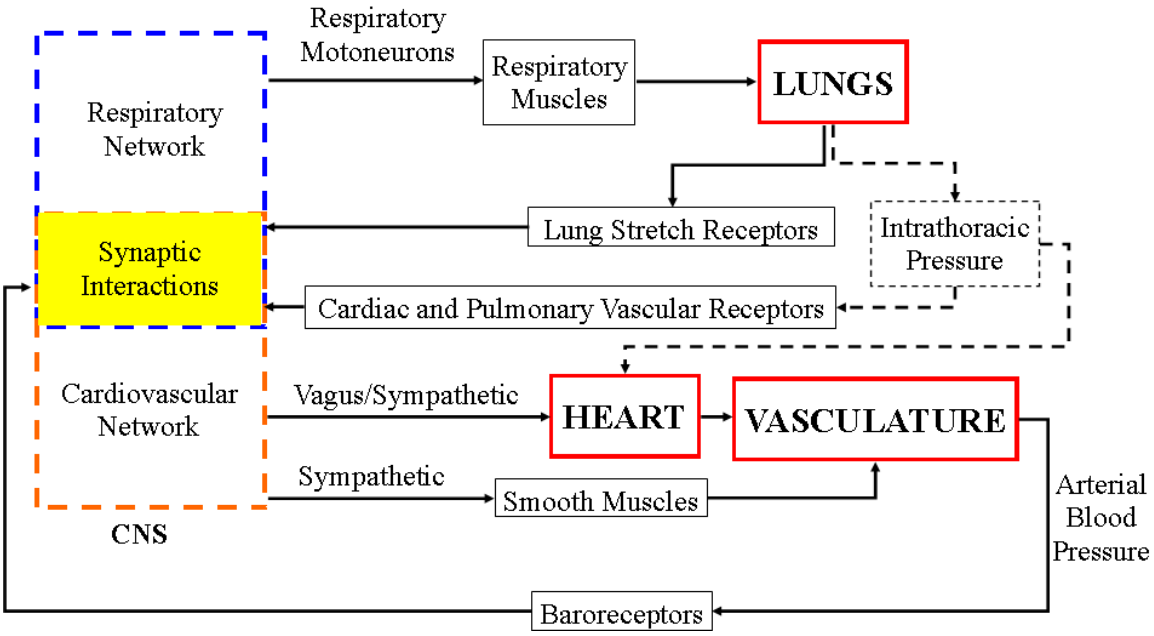

Figure 5.1 An illustration of major rhythmic processes regulating cardiovascular homeostasis.

Orthostatic stress is known to increase pooling of blood in the lower body, resulting in reduction in venous return and central blood volume. Consequently, baroreflex-mediated increase in HR and vasoconstriction are evoked to compensate for the reduction in central blood volume and to maintain blood pressure [36]. Failure to induce these compensations may result in OI, i.e., the inability to maintain blood pressure with the eventual loss of consciousness upon upright posture. Cardiovascular and cardiorespiratory coupling have

been explored during orthostatic stress [108, 110, 111, 115] and preceding syncope [31, 109, 122, 123, 125] using different nonlinear methods, with an aim of understanding and preventing orthostatic intolerance. However, there are limitations in those studies, such as lack of respiratory variability information [31, 109-111, 125], lack of coupling direction [122], and that require prior assumptions of the cardiovascular system [115]. In addition, reduction in blood volume has been considered contributing to the occurrence of OI [67, 72], particularly in astronauts following long term spaceflight, however, studies concerning the effects of combining orthostatic stress and dehydration on cardiovascular and cardiorespiratory interactions are rare. Furthermore, none of the previous studies investigating causal relationship among cardiovascular and respiratory oscillations [108, 123] has addressed the effects of dehydration.

In this study, the phase synchronization approach was applied to investigate effects of different gravitational environments and reduced blood volume on cardiovascular and cardiorespiratory couplings in a ground-based simulation of space exploration to obtain more information concerning spaceflight induced orthostatic intolerance. Changes in phase relationships among SBP, R-R intervals (RRI) and RESP were tested during graded head-up tilt (HUT) with normal (euhydration [EUH]) and reduced (dehydration [DEH]) blood volume using the phase synchronization approach. We hypothesized that both dehydration and orthostatic stress, as physiological stressors, would reduce cardiovascular and cardiorespiratory couplings.

5.2 Materials and Methods

This study was part of a broader experimental design testing whether upright LBPP would be comparable to HUT in modeling physiological responses to partial gravities

during both EUH and DEH. Details of experimental protocols and results concerning cardiovascular responses to HUT and upright LBPP are reported in sections 3.2 & 3.3. For the present study, only HUT data were used.

5.2.1 Subjects

See section 3.2.1 for details.

5.2.2 Experimental Protocol

Briefly, the experimental protocol was as follows. Subjects participated in two experimental sessions separated by 7 days. Subjects were euhydrated during one session and dehydrated during the other. Acute DEH was induced by intravenous furosemide administration (0.5 mg furosemide per kilogram body weight). The order of EUH and DEH sessions was counterbalanced across subjects. During each session, subjects were tilted from supine (T0) to 10º (T10), 20º (T20) and 80º (T80) to simulate standing in the gravitational environments of 0 g (spaceflight), 1/6 g (Moon), 3/8 g (Mars) and 1 g (Earth), respectively. Tests were terminated when experimental protocols were completed or subjects developed presyncopal symptoms (SBP < 70 mmHg, HR drop > 20 beats per minute, lightheadness, dizziness or nausea).

5.2.3 Instrumentation and Data Acquisition

Standard lead II electrocardiogram (Model 90623A, SpaceLabs Inc., Redmond, WA) was continuously monitored and recorded. Continuous BP was obtained at the middle finger using photoplethysmography (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands) with the hand positioned at heart level. In addition, brachial artery BP was measured periodically using a manometer (UA-767, A&D Medical, San Jose, CA) placed

around the upper arm for the calibration of continuous BP. Respiration was derived from thoracic impedance (UFI Model 2994D, Morro Bay, CA). The angle of the tilt table was recorded by an accelerometer (Crossbow, Jameco, CA). All data were collected by computer acquisition software (WinDAQ, DATAQ Instruments, Akron, OH) at 1000 Hz with subsequent analysis using MATLAB (R2012b, Mathworks, Natick, MA).

5.2.4 Data Analysis

In contrast to approaches investigating signal amplitudes, the phase synchronization approach investigates phases of oscillations directly [33]. The concept of phase synchronization is taken from studies of two weakly interacting oscillators [32, 130, 131]. Generally, weak or moderate interaction only affects phases of oscillators but not their amplitudes, and as the interaction strength increases, phases of oscillators are affected first, followed by correlation between amplitudes [32, 130, 131]. Therefore, the phase synchronization approach is appropriate to study coupling between cardiovascular and respiratory systems, in which case, coupling is usually weak or moderate [128, 132], in terms of both strength [32] and direction [130] of the interactions. In addition, the phase synchronization approach requires no priori assumptions of the cardiovascular system [130]. The steps of this approach are described in detail below.

Preprocessing. Data were summarized as three-min averages (Figure 3.1) at each tilt angle during both EUH and DEH. The times of occurrence of R wave peaks were calculated using the Pan-Tompkins algorithm [133]. Then RRI was calculated as the duration between successive R peaks. The local maximum of the BP waveform within each heartbeat was designated as SBP. After removing artifacts by visual inspection, the resulting RRI and SBP time series were resampled at 4 Hz using the cubic spline

interpolation method. Respiratory rate (f_R) was determined by identifying local minima of the respiratory waveform (i.e., the start of expiration). The respiratory signal was then down-sampled to 4 Hz to obtain corresponding sampling times as in the RRI and SBP time series. To estimate phase coupling in sympathetic and vagal branches of the autonomic nervous system, time series were band pass filtered in low- (LF, 0.04-0.15 Hz) and high- (HF, 0.15-0.4 Hz) frequencies, respectively [78]. A Butterworth forward and backward zero-phase shift filter was used to avoid altering the phase of the time series. It is worth noting that by obtaining LF and HF components separately, we were able to analyze synchronization among RESP, SBP and RRI in both LF and HF components, an outcome that would not be possible when defining phases from raw signals.

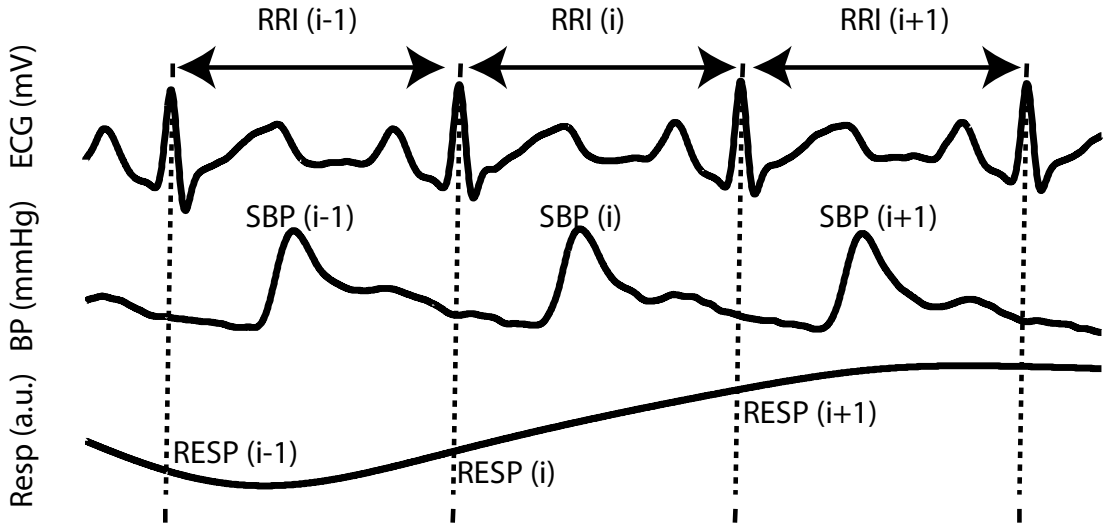

Figure 5.2 An illustration of time series measurement from physiological signals. Measurement of R-R intervals (RRI), systolic blood pressure (SBP) and respiration (RESP) variability time series from electrocardiogram (ECG), blood pressure (BP) and respiratory waveform (Resp).

*Phase extraction via the Hilbert transform approach.*The Hilbert transform [131, 134] was used to extract phase, resulting in time series of phases of RRI, SBP and RESP signals, $\varphi(t)_{RRI}$, $\varphi(t)_{SBP}$ and $\varphi(t)_{RESP}$. An illustration of this procedure is as follows,

let $x(t)$ be the filtered physiological signal within the given frequency range, then the complex analytic extension of $x(t)$ is given by

$$\vartheta(t) = x(t) + i\hat{x}(t), \quad \textbf{(5.1)}$$

where the imaginary part, $\hat{x}(t)$, is generated by the Hilbert transform of the signal $x(t)$

$$\hat{x}(t) = H(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{+\infty} \frac{x(\tau)}{t-\tau} d\tau, \quad \textbf{(5.2)}$$

where *P.V.* is the Cauchy principal value of the integral. The analytic signal, $\vartheta(t)$ is then projected on the unit circle

$$z(t) = \frac{\vartheta(t)}{\|\vartheta(t)\|} = e^{i\varphi(t)}, \quad \textbf{(5.3)}$$

where $\|\vartheta(t)\|$ is the modulus of $\vartheta(t)$. The phase $\varphi(t)$ can be extracted as the angle of $z(t)$.

Phase synchronization index. A phase synchronization analysis [32] was performed between SBP and RRI (SBP-RRI), RESP and RRI (RESP-RRI), and RESP and SBP (RESP-SBP). Firstly, phase differences between each pair of parameters were constructed: $\Delta\varphi(t)_{SBP-RRI} = \varphi(t)_{SBP} - \varphi(t)_{RRI}$; $\Delta\varphi(t)_{RESP-RRI} = \varphi(t)_{RESP} - \varphi(t)_{RRI}$; $\Delta\varphi(t)_{RESP-SBP} = \varphi(t)_{RESP} - \varphi(t)_{SBP}$. A phase synchronization index (λ) was then defined as

$$\lambda = \sqrt{\langle sin\, \Delta\varphi(t)\rangle^2 + \langle cos\, \Delta\varphi(t)\rangle^2}, \quad \textbf{(5.4)}$$

where $\Delta\varphi(t)$ describes phase differences and $\langle\ \rangle$ denotes averaging over time. In the present study, the phase synchronization index was calculated from 40 sec moving average windows with 50% overlap, which were then averaged over each three min segment. An $\lambda = 0$ indicated independent phases, i.e., a complete lack of interaction, and an $\lambda = 1$ indicated perfect interaction [32].

Figure 5.3 shows an example of the analysis procedure above. The filtered one minute data (thin line, a-c) in the HF range, the instantaneous amplitudes (thick line, a-c) and instantaneous phases (d-f) of the Hilbert transform of one euhydrated male subject at supine rest are shown. The saw-tooth shape traces (d-f) indicate phase evolution of physiological signals, where φ_{RESP} slightly precedes φ_{RRI}, and φ_{SBP} slightly leads φ_{RRI}. In addition, the frequency distributions of cyclic relative phase differences among RRI, SBP and RESP at supine (g-i, *left*) and at T80 (g-i, *right*) are shown for the same subject in EUH. The narrow distributions of phase differences at supine imply high synchronization between each pair of signals, while the wide, uniform distributions of phase differences at T80 demonstrate that upright posture reduced the synchronization among RRI, SBP and RESP.

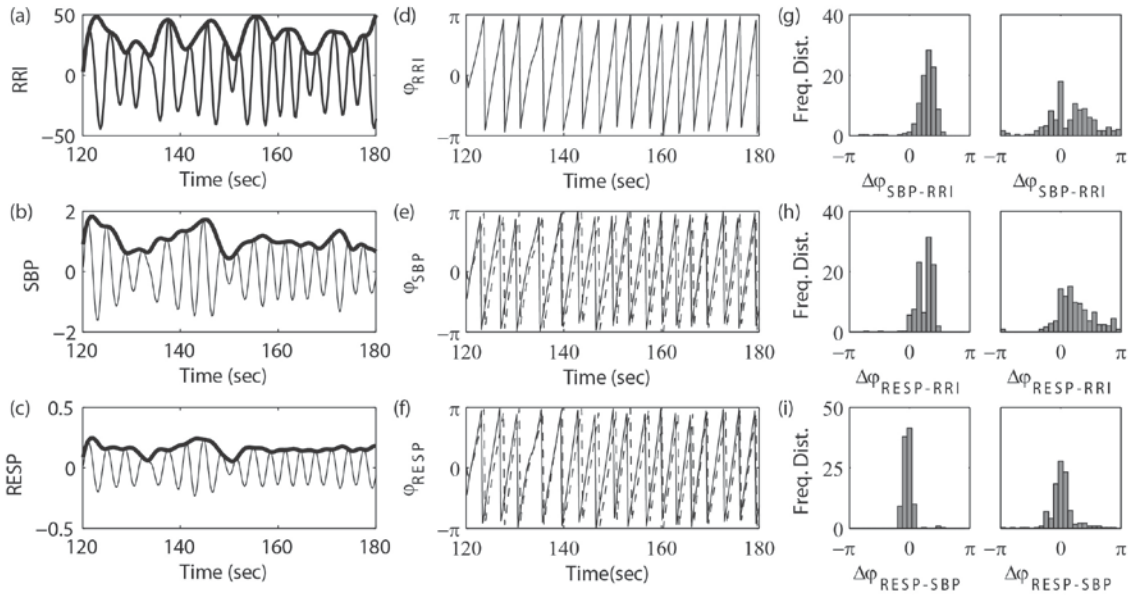

Figure 5.3 A representative illustration of phase synchronization analysis procedure. One minute, filtered data in the high-frequency (0.15-0.4 Hz) range (*thin line*, a-c), the instantaneous amplitudes (*thick line*, a-c) and instantaneous phases (d-f) after the Hilbert transform, and the phase difference distributions at supine rest (g-i, *left*), as well as the phase difference distributions at 80º head-up tilt (g-i, *right*) of one euhydrated male subject are shown. Dotted lines in (e) and (f) indicate instantaneous phase of RRI. RRI,

R-R intervals, ms; SBP, systolic blood pressure, mmHg; RESP, respiration, a.u.; $\boldsymbol{\varphi_{RRI}}$, phase of R-R intervals, radians; $\boldsymbol{\varphi_{SBP}}$, phase of systolic blood pressure, radians; $\boldsymbol{\varphi_{RESP}}$, phase of respiration, radians; $\Delta\boldsymbol{\varphi_{SBP-RRI}}$, phase differences between R-R intervals and systolic blood pressure, radians; $\Delta\boldsymbol{\varphi_{RESP-RRI}}$, phase differences between respiratory trace and R-R intervals, radians; $\Delta\boldsymbol{\varphi_{RESP-SBP}}$, phase differences between respiratory trace and systolic blood pressure, radians; Freq. Dist., frequency distribution, %.

Directionality index. A directionality index (d) was also calculated to determine which parameter influenced the coupling relationship more strongly [120, 130]. Generally, let $\varphi_1(t)$ and $\varphi_2(t)$ be phases of two signals. The basic idea behind this method is that phase increments over a certain temporal window of length τ

$$\Delta_{1,2} = \varphi_{1,2}(t+\tau) - \varphi_{1,2}(t), \quad \textbf{(5.5)}$$

can be considered as being generated by an unknown two-dimensional noisy map

$$\Delta_{1,2} = \omega_{1,2}\tau + \tilde{F}_{1,2}\left(\varphi_{2,1}, \varphi_{1,2}\right) + \varepsilon_{1,2}, \quad \textbf{(5.6)}$$

where $\omega_{1,2}$ are the natural frequencies, $\tilde{F}_{1,2}$ is the coupling term, and $\varepsilon_{1,2}$ are the noisy perturbations. To estimate the deterministic term $\tilde{F}_{1,2}$ of the two-dimensional noisy map, a finite Fourier series,

$$F_{1,2} = \sum_{n,m} e^{i(n\varphi_1 + m\varphi_2)}, \quad \textbf{(5.7)}$$

is used to fit the function $\tilde{F}_{1,2}$ in a least mean square sense. In our calculation, we chose $n, m < 4$. To measure how strongly an oscillator is driven and how sensitive it is to being driven, the cross-dependency coefficients are then computed by

$$c_{1,2}^2 = \int_0^{2\pi}\int_0^{2\pi}\left(\partial F_{1,2}/\partial\varphi_{2,1}\right)^2 d\varphi_1 d\varphi_2. \quad \textbf{(5.8)}$$

And finally, a directionality index is obtained as

$$d_{1,2} = (c_2 - c_1)/(c_1 + c_2), \quad \textbf{(5.9)}$$

which ranges from 1 (oscillator 1 drives oscillator 2) to -1 (oscillator 2 drives oscillator 1) [114, 123, 127, 130]. Therefore, in our study, a positive value of $d_{SBP-RRI}$ indicates that

SBP drives RRI (i.e., feedback control) and a negative value indicates that RRI drives SBP (i.e., feed-forward control). The same interpretation can also be applied to $d_{RESP-RRI}$ and $d_{RESP-SBP}$.

Data analysis with surrogate data. To exclude the possibility that the synchronization patterns detected for different orthostatic stress levels (i.e., T0, T10, T20 and T80) and plasma volume conditions (i.e., EUH vs. DEH) appeared by chance, surrogate data analysis [135] was conducted. Specifically, we analyzed the phase synchronization between the original physiological signals (λ_{ori}) and the phase synchronization between one original signal and one surrogate signal (λ_{surr}). The surrogate signal was obtained by substituting the Fourier phases in the original signals with random phases in the range $[0, 2\pi)$ with a uniform distribution, while preserving the amplitude of the Fourier coefficients [135]. One hundred surrogate datasets were generated from each original signal. The phase synchronization indices between the 100 surrogate datasets of one original signal and each of the other two original signals were computed using the method described above. The 95th percentile of the phase synchronization indices was chosen as the surrogate phase synchronization index for subsequent statistical analysis. Details of the surrogate data analysis are shown in APPENDIX B.

Statistical Analysis

The normality of the distribution was assessed using the Kolmogorov-Smirnov test. A two-tailed, paired t-test was used to determine the significance of phase synchronization indices computed using original signals over those computed using surrogate signals. A two-way mixed model ANOVA was used to determine the effects of stress (T0, T10, T20 and T80) and condition (EUH vs. DEH) on cardiovascular variables, phase

synchronization indices and directionality indices. When significant effects were observed, Tukey's post-hoc analysis was performed to estimate differences between pairwise comparisons. Significance was accepted at $p < 0.05$. Analyses were completed using SAS 9.3 (SAS Institute Inc., Cary, NC). Values are shown as mean ± standard error of the mean (SEM).

5.3 Results

As stated in 3.3, one male subject was not involved in DEH sessions since his BP after furosemide infusion was above 140/90 mmHg, and another male subject was excluded due to him developing orthostatic intolerance symptoms during a low level of orthostatic stress in DEH. Therefore, data from 10 subjects (four men, six women) are reported. Data from one male and one female subject at T80 during DEH were excluded because shortly after starting data collection at T80, these subjects developed presyncopal symptoms.

5.3.1 Hemodynamic Parameters and Respiration

Figure 5.4 shows hemodynamic responses to HUT during both EUH and DEH. Heart rate (main effect of stress, $p < 0.0001$) increased, and SBP decreased (main effect of stress, $p < 0.0001$) with increasing tilt angle. Dehydration significantly elevated HR at each stress (main effect of condition, $p < 0.0001$). Respiratory rate was not affected by tilt or hydration status.

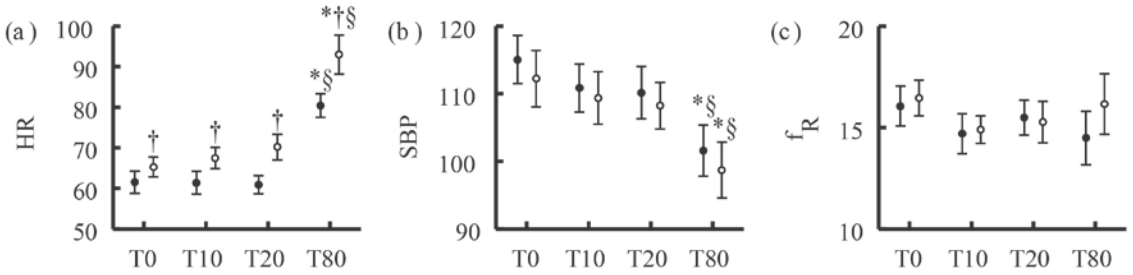

Figure 5.4 Heart rate (a), systolic blood pressure (b) and respiratory rate (c) at supine rest (0º [T0]) and in response to head-up tilt (10º [T10], 20º [T20] and 80º [T80]) under euhydration (*solid circle*) and dehydration (*open circle*) conditions.
HR, heart rate, beats per minute; SBP, systolic blood pressure, mmHg; f_R, respiratory rate, breaths per minute. * Significantly different from T0, $p < 0.05$; § significantly different from adjacent stress, $p < 0.05$; † significantly different from euhydration, $p < 0.05$.

5.3.2 Surrogate Data Analysis

Table 5.1 shows differences between the phase synchronization index computed using original and surrogate data ($\lambda_{ori} - \lambda_{surr}$). Phase synchronization indices related to the LF-component of RESP, i.e., $\lambda_{RESP-RRI,LF}$ and $\lambda_{RESP-SBP,LF}$, were not significantly greater than those computed using surrogate data. However, significantly greater phase synchronization indices were obtained for signals related to the HF-component of RESP, i.e., $\lambda_{RESP-RRI,HF}$ and $\lambda_{RESP-SBP,HF}$. In addition, $\lambda_{SBP-RRI,LF}$ and $\lambda_{SBP-RRI,HF}$ generated from original data were significantly greater than those computed using surrogate data. Therefore, in the remainder of the text, we report only those parameters that quantify cardiovascular coupling in LF and HF ranges, and those that quantify cardiorespiratory (i.e., cardiac-respiratory and vascular-respiratory) coupling in the HF range.

Table 5.1 Differences (∆) of phase synchronization index computed from orignal (λ_ori) and surrogate data (λ_surr)

$\lambda_{ori} - \lambda_{surr}$	Euhydration				Dehydration			
	T0	**T10**	**T20**	**T80**	**T0**	**T10**	**T20**	**T80**
$\Delta\lambda_{SBP-RRI,LF}$	0.24 ± 0.04†	0.26 ± 0.04†	0.21 ± 0.04†	0.31 ± 0.04†	0.22 ± 0.05*	0.21 ± 0.04*	0.19 ± 0.04*	0.27 ± 0.07*
$\Delta\lambda_{RESP-RRI,LF}$	0.09 ± 0.05	0.13 ± 0.04	0.08 ± 0.04	0.03 ± 0.02	0.02 ± 0.07	0.04 ± 0.04	0.03 ± 0.03	-0.00 ± 0.03
$\Delta\lambda_{RESP-SBP,LF}$	0.15 ± 0.04	0.09 ± 0.05	0.06 ± 0.03	0.08 ± 0.03	0.09 ± 0.04	0.06 ± 0.03	0.03 ± 0.04	0.11 ± 0.04
$\Delta\lambda_{SBP-RRI,HF}$	0.42 ± 0.03†	0.42 ± 0.03†	0.48 ± 0.02†	0.38 ± 0.05†	0.41 ± 0.04†	0.44 ± 0.05†	0.46 ± 0.03†	0.27 ± 0.06*
$\Delta\lambda_{RESP-RRI,HF}$	0.43 ± 0.04†	0.38 ± 0.03†	0.41 ± 0.03†	0.24 ± 0.05*	0.39 ± 0.03†	0.41 ± 0.02†	0.32 ± 0.04†	0.24 ± 0.04†
$\Delta\lambda_{RESP-SBP,HF}$	0.39 ± 0.04†	0.41 ± 0.03†	0.41 ± 0.05†	0.35 ± 0.06†	0.33 ± 0.04†	0.40 ± 0.05†	0.34 ± 0.05†	0.38 ± 0.08*

Values are mean ± SEM. RRI, R-R intervals; SBP, systolic blood pressure; RESP, respiratory trace; LF, low frequency (0.04-0.15 Hz); HF, high frequency (0.15-0.4 Hz). Significant greater phase synchronization index when computed using original data compared with that using surrogate data, * $p < 0.01$; † $p < 0.001$.

5.3.3 Phase Synchronization Index and Directionality Index in Low- and High-Frequency Ranges

Figure 5.5 shows phase synchronization index (λ, a-d) and directionality index (d, e-h) of SBP-RRI, RESP-RRI and RESP-SBP in LF and HF regions. Compared to T0, increasing tilt angle increased $\lambda_{SBP-RRI,LF}$ (main effect of stress, p = 0.0001) and decreased $\lambda_{SBP-RRI,HF}$ (main effect of stress, p = 0.0006) and $\lambda_{RESP-RRI,HF}$ (main effect of stress, p < 0.0001) during both EUH and DEH. Orthostatic stress tended to reduce $\lambda_{RESP-SBP,HF}$ (main effect of stress, p = 0.0513). With respect to T0, $d_{SBP-RRI,LF}$ (main effect of stress, p = 0.0016) and $d_{RESP-SBP,HF}$ (main effect of stress, p =0.0157) decreased, while $d_{RESP-RRI,HF}$ remained unchanged throughout graded HUT during both EUH and DEH. In addition, HUT appeared to have different effects on $d_{SBP-RRI,HF}$ in different hydration conditions (condition by stress interaction, p = 0.0298). However, post-hoc tests indicated that neither HUT nor hydration status affected $d_{SBP-RRI,HF}$. With respect to EUH, DEH reduced $\lambda_{SBP-RRI,HF}$ (main effect of condition, p = 0.0146), $\lambda_{RESP-RRI,HF}$ (main effect of condition, p = 0.0063) and $\lambda_{RESP-SBP,HF}$ (main effect of condition, p = 0.0386), and had no significant effect on $\lambda_{SBP-RRI,LF}$. In addition, DEH had no significant effects on $d_{SBP-RRI,HF}$, $d_{RESP-RRI,HF}$ and $d_{RESP-SBP,HF}$, but significantly decreased $d_{SBP-RRI,LF}$ (main effect of condition, p = 0.0179).

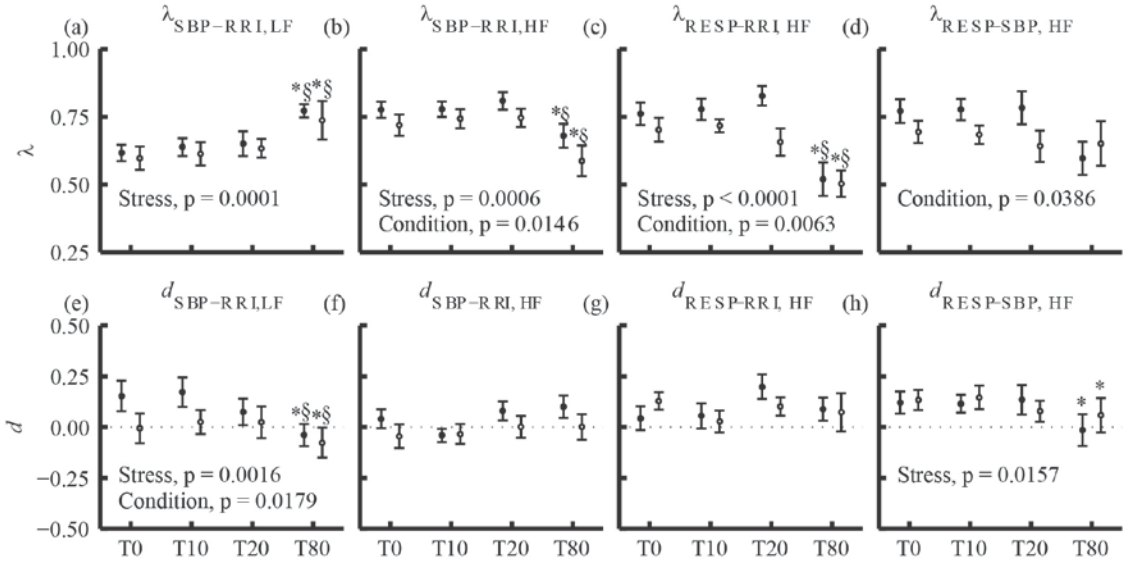

Figure 5.5 Phase sychronization index (λ, a-d) and directionality index (d, e-h) between systolic blood pressure and R-R interval (SBP-RRI), respiration and R-R interval (RESP-RRI) and respiration and systolic blood pressure (RESP-SBP) in low- (LF, 0.04-0.15 Hz) and high-frequency (HF, 0.15-0.4 Hz) ranges in response to head-up tilt [0º (T0), 10º (T10), 20º (T20) and 80º (T80)] under euhydration (*solid circle*) and dehydration (*open circle*) conditions.
Stress, main effect of stress level; Condition, main effect of condition (euhydration vs. dehydration). * Significantly different from T0, $p < 0.05$; § significantly different from adjacent stress, $p < 0.05$.

5.4 Discussion

Two nonlinear indices, the phase synchronization index (λ) and the directionality index (d), were used to determine the presence and causal relationship of cardiovascular and cardiorespiratory couplings at rest and in response to orthostatic stress and to dehydration. The main findings of the present study are 1) the phase synchronization of variables related to respiration did not exceed that occurring by chance in the LF range; 2) dehydration reduced phase synchronization indices among all variables in the HF range and $d_{SBP-RRI}$ in the LF range; and 3) orthostatic stress increased $\lambda_{SBP-RRI}$ and decreased $d_{SBP-RRI}$ in the LF range, and decreased $\lambda_{SBP-RRI}$, $\lambda_{RESP-RRI}$ and $d_{RESP-SBP}$ in the HF range.

5.4.1 Validity of Utility of the Phase Synchronization Approach in Cardiovascular Coupling Analysis

In the present study, phase synchronization between SBP and RRI oscillations were studied in both LF and HF ranges, based on the assumption that SBP oscillations and RRI oscillations are generated by different central neural structures involved in autonomic cardiovascular regulation. Concerns may exist since some investigators assumed that RRI oscillations are just produced by resonance phenomenon due to SBP oscillations. Specifically, it is assumed that respiratory sinus arrhythmia is caused by blood pressure oscillations in the HF range [136]. However, the findings that respiratory SBP oscillations are resulted almost entirely from the direct effect of centrally mediated heartbeat fluctuations in dogs [137], and that respiratory sinus arrhythmia can actually contribute to respiratory arterial pressure fluctuations in humans [138] support our hypothesis, i.e., RRI oscillations in the HF range are not simply a result of baroreflex buffering of SBP oscillations. Some facts also support a central origin for LF fluctuations of RRI. Cooley *et al.* [139] found that LF components of RRI oscillations were restored without any change in the LF component of SBP oscillations, using the left ventricular assist device in severe heart failure patients. Taylor *et al.* [138] found that elimination of the LF component of RRI oscillations by fixed-rate cardiac pacing did not change LF blood pressure oscillations. These different responses of SBP and RRI to external stimuli indicate that different centers are responsible for generation of LF cardiovascular oscillations. In addition, an inconsistent relationship between LF oscillations of SBP and RRI in response to lower body negative pressure [140] suggests that a complex interaction of regulatory mechanisms determines the link between LF fluctuations.

5.4.2 Surrogate Data Analysis

Respiration did not synchronize with RRI and SBP in the LF range, which is consistent with other studies [118, 119]. Cysarz *et al.* [117] indicated that respiratory oscillations did not contain a LF component during spontaneous breathing, and therefore, the cardiorespiratory interaction was desynchronized. Badra *et al.* [34] also found that respiratory frequency had no effect on LF autonomic rhythms, indicating that LF rhythms are generated by mechanisms independent of respiratory rhythm generators. Thus, the cardiorespiratory desynchronization in the LF range was expected since our subjects were allowed to breathe spontaneously, and therefore, the breathing frequency was mainly in the HF band. Coupling between SBP and RRI, however, significantly exceeded those occurring by chance in both LF and HF ranges. The significantly high value of the phase synchronization index between SBP and RRI in the LF range indicated a high correlation within the cardiovascular system in healthy humans, which is consistent with other studies [118, 119, 129]. In addition, it has been shown that partialization of respiratory effects using a partial coherence method reduced coherence between RRI and SBP in the HF range [34], therefore, the significant phase synchronization between RRI and SBP in the HF range indicated that cardiovascular interaction could be respiratory driven [118, 119].

5.4.3 Cardiovascular Coupling Analysis

The analysis of causal relationships [31, 108-111, 123] and coupling strength [115, 118, 119, 123] between SBP and RRI provides information concerning the cardio-vagal baroreflex, which is essential to maintain blood pressure in response to orthostatic stress. Using a cross-conditional entropy method, Porta *et al.* [110] reported that the causal

relationship changed from RRI leading SBP at supine rest, to SBP driving RRI during HUT. Ocon *et al.* [123] also illustrated a dominant feed-forward relationship at supine in healthy subjects using the phase synchronization method. Similar results have been reported using other mathematical approaches [31, 109, 111]. In contrast, our results indicate a causal relationship from SBP to RRI in the LF range, but a bidirectional relationship in the HF range, at supine rest. Although similar bivariate methods were used in the present study and other studies [31, 109-111, 123], we explored the coupling strength and causal relationship in the LF and HF ranges separately. Results from other studies [34, 117-119] and the present study indicated that respiration did not interact with RRI and SBP in the LF range. Faes *et al.* [108] demonstrated that cardiovascular feed-forward and feedback mechanisms were balanced at supine rest by excluding respiratory effects using a multivariate information domain approach, which is not consistent with their previous studies using similar protocols, but with different bivariate analysis methods [31, 110, 111]. Faes *et al.* [108] pointed out that respiration may induce a feed-forward mechanism contributing to the observed RRI driving SBP in those studies [31, 110, 111]. Therefore, the predominance of the feedback causal relationship we observed in the LF range may reflect a relationship that is independent of the main rhythms of respiration.

Previous studies [31, 108-111, 123] have shown increased SBP driving RRI with increased tilt angle, in contrast, we observed that the moderate unidirectional SBP driving RRI in the LF range was reduced and converted to bidirectionally driven by both HUT and DEH, while a bidirectional relationship in the HF range was maintained throughout HUT and DEH. Pereda *et al.* [114] previously indicated that parasympathetic blockade

via atropine administration increased the dependency of SBP on RRI in the LF range, but had no effect on the bidirectional causal relationship between SBP and RRI in the HF range, in male rats. Therefore, results from the present study and those from our previous report [67], indicated reduced vagal outflow in response to both HUT and DEH. Different responses of the causal relationship between SBP and RRI with HUT in the present study and other studies [31, 108-111, 123] might be due to differences in experimental protocols. In our study, subjects were exposed to orthostatic stress for a much longer time period (~45 min) compared with other studies (~10 min). It has been shown that a prolongation of passive HUT may lead to orthostatic intolerance [109, 122, 123, 125, 126]. Indeed, a significant SBP drop was observed throughout HUT during both EUH and DEH in our study, and seven of our ten subjects had presyncopal symptoms by the end of HUT test during DEH. It has been shown that patients with a history of vasovagal syncope demonstrated diminished SBP driving RRI using a directionality index [123], or reduced information transferred from SBP to RRI using a corrected conditional entropy method [109] and an information decomposition strategy [125], indicating a loss of baroreflex regulation preceding syncope. Therefore, our results are more applicable to situations where the stability of circulation is challenged to the point of approaching syncope and thus may be more relevant in prediction of orthostatic intolerance. In addition, it is possible that differences in the causal relationship between SBP and RRI in the present study, compared to previous studies [31, 108-111, 123], might arise from methodological differences. The application of our phase focused method on filtered signal components may affect the interdependence between signals, since interactions between LF and HF components of each signal were not considered. Therefore, further

research should be performed to assess effects of different methodologies.

In addition to the changes in causal relationships, changes in coupling strength between SBP and RRI have been observed in subjects approaching syncope using different methods. Ocon *et al.* [123] reported that the coupling strength between SBP and RRI reduced preceding faint in patients with a history of vasovagal syncope, revealed by the phase synchronization approach, indicating an impaired cardiovagal integrity. Wang *et al.* [122] utilized a bispectral analysis to observe that coupling between SBP and RRI decreased during tilt, and was smaller in tilt-positive with respect to tilt-negative healthy subjects. It has been suggested that LF oscillations are determined by both sympathetic and parasympathetic activities, with HF oscillations determined by vagal activity only [78]. Thus, in our study, the augmentation of $\lambda_{SBP-RRI,LF}$ reflected a case in which sympathetic compensation overwhelmed the parasympathetic effects before the collapse of cardiovascular regulation, while the reduction of $\lambda_{SBP-RRI,HF}$ indicated vagal withdrawal.

5.4.4 Cardiorespiratory Coupling Analysis

In normal, unstressed, physiological conditions (e.g., at supine in EUH), the cardiovascular system is closely tied to the respiratory system, as indicated by high phase synchronization indices related to the HF-component of RESP. Decreased cardiac-respiratory coupling (i.e., RESP-RRI) has been observed during stressful conditions, such as orthostatic stress [108, 115], mental challenge [118, 121] and pregnancy [119]. However, decreased [118, 119], unchanged [108] and increased [115] vascular-respiratory coupling (i.e., RESP-SBP), have been obtained during different physiological conditions. Our results indicated that HUT reduced cardiac-respiratory, and did not alter

vascular-respiratory, coupling, which is consistent with Faes *et al.* [108], while DEH significantly reduced both cardiac- and vascular-respiratory interactions. Bartsch *et al.* [116] found that cardiac-respiratory phase synchronization was high when sympathetic activity was reduced and weak when sympathetic tone was dominant during different sleep stages using a phase synchrogram method. Niizeki *et al.* [121] indicated that the phase synchronization index of cardiac-respiratory coupling was positively related to parasympathetic status. The decreased cardiac-respiratory coupling, i.e., $\lambda_{RESP-RRI,HF}$, during DEH and HUT is consistent with DEH- and HUT-induced sympathetic excitation and vagal withdrawal determined in our previous report [67] and also consistent with cardiorespiratory decoupling before syncope [126]. The DEH-induced, but not HUT-induced, reduction in vascular-respiratory interaction, i.e., $\lambda_{RESP-SBP,HF}$, indicated that the respiratory effect on stroke volume was more detectable in response to DEH-induced acute overall reduction, compared with tilt-induced caudal shift, of blood volume. The difference between HUT and DEH induced changes in $\lambda_{RESP-SBP,HF}$ imply that DEH exacerbated the orthostatic stress induced desynchronization among RESP, RRI and SBP by reducing mechanical effects of respiration on SBP. In addition, we observed that the causal relationship was always from RESP to RRI and SBP in response to both HUT and DEH, consistent with the fact that RESP interacts with cardiovascular variables as an external oscillator [108, 123].

5.4.5 Limitations

A limitation of the approach used in the present study is that it requires windowing of the original data and therefore, choice of window size can affect the exact value of the synchronization indices. In order to eliminate a bias induced by choice of window size,

we used several different windows ranging from 10s to 100s. Analysis using these different window sizes all resulted in the same conclusion, although the exact values were different. In addition, in the case of perfect synchrony (i.e., $\lambda = 1$), it is not possible to separate the effect of interaction from the internal dynamics of autonomous systems [130], therefore, the directionality index cannot be obtained in this situation. However, none of the oscillations in our study were perfectly synchronized.

5.5 Conclusions

In summary, we utilized the phase synchronization method to quantify cardiovascular and cardiorespiratory coupling in response to orthostatic stress and dehydration. We found that orthostatic stress resulted in desynchronization among heart rate, blood pressure and respiration, and dehydration exacerbated this disassociation. Dehydration also reduced involvement of baroreflex regulation, which may contribute to the increased occurrence of orthostatic intolerance following acute blood volume reduction.

CHAPTER 6 SUMMARY AND PERSPECTIVES

6.1 Summary

The purpose of this dissertation was to investigate spaceflight related cardiovascular regulation, with the aim of protecting astronauts against the effects of spaceflight induced cardiovascular deconditioning, in terms of simulation of partial gravity environment, designing gravity-based countermeasure to OI and understanding causal relationships of cardiovascular regulation in response to orthostatic stress and dehydration. Specifically, in this dissertation, (1) a new model (upright LBPP) simulating cardiovascular responses to partial gravity has been assessed (see CHAPTER 3); (2) the ability of a short AG exposure in postponing the occurrence of OI symptoms has been tested (see CHAPTER 4); and (3) a new signal processing method has been applied to better understand the nonlinear information transfer among different cardiovascular subsystems in response to graded orthostatic stress and dehydration (see CHAPTER 5).

The major novel findings of this dissertation contribute to spaceflight physiology research in the following aspects:

First, this dissertation has provided a new ground-based simulation model of cardiovascular responses to partial gravity, thereby freeing subjects to be active. In CHAPTER 3, to determine whether upright LBPP provides a suitable space mission simulation, the cardiovascular responses of euhydrated and dehydrated men and women to orthostatic stress induced by HUT and upright LBPP, representing standing in lunar, Martian, and Earth gravities, were investigated. Results indicated that cardiovascular responses induced by upright LBPP are comparable to those induced by HUT, and

suggest the potential use of upright LBPP in future studies investigating cardiovascular responses to different activities in partial gravity.

Second, this dissertation has determined the beneficial effects of a short AG exposure in reducing the occurrence of OI in cardiovascularly deconditioned men and women. In CHAPTER 4, cardiovascular responses to orthostatic stress in 16 cardiovascularly deconditioned subjects (9 men and 7 women) were tested, once following a short (~90 minutes) AG exposure and once following a short (~90 minutes) HDBR exposure. Results indicated that compared to HDBR, a short AG exposure increased OTL in both men and women; a short AG exposure increased men's low frequency (0.04-0.15 Hz) power of systolic blood pressure (SBP_{LF}), but did not change women's SBP_{LF} responses to orthostatic stress. In addition, supine low frequency phase delay ($Phase_{LF}$) between systolic blood pressure and RR intervals became more negative following HDBR, but did not change following AG, in response to 70º HUT, reflecting improved baroreflex activity at milder level of orthostatic stress after AG. These results show that a short AG exposure increased both sympathetic and baroreflex responsiveness to orthostatic stress in cardiovascularly deconditioned men and women, which may contribute to the AG-induced increased OTL.

Finally, this dissertation has provided directionality information regarding interactions among cardiovascular oscillations in a ground-based simulation of spaceflight. In CHAPTER 5, the causal relationship and coupling strength of cardiovascular, cardiac-respiratory, vascular-respiratory interactions in response to orthostatic stress and dehydration were investigated by a thorough analysis of interactions among RRI, SBP and RESP, respectively. Results show that orthostatic stress disassociated interactions

among RRI, SBP and respiration, and that dehydration exacerbated the disconnection. These results of the present study also indicate that loss of causality from SBP to RRI seems to be able to early identify the onset of presyncope. In addition, the phase synchronization method used in this dissertation can deal with closed-loop interactions without priori assumptions and is able to capture both linear and nonlinear interactions without specifying a model of the observed interactions. This is important in furthering our understanding of mechanisms contributing to neutrally-mediated syncope and assessing interventions for preventing OI.

6.2 Perspectives

Future studies would focus on the following aspects:

First, as have mentioned in section 2.4.1, exercise alone or in combination with other procedures is a very important countermeasure to OI and crucial approach to maintain fitness of astronauts. Knowledge of changes in cardiovascular responses during exercise (e.g., walking, running and directed activities) in different gravitational environments (e.g., lunar and Martian gravity) is important, since such knowledge would provide guidelines for future Mars exploration and countermeasure design. So far, there is only one study investigating hemodynamic and biomechanical parameters during walking in simulated lunar and Martian gravity using upright LBPP [63]. Future studies need to be conducted to better understand the cardiovascular responses (e.g., autonomic cardiovascular regulation) and possible interactions with other physiological subsystems (e.g., cardio-locomotion interaction) to voluntary exercise in simulated partial gravitational environments. This would not only benefit astronauts in terms of efficient countermeasure design and better protection, but also clinical patients (e.g., obese

individuals, elderly subjects and injured athletes).

Second, as shown in section 4.3.1, although a short AG exposure improves orthostatic tolerance limit in cardiovascularly deconditioned men and women, the effect of a short AG exposure on female's cardiovascular function is not as good as those in male. Therefore, gender differences in response to AG exposure would be an interesting focus of future studies. This would provide us with useful information about potential countermeasure design for female astronauts. Another focus of future studies is to design and test potential countermeasure to OI by combining a short AG exposure and other countermeasures (e.g., exercise).

Finally, multivariate signal processing methods need to be applied in future studies to assess information transfer among cardiovascular oscillations. As shown in CHAPTER 5, although the phase synchronization approach provides more information in addition to conventional methods, it is a bivariate signal processing method in essence. Future studies of multivariate analysis (e.g., information domain approach [108]) may make it possible to differentiate between direct coupling (from one time series to another) and indirect coupling (effects mediated by one or more other time series), and improve our knowledge regarding interacting regulatory subsystems. Furthermore, effects of different gravitational environments [23, 67], and interventions to OI, such as AG [25], on cardiovascular regulation have been analyzed using conventional methods. In an application view, future studies may be needed to assess the effects of simulated partial gravities (see section 2.5 for more information) and existing interventions to OI (see section 2.4 for more information) on causal relationship and strength of interactions among cardiovascular and respiratory oscillations to gain more insight into the question.

APPENDIX A LIST OF ABBREVIATIONS OR SYMBOLS

AG	Artificial Gravity
ANOVA	Analysis of Variance
BEI	Baroreflex Effectiveness Index
BP	Blood Pressure
BRS	Baroreflex Sensitivity
BW	Body Weight
CFV	Cerebral Flow Velocity
CO	Cardiac Output
COH_{LF}	Coherence in Low Frequency Range
CVLM	Caudal Ventrolateral Medulla
DBP	Diastolic Blood Pressure
DBP_{LF}	Low Frequency Spectral Power of Diastolic Blood Pressure Oscillations
DEH	Dehydration
ECG	Electrocardiogram
ET	Early Tilt
EUH	Euhydration
f_R	Respiratory Rate
g	The Strength of A Gravitational Field
GABA	γ-Aminobutyric Acid
$Gain_{LF}$	Transfer Function Gain in Low Frequency Range
+Gz	Gravitational Force Directed Head-to-foot

Hb	Hemoglobin
Hct	Hematocrit
HDBR	Head-down Bed Rest
HF	High Frequency
HR	Heart Rate
HUT	Head-up Tilt
IAG	Intermittent Artificial Gravity
LBNP	Lower Body Negative Pressure
LBPP	Lower Body Positive Pressure
LF	Low Frequency
LT	Late Tilt
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
NA	Nucleus Ambiguous
NASA	National Aeronautics and Space Administration
NTS	Nucleus Tractus Solitarius
N_{seq}	Normalized Number of Baroreflex Sequences
N_{ramps}	Normalized Number of Systolic Blood Pressure ramps
OI	Orthostatic Intolerance
OTL	Orthostatic Tolerance Limit
$P_{ET}CO_2$	Partial Pressure of End-tidal Carbon Dioxide
$Phase_{LF}$	Transfer Function Phase in Low Frequency Range
PLT	Presyncopal Limit Test

POGO	Partial Gravity Simulator
PSD	Power Spectral Densities
PV	Plasma Volume
%ΔPV	Percentage Change in Plasma Volume
RESP	Respiration
RRI	R-R Intervals
RR_{HFnu}	Normalized High Frequency Spectral Power of R-R Interval Oscillations
$RR_{LF/HF}$	Ratio of Low to High Frequency Spectral Power of R-R Interval Oscillations
RVLM	Rostral Ventrolateral Medulla
SBP	Systolic Blood Pressure
SBP_{HF}	High Frequency Spectral Power of Systolic Blood Pressure Oscillations
SBP_{LF}	Low Frequency Spectral Power of Systolic Blood Pressure Oscillations
SEM	Standard Error of the Mean
SV	Stroke Volume
TPR	Total Peripheral Resistance
Z_{ABD}	Normalized Abdominal Impedance
Z_{THX}	Normalized Thoracic Impedance

APPENDIX B SURROGATE DATA ANALYSIS

Given a time series, $x(t)$, of N values taken at regular intervals of time $t = t_0, t_1, \dots, t_{N-1} = 0, \Delta t, \dots, (N-1)\Delta t$, apply the discrete Fourier transform operator, $\mathcal{F}$, to obtain

$$X(f) = \mathcal{F}\{x(t)\} = \sum_{n=0}^{N-1} x(t_n) e^{i2\pi f n \Delta t}. \quad \textbf{(B.1)}$$

This complex valued Fourier transform can be further written as $X(f) = A(f)e^{i\emptyset(f)}$, where $A(f)$ is the amplitude and $\emptyset(f)$ is the phase. $X(f)$ is evaluated at the discrete frequencies $f = -N\Delta f/2, \dots, -\Delta f, 0, \Delta f, \dots, N\Delta f/2$, where $\Delta f = 1/N\Delta t$.

A phase-randomized Fourier transform $\check{X}(f)$ is made by rotating phase $\emptyset$ at each frequency f by an independent random variable φ which is chosen uniformly in the range $[0, 2\pi)$. That is,

$$\check{X}(f) = A(f)e^{i[\emptyset(f)+\varphi(f)]}, \quad \textbf{(B.2)}$$

and from (B.2) the surrogate time series is given by the inverse Fourier transform:

$$\check{x}(t) = \mathcal{F}^{-1}\{\check{X}(f)\} = \mathcal{F}^{-1}\{X(f)e^{i\varphi(f)}\}. \quad \textbf{(B.3)}$$

By construction, $\check{x}(t)$ will have the same power spectrum as the original data set $x(t)$.

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Publications Related to This Dissertation

1. **Qingguang Zhang**, Joyce M. Evans, Michael B. Stenger, Fritz B. Moore and Charles F. Knapp. Autonomic cardiovascular responses to orthostatic stress after ninety minutes artificial gravity exposure compared with head-down bed rest in hypovolemic men and women. (Ready for submission)

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3. **Qingguang Zhang**, Charles F. Knapp, Michael B. Stenger, Abhijit R. Patwardhan, Samy C. Elayi, Siqi Wang, Vladimir I. Kostas and Joyce M. Evans. Simulations of gravitational stress on normovolemic and hypovolemic men and women. Aviation, Space and Environmental Medicine, 2014, 85 (4): 407-413.