# Cardiovascular Deconditioning Resulting from 28-hour Bed-rest and the Efficacy of the Fluid Loading Countermeasure

by

Anna Grinberg

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## AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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## ABSTRACT

This study tested the hypotheses that 1) 28h head-down bed-rest (HDBR) would result in significant hypovolemia and cardiovascular deconditioning, and that 2) NASA's fluid loading protocol (ingestion of 15 ml/kg water with a 1g NaCl for every 125ml of water consumed) would restore normovolemia and prevent cardiovascular deconditioning resulting from 28h HDBR. Nine healthy men were tested in 5 testing scenarios, with a progressive lower body negative pressure (LBNP) protocol performed before and after each scenario to measure the subjects' cardiovascular responses to orthostasis. Subjects were tested in two 28h HDBR conditions, without fluid loading (NFL) and with fluid loading (FL), as well as in three 4-hour control conditions to isolate the effects of circadian rhythm, HDBR, and fluid loading.

After 28h NFL HDBR, plasma volume was reduced by 8%. There were no symptoms of syncope during orthostatic testing following 28h NFL HDBR, however cardiovascular deconditioning was apparent as there were significant increases in heart rate, reductions in central venous pressure, and reductions in portal vein diameter during LBNP testing. There were no changes in stroke volume, cardiac output, systemic vasoconstriction, cardiac measures, and arterial and cardiopulmonary baroreflex responses, and no evidence of splanchnic or venous pooling.

This study also found that NASA's fluid loading protocol was ineffective at restoring normovolemia after 28h HDBR, as there were no

differences in plasma volume between 28h FL HDBR post and 28h NFL HDBR post tests ($p=0.22$). Cardiovascular deconditioning was not prevented by fluid loading as the heart rate response remained elevated and central venous pressure remained reduced after 28h FL HDBR. In addition, four of the nine subjects experienced nausea during administration of the fluid loading protocol prescription and two subjects vomited, further evidence that NASA's fluid loading protocol is not effective at preventing orthostatic hypotension.

Investigation of control models verified that deconditioning was the result of HDBR. It was also concluded that circadian rhythm did not affect the measured cardiovascular responses and the fluid loading protocol was ineffective at increasing blood volume in the absence of HDBR.

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| | |
|---|---|
| ANOVA | ANalysis of VAriance |
| CVP | Central Venous Pressure |
| DBP | Diastolic Blood Pressure |
| EDD | End-Diastolic Diameter |
| EDV | End-Diastolic Volume |
| ESD | End-Systolic Diameter |
| ESV | End-Systolic Volume |
| FL | Fluid Loading |
| HDBR | Head-down Bed-rest |
| HF | High Frequency |
| HR | Heart Rate |
| IVC | Inferior Vena Cava |
| LBNP | Low Body Negative Pressure |
| MAP | Mean Arterial Pressure |
| MF | Medium Frequency |
| NA | Not Applicable |
| NFL | Non-Fluid Loading |
| PP | Pulse Pressure |
| SBP | Systolic Blood Pressure |
| TPR | Total Peripheral Resistance |

# 1 INTRODUCTION

A common cardiovascular response experienced by astronauts returning from spaceflight is orthostatic hypotension. The term *orthostatic hypotension* describes the fall in blood pressure that arises due to postural stress and can lead to presyncope and syncope. Presyncope is generally defined as a fall in systolic blood pressure to 70 mmHg and may be accompanied by dizziness, narrowing of vision and fainting. The condition is common in young women and the elderly on Earth; however the susceptibility of healthy astronauts to orthostatic hypotension and presyncope is exacerbated upon return to spaceflight. Buckey and colleagues reported that up to 64% of astronauts experience orthostatic hypotension following exposure to microgravity (Buckey et al. 1996). Meck and colleagues found that short duration (2 week) flights resulted in 20% of astronauts experiencing presyncope, whereas the rate rose to 83% following long duration (129-190 day) spaceflight (Meck et al. 2001).

Many studies have worked to identify the mechanisms that lead to orthostatic hypotension following exposure to microgravity in an otherwise healthy population of astronauts. Hypovolemia is suspected to play an important role in cardiovascular deconditioning following exposure to microgravity. This may lead to increased peripheral resistance, reduction in vasoconstrictor reserve, attenuated arterial baroreflex, and changes in cardiac function. Other effects of bed-rest, such as compliance and changes in systemic blood distribution, are not thought to be directly linked to hypovolemia but result from bed-rest deconditioning. Together, these vascular changes may

increase susceptibility to orthostatic hypotension but identification of their link with hypovolemia requires further study.

Countermeasures have been developed to reduce and remove the risk of orthostatic hypotension following spaceflight. NASA has created a countermeasure that attempts to restore lost plasma volume by administration of salt tablets and water prior to re-entry. There are questions regarding the efficacy of this countermeasure which still need to be addressed.

Ground-based analogues have been developed that allow researchers to investigate cardiovascular deconditioning in a controlled, economical, and flexible laboratory setting. Six-degree head-down bed-rest (HDBR) has been shown to be an analogue to spaceflight as it removes the gravity vector directed from the head to the feet and induces a similar cephalad fluid shift as seen in spaceflight (Fortney Schneider and Greenleaf 1996).

This study investigated the effects of hypovolemia on cardiovascular deconditioning and the efficacy of NASA's fluid loading countermeasure in restoring both plasma volume and preventing cardiovascular deconditioning using the 6° HDBR model.

## 1.1 SPACEFLIGHT AND BED-REST INDUCED HYPOVOLEMIA

One of the earliest observations of space physiology was that microgravity causes a fluid shift towards the upper body. In space astronauts exhibit 'chicken' legs and puffy faces since gravity no longer pushes blood towards the feet. With the cephalad displacement of fluid, water and sodium are excreted

from the kidney resulting in hypovolemia within the first hours of spaceflight. The Spacelab Life Science Missions reported a 10% plasma volume reduction during spaceflight (Sawka 1999). Bed-rest results corroborate the results from spaceflight (Vernikos and Convertino 1994, Waters 2004). This cephalad fluid shift results in immediate increases in central venous pressure, stroke volume and cardiac output. Within a few hours of exposure there is salt and water diuresis, reducing central venous pressure and stroke volume below supine values (Sawka 1999).

Natochin and colleagues (1991) hypothesized that adaptation to microgravity causes a resetting of the fluid volume regulation system. The renal response to antidiuretic hormone, aldosterone, urodilatin, and atrial natriuretic peptide may be reset with spaceflight and bed-rest. The central venous pressure set point also appears to be lower with exposure to microgravity. Urine output during saline loading in ambulatory subjects was not altered by head-down bed-rest. These factors may increase water clearance, and result in a lower set-point for body fluid balance.

Convertino and Cooke hypothesize that the effects of hypovolemia may be a major factor in onset of presyncope following spaceflight by leading to reductions in vasoconstrictor reserve (Convertino and Cooke 2007). They speculate that if fluid volume is effectively restored, vasoconstrictor reserve will also be restored, providing adequate increases in peripheral resistance and prevention of orthostatic hypotension.

Hypovolemia is suspected to cause attenuation of cardiopulmonary and arterial baroreflex function, impairing baroreflex mediated responses to maintain blood pressure during orthostatic stress (Iwasaki et al. 2000, Vernikos and Convertino 1994). It is also suspected to negatively impact cardiac function by reducing preload (Perhonen et al 2001).

## 1.2 REDUCTION IN VASOCONSTRICTOR RESERVE

Diminished vascular function is suspected to be a major contributor to orthostatic hypotension. Following spaceflight and bed-rest, a reduced vasoconstrictor response is often seen during orthostatic challenge compared to pre-flight. In a group of astronauts returning from scientific missions on Spacelab, there were no statistical differences in circulating blood volume, blood pooling in the legs, reduced cardiac filling, stroke volume, or tachycardia compared to their pre-flight responses. However, only five of the 14 astronauts were able to successfully complete a 10-minute stand test that each had completed pre-flight The feature that distinguished finishers from nonfinishers was the ability to maintain mean arterial pressure and increase their vascular resistance response (Buckey et al 1996). Similar results relating reduced peripheral resistance responses to syncope following spaceflight were found in studies of astronauts by Fritsch-Yelle (1996), Meck (2004), and Waters (2002). In a 2-week bed-rest study, Zhang and colleagues found that an increase in vasoconstrictor outflow to muscle was attenuated during orthostatic testing following bed-rest. Cerebral autoregulation was also impaired, illustrated by an earlier and greater fall in cerebral blood flow velocity during orthostasis after

bed-rest (Zhang 2001). Arbeille and colleagues expanded these results, measuring femoral, renal, and cerebral vascular resistance in cosmonauts during short term and 3-month space travel. At rest, post-flight femoral and renal vascular resistances were reduced and cerebral resistance didn't differ statistically from pre-flight values. Cerebral blood flow was maintained at pre-flight values. During orthostatic challenge femoral vascular resistance was attenuated following spaceflight and the ratio between cerebral and femoral blood flow velocity was attenuated (Arbeille 1995). These studies suggest that failure to elevate peripheral resistance may contribute to a reduction in orthostatic tolerance after exposure to microgravity.

Convertino describes syncopal and nonsyncopal responses as two different physiological states. During a nonsyncopal response there is an increase in sympathetic stimulation that leads to an increase in total peripheral resistance (TPR) enabling mean arterial pressure to be maintained. During severe hypotension there is an abrupt sympathetic withdrawal and subsequent vasodilation, causing a loss of blood pressure control and onset of syncope (Convertino and Cooke 2006).

One hypothesis regarding onset of syncope following exposure to microgravity and the reduced TPR response in nonfinishers is that bed-rest and spaceflight induce a change in vasoconstrictor reserve. Vasoconstrictor reserve is defined as the difference between baseline and maximum vasoconstriction. Rather than changes in vascular responsiveness, syncope may result when an

individual does not have enough vasoconstrictor reserve to enable adequate TPR (Convertino and Cooke 2006).

There is evidence supporting the idea that each individual has a finite maximal vasoconstriction which is unchanged with exposure to microgravity. In a study investigating use of maximal exercise as a countermeasure, Convertino and colleagues showed that maximal vascular resistance does not change with 16 days of bed-rest (Engelke et al.1996, Convertino and Cooke 2007).

Rather than a change in maximal vasoconstriction, there is evidence that exposure to microgravity elevates supine total peripheral resistance compared to pre-flight or bed-rest. This elevation is thought to be due to hypovolemia and a lower circulating blood volume. An elevated baseline peripheral resistance results in a smaller capacity to constrict resistive vessels and therefore a lower vasoconstrictor reserve (Convertino and Cooke 2006). Convertino studied 8 subjects in 16 day bed-rest with orthostatic testing occurring pre and post-bed-rest. As orthostatic stress was increased, central blood volume was reduced, and peripheral vascular resistance was increased until maximal vasoconstriction was achieved (seen as a plateau in forearm vascular resistance with increasing orthostatic stress). Maximum vasoconstriction was unaltered with bed-rest, but it was reached at a lower orthostatic stress compared with pre-flight. Baseline resistance was elevated, reducing the vasoconstrictor reserve available to support orthostasis (Convertino 1999).

Another method to examine reduced vasoconstrictor reserve is to investigate the cardiopulmonary baroreflex-mediated changes in forearm vascular resistance in response to changes in central venous pressure (ΔFVR/ΔCVP). Convertino and colleagues (1994) found that this relationship was elevated following exposure to microgravity. Vascular responsiveness (the ability to vasoconstrict) is not compromised, as this would be reflected by attenuation of the ΔFVR/ΔCVP relationship.

Investigators have examined if hypovolemia is a primary cause of the reduction in vasoconstrictor reserve. Thompson and colleagues induced a 16% reduction in plasma volume by furosemide administration. Acute hypovolemia was associated with at 25% reduction in supine total peripheral resistance compared with normovolemia and an upward shift of the vasoconstriction response to orthostatic stress (Thompson 1990). Fu and colleagues induced an 11% reduction in plasma volume by furosemide and found it was related to a 67% reduction in vasoconstrictor reserve and a 35% reduction in tolerance time (Fu et al 2004). Vernikos and Convertino (1994) found that 7 days of bed-rest induced a 12% plasma volume reduction. The cardiopulmonary baroreflex response (ΔFVR/ΔCVP) following bed-rest had a higher slope than pre-bed-rest conditions. This indicates a greater utilization of the vasoconstrictor reserve, and that subjects are operating closer to their maximal vasoconstriction limit. Plasma volume restored by fludrocortisone also restored cardiopulmonary baroreflex function. Oral administration of salt and water did not restore plasma volume fully, nor did it restore cardiopulmonary baroreflex function. These data

provide evidence that hypovolemia may be responsible the reduction in vasoconstrictor reserve.

These findings raise the possibility that hypovolemia resulting from bed-rest and spaceflight may be a primary mechanism of orthostatic intolerance via reductions in vasoconstrictor reserve. Research is needed to investigate if similar results can be found with short term bed-rest and whether or not NASA's fluid loading protocol can prevent the reduction in vasoconstrictor reserve.

## 1.3 ARTERIAL BAROREFLEX ATTENUATION

Attenuated arterial baroreflex control of arterial blood pressure may lead to a reduced tachycardia response with orthostasis and be a cause of orthostatic hypotension following spaceflight and bed-rest. Fritsch-Yelle and colleagues found that orthostatic tolerance was decreased in 16 astronauts who experienced 4-5 days of spaceflight and this was accompanied by an attenuated baroreflex. The slope, range, and operational points of the carotid transmural pressure-sinus node response were reduced post-flight compared to pre-flight. The results illustrated functionally relevant reductions in parasympathetic and increases in sympathetic influences on arterial pressure control after spaceflight (Fritsch-Yelle et al 1994).

Harrison has suggested that central blood volume changes may be responsible for altered carotid baroreflex responses because head down tilt reduces and head up tilt increases R-R interval responses to neck pressure

stimuli (Harrison 1986). It is not known whether attenuated arterial baroreflex function following exposure to microgravity is due to hypovolemia or a unique adaptation of the autonomic nervous system.

Iwasaki et al. examined the link between arterial baroreflex attenuation and hypovolemia by comparing the effects of acute hypovolemia and bed-rest (deconditioning plus hypovolemia). Nine healthy subjects underwent 2 weeks of 6º HDBR as well as acute hypovolemia induced by furosemide. Both protocols induced the same reductions in plasma volume. Power spectral and transfer function analysis investigated the relationship between R-R interval and systolic blood pressure (SBP). Iwasaki et al. found that there were no significant differences between the effects of the 2 protocols in terms of cardiac filling pressures, arterial pressure, cardiac output, or stroke volume. Normalized high frequency power in the 0.15-0.25 Hz region of the R-R interval signal was attenuated to the same degree in both protocols, illustrated similar degree of vagal withdrawal. Arterial-cardiac sensitivity, assessed by SBP to RR-interval transfer function gain, was reduced to a similar extent in both conditions. The ability of the baroreflex to alter blood pressure by modifying systemic flow, assessed by BP to cardiac output, was also reduced by both protocols. These results support the hypothesis that reductions in plasma volume may be a primary contributor to arterial baroreflex attenuation following bed-rest (Iwasaki et al 2000).

Convertino and colleagues (1990) studied carotid baroreceptor-cardiac reflexes in 11 healthy men before, during, and after 30 days of bed-rest to test

the hypothesis that baroreflex malfunction contributes to orthostatic intolerance and is related to hypovolemia. Sigmoidal baroreflex responses were provoked with a neck chamber device. Baseline R-R intervals and the minimum, maximum, range and maximum slope of the RR interval-carotid pressure relationships were reduced with bed-rest. The four subjects who experienced syncope during standing following bed-rest exhibited larger reductions in slope than the six subjects who completed the stand test. Non-syncopal subjects maintained the same systolic pressure before and after bed-rest, whereas the syncopal subjects experienced a significant reduction. Although plasma volume declined by 15% there were no significant correlations between hypovolemia and changes of baroreflex response; plasma volume reductions stabilized after 2 days but baroreflex sensitivity continued to deteriorate throughout bed-rest. Bed-rest leads to substantial and progressive impairment of baroreflex function and that the progressive impairment of baroreflex malfunction is related significantly to the occurrence of orthostatic hypotension (Convertino 1990).

The different results found by Convertino and Iwasaki may be attributed to the method of arterial baroreflex investigation. Transfer function analysis is thought to reflect both the aortic and carotid-cardiac loops of arterial baroreflex control, however the neck chamber technique reflects only the carotid-cardiac loop. It is possible that the loops adapt uniquely and independently to spaceflight and bed-rest. The neck chamber technique is considered to investigate the static index of baroreflex function whereas the transfer function

gain provides insight into the dynamic properties of baroreflex function (Iwasaki et al 2000). The conflicting results may also be due to differences in acute and long term changes in blood volume.

Vernikos and Convertino (1994) investigated the effects of hypovolemia on arterial baroreflex function. Arterial baroreflex was attenuated following 7 days of bed-rest and a 12% reduction in plasma volume. When fluid volume was restored fully by fludocortisone administration, arterial baroreflex function was restored. Fluid volume restoration was also attempted by saline loading, however the prescription was insufficient to fully restore plasma volume to pre-bed-rest levels (Vernikos and Convertino 1994).

In order to gain insight into the rapidity of baroreflex attenuation and to further investigate the role of hypovolemia, a short duration bed-rest study examining the effects of fluid loading would be valuable.

## 1.4 ALTERED SYSTEMIC BLOOD DISTRIBUTION

There is a growing body of evidence that suggests systemic blood flow distribution is altered following exposure to microgravity. Increased vasoconstriction during orthostasis enables blood to be directed to the cerebral vasculature to maintain orthostasis. However, it appears that vasoconstriction responses vary across different various body compartments as a result of cardiovascular deconditioning following bed-rest. Specifically, there is evidence for different vasoconstriction responses between the systemic

circulation and the splanchnic region. Differential vasoconstriction may hinder cerebral blood flow availability and be associated with orthostatic intolerance.

Arbeille and colleagues (2005) identified the portal vein as a valuable blood vessel to image as it is the only output from the splanchnic vascular area. Increases in portal flow reflect reductions in splanchnic vasoconstriction. This study also found that portal flow was increased following 90 days of bed-rest. This indicates that splanchnic arterial vasoconstriction was reduced with bed-rest, which was associated with orthostatic intolerance.

Fischer and colleagues (2007) found that total peripheral resistance was not altered with 4 hour bed-rest, but splanchnic blood flow was increased at each level of orthostatic stress after bed-rest compared to pre-bed-rest. This impaired ability to distribute blood away from the splanchnic region during orthostasis was associated with reductions in stroke volume and heart rate, and affected the ability to control arterial blood pressure.

Compliance has been investigated by various space and bed-rest studies in the past as a source of blood pooling in the venous system. Compliance increases following exposure to bed-rest and spaceflight, allowing for increased pooling and reductions in venous return. Compliance is thought to be an adaptation independent of hypovolemia and linked to muscle atrophy (Convertino 1989, Louisy 1997).

Further investigation is needed to clarify altered systemic blood distribution with exposure to bed-rest. Knowledge of compliance and

differential vasoconstrictor responses also provide additional insight into systemic blood flow changes with bed-rest, and will be useful to provide understanding of the other measured variables.

## 1.5 Reduction in Cardiac Function

Cardiac muscle is extremely plastic and adapts to changes in volume or pressure loading. Spaceflight and bed-rest are models where the heart experiences unloading due to hypovolemia. The heart experiences a lower filling pressure, resulting in reduced pre-load and myocardial work. Perhonen and colleagues (2001) have shown that exposure to bed-rest and spaceflight has led to cardiac atrophy. M-mode echocardiography performed on Skylab-4 astronauts showed an 8% reduction in LV mass in four astronauts during an 84-day mission (Henry 1977). Using the bed-rest model, left ventricular (LV) mass decreased in 5 sedentary men by 8% in 6 weeks, and 15.6% in 12 weeks. No changes were seen in the control population who were ambulatory for the same duration. The reduction in LV mass was associated with a reduction in wall thickness. LV end-diastolic volume was decreased by 14% after 2 weeks and was sustained until bed-rest completion. This study utilized magnetic resonance imaging for cardiac measures, providing more precise measurements than echocardiography performed previously. Both bed-rest and spaceflight resulted in reductions in cardiac output and stroke volume which may result in orthostatic hypotension (Perhonen et al 2001).

Cardiac atrophy was studied in direct reference to hypovolemia to determine if reductions in stroke volume are the due a cardiac adaptation to

head-down bed-rest or to acute hypovolemia alone. With hypovolemia alone baseline LV end-diastolic volume decreased by 7%, whereas after 2 weeks of bed-rest it decreased by 20%. Therefore both hypovolemia and cardiovascular deconditioning play a role in reduced cardiac function.

Though significant cardiac remodeling does not likely occur within the first day of bed-rest, heart function may be reduced due to hypovolemia. Lollgen (1984) found no changes in stroke volume and ejection fraction with 2 hours of bed-rest, despite an increase in preload, indicating left ventricular function was not disturbed . However, these durations were too short to induce hypovolemia. Waters and colleagues (2004) studied the effects of fluid volume restoration following 14 days of bed-rest. With fluid restoration there were no changes in end-systolic and end-diastolic diameters, stroke volume or ejection fraction compared to pre-bed-rest conditions. However, the effects of bed-rest without fluid volume restoration were not examined so the extent of deconditioning that occurred with bed-rest is not known.

A short-duration bed-rest study in which both hypovolemia and fluid volume restoration are examined is needed to clarify the role of hypovolemia on reduced cardiac function in the absence of cardiac remodeling.

## 1.6 HYPOVOLEMIA AND FLUID-VOLUME RESTORATION

In order to identify the impact of hypovolemia as a primary mechanism affecting orthostatic hypotension, two studies have investigated whether a fluid loading protocol might restore fluid volume lost during bed-rest and improve

the cardiovascular response to orthostasis. These studies have yielded conflicting outcomes.

Vernikos and Convertino (1994) studied the effects of fluid volume replacement following 7 days of 6º head-down bed-rest using saline in a dosage of 8g of NaCl and 960 ml water. Saline loading was ineffective at both restoring plasma volume to baseline levels and preventing orthostatic hypotension. Plasma volume decreased by 12% with 7 days of bed-rest, but was not restored by saline. Four of the five subjects treated with the saline countermeasure experienced syncopal symptoms with failure to maintain stroke volume and mean arterial pressure during the post-bed-rest orthostatic challenge. This was accompanied by compromised arterial and cardiopulmonary baroreflexes. Heart rate was increased following bed-rest despite saline loading and a reduction in peripheral resistance for the same drop in central venous pressure after bed-rest suggested that vasoconstrictor reserve was reduced. This study was limited by the lack of comparison between subjects' natural response to bed-rest and their response following fluid load (Vernikos and Convertino 1994). Also, a pre-determined fluid load was given to all subjects despite variation in body mass, with subjects receiving less salt and water then prescribed by NASA's official fluid loading protocol. This may have prevented full volume restoration in subjects whose weight exceeded 64 kg (Waters et al 2004).

Waters and colleagues (2005) found promising results with fluid loading as a countermeasure prior to orthostatic challenge after 12 days of 6º head-down bed-rest. Fluid loading was achieved with the standard NASA fluid loading

protocol of oral consumption of 1 g salt tablet per 125 ml water with a total volume of 15ml/kg within a 2 hour period prior to orthostatic challenge. The average blood and plasma volume losses, respectively, of 5% and 8% following bed-rest were restored to baseline with the fluid load. No subject experienced syncope. Heart rate increased following bed-rest despite the fluid load. All other measured variables (mean arterial pressure, cardiac output, stroke volume, ejection fraction, central venous pressure, and peripheral resistance) did not change with bed-rest. These results suggest that the latter cardiovascular variables and orthostatic hypotension are affected secondarily to hypovolemia. The important limitation of this study is the lack of investigation into the subject's natural bed-rest responses without fluid load because responses to orthostatic stress were only measured following fluid load. Therefore it cannot be said that fluid loading improved the subjects' responses to orthostatic stress following bed-rest.

To clarify the cardiovascular responses to bed-rest and its resulting hypovolemia as well as the efficacy of NASA's fluid loading protocol, the following study was performed.

## 2 PURPOSE OF STUDY

A 28h bed-rest study will be performed with 3 objectives:

1. To study the cardiovascular effects of 28h bed-rest, with specific consideration to the magnitude of the resulting plasma hypovolemia and mechanisms contributing to cardiovascular deconditioning.

2. To examine the effectiveness of NASA's fluid loading protocol on restoring lost plasma volume to its initial value after 28h bed-rest and to determine if this countermeasure reduces susceptibility to cardiovascular deconditioning.

3. To isolate the circadian effects of head-down bed-rest and fluid loading on plasma volume and cardiovascular deconditioning by three 4-hour control protocols.

# 3 HYPOTHESES

Plasma Volume

1. Plasma volume will be significantly reduced from baseline following 28 hour bed-rest.
2. Plasma volume will be restored by NASA's fluid loading protocol.

Cardiovascular Deconditioning

1. Cardiovascular deconditioning will result following 28 hour bed-rest, manifested by reductions in stroke volume, cardiac output and central venous pressure, as well earlier onset of tachycardia during orthostatic challenge post bed-rest.
2. Some subjects will experience pre-syncopal symptoms (loss of blood pressure maintenance) during post bed-rest orthostatic testing.
3. Cardiovascular deconditioning will be prevented with fluid loading. Post bed-rest stroke volume, cardiac output, central venous pressure, and onset of tachycardia will not significantly differ from pre bed-rest results.
4. Subjects who experienced pre-syncopal symptoms during the non-fluid-loading protocol will not experience pre-syncopal symptoms with fluid loading.

Vasoconstrictor reserve and Peripheral Resistance

1. Vasoconstrictor reserve will be reduced with 28h bed-rest. This will be illustrated by an increased baseline peripheral resistance, an

increased slope of peripheral resistance response with levels of orthostatic challenge, and an attenuated cardiopulmonary baroreflex response (Δ forearm vascular resistance/ Δ central venous pressure).

2. Vasoconstrictor reserve will be restored with fluid loading. The baseline peripheral resistance, the peripheral resistance response to orthostatic challenge, and the cardiopulmonary baroreflex response following bed-rest and fluid loading will not significantly differ from baseline.

Arterial Baroreflex Sensitivity

1. Arterial baroreflex sensitivity will be attenuated following 28 hour bed-rest.
2. Arterial baroreflex responses will be restored with fluid loading.

Cardiac Measures

1. Ejection fraction will be reduced with 28 hour bed-rest and orthostatic challenge.
2. Ejection fraction at baseline and during orthostasis will be restored with fluid loading.

Systemic Blood Distribution

1. Splanchnic vasoconstriction and total peripheral vasoconstriction responses will differ; splanchnic vasoconstriction is expected to decrease following bed-rest where as total peripheral resistance is expected increase.

2. Fluid loading will not restore differential vasoconstriction responses between the total peripheral resistance and splanchnic resistance.
3. Venous compliance will be increased with 28 hour bed-rest.
4. Venous compliance will not be restored with fluid load.

# 4 METHODOLOGY

## 4.1 SUBJECTS

This study examined 9 male subjects between the ages of 18 to 33 years old. Subjects completed a medical questionnaire prior to participation, which informed the examiners of their health status, prescribed medications, time of last blood donation, and fitness level. All subjects were free of cardiovascular illness. None of the subjects had taken prescription or non-prescription medication during the days prior to testing. The regular daily activity levels of the subjects varied considerably, with subjects' lifestyles ranging from low fitness levels to daily training.

Prior to testing, subjects were familiarized with the laboratory equipment and the study protocol to reduce stress during the first test. Subjects were screened for portal vein imagery before their first test. All subjects read and signed a consent form authorized by the University of Waterloo's Office of Research Ethics prior to participation.

## 4.2 PROTOCOL

This study had a randomized crossover design; all subjects completed each treatment and control. The protocol included two 28h head-down bed-rest (HDBR) treatment tests: 28h FL HDBR (with fluid loading) and 28h NFL HDBR (without fluid load), as well as three 4-hour control tests: 4h S NFL (seated), 4h S FL (seated with fluid load), and 4h HDBR. These tests are described in detail in Section 4.3. Table 4.2-1 below indicates the order in

which each subject performed each test (1= first test completed and 5= last test completed), and confirms that the randomized cross-over design was performed.

**Table 4.2-1: Order of Test Completion**

| Subject | 28h NFL HDBR | 28h FL HDBR | 4h S NFL | 4h S FL | 4h HDBR |
|---|---|---|---|---|---|
| 1 | 1 | 5 | 4 | 2 | 3 |
| 2 | 5 | 4 | 2 | 1 | 3 |
| 3 | 3 | 4 | 5 | 1 | 2 |
| 4 | 5 | 4 | 3 | 1 | 2 |
| 5 | 2 | 1 | 5 | 3 | 4 |
| 6 | 2 | 5 | 3 | 4 | 1 |
| 7 | 5 | 4 | 3 | 1 | 2 |
| 8 | 4 | 5 | 1 | 3 | 2 |
| 9 | 4 | 5 | 2 | 3 | 1 |

It is noted that the 28h FL HBDR test was frequently performed later in the testing sequence. Several constraints determined the order in which testing was performed. Subjects' school and work schedules played a large role. Three participants were competitive swimmers, and test scheduling took into consideration their training and competition obligations due to the time commitment of each test and the volume of blood collected during each test. Another major determinant of test order was that the data from two of the 4-hour tests (4h S NFL and 4h HDBR) were shared with another project. Data collection for the other study began prior to data collection from this study, meaning that often data from the two aforementioned tests was collected prior to the 28h NFL HDBR, 28h FL HDBR, and 4h S FL tests.

Each testing session began at 7 am to control for the effects of circadian rhythm. At least 1 day of recovery was required after a 4-hour test and 7 days after a 28h test before another test was performed. This allowed for red blood cell replacement after blood collection.

## 4.3 28H TREATMENT MODELS

The subjects were tested under the conditions specified by two 28h treatment models:

1. 28h non-fluid loading HDBR model (28h NFL HDBR)

2. 28h fluid loading HDBR model (28h FL HDBR)

The 28h NFL HDBR model was designed to investigate the natural cardiovascular response to orthostasis following 28 hours of bed-rest. The 28h FL HDBR test was designed to examine the cardiovascular responses to orthostatic stress following 28 hours of bed-rest while employing NASA's fluid loading protocol during the final 2.5 hours of bed-rest exposure.

The 28h test protocols are illustrated by Figure 4.3-1. Upon arriving at the lab, the subject was prepared for the test with measurements of height and weight, morning urine collection, and electrode placement for ECG and impedance plethysmography. The subject's baseline total blood and plasma volumes were measured during the carbon-monoxide (CO) re-breathe. Baseline cardiovascular responses to cardiovascular stress were then collected during a low-body negative pressure (LBNP) test. The subjects then underwent 28 hours of 6° HDBR. After HDBR, a second "Post" LBNP test examined the effects of

the protocol. During the fluid loading model, the prescribed treatment took place between 25.5 and 27.5 hours, with the intention to restore plasma volume to its pre-bed-rest values.

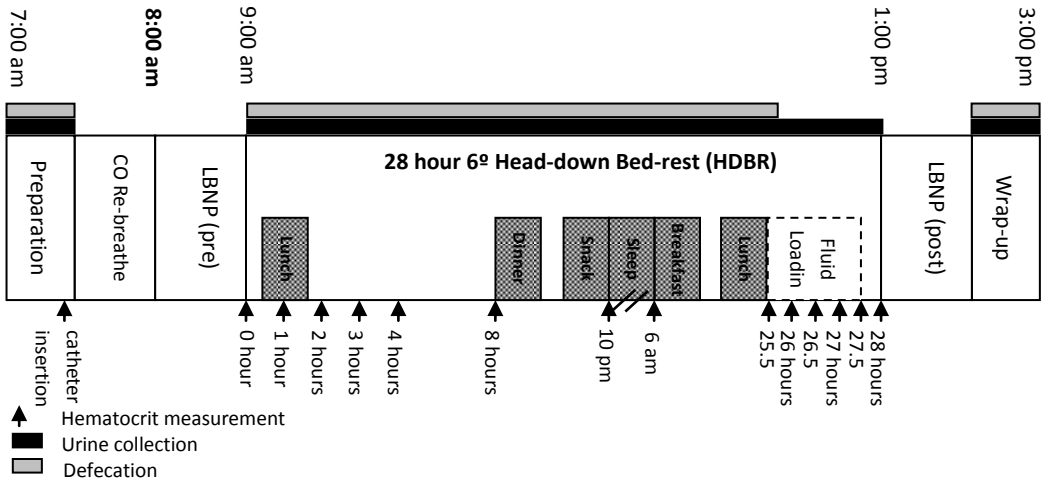


**Figure 4.3-1: Timeline for 28h HDBR Treatment Models**

Food consumption was scheduled after blood sampling and at least 3 hours prior to portal imagery in order to minimize the effects of food intake on these measurements. Subjects' sleep schedules were planned for 8 hours, between 10 pm and 6 am. Blood draws for hematocrit measurements are indicated by arrows in Table 4.2-1. Times when urine collection and defecation were permitted are also indicated. Urine collection was permitted throughout testing, except during LBNP testing and the CO re-breath. Defecation was not allowed within the final 4 hours of HDBR to prevent exposure to ambulation and gravity. Following the post-LBNP test, height and weight was measured and a final urine collection.

## 4.4 4-HOUR CONTROL MODELS

The subjects were also tested under the conditions specified by three 4-hour control models:

1. 4-hour seated non-fluid loading model (4-hour S NFL)
2. 4-hour seated fluid loading model (4-hour S FL)
3. 4-hour HDBR non-fluid loading model (4-hour HDBR)

The 4-hour S NFL and 4-hour HDBR models were used to determine whether the cardiovascular changes in the 28 hour models were affected by the time of day. The 4h S FL model was compared to the 4-hour S NFL model to isolate the effects of the fluid loading protocol in the non-HDBR condition. The 4-hour S FL protocol was also compared with 28h FL HDBR results to isolate the effects of bed-rest in the 28h tests.

Figure 4.4-1 illustrates the timeline for the 4 hour control models. Testing was designed to mimic the 28 hour tests, with fluid loading scheduled between 1.5 and 3.5 hours. Lunch was scheduled after the first blood sample (0 hour) taken during the bed-rest or seated period, to control for its effects on hormones and portal blood flow measurements. Blood draws for hematocrit measurements and times for urine collection and defecation are also indicated in Table 4.4-1. Defecation is not permitted within the 4 hour model to prevent exposure to ambulation and gravity.

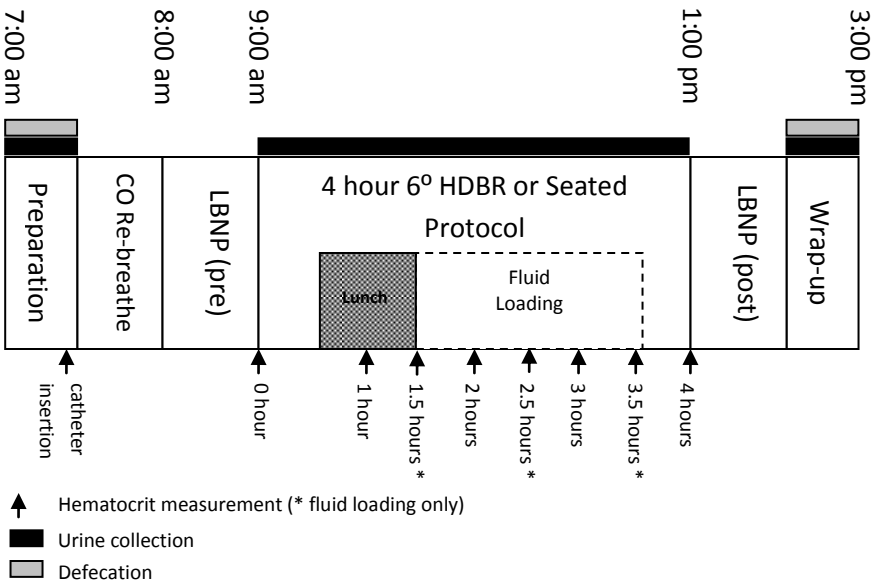


**Figure 4.4-1: Timeline for 4-hour Control Models**

## 4.5 LOWER-BODY NEGATIVE PRESSURE BOX (LBNP) TESTING

Lower-body negative pressure (LBNP) testing is a method of exposing a subject to the cardiovascular effects of orthostasis in a safe manner. The LBNP box is composed of a box with an opening for the subject to enter feet-first with an attachment for a kayak skirt that creates a seal at the subject's waist. The closed system is connected to a vacuum which regulates pressure inside the box via a voltage regulator. One of the major advantages of this method of orthostatic testing compared to tilt or stand tests is that it removes the effects of the muscle pump, therefore allowing examination of blood pressure regulation solely by cardiovascular responses. It is also safe since the subject remains in the supine position and the suction can be immediately removed if pre-syncopal symptoms arise, eliminating the risk of falling.

This study used a progressive LBNP test to measure its subjects' response to orthostatic challenge: 0 mmHg (5min), -10 mmHg (3 min), -20 mmHg (5 min), -30 mmHg (3 min), and -40 mmHg (5 min). Subjects were placed in the box in the supine position for at least 20 min prior to the start of the LBNP test during equipment set-up to stabilize their physiological condition. Signals directly indicating the stability of blood pressure regulation, such as heart rate, mean arterial pressure, and systolic blood pressure, were monitored continuously throughout the progressive test for monitoring of the subject's condition and data collection. Brachial blood flow and cardiac output were also measured continuously. Aortic blood flow and cardiac impedance, used in calculations of cardiac output, were measured at specific times throughout each level as described in Figure 4.5-1. Ultrasound imaging of portal vein and inferior vena cava were measured separately from aortic blood flow to prevent acoustical interference. Splanchnic impedance was collected during this time since it was used in conjunction with portal vein measurements to describe splanchnic hemodynamics. Blood was collected between 3:15 and 5:00 min of 0, -20 and -40 mmHg segments, and therefore this was the only time when central venous pressure was not collected due to use of the same catheter. Echocardiography was tested at 0 and -40 mmHg. In order to reduce stress on the subject, echocardiography at 0 mmHg was done before the LBNP test commenced (during the setup period), and at -40 mmHg following a 1 min recovery period at 0 mmHg following the progressive test in order to minimize stress on the subject. In the event the subject was unable to complete the LBNP

test, echocardiography was performed at -30 mmHg instead, or not collected. The LBNP protocol used in this study is described by Figure 4.5-1.

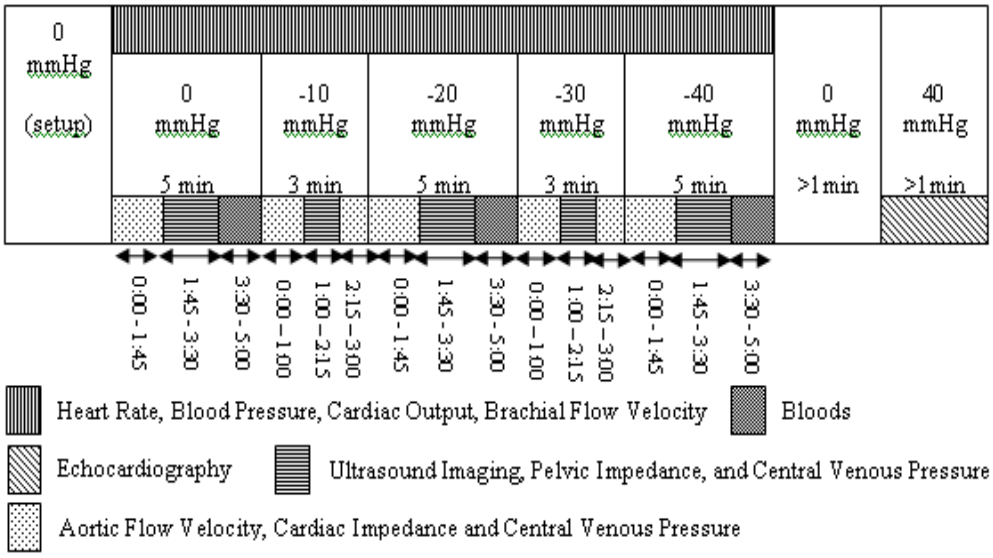


**Figure 4.5-1: LBNP Testing Protocol**

### 4.5.1 Test Termination Criteria

In order to protect the subjects, the following procedures were in place to terminate LBNP testing before syncope occurred:

1. Fall in systolic blood pressure < 70 mmHg (or reduced by 25 mmHg from baseline)
2. Rapid drop in mean arterial pressure or heart rate
3. Symptoms of stomach awareness, nausea, sweating, narrowing of visual field, or dizziness

During bed-rest period, many subjects experienced back pain, restlessness, and light headaches. The subjects had to option to abort the test at any point if they felt extreme discomfort; however no subjects chose to end the test prematurely.

## 4.6 HDBR AND SEATED POSITIONS

In protocols using the HDBR position, the subject was placed in a 6° head-down position for the duration of the test. The objective of this position was to mimic microgravity by inducing a fluid shift from the feet towards the upper body. The subject remained in this head-down position for all activities including eating, drinking, sleeping, and urination. The only exception during the 28 hour tests was defecation, when the subject was permitted to use the washroom. No exercise was performed by the subjects during the testing period. The HDBR position is shown in Figure 4.6-1.

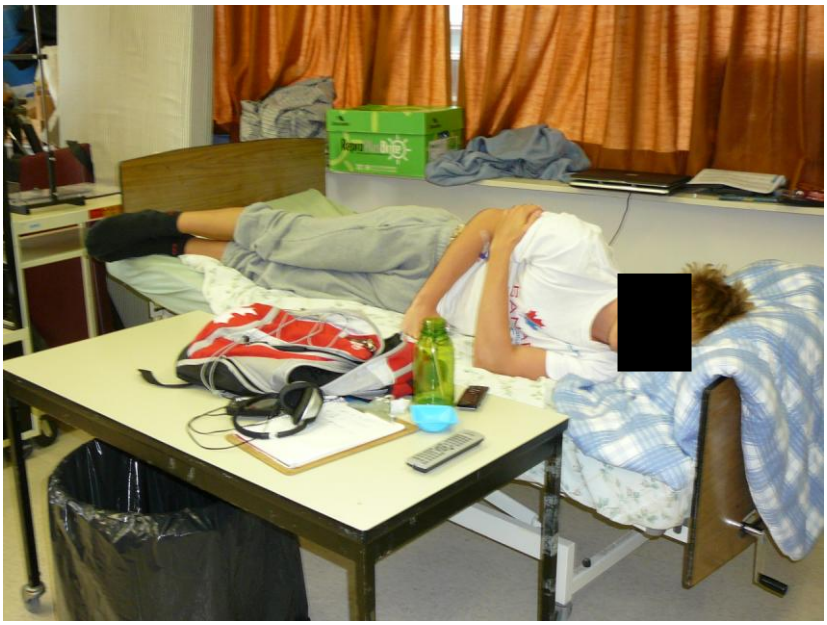

**Figure 4.6-1: HDBR Position**

During seated tests the subject was seated in a comfortable chair with their head and torso above their legs. The subject remained in the seated position for all activities including eating, drinking, sleeping, and urination. No

exercise was performed in the seated position. The seated position is illustrated in Figure 4.6-2.

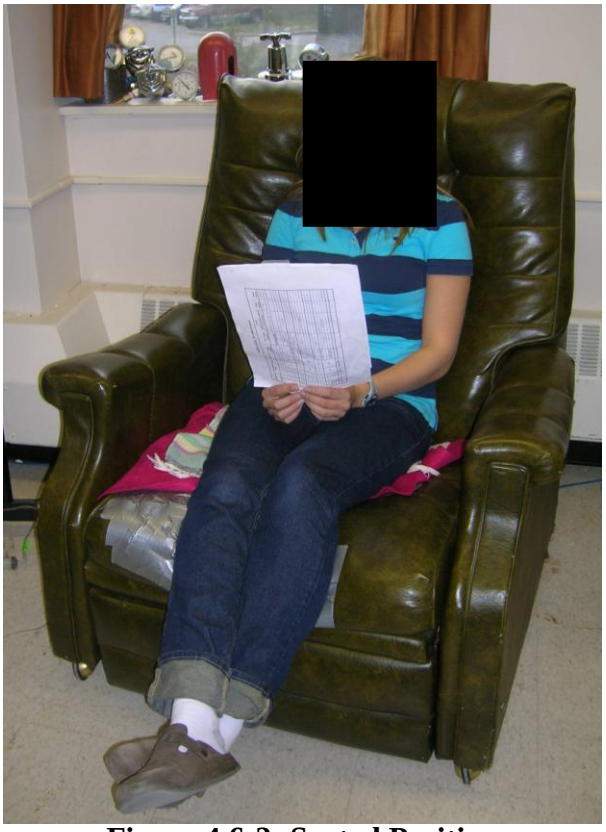

**Figure 4.6-2: Seated Position**

## 4.7 FLUID LOADING PROTOCOL

During protocols involving fluid loading, subjects underwent NASA's fluid loading protocol. The intent of this protocol was to restore lost fluid volume resulting from bed-rest with a hypotonic solution that could be easily performed by astronauts returning from space. Water and salt was ingested in a dosage of 15 ml/kg with a 1g salt tablet for each 125 ml water consumed (Waters 2004). The fluid loading protocol was performed between 1.5h and 3.5h (during the 4h protocol) and 25.5h and 27.5h (during the 28h protocol), as seen in Figures 4.3-1 and 4.4-1. Salt and water were administered in equal intervals across the fluid loading period. If the subject experienced stomach upset or nausea, fluid loading was paused or aborted to prevent loss of fluid by vomiting and subject discomfort. The impact of nausea is discussed thoroughly in Section 5.2.

**Figure 4.7-1: Fluid Load Salt Tablets and Graduated Water Bottle**

## 4.8 ADDITIONAL TESTING CONSIDERATIONS

### 4.8.1 PRE-TESTING CONSIDERATIONS

Subjects were asked to complete a food diary for the three days prior to testing in order to gauge their sodium intake. For 24 hours prior to testing, subjects were asked to abstain from alcohol, caffeine, and heavy exercise. They were also provided with a list of sodium-rich foods to avoid. It was requested that the subject consume the same breakfast prior to each test. The recommended breakfast was two slices of bread with 1 tbsp jam. The subject was required to consume 5 ml of water per kg body weight the night before and the morning of testing to achieve a hydrated state, as done by Waters and colleagues (2004). During the 28h HDBR tests, the subject also consumed 5 ml/kg of water before bedtime and after waking up while in the lab. Monitoring of these requests was accomplished by a series of questions posed to the subject before the commencement of testing, and the submission of their food diary.

### 4.8.2 SLEEP

A pre-determined sleep schedule was adopted to control for circadian rhythm and provide a control measure for blood analysis. An 8 hour block of time between 10 and 6 am was allocated for sleep. Sleep was not permitted at any other time.

In practice, subjects often fell asleep between 10:30-11:00 pm and awoke between 6:30-7:00 am, although the "lights-out" occurred on time. Most of the subjects were accustomed to going to sleep later, and had not adapted before the study to the prescribed sleep schedule. It is also important to mention

that it was common for subjects to wake-up during the night due to back pain or discomfort, or to urinate. An investigator stayed overnight with the subjects in order to facilitate toiletry or discomfort needs.

### 4.8.3 FOOD AND WATER INTAKE

Breakfast, lunch, dinner, and snacks were provided to the subjects during each 28h test period, and lunch was provided during each 4h test. The menu was based on a standard 2500 calorie daily intake for males with a minimum sodium intake of 4g (174 mEq), similar to the diet used by Waters and colleagues (2004). It is described in Table 4.8-1. Each subject was given the same menu regardless of body size or daily physical activity. This approach was intended to keep caloric and salt constant across all subjects during each test and avoid addressing daily variation in subjects' weights. It was supported by the fact that none of the subjects were exercising during the experiment period.

**Table 4.8-1: Menu**

| Meal | Item | Applicable Tests |
|---|---|---|
| Breakfast | • 2 pc multigrain toast with 1 tbsp jam<br>• 5 ml/kg water<br>• (or breakfast chosen by subject) | • To be consumed at home by the subject prior to a 4h or 28h test<br>• Provided to subject during 28h NFL/FL HDBR tests |
| Lunch | • Turkey sandwich (2 pcs multigrain bread, mayonnaise, 2 turkey slices, tomato slice, lettuce) | • All tests |
| Afternoon Snack | • Harvest Crunch granola bar<br>• Orange Juice | • 28h NFL/FL HDBR |
| Dinner | • Spaghetti with meat balls<br>• Yoghurt (fruit bottom)<br>• 500 ml Gatorade<br>• 2 g NaCl | • 28h NFL/FL HDBR |
| Evening Snack | • Apple<br>• Orange Juice | • 28h NFL/FL HDBR |

It should be noted that the breakfast described in Table 4.8-1 is the suggested breakfast, however if subjects consumed a different breakfast in the morning of their first test, all subsequent breakfasts throughout testing were based on their first breakfast.

A general observation was that subjects had an appetite during the first hours of the 28h bed-rest sessions, but their appetite diminished by dinnertime on the first day or breakfast on the second day of testing. Occasionally it was a struggle for subjects to finish dinner due to lack of appetite. No subjects complained that they wanted more food, and all subjects finished their meals.

Water intake during the HDBR or seated portion of testing was controlled between hour 0 - hour 4 (4h and 28h tests) and hour 24 - hour 28 (28h tests). This was to ensure subjects remained hydrated, but only ingested the water prescribed by the fluid loading protocol during the fluid loading period, and also to maintain consistency in fluid intake during these time periods across each of the subjects' tests. Subjects' water intake during the first completed test was ad libitum between hour 0 - hour 1.5, and hour 24 - hour 25.5 (no water was ingested between 1.5 - 4 hours or 25.5 - 28 hours except for the prescribed fluid load). During subsequent tests the subjects were allowed to consume the amount of water consumed in their first session ± 25%. During 28h tests, subjects could consume water freely between hour 4 and hour 24, in addition to the fluid prescribed by meal, and morning and evening hydration. Each evening and morning (including the night prior to testing and the night

spend in the lab), subjects consumed 5 ml/kg water in an effort to maintain hydration.

## 4.9 MEASURED VARIABLES

Table 4.9-1 below summarizes the raw data and that will be collected during testing its associated hardware.

**Table 4.9-1: Measured Variables**

| Measured Variable | Method |
|---|---|
| Basic Cardiovascular Indicators | |
| ECG | Colin |
| Blood pressure (SBP, DBP, MAP, PP) | Finometer |
| Manual blood pressure | Sphygmomoneter |
| Vascular Data | |
| Cardiac output | Finometer |
| Aortic flow velocity | Doppler multigon (2 MHz), peak flow velocity |
| Brachial flow velocity | Doppler multigon (4 MHz), mean flow velocity |
| Splanchnic impedance | Impedance plethysmography |
| Central venous pressure | Pressure transducer connected via water to catheter in antecubical vein; digital signal collected via Colin |
| Lower body negative pressure | Portable manometer connected to LBNP internal volume |
| Brachial diameter | Ultrasound (4 MHz probe; 2-D and M-mode) |
| Aortic diameter | Echocardiography (2 MHz probe; 2-D Parasternal Long Axis of Left Ventricle) |
| Left ventricular function (ejection Fraction, stroke volume) | Echocardiography (2 MHz probe; M-mode view of Parasternal Long Axis of Left Ventricle) |
| Portal diameter and flow velocity | Ultrasound (2 MHz probe; 2-D and Doppler) |
| Inferior vena cava diameter | Ultrasound (2 MHz probe; 2-D) |
| Blood and Plasma Volume Measurement | |
| Blood volume measurement | Carbon monoxide rebreathe and hematocrit measurements |
| Urine volume | Urine collection (ml) in graduated bottle and weight measurement |
| Water intake | Fluid intake measurement (ml) in graduated bottle |

The LBNP test set-up is illustrated in Figures 4.9-1 and 4.9-2.

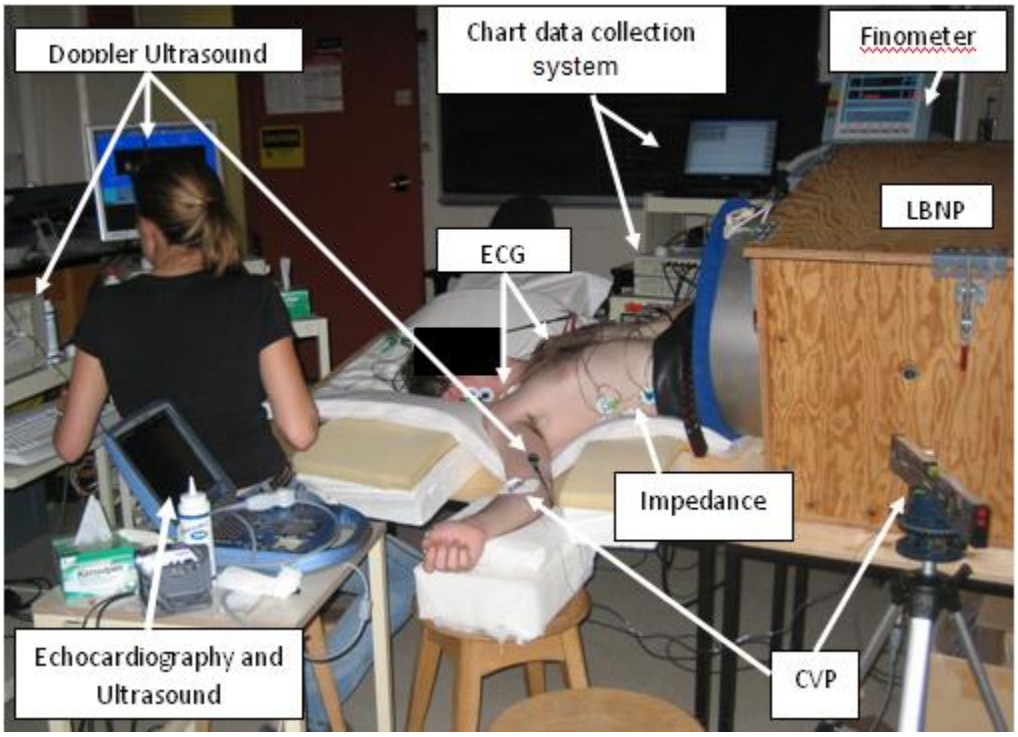


**Figure 4.9-1: LBNP Test Setup View 1**

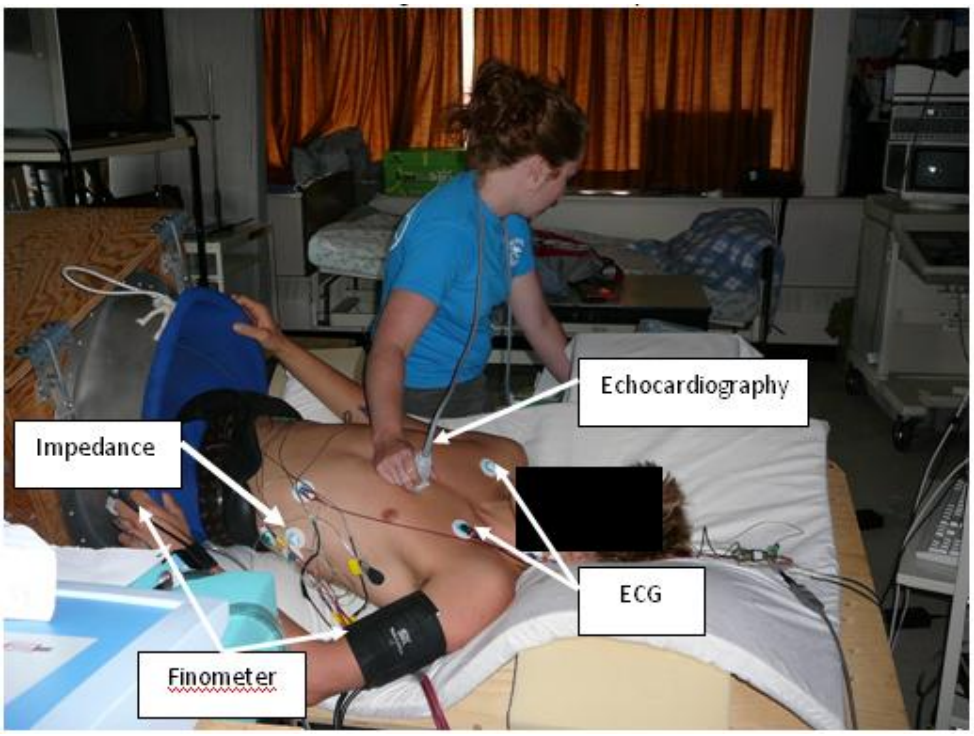


**Figure 4.9-2: LBNP Test Setup View 2**

### 4.9.1 BLOOD PRESSURE AND HEART RATE

Heart rate was continuously collected via the Colin using a 3 lead ECG. Electrodes were placed on the left and right upper chest below the collarbone with a grounding lead on the lower left abdomen, on the rib cage. An excellent ECG signal was required as each "R" peak was identified in order to analyze data on a beat-by-beat basis.

The Finometer provided a continuous arterial blood pressure signal and an estimation of cardiac output (Q). MAP, SBP, DBP, and PP were determined from the blood pressure signal. The Finometer calibrated intermittently with "Physiocal", which interrupted the continuous blood pressure data. Physiocal was turned off during the 0 mmHg, -20 mmHg, and -40 mmHg segments of 28 hour tests to provide continuous data sets for spectral analysis of these data (which were used to analyze arterial baroreflex sensitivity as described in Section 4.9.9). A manual blood pressure measurement using a sphygmanometer was performed in the supine position before testing commenced as an additional Finometer calibration.

The Finometer also provided a visual indication of heart rate and blood pressure trends over time and was used to monitor the subject's condition during the LBNP test and be aware if pre-syncopal symptoms were developing.

### 4.9.2 AORTIC AND BRACHIAL BLOOD FLOW

Aortic blood flow data were collected to measure stroke volume and cardiac output at each level of LBNP during pre and post bed-rest testing. The 2 MHz probe was used to measure peak blood flow velocity from the ascending aorta. Aortic blood flow velocity was used in the calculations of stroke volume and cardiac output as described by the following equations:

$$StrokeVolume = AorticMeanFlowVelocity * \pi * \left(\frac{Diameter}{2}\right)^2$$

$$CardiacOuput = StrokeVoume * HR$$

Aortic blood flow velocity data were collected during each level as described in Figure 4.5-1 to prevent interference with ultrasound imaging. Data was collected after a minute of exposure to each level of LBNP stress to ensure the body had fully adapted. Stroke volume and cardiac output measurements were divided by the subject's body surface area (BSA) to create a normalized variable for statistical analysis, as done by Waters and colleagues (2005).

$$BSA = 0.007184 * (weight)^{0.425} * (height)^{0.725}$$

Doppler ultrasound was used to obtain measurements of brachial blood flow velocity. The 4MHz probe was used to measure mean blood flow velocity through the brachial artery. Data were collected throughout each level of LBNP testing except when blood was drawn, as described in Figure 4.5-1. Room temperature was maintained around 21°C to control for skin blood flow, which affected brachial blood flow measurements. Brachial blood flow was calculated

using brachial blood flow velocity with the equation on the following page, with the cos45° factor correcting for probe angle.

$$BrachialBloodFlow = \left[\cos(45) * BrachialMeanFlowVelocity\right] * \pi * \left(\frac{Diameter}{2}\right)^2 * HR$$

Brachial blood flow data was divided by the subject's arm volume to normalize the data for statistical comparison. Arm volume was measured by the water displaced by the arm between the subject's elbow and wrist (water displaced by whole forearm minus water displaced by hand).

### 4.9.3 CENTRAL VENOUS PRESSURE

Central venous pressure provided an indication of blood return to the heart and was used to explain reductions in stroke volume and cardiac output, as well as changes in compliance and cardiopulmonary baroreflex response with bed-rest (Fischer et al 2007, Waters et al 2005, Vernikos and Convertino 1994). During LBNP testing, a pressure transducer (TranStar 152 cm Single Monitoring Kit) was connected to the catheter inserted in the antecubital vein via a continuous column of water. The pressure transducer was mounted on a laser level to place the transducer at the level of the heart, which provided a continuous indication of venous pressure at heart level. CVP data was continuously collected throughout LBNP testing except during blood draws at the during the final 1.5 minutes of 0 mmHg, -20mmHg, and -40 mmHg LBNP levels, as described in Figure 4.5-1. A visual description of the CVP apparatus can be sound in Figures 4.9-1 and 4.9-2.

### 4.9.4 ECHOCARDIOGRAPHY

Echocardiography was used to study changes in cardiac performance with exposure to bed-rest and orthostasis. Data was collected using a 2 MHz ultrasound probe at 0 and -40 mmHg pre and post bed-rest. The 2-D parasternal long axis view of the left ventricle was used to measure the diameter of the aorta for calculation of cardiac output using aortic flow velocity. M-mode of the parasternal long axis was used to calculate ejection fraction. Ejection fraction was defined as the percentage of blood volume pumped out of the left ventricle per beat. Left ventricular end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated from left ventricular end-systolic and end-diastolic diameters and used to determine ejection fraction using the following equation:

$$EjectionFraction = \frac{EDV - ESV}{EDV} * 100\%$$

The M-mode view of the parasternal long axis was also used to calculate fractional shortening, which describes the ratio of left ventricular diastolic diameter to left ventricular systolic diameter. A decrease in fractional shortening usually precedes a reduction in ejection fraction., and therefore fractional shortening can provide additional information on changing left ventricular performance. Fractional shortening was calculated by the following equation using end-diastolic diameter (EDD) and end-systolic diameter (ESD).

$$FractionalShortening = \frac{EDD - ESD}{EDD} * 100\%$$

During echocardiography, the table was tilted so that the subject's right shoulder was elevated, shifting the lungs to obtain a clear view of the heart. In order to reduce stress on the subject and to change the subject's position, the -40 mmHg measurement was taken after a brief recovery period at 0 mmHg, as described in Figure 4.5-1. The measurements were made after a minute of re-exposure to -40mmHg to ensure adaptation to the level of orthostatic stress. Examples of 2-D and M-mode views of the parasternal long axis of the left ventricle are available in Figures 4.9-3 and 4.9-4.

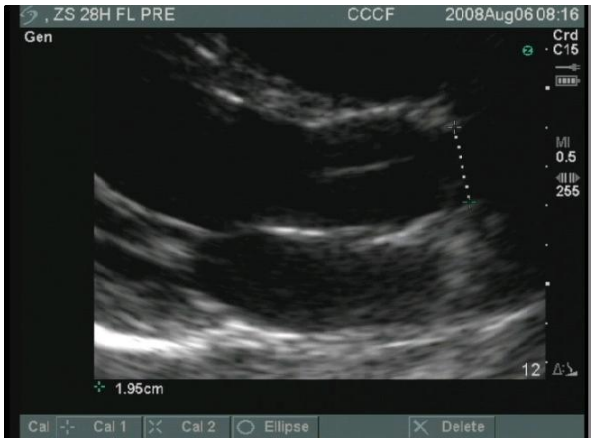


**Figure 4.9-3: 2-D Echocardiography Image of Aortic Diameter**

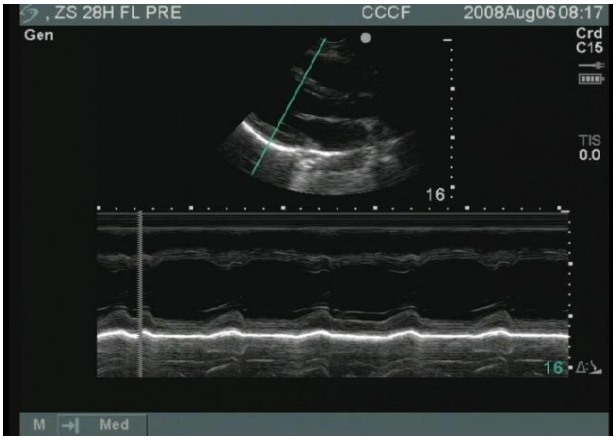


**Figure 4.9-4: M-Mode Echocardiography to Measure Ejection Fraction and Fractional Shortening**

### 4.9.5 PORTAL VEIN, IVC AND BRACHIAL ULTRASOUND IMAGING

Portal diameter and flow were used to examine splanchnic resistance and systemic blood distribution. At each level of LBNP ultrasound images of portal vein diameter and portal vein Doppler flow velocity were obtained using the 2 MHz probe to image between the ribs from a right lateral view. Blood flow through the portal vein was calculated from diameter imagery and Doppler flow velocity by the following equation:

$$PortalVein\,Flow = PortalFlow\,Velocity * \pi\left(\frac{PortalDiameter}{2}\right)^2 * HR$$

Portal vein flow velocity was measured at the end of exhalation for consistency due to tissue movement. Subjects had not eaten for 3 hours prior to imaging in order to minimize the effects of digestion. Figure 4.9-5 provides an example of portal vein imaging.

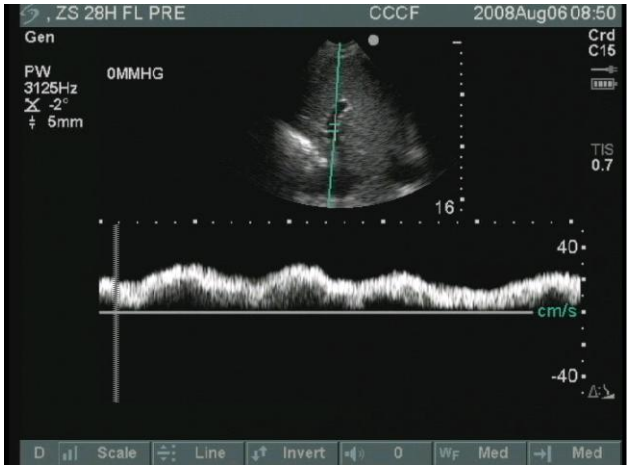


**Figure 4.9-5: M-mode Ultrasound of Portal Vein Flow Velocity**

Inferior vena cava diameter (IVC) measurements provided an indication of venous return to the heart. Venous return is known to reduce with increasing

LBNP and bed-rest, and reductions in IVC diameter have provided evidence that supports direct right atrial pressure data. IVC diameters were obtained using the 2 MHz ultrasound probe with a right lateral view through the ribcage. An illustration is provided in Figure 4.9-6 below. All diameter measurements were taken during systole.

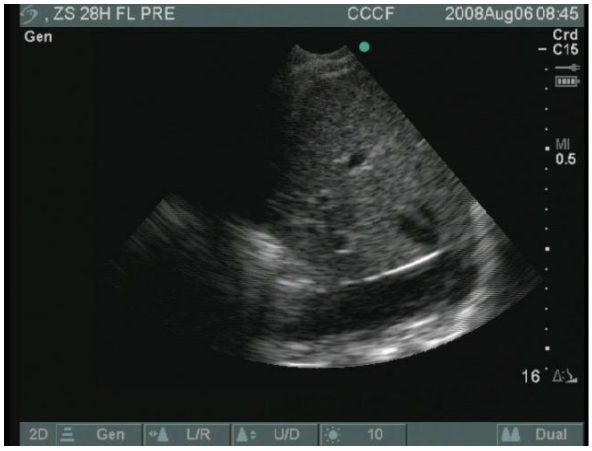


**Figure 4.9-6: Ultrasound Image of Inferior Vena Cava Diameter**

Brachial diameter was used in brachial blood flow calculations as described in Section 4.9.2. Diastolic brachial diameter was measured at 0 mmHg before commencement of the LBNP protocol using the 4 MHz probe. The probe was placed above the elbow of subject's right arm, in the same location where brachial blood flow velocity was measured during LBNP testing. Due to technical limitations, brachial diameter was not measured during the LBNP testing. An example of brachial imaging is provided in Figure 4.9-7.

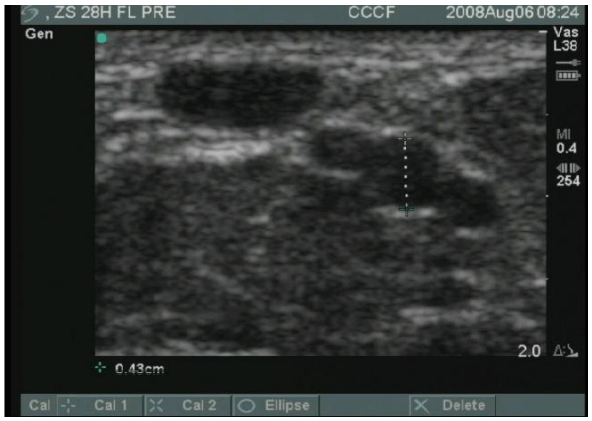


**Figure 4.9-7: Ultrasound Image of Brachial Diameter**

### 4.9.6 TOTAL PERIPHERAL RESISTANCE

Total peripheral resistance (TPR) was estimated using cardiac output, mean arterial pressure, and central venous pressure, using the following equation below. Individual subject's data was normalized using their body surface area, using the equation described in Section 4.9.2.

$$TPR = \frac{MAP - CVP}{Q}$$

### 4.9.7 ARTERIAL AND VENOUS COMPLIANCE

Arterial and venous compliance were measured during LBNP testing. Compliance was defined as the ratio of changes in volume to changes in pressure. Venous compliance was measured as the ratio between the overall change in IVC diameter to the overall change in CVP measured between 0 and -40 mmHg. An increase in this ratio reflected an increased venous compliance. Arterial compliance was calculated as the ratio of the overall change in stroke volume to the overall change in pulse pressure between 0 and -40 mmHg. An increase in this ratio reflected an increase in arterial compliance.

### 4.9.8 SPLANCHNIC IMPEDANCE

Splanchnic impedance provided an indication of systemic fluid shifts occurring during application of lower body negative pressure, and was investigated by measuring the changes in resistance of the splanchnic segment. An increase in R was expected with orthostasis and LBNP since these conditions cause blood to move away from the splanchnic cavity towards the feet (Taneja 2007). Electrodes were placed between the xyphoid process and iliac crest to isolate regional blood volume shifts in the splanchnic compartment. Splanchnic impedance was measured during ultrasound imaging of the portal vein and inferior vena cava since these measurements were collectively used to describe fluid shifts. This is reflected in the timeline available in Figure 4.5-1.

### 4.9.9 ARTERIAL BAROREFLEX

The closed-loop arterial baroreflex regulation of blood pressure was investigated by transfer function analysis of continuous beat-by-beat R-R interval and systolic blood pressure data (SBP). Three- minute data sets were collected during 0, -20, and -40 mmHg levels of pre and post LBNP tests. Transfer function analysis yielded mean gain, phase, and coherence values of the SBP and R-R interval signals in the low (0.05-0.15 Hz) and high frequency ranges (0.15-0.25 Hz) (Iwasaki et al 2000). The low frequency range provided insight into sympathovagal balance, whereas the high frequency range studied cardiac vagal modulation of blood pressure. The transfer function gain reflected changes in R-R interval in response to changes in SBP mediated by baroreflex function. Phase reflected the response time between spontaneous fluctuations

in SBP and changes in R-R interval control (Iwasaki et al 2000). Coherence indicated the degree of linearity between the two signals and therefore provided an indication of the merit of the gain and phase results; in order for transfer function analysis to be relevant the coherence was required to be at least 0.5 on a scale between zero and one. This method was noninvasive and could be applied repeatedly within a short period of time. The method is highly correlated with traditional phenylephrine method of investigating baroreflex sensitivity (Robbe et al 1987). These data were divided by the total spectral power to provide a normalized value for statistical purposes (Montano et al 1994, Pagani et al 1986).

The primary limitation of this method was the short time period available during LBNP testing for collection of R-R Interval and SBP signals, since longer data sets provide a better indication of baroreflex function. In order to increase the accuracy of transfer function analysis and maximize continuous dataset length, the Finometer's "Physiocal" was turned off during the data collection period (between 1:30 - 4:30 minutes of the 5 minute time period at 0, -20, and -40 mmHg). Physiocal was on during the first 1:30 minutes and final 30 seconds of the LBNP level to ensure accurate cardiac output data, which was important for another project that used the same data collection. If data points were missing during the last 30 seconds of each level (when Physiocal was turned on), the missing data points were estimated by linear interpolation from surrounding data points in order to have a continuous dataset.

### 4.9.10 CARDIOPULMONARY BAROREFLEX

The cardiopulmonary baroreflex was examined in two ways. One method analyzed the relationship of forearm vascular resistance (FVR) versus central venous pressure (CVP). FVR was calculated as mean arterial pressure divided by brachial blood flow. Vernikos and Convertino (1994) showed that an attenuated cardiopulmonary baroreflex response was reflected by an increased slope of the FVR-CVP response. Changes in cardiopulmonary baroreflex response were also examined by changes in supine brachial diameter, brachial blood flow velocity and brachial blood flow with orthostatic stress. A significant difference in these variables in the supine position after bed-rest indicated resetting of the cardiopulmonary baroreflex. A change in the characteristic response curve of these variables with increasing orthostatic stress following bed-rest illustrated a change in cardiopulmonary baroreflex sensitivity due to bed-rest exposure.

### 4.9.11 TOTAL BLOOD VOLUME AND PLASMA VOLUME

A carbon monoxide (CO) rebreathe was performed at the beginning of each test to determine each subject's baseline total blood volume and plasma volume, in accordance with the methodology described by Burge and Skinner (1995). This method used CO to estimate total blood volume due to hemoglobin's strong affinity for the molecule. After a small dosage of CO was administered, a blood sample was analyzed to estimate the ratio of CO-bound hemoglobin, total hemoglobin, and hematocrit. These values were used to calculate total blood and plasma volume. Blood and plasma volume calculations depended on

pressure and temperature, which were recorded at commencement of the CO re-breathe protocol. The CO-rebreathe method shows a 99% correlation to plasma volumes measured using 125I-labeled albumin (Burge and Skinner 1995).

The protocol began with the subject breathing pure oxygen for ten minutes on an open loop system to expel nitrogen from their blood. The subject was then switched to a closed loop system where the exhaled oxygen was recirculated. After the subject became accustomed to the increased resistance of breathing on the closed loop system, a small dose (~20 ml) of CO was inserted into the closed loop system. After 5 minutes of quiet breathing, which ensured maximal CO-Hb binding, a small blood sample was taken. A second blood sample was taken 5 minutes after a test dosage of ~70 ml administered. CO dosages varied slightly according to body size, with greater doses administered to larger individuals (i.e. 60 versus 80 ml). Carbon dioxide absorbers protected the subject from inhaling high concentrations of $CO_2$. During the rebreathe, the subject was only able to breathe from the system via a mouthpiece and biological filter. Nose clips and taping of the mouthpiece to the mouth prevented intake of outside air. The subject's oxygen supply was constantly monitored. If the subject needed to abort the test, the nose clips were immediately removed and the test finished. There was one instance where a test was aborted prematurely due to subject discomfort.

Monitoring of hematocrit throughout the protocol allowed for changes of blood and plasma volume to be easily tracked without the need for additional CO rebreathe tests. Blood was collected from a catheter inserted in the

antecubital vein of the right arm. The catheter was inserted prior to the CO rebreathe test and removed following completion of the post-LBNP session. The catheter was also removed during the overnight portion of 28h testing for subject comfort. The timing of hematocrit measurements are indicated as arrows in Figures 4.3-1 and 4.4-1. Approximately 1 ml of blood was taken per hematocrit sample (often as part of a larger sample for hormones and catecholamines for another study). Following sampling, the catheter and stop cock were flushed with saline to prevent clotting. Hematocrit was measured using the microcapillary centrifuge method with each sample examined in triplicate. After averaging, the hematocrit value for each time point was multiplied by 0.91 ("$F_{cell}$ ratio") to correct vein hematocrit to whole body hematocrit (Burge and Skinner 1995).

### 4.9.12 URINE COLLECTION

Urine output was monitored to support plasma volume data throughout bed-rest in conjunction with fluid intake. Urine was collected throughout the testing period, starting with the first urination after wake-up (before the subject arrived in the laboratory) and ending with the final urine collection following the post-LBNP test. During HDBR tests urine was collected in the head-down position. Collected urine was weighed to monitor urine output throughout the testing period. Total urine output was calculated to assess differences in fluid balance (water excretion to the bladder) between 28h NFL HDBR and 28h FL HDBR.

### 4.9.13 WATER INTAKE

As described in Section 4.8.3, water intake was controlled throughout the testing period. These data were used to support plasma volume data throughout bed-rest in conjunction with urine collection. Water intake included morning and evening hydrations (5 ml/kg), regulated water consumption between Hour 0 - Hour 4 and Hour 24 - Hour 28 (determined from first test ± 25%), and ad libitum water intake during Hour 4 - Hour 24. Water was accurately measured to the prescribed amount in a graduated water bottle before being given to the subject and noted in the test data sheet. If the subject was still thirsty, they were given up to 25% more water, and their additional consumption was noted. As a general observation, subjects often consumed the exact amount of water prescribed to them, but their thirst reflex varied between tests. Often subjects would either force themselves to consume the prescribed amount or remain thirsty, despite a consistent activity level and food intake across all tests.

### 4.9.14 STATISTICS

In order to study the hypotheses regarding the effects of 28h NFL HDBR on cardiovascular deconditioning, LBNP Pre and Post test data was be compared by a 2-way ANOVA (Pre/Post, LBNP), analyzed as mean ± standard error. Pre-planned comparisons (Pre vs. Post response in the supine position, Pre vs. Post response at -40 mmHg, and 0 mmHg Post vs. -40 mmHg Post responses) were performed to clarify statistical differences, which were especially relevant in the event of a significant interaction effect between bed-rest and LBNP. Significant changes were defined as having $p < 0.05$.

Hypotheses regarding the efficacy of fluid loading on preventing cardiovascular deconditioning that arises following 28h NFL HDBR were studied by comparing LBNP testing data from 28h NFL HDBR Post and 28h FL HDBR Post tests. A 2-way ANOVA (NFL Post/FL Post, LBNP) were performed with significance determined as $p<0.05$. Pre-planned comparisons (NFL Post vs. FL Post response in the supine position and NFL Post vs. FL Post response at -40 mmHg) were also performed to clarify statistical differences, which were especially relevant in the event of a significant interaction effect between bed-rest and LBNP. A 2-way ANOVA comparing 28h NFL HDBR Pre and 28h FL HDBR Pre tests were also performed (FL Pre/NFL Pre, LBNP) to ensure there were no differences in baseline conditions.

The 4-hour control models were used to isolate the effects of circadian rhythm, HDBR, and fluid loading on the 28h NFL HDBR and 28h FL HDBR tests. To isolate the effects of circadian rhythm, two statistical analyses were performed. A 2-way ANOVA comparing the Pre versus Post responses of the 4-hour S NFL test (4SNFL Pre/4SNFL Post, LBNP) examined if there were any circadian effects in the seated position. This was followed by a second 2-way ANOVA comparing the 4-hour S NFL Post and 4-hour HDBR Post responses ( 4h SNFL Post/4h HDBR Post, LBNP), which indicated if there was a circadian effect on bed-rest. If there were differences when comparing the S NFL Post and HDBR Post tests, the differences can be attributed to HDBR and not circadian rhythm. Only cardiovascular variables that were changed during 28h NFL HDBR and 28h FL HDBR were investigated by the control models.

To isolate the effects of fluid loading a single 2-way ANOVA was performed which compared the 4-hour S NFL Post and 4-hour S FL Post test responses (NFL Post/FL Post, LBNP). This test concluded whether or not the fluid loading protocol had an effect on blood volume in the absence of HDBR. Responses from the 4-hour S NFL Pre and 4-hour FL Pre tests were compared to ensure there were no differences in baseline conditions.

To isolate the effects of HDBR, a single 2-way ANOVA was performed comparing 4-hour SFL Post and 28h FL HDBR Post test responses (4/28, LBNP). This test indicated if there were any effects of bed-rest on the fluid loading protocol. Responses from the 28h FL HDBR Pre and 4-hour SFL Pre tests were compared to ensure there are no differences in baseline conditions.

# 5 RESULTS

## 5.1 EFFECT OF 28H HDBR ON CARDIOVASCULAR DECONDITIONING

This analysis investigated the hypotheses regarding the effects of exposure to 28 hours of 6° HDBR on the cardiovascular system. Specifically, it examined the degree of hypovolemia resulting from 28h NFL HDBR, as well as the hypothesized reductions in stroke volume and cardiac output, elevated TPR response, attenuated arterial and cardiopulmonary baroreflex responses, reductions in cardiac function, and reductions in splanchnic vasoconstrictor response. This analysis compared subjects' responses from Pre and Post LBNP testing. Relevant data are illustrated within the following section. Tables summarizing raw data (in the form of mean ± standard error), statistical analyses results, and sample sizes are included in Appendices A1, B and C respectively.

### 5.1.1 HYPOVOLEMIA

The 28h NFL HDBR protocol resulted in significant hypovolemia. Following 28h HDBR, total blood volume was significantly reduced by 7%, from 5641.6 ml ± 425.2 to 5246.5 ml ± 378.6 ($p<0.05$). Plasma volume fell by 11.5%, from 3431.9 ml ± 271.7 to 3036.8 ml ± 225.1 ($p<0.05$). Figure 5.1-1 shows the changes in total blood volume and plasma volume throughout the 28h HDBR period. From the graph, it can be seen that hypovolemia occurred within the early hours of bed-rest exposure. After 8 hours, total blood volume was reduced to 5027.3 ml ± 346.3, a reduction of 10.9%. Later time points (25.5, 26, 26.5,

27, and 27.5 hour time points) show that total blood and plasma volume remained diminished after 8 hours.

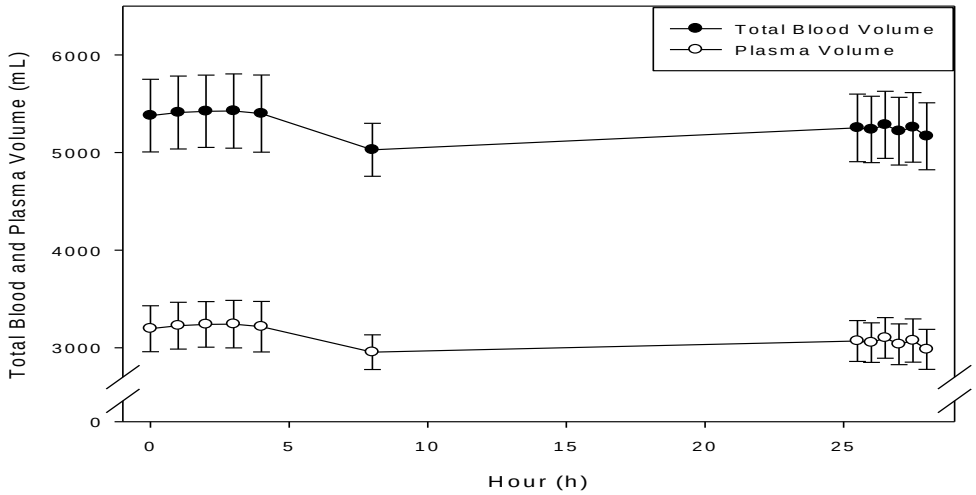


**Figure 5.1-1: Total Blood Volume and Plasma Volume Trends Throughout 28h NFL HDBR (n=8); TBV HDBR $p<0.05$, PV HDBR $p<0.05$**

Changes in hematocrit and weight also provided evidence of hypovolemia resulting from 28h NFL HDBR. Hematocrit was increased from 39.61% ± 0.74 to 42.31% ± 0.63 across all 9 subjects ($p<0.05$). Subjects' weights decreased by 1.2 kg ± 0.5 following 28h NFL HDBR, reflecting the blood volume loss following bed-rest ($p=0.04$).

### 5.1.2 BLOOD PRESSURE RESPONSE

All subjects were able to successfully complete the LBNP before and after 28h HDBR. Mean arterial pressure (MAP) was maintained throughout LBNP testing following 28h HDBR. MAP was measured as 87.6 mmHg ± 1.6 in the supine position during pre testing and there were no significant effects of orthostatic stress ($p=0.50$) or bed-rest ($p=0.95$) on MAP. Comparison of pre and post supine MAP ($p=0.87$) as well as pre and post MAP during maximal

orthostatic stress (p=0.63) indicated no changes with HDBR. However it must be noted that MAP did decrease significantly between supine and maximum orthostatic stress during post testing, illustrating that blood pressure was not perfectly maintained during orthostatic stress following bed-rest exposure. Systolic blood pressure (SBP) was also unaffected by bed-rest (p=0.88). After HDBR, both supine SBP (p=0.65) and SBP during maximum stress (p=0.54) were not different from their respective pre values. SBP was significantly reduced by increasing orthostatic stress ($p<0.05$). Diastolic blood pressure (DBP) was maintained constant between pre and post bed-rest testing (p=0.94) and with LBNP (p=0.17). Figure 5.1-2 illustrates the MAP, SBP, and DBP responses to orthostatic stress pre and post 28h HDBR.

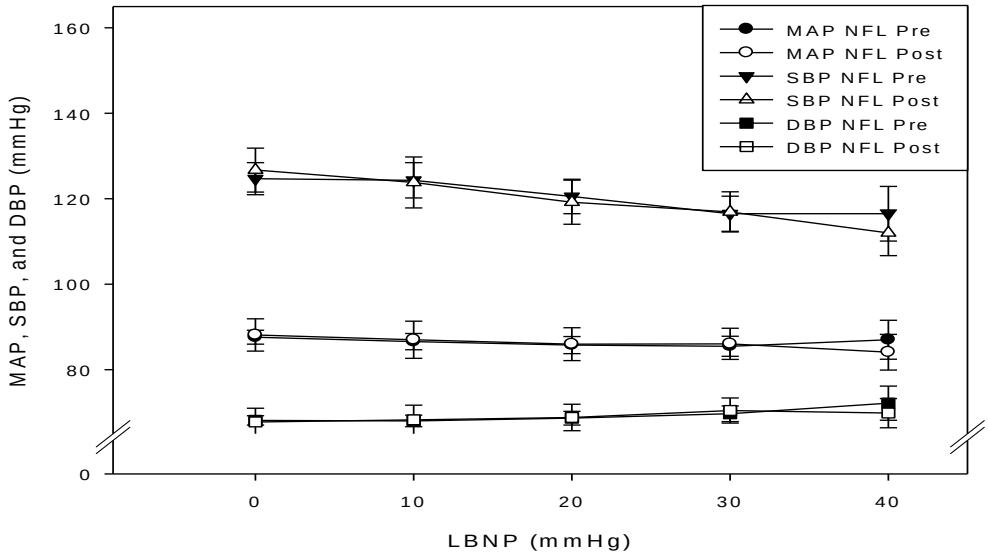


**Figure 5.1-2: MAP, SBP, and DBP Responses during LBNP Testing Pre and Post 28h NFL HDBR (n=9); MAP HDBR p=0.95, MAP LBNP p=0.50, SBP HDBR p=0.88, SBP LBNP p<0.05, DBP HDBR p=0.94, DBP LBNP p=0.17**

Pulse pressure (PP) was not altered by 28h HDBR (p=0.86), but was significantly reduced with increasing orthostatic stress ($p<0.05$). Comparison of

PP in the supine position before and after bed-rest yielded no differences (p=0.38). Similarly comparison of PP pre and post bed-rest at maximum orthostatic stress did not yield statistical differences (p=0.47). PP was significantly reduced during maximum orthostatic stress compared to the supine condition following 28h HDBR (p<0.05). The PP response is illustrated in Figure 5.1-3.

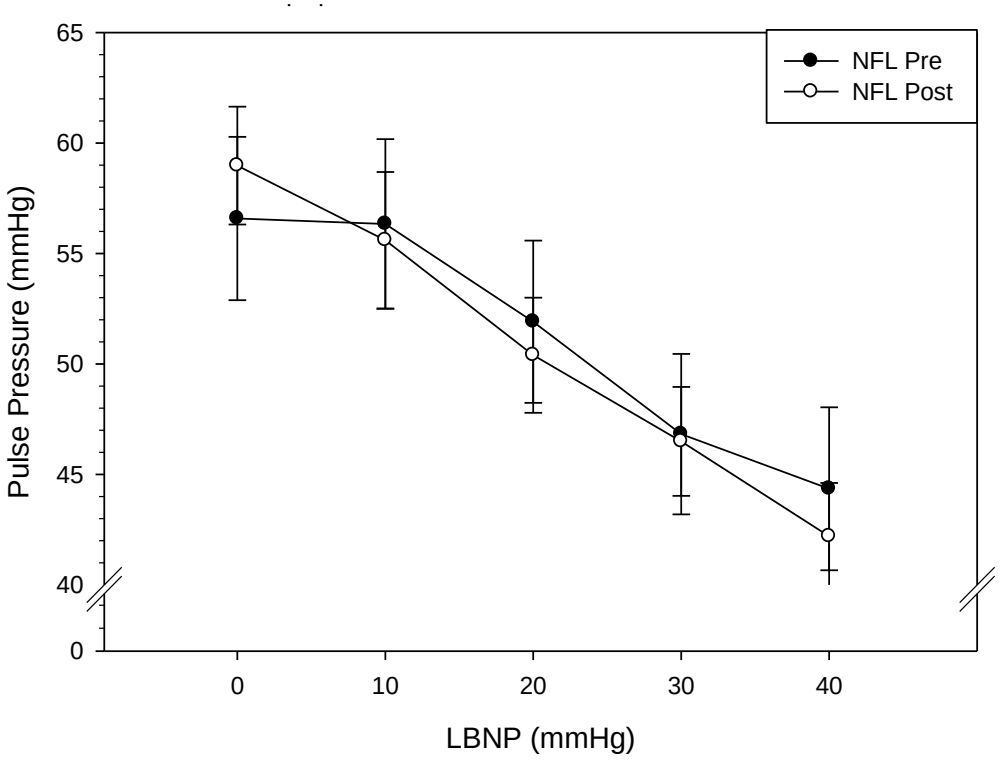


**Figure 5.1-3: PP Response during LBNP Testing Pre and Post 28h NFL HDBR (n=9); HDBR p=0.38, LBNP p<0.05, LBNP-HDBR interaction p<0.05**

### 5.1.3 HEART RATE RESPONSE

Evidence of cardiovascular deconditioning was apparent following 28h HDBR when examining heart rate response. Subjects' overall heart rate responses post-HDBR were not significantly different (p=0.22), but there was a significant interaction between bed-rest and orthostatic stress such that the elevation in heart rate post-HDBR became more apparent at higher levels of LBNP (p=0.01). The heart rate response is shown in Figure 5.1-4.

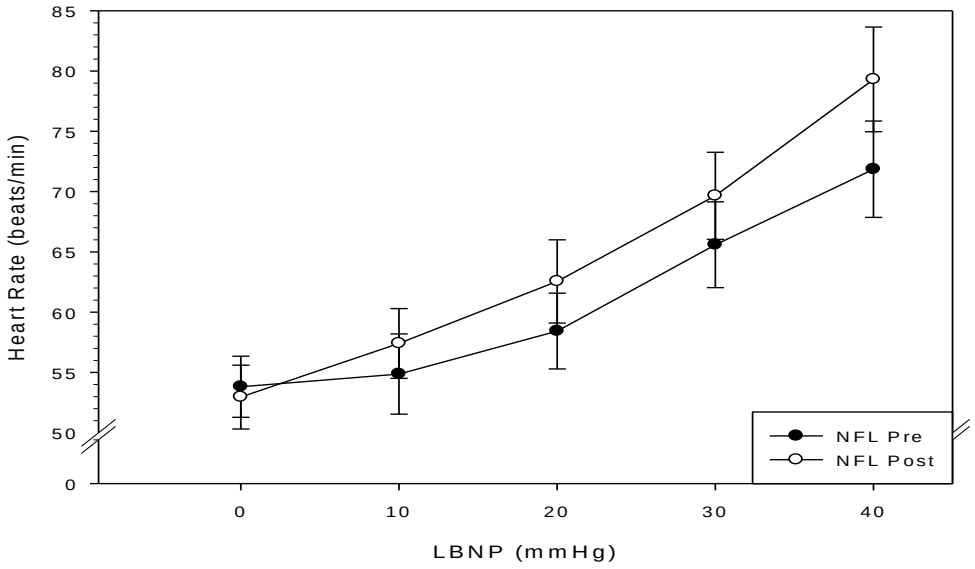


**Figure 5.1-4: Heart Rate Response during LBNP Testing Pre and Post 28h HDBR (n=9); HDBR p=0.22, LBNP p<0.05, LBNP-HDBR interaction p<0.05**

Analysis of R-R interval showed a similar trend to heart rate, with R-R interval diminished at the higher levels of orthostatic stress post-HDBR compared to pre-HDBR (p<0.05).

### 5.1.4 ARTERIAL HEMODYNAMICS

Analysis of stroke volume and cardiac output showed no cardiovascular deconditioning following exposure to 28h HDBR. The stroke volume response was unchanged with bed-rest ($p= 0.29$), and reduced with increasing orthostatic stress ($p<0.05$). Pre and post values of stroke volume are illustrated in Figure 5.1-5.

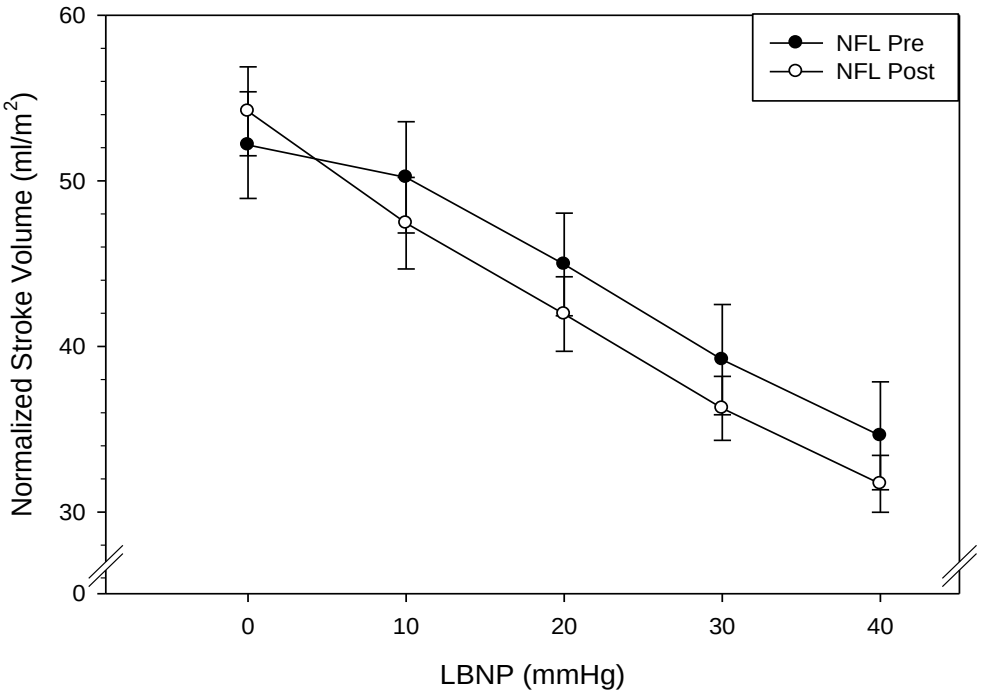


**Figure 5.1-5: Normalized Stroke Volume Response during LBNP Testing Pre and Post 28h HDBR (n=9); HDBR p=0.29, LBNP p<0.05**

The cardiac output resembled the stroke volume response. Cardiac output was not significantly affected by 28h HDBR (p=0.67), and was reduced with increasing orthostatic stress (p<0.05). The cardiac output response pre and post 28h HDBR is illustrated in Figure 5.1-6.

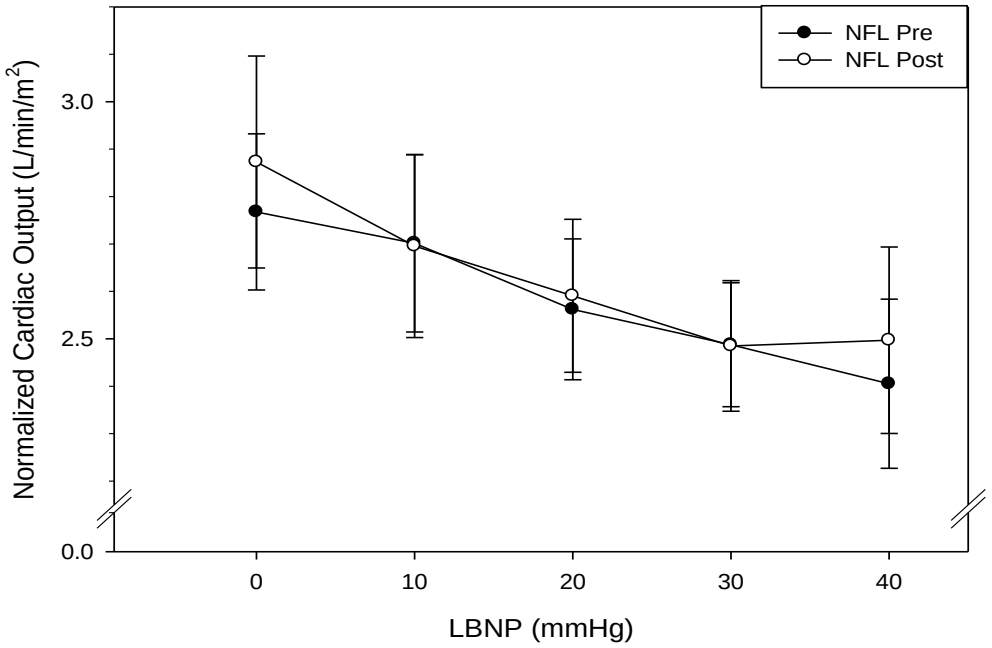


**Figure 5.1-6: Normalized Cardiac Output Response during LBNP Testing Pre and Post 28h HDBR (n=9); HDBR p=0.67, LBNP p<0.05**

The total peripheral resistance (TPR) response was maintained following bed-rest. There were no statistical differences between TPR pre and post 28h NFL HDBR responses (p=0.87). TPR increased with orthostatic stress, increasing from 29.54 mmHg/(ml/[min*$m^2$]) ± 2.00 to 35.50 mmHg/(ml/[min*$m^2$]) ± 2.84 during post bed-rest testing (p<0.05). The TPR response during orthostatic stress pre and post 28h NFL HDBR is illustrated in Figure 5.1-7.

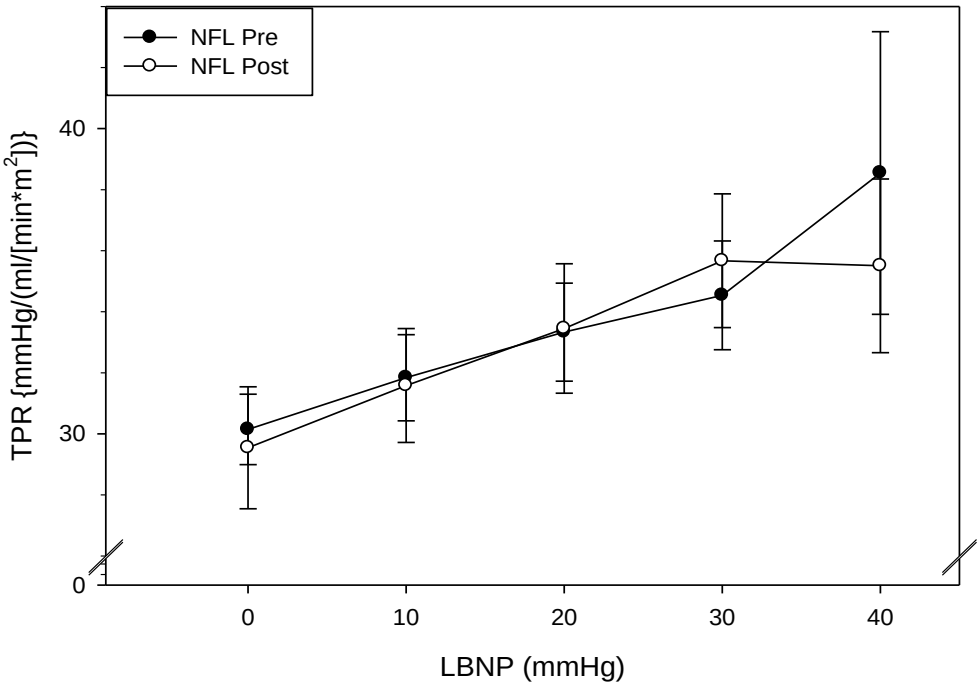


**Figure 5.1-7: Total Peripheral Resistance Response during LBNP Testing Pre and Post 28h HDBR (n=8); HDBR p=0.87, LBNP p<0.05**

Arterial compliance, calculated as normalized stroke volume divided by pulse pressure, was unchanged with 28h HDBR (p=0.24). Arterial compliance prior to 28h HDBR was 1.72 $(ml/m^2)/mmHg \pm 0.25$, which was not significantly different from the post 28h HDBR value of 1.22 $(ml/m^2)/mmHg \pm 0.18$ ($p<0.05$).

### 5.1.5 VENOUS HEMODYNAMICS

Venous hemodynamics showed evidence of cardiovascular deconditioning following exposure to 28h HDBR. Central venous pressure (CVP) was significantly reduced by bed-rest ($p=0.01$) and with orthostatic stress ($p<0.05$). CVP also exhibited an interaction between bed-rest and LBNP, as the reduction in CVP at higher levels of LBNP became more apparent post-HDBR ($p<0.05$). Figure 5.1-8 illustrates the CVP response pre and post 28h HDBR during orthostatic testing.

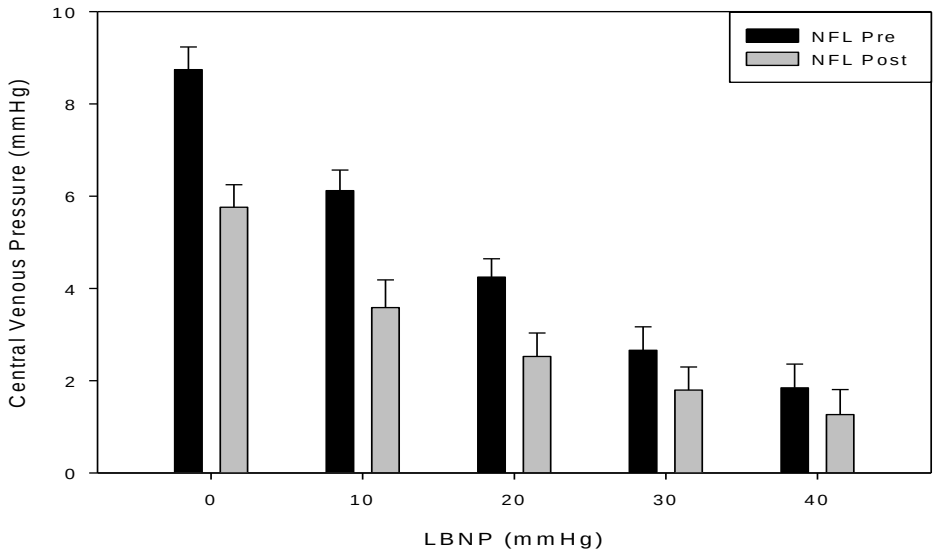


**Figure 5.1-8: Central Venous Pressure Response during LBNP Testing Pre and Post 28h HDBR (n=8); HDBR $p<0.05$, LBNP $p<0.05$, LBNP-HDBR interaction $p<0.05$)**

Unlike CVP, inferior vena cava (IVC) diameter was not significantly reduced after exposure to 28h NFL HDBR ($p=0.19$). However, IVC diameter was reduced with increasing orthostatic stress ($p<0.05$). The IVC diameter response pre and post 28h HDBR is illustrated in Figure 5.1-9.

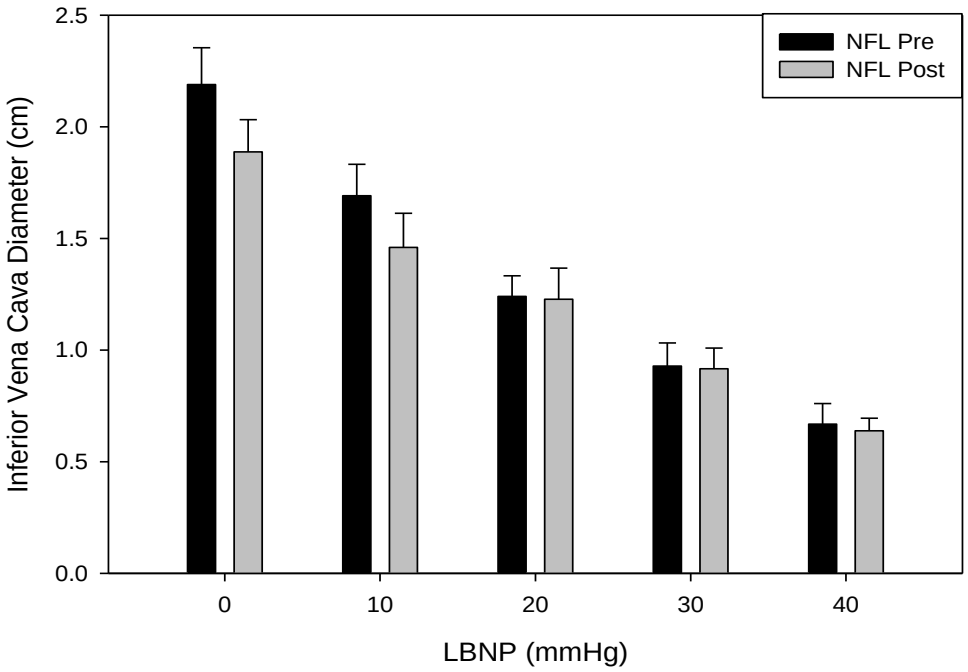


**Figure 5.1-9: Inferior Vena Cava during LBNP Testing Pre and Post 28h NFL HDBR (n=9); HDBR p=0.19, LBNP p<0.05**

Venous compliance data, calculated as delta IVC diameter divided by delta CVP (where delta values are measured as the value at 0 mmHg minus the value at -40 mmHg), did not show evidence of cardiovascular deconditioning after bed-rest. The venous compliance response during orthostatic testing pre and post 28h NFL HDBR is illustrated in Figure 5.1-10.

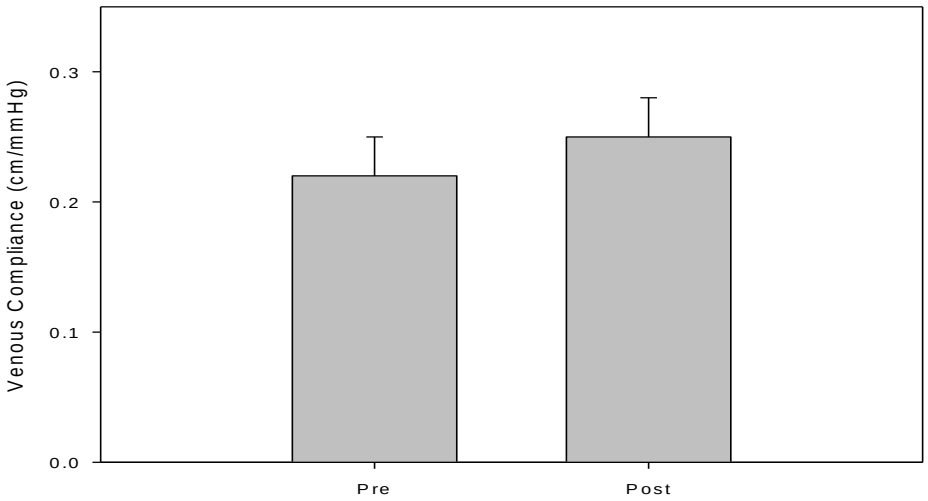


**Figure 5.1-10: Venous Compliance Response during Pre and Post 28h NFL HDBR Testing (n=6); HDBR p=0.31)**

### 5.1.6 SPLANCHNIC HEMODYNAMICS

Splanchnic hemodynamics were affected by 28h NFL HDBR. Portal vein diameter was significantly diminished with bed-rest ($p<0.05$) and orthostatic stress ($p<0.05$), and there was a significant interaction between bed-rest and orthostatic stress where the reduction in portal diameter at higher levels of LBNP was more pronounced post-HDBR ($p=0.04$). This reduction in portal vein diameter with bed-rest indicates that splanchnic vasoconstriction increased with bed-rest. Figure 5.1-11 illustrates the portal vein diameter response during LBNP testing pre and post 28h bed-rest.

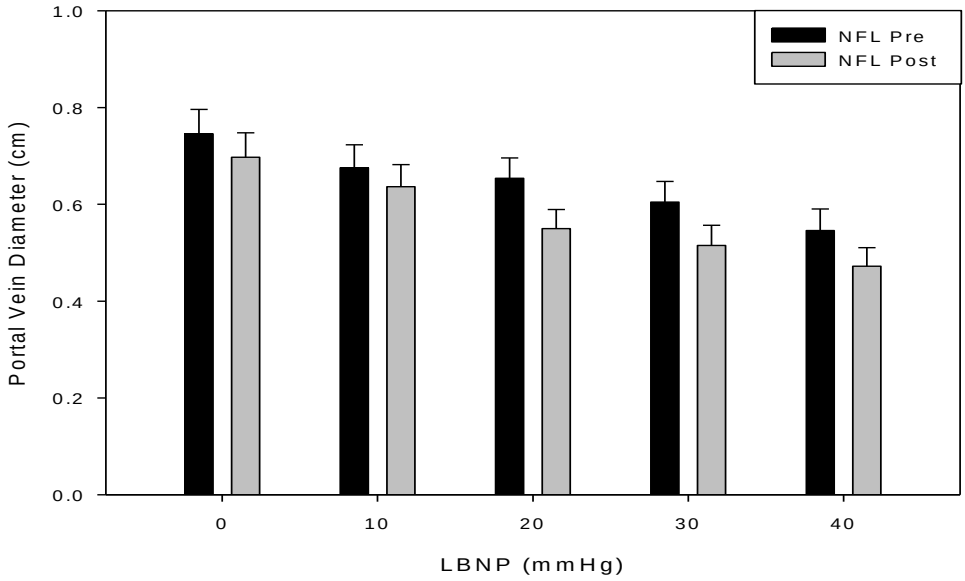


**Figure 5.1-11: Portal Vein Diameter Response Pre Response during Pre and Post 28h NFL HDBR Testing (n=9); HDBR $p<0.05$, LBNP $p<0.05$**

Portal blood flow was significantly reduced following 28h NFL HDBR ($p<0.05$), reflecting an increase in splanchnic vascular resistance after bed-rest. These data are consistent with the portal diameter data described previously, suggesting an increase in splanchnic vascular resistance after bed-rest. However, portal blood flow was not reduced with increasing orthostatic stress

(p=0.28), as would be expected given the portal vein diameter data. Although the portal blood flow results may be physiological, the unexpected results are more likely attributed to measurement error, which is fully described in the "Limitations" section of this document. Figure 5.1-12 illustrates the portal vein blood flow response during LBNP testing pre and post 28h bed-rest.

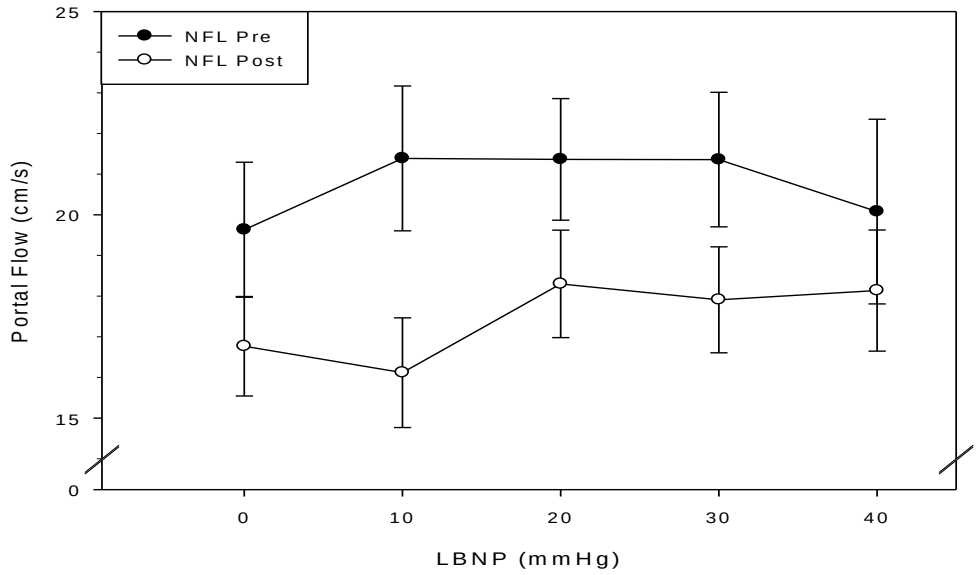


**Figure 5.1-12: Portal Vein Blood Flow Response during Pre and Post 28h NFL HDBR Testing (n=9); HDBR p<0.05, LBNP p=0.28**

Splanchnic impedance measurements indicated that there were no changes in blood volume in the splanchnic region between pre and post 28h HDBR, further evidence that splanchnic pooling did not occur following bed-rest exposure. Splanchnic impedance was unchanged following 28h HDBR (p=0.37). Splanchnic impedance was increased with increasing orthostatic stress (p<0.05), illustrating that the splanchnic vasoconstriction seen in the portal vein diameter date led to a reduction of splanchnic blood volume during

orthostasis. The splanchnic impedance response during orthostatic testing pre and post 28h NFL HDBR is illustrated in Figure 5.1-13.

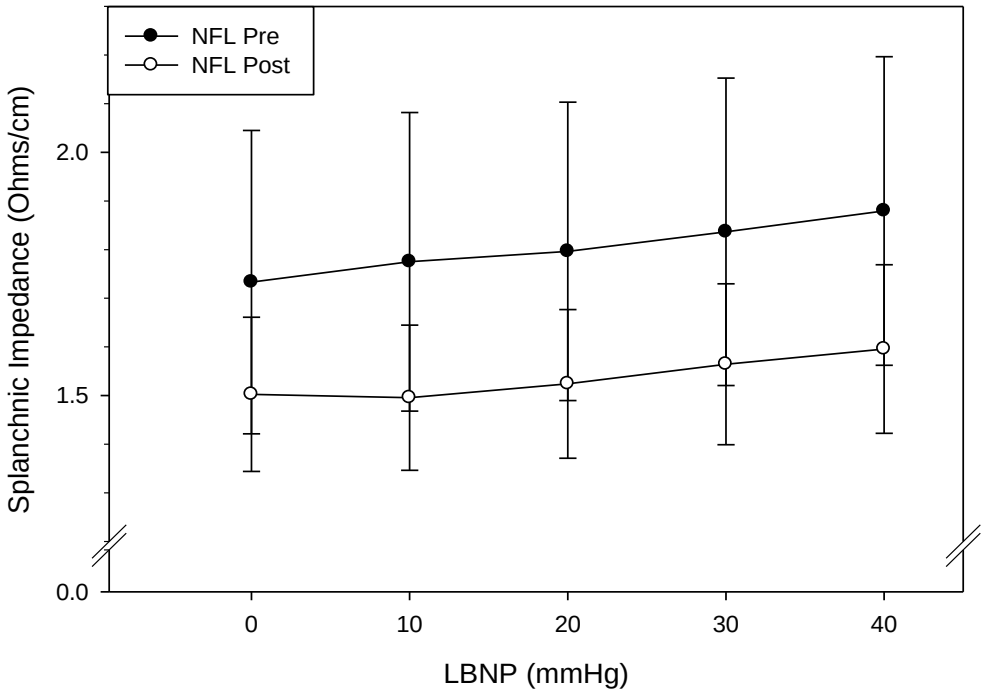


**Figure 5.1-13: Splanchnic Impedance Response Pre and Post 28h HDBR (n=8); HDBR p=0.37, LBNP p<0.05**

### 5.1.7 LEFT VENTRICULAR PERFORMANCE

Left ventricular performance was not affected by 28h HDBR. Ejection fraction was unchanged following exposure to 28h HDBR (p=0.19). Ejection fraction was significantly reduced by orthostatic challenge, falling from 79.5% ± 1.1 to 72.2% ± 1.4 during post orthostatic testing (p<0.05). The ejection fraction responses pre and post bed-rest at 0 and -40 mmHg are illustrated in Figure 5.1-14.

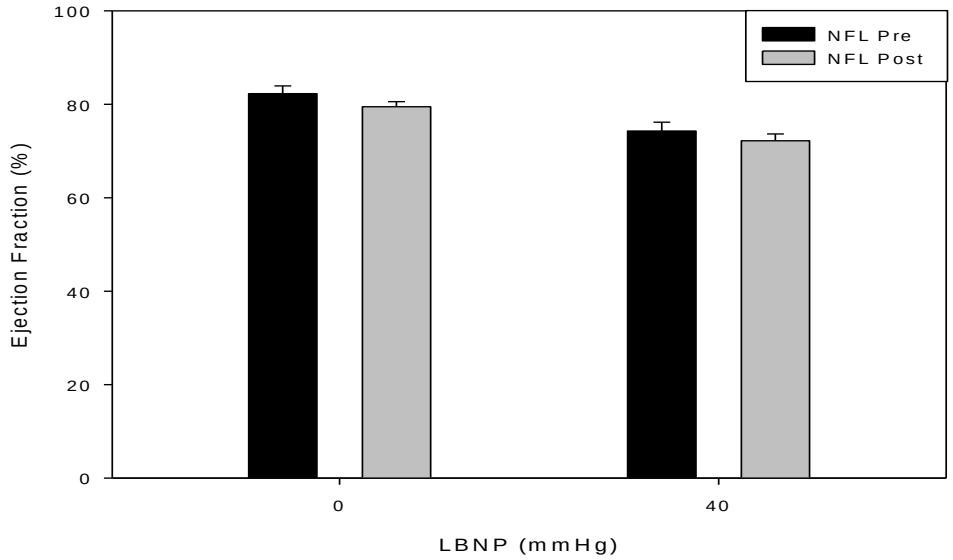


**Figure 5.1-14: Ejection Fraction Response following 28h HDBR (n=8); HDBR p=0.19, LBNP p<0.05**

Like ejection fraction, fractional shortening was also not affected by 28h HDBR (p=0.17), but was reduced during orthostatic challenge (p<0.05). Fractional shortening was reduced from 41.2% ± 1.0 to 34.9% ± 1.2 during post LBNP testing. Therefore bed-rest induced hypovolemia did not cause impaired left ventricular performance. Figure 5.1-15 illustrates the fractional shortening response pre and post bed-rest in both the supine position and during maximum orthostatic stress.

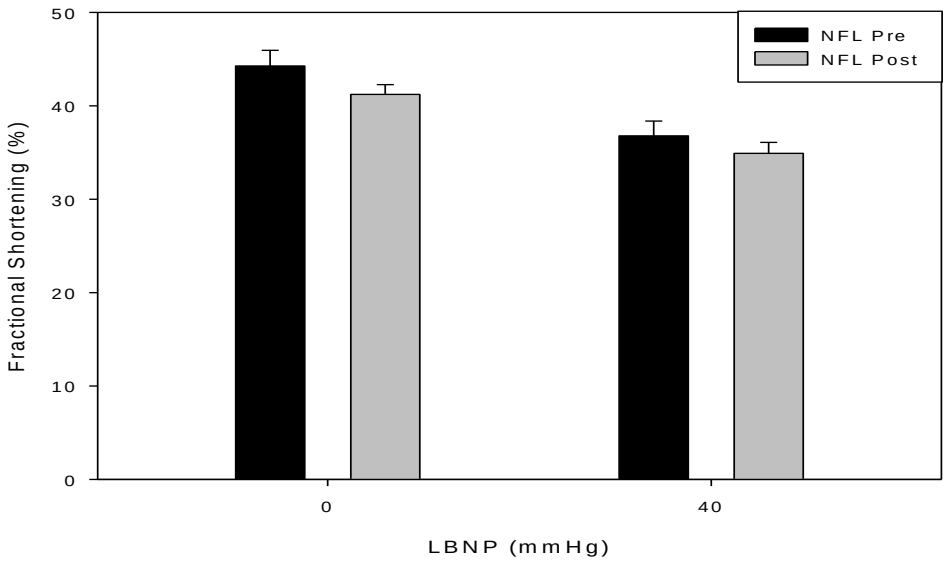


**Figure 5.1-15: Fractional Shortening Response Pre and Post 28h HDBR (n=7); HDBR p=0.17, LBNP p<0.05**

### 5.1.8 ARTERIAL BAROREFLEX

Transfer function analysis was performed on the SBP and R-R Interval data of 7 of the 9 subjects. The remaining 2 subjects had nonstationary datasets with rapid R-R Interval peaks. The software analyzed these peaks as a discontinuity in the data signal, or a nonlinearity, which is a violation of the criterion required to perform transfer function analysis, and prevented the analysis to be performed. Figure 5.1-16 illustrates an example of a "discontinuity" in a RR-Interval dataset which prevented analysis.

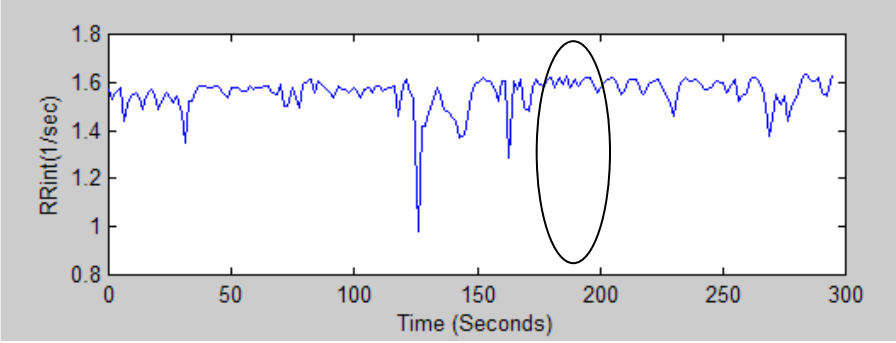


**Figure 5.1-16: Discontinuity in an Individual Subject's R-R Interval Signal**

SBP datasets were linearly interpolated where "Physiocal" had been performed by the Finometer, in order to obtain longer datasets. The noninterpolated datasets were also analyzed and compared to the interpolated datasets, with no significant difference in coherence values between the two methods of analysis. Since the noninterpolated datasets could be up to 80 data points shorter, the interpolated data were used to provide more data points for the analysis. Figures 5.1-17, 5.1-18, and 5.1-19 illustrate an individual's raw arterial SBP and RR-interval data signals sampled in the supine position, the

signals' autospectra (pxx and pyy), and transfer function analysis results of gain (pxy and txy), phase (phi) and coherence (cxy) respectively.

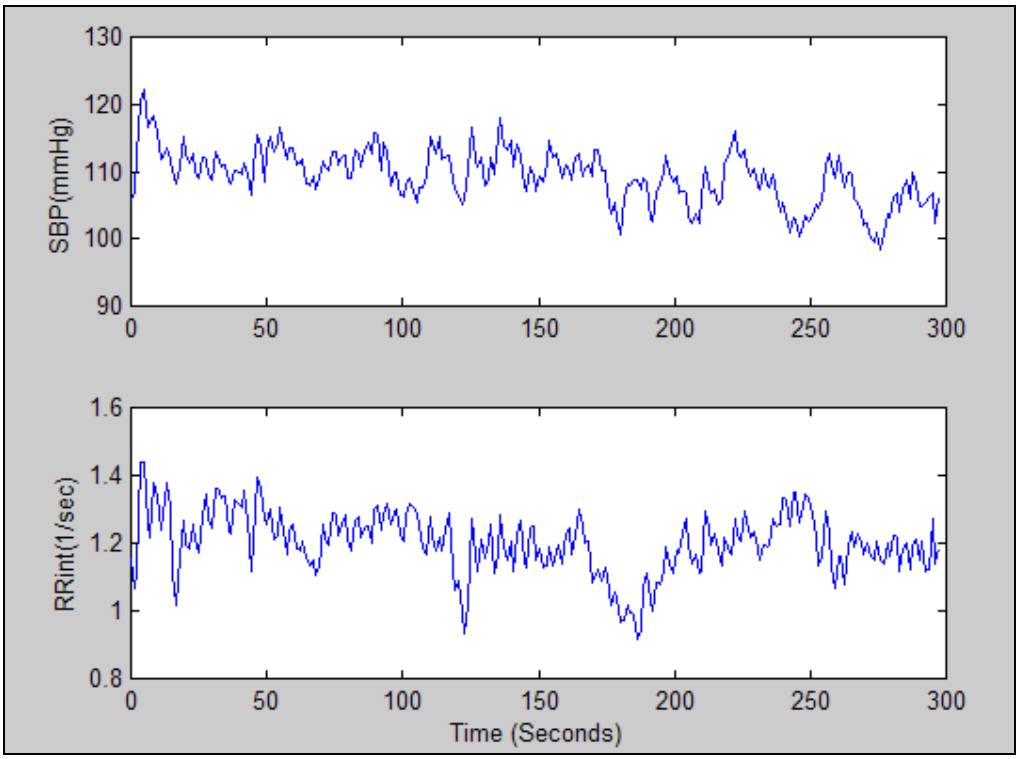


**Figure 5.1-17: SBP and R-R Interval Data for an Individual Subject**

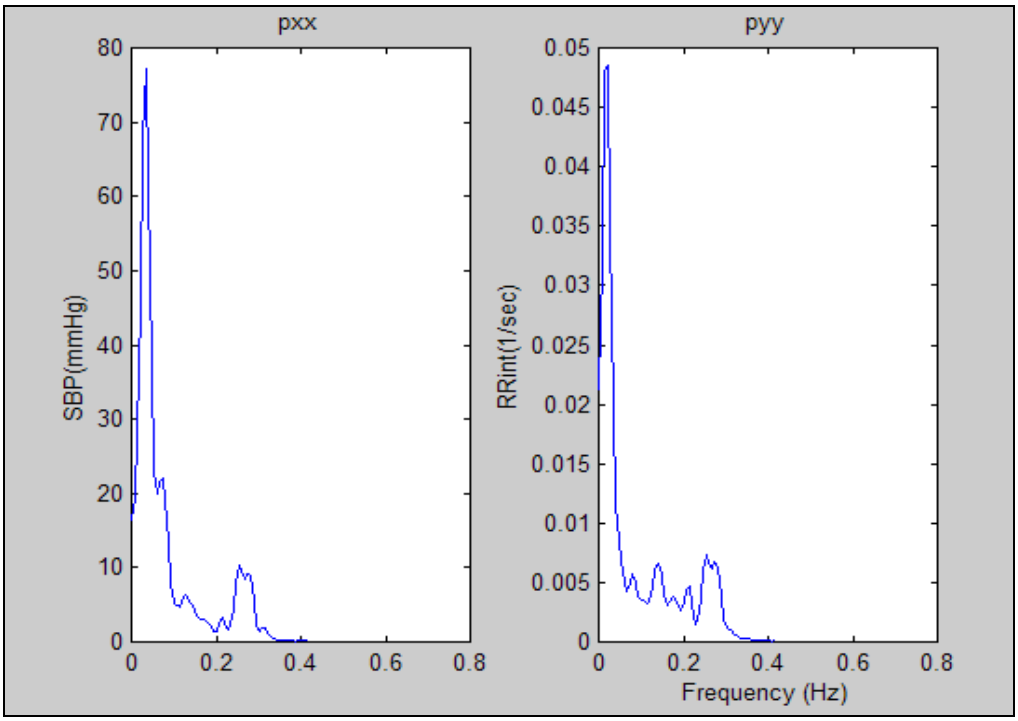


**Figure 5.1-18: Autospectra of SBP (pxx) and R-R Interval (pyy) Signals for an Individual Subject**

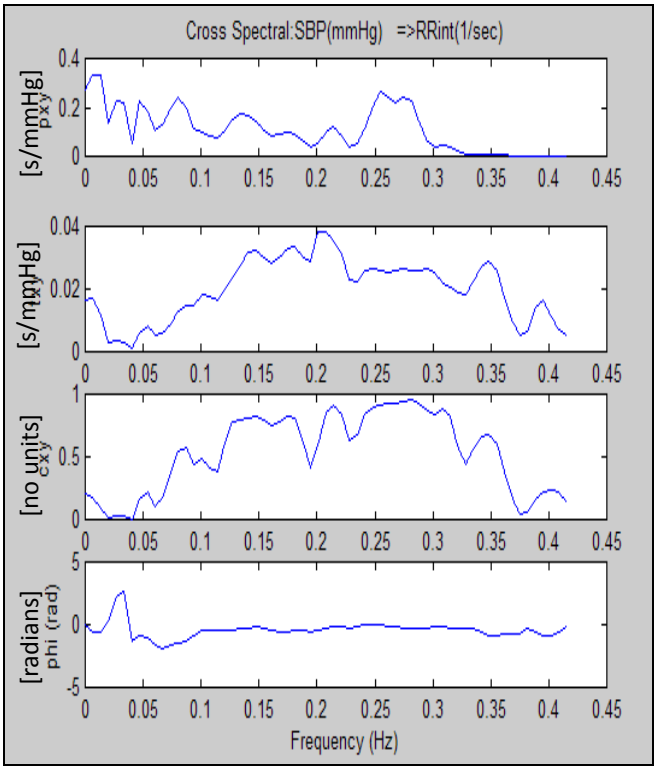


**Figure 5.1-19: Gain (pxy), Normalized Gain (txy), Coherence (cxy), and Phase (phi) Results for an Individual Subject**

Twenty-eight hour bed-rest did impact arterial baroreflex sensitivity. There were no significant differences in MF gain with 28h HDBR (p=0.88), which was determined in the frequency range from 0.05-0.15 Hz when coherence was greater than 0.5. This illustrated that the sympathovagal balance of arterial blood pressure modulation had not changed with bed-rest exposure. Similarly, there were no significant differences in HF gain following 28h HDBR (p=0.37), which was determined in the frequency range of 0.15-0.40 Hz. These data illustrate there were no changes in cardiac vagal modulation. Figures 5.1-20 and 5.1-21 illustrate the MF and HF gain responses pre and post bed-rest at 0, -20, and -40 mmHg.

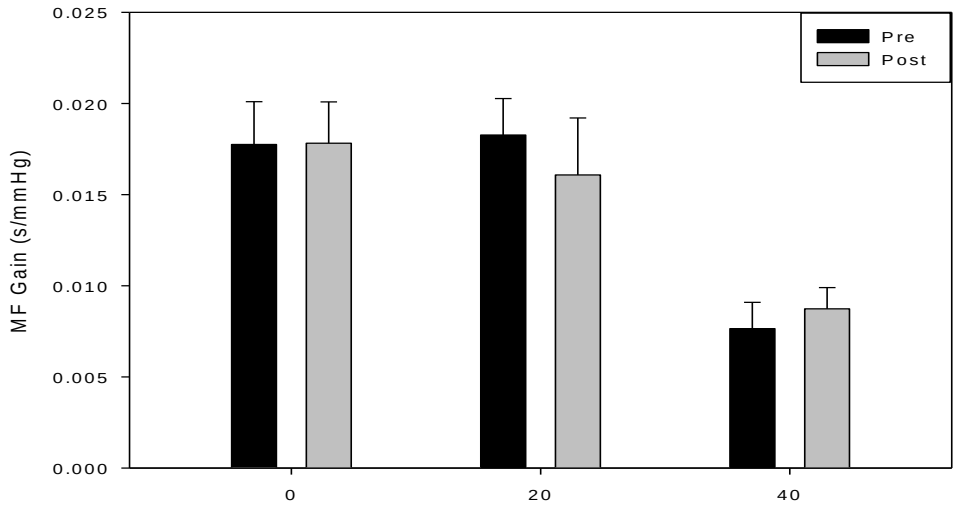


**Figure 5.1-20: Mid-Frequency Gain Response during LBNP Testing Pre and Post 28h NFL HDBR (n=7); HDBR p=0.88, LBNP p<0.05**

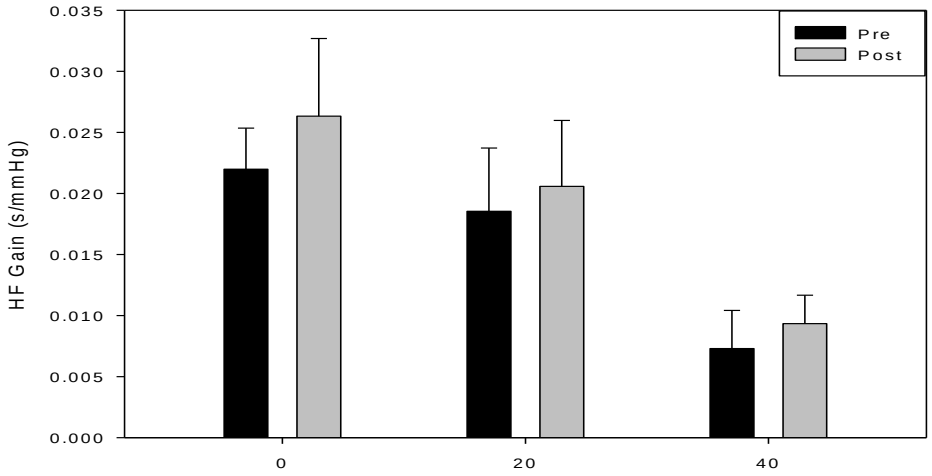


**Figure 5.1-21: High-frequency Gain Response during LBNP Testing Pre and Post 28h NFL HDBR (n=7); HDBR p=0.37, LBNP p<0.05**

Analysis of MF and HF phase responses also illustrated that arterial baroreflex sensitivity was not changed with 28h HDBR. There were no significant changes in MF phase (p=0.45) or HF phase (p=0.09) between pre and post 28h bed-rest data. This illustrated that the R-R interval time response to changes in SBP was not faster or slower as a result of bed-rest exposure. Figures 5.1-22 and 5.1-23 illustrate the MF and HF phase responses pre and post bed-rest at 0, -20, and -40 mmHg.

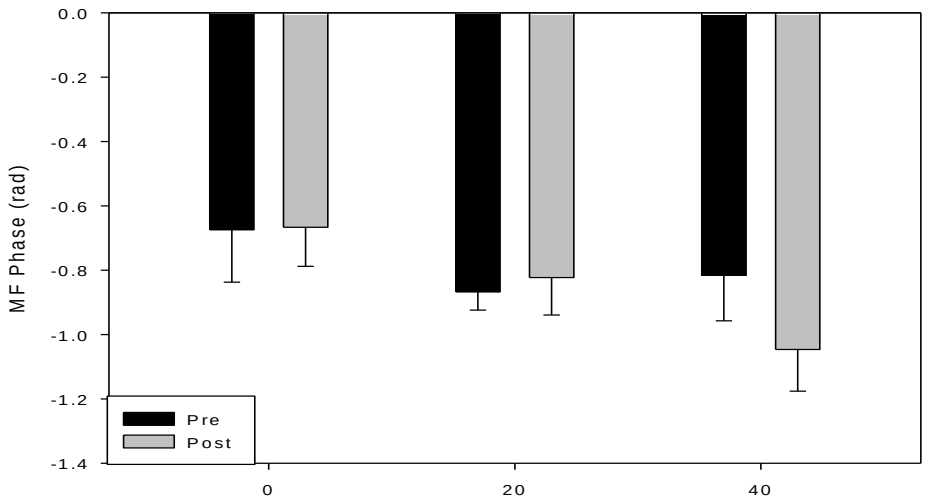


**Figure 5.1-22: Mid-frequency Phase Response during LBNP Testing Pre and Post 28h NFL HDBR (n=7); HDBR p=0.45, LBNP p<0.05**

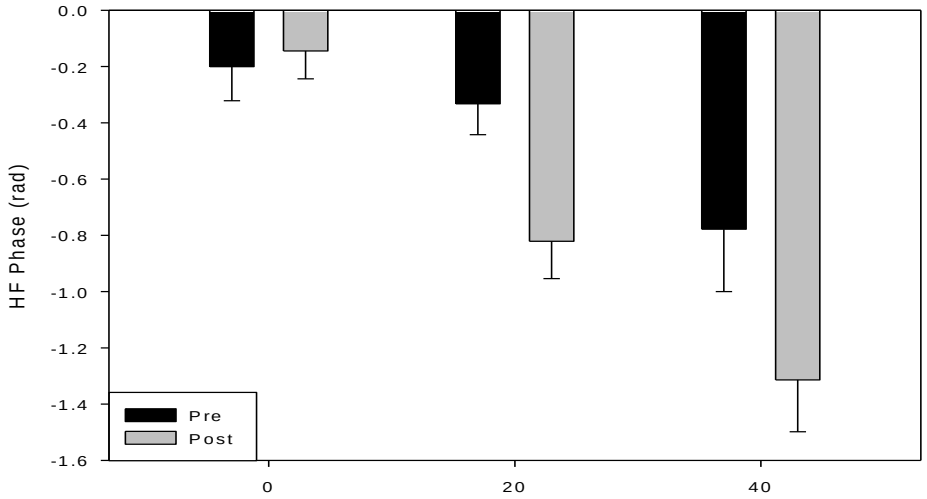


**Figure 5.1-23: High-frequency Phase Response during LBNP Testing Pre and Post 28h NFL HDBR (n=7); HDBR p=0.09, LBNP p<0.05**

There was a significant effect of orthostatic stress on arterial baroreflex sensitivity with orthostatic stress. Arterial baroreflex sensitivity decreased with increasing orthostatic stress with reductions in the response magnitude of both MF gain ($p<0.05$) and HF gain ($p<0.05$), as well as increased time delay shown as an increased negative HF phase response ($p<0.05$).

It must be noted that coherence was less than 0.5 in the HF region during -20 and -40 mmHg (data available in Appendix A1), perhaps due to a low signal to noise ratio. At -20 mmHg, 3 subjects had coherence less than 0.5 in the HF region during pre testing, and 4 subjects during post testing. At -40 mmHg, all of the 7 subjects had coherence less than 0.5 in the HF region during pre testing, and 4 subjects during post testing.

### 5.1.9 CARDIOPULMONARY BAROREFLEX

The cardiopulmonary baroreflex was analyzed by measuring brachial blood flow velocity and brachial diameter, and using these variables to compute brachial blood flow. Analysis of brachial blood flow velocity showed a reduction with increasing LBNP ($p<0.05$), which is expected and mirrors the systemic vascular resistance response to increasing orthostatic stress discussed earlier. The brachial blood flow velocity response to orthostatic stress is illustrated in Figure 5.1-24. Conversely, analysis of brachial blood flow indicated that this variable was maintained constant throughout orthostatic stress ($p=0.65$), as illustrated in Figure 5.1-25. Brachial blood flow is expected to show a similar reduction with increasing stress as seen in brachial blood flow velocity. Brachial diameter is included in the calculation of brachial blood flow, and may be a source of error in brachial blood flow calculations at levels of LBNP above 0 mmHg since brachial diameter was only measured in the supine position. Therefore, measurements of brachial blood flow during orthostatic stress should be discarded. However the decrease in brachial blood flow

velocity provides an indication that the cardiopulmonary baroreflex is causing forearm vasoconstriction during increasing LBNP.

These data also indicate that cardiopulmonary baroreflex function was unchanged after 28h NFL HDBR. Comparing supine values pre and post bed-rest, brachial blood flow velocity (p=0.11), brachial blood flow (p=0.06), and brachial diameter (p=0.72) were unchanged in the supine position. Therefore the cardiopulmonary baroreflex is performing at the same baseline flow following 28h NFL HDBR; there is no attenuation of the cardiopulmonary baroreflex following 28h NFL HDBR. The brachial blood flow velocity, brachial blood flow, and brachial diameter responses following bed-rest are illustrated in Figures 5.1-24, 5.1-25, and 5.1-26 respectively.

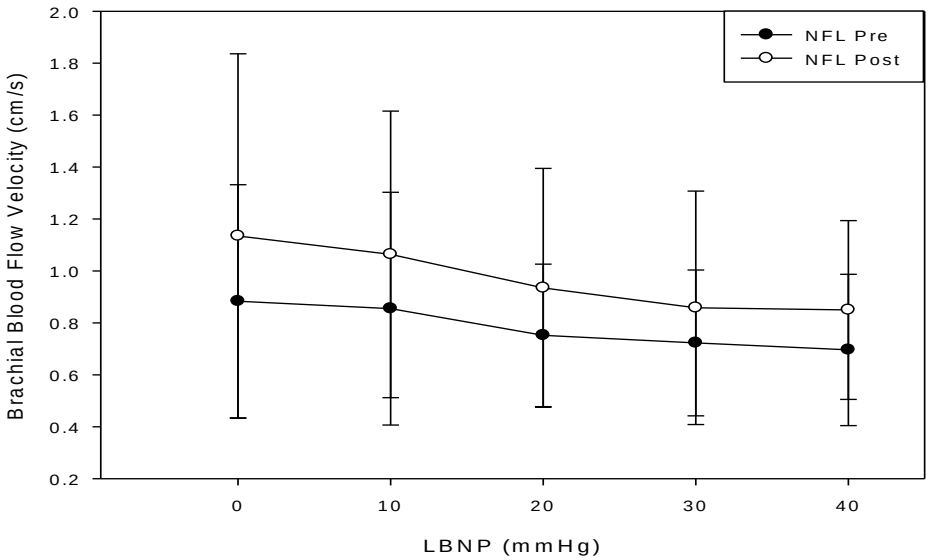


**Figure 5.1-24: Brachial Blood Flow Velocity Response during LBNP Testing Pre and Post 28h NFL HDBR (n=8); HDBR p=0.11, LBNP p<0.05**

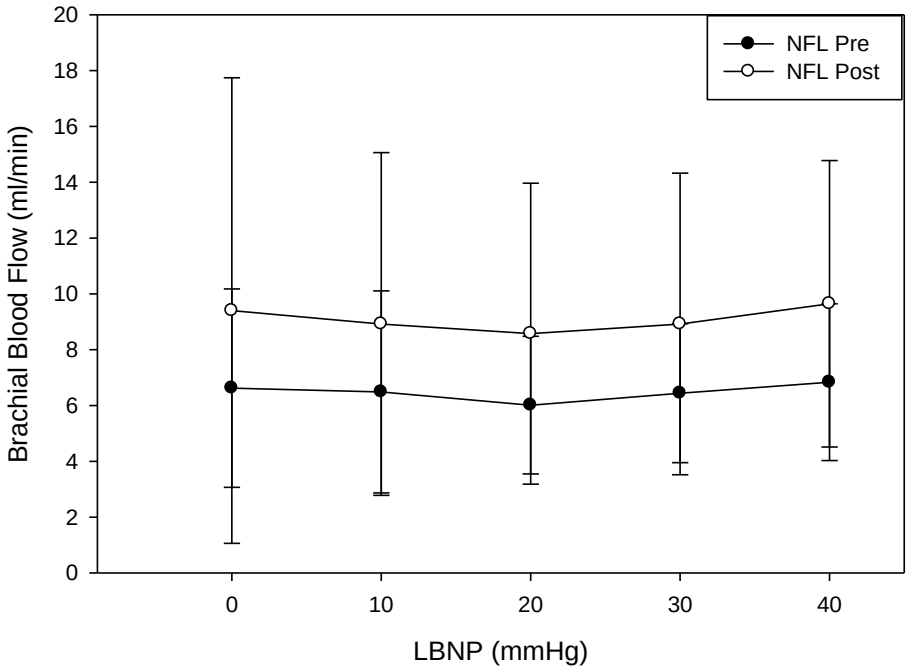


**Figure 5.1-25: Brachial Blood Flow Response during LBNP Testing Pre and Post 28h NFL HDBR (n=8); HDBR p=0.09, LBNP p<0.65**

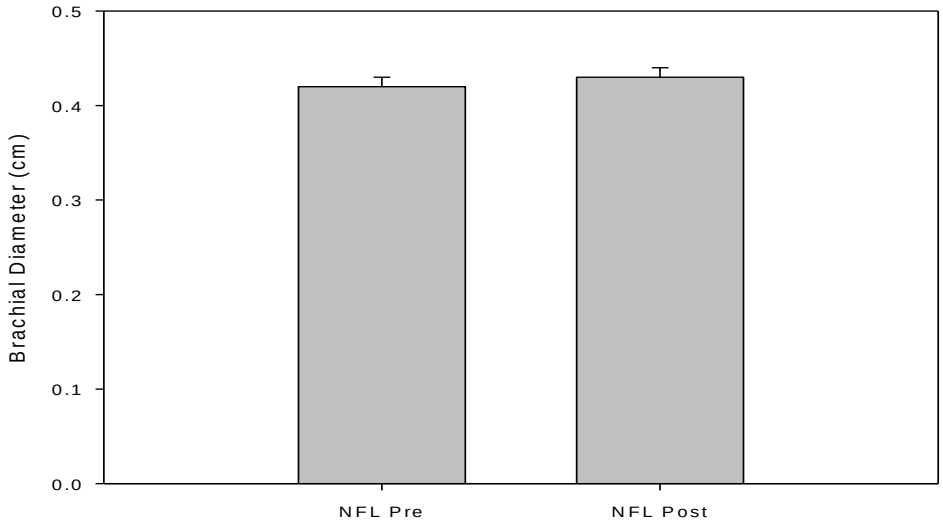


**Figure 5.1-26: Diameter Response during LBNP Testing Pre and Post 28h NFL HDBR (n=9); HDBR p=0.72**

### 5.1.10 SUMMARY OF 28H HDBR EFFECTS ON CARDIOVASCULAR DECONDITIONING

Contrary to the hypotheses, no subjects experienced orthostatic hypotension following 28h bed-rest, and there was no evidence of loss of blood pressure maintenance following 28h HDBR. Examination of MAP, SBP, DBP, and PP showed that these cardiovascular variables were unchanged with bed-rest. Although SBP and PP were reduced with increasing orthostatic stress, they did not limit overall MAP maintenance after exposure to bed-rest.

As hypothesized, hypovolemia resulted from exposure to 28h NFL HDBR. This was illustrated by significant falls in total blood volume and plasma volume and increases in hematocrit, as well as indirectly from a reduction in weight.

Following bed-rest, the heart rate response to orthostatic stress was significantly elevated, which was also in line with the hypotheses. However, stroke volume and cardiac output were not significantly affected by bed-rest, though both variables diminished with orthostatic stress. TPR was also unaffected by bed-rest, which was contrary to the hypothesis, as the vasoconstriction response increased similarly during orthostatic stress before and after bed-rest.

CVP was significantly reduced following bed-rest, as expected, and IVC diameter tended to diminish as well indicating the impact of hypovolemia (reduction was not significant). However there was no evidence of the

hypothesized venous pooling, as central venous compliance was unchanged by 28h HDBR.

Splanchnic hemodynamics were altered by 28h bed-rest, however not as expected. Portal vein diameter was reduced after bed-rest exposure, and reduced further during orthostatic stress. Portal blood flow was also reduced following bed-rest, supporting the finding of increased splanchnic vasoconstriction following bed-rest. However portal blood flow was not reduced with orthostatic stress, casting suspicion on the validity of this measurement. Splanchnic impedance was not affected by bed-rest, illustrating that there was not a significant change in blood volume in the splanchnic vasculature due to bed-rest exposure. There was no evidence of the hypothesized splanchnic pooling after 28h HDBR.

The hypothesized reduction of left ventricular function did not arise following 28h NFL HDBR, rather it was maintained. Ejection fraction and fractional shortening were not affected by bed-rest with responses in the supine and during maximum orthostatic stress unchanged between pre and post testing.

Arterial baroreflex sensitivity was also unaffected by 28h NFL HDBR, which was not in accordance with the hypothesized result. There were no significant differences in magnitude or time delay responses in the mid-frequency and high-frequency regions. This information is evidence that there are no changes in vagal or sympathetic modulation of heart rate response due to short-term hypovolemia.

Cardiopulmonary baroreflex was also unchanged by exposure to 28h NFL HDBR. Supine brachial diameter, brachial blood flow velocity, and brachial blood flow were unchanged following bed-rest.

## 5.2 EFFICACY OF FLUID LOADING PROTOCOL

This analysis investigated the hypotheses that the hypovolemia and cardiovascular deconditioning resulting from 28h NFL HDBR are restored by the fluid loading protocol. Subjects completed a 28h HDBR protocol similar to that used to investigate the effects of bed-rest alone (described in Section 5.1), with the fluid loading protocol performed between 25.5 and 27.5 hour time points. This section compares the subjects' responses during NFL HDBR and FL HDBR Post LBNP testing to determine the effects of the fluid loading protocol. Tables summarizing raw data (in the form of mean ± standard error), statistical analyses results, and sample sizes are included in Appendices A2, B and C respectively. Pre data from the 28h NFL HDBR and 28h FL HDBR were also compared to ensure there were no statistical differences in baseline testing for the two models; the statistical results of these tests can also be found in Appendix B.

### 5.2.1 SUBJECTS' RESPONSE TO FLUID LOADING PROTOCOL

During the fluid loading protocol, 4 of the 9 subjects experienced symptoms of nausea. Two subjects could not complete their prescription of NaCl and water due to the nausea, and two subjects (1 of whom did not complete the fluid loading protocol) vomited prior to post bed-rest orthostatic testing. Vomiting reduced the efficacy of the fluid loading protocol by reducing ingested fluid volume and causing additional discomfort and nausea. The data of the two subjects who did not complete the fluid loading protocol are not included in the following comparison of NFL and FL post tests. Table 5.2-1 summarizes fluid

loading protocol data for each subject. Subjects who did not complete fluid loading are highlighted.

**Table 5.2-1: Summary of Subjects' Fluid Loading Data during 28h FL HDBR**

| Subject | Prescribed NaCl Intake (g) | Prescribed Water Intake (ml) | Ingested NaCl (g) | Ingested Water (ml) | Fluid Load Completion (%) | Onset of Nausea | Vomiting |
|---|---|---|---|---|---|---|---|
| 1 | 8.8 | 1102.5 | 8.8 | 1102.5 | 100 | No | No |
| 2 | 8.5 | 1057.5 | 8.5 | 1057.5 | 100 | No | No |
| 3 | 8.7 | 1084 | 8.7 | 1084 | 100 | No | No |
| 4 | 9.1 | 1143 | 9.1 | 1143 | 100 | No | No |
| 5 | 9.3 | 1167 | 9.3 | 1167 | 100 | Yes | No |
| 6 | 9.3 | 1167 | 8.2 | 1125 | 96 | Yes | Yes |
| 7 | 7.4 | 922 | 2.9 | 500 | 54 | Yes | No |
| 8 | 9.8 | 1225.5 | 9.8 | 1225.5 | 100 | Yes | Yes |
| 9 | 9.6 | 1201.5 | 9.6 | 1201.5 | 100 | No | No |

### 5.2.2 EFFECT OF FLUID LOADING ON PREVENTING HYPOVOLEMIA

Comparing the overall changes in blood volume that occurred during fluid-loading and non-fluid loading tests showed that fluid loading was not successful in restoring hypovolemia resulting from 28h NFL HDBR. After 28h FL HDBR, total blood volume was reduced by 380.1 ml ± 74.3, which was similar to the amount of total blood volume lost during 28h NFL HDBR, 390.3 ml ± 66.8 ($p=0.29$). Plasma volume was reduced similarly, with no significant differences between NFL Post and FL Post tests ($p=0.22$). Figure 5.2-1 shows the total blood volume trends throughout 28h NFL HDBR and 28h FL HDBR.

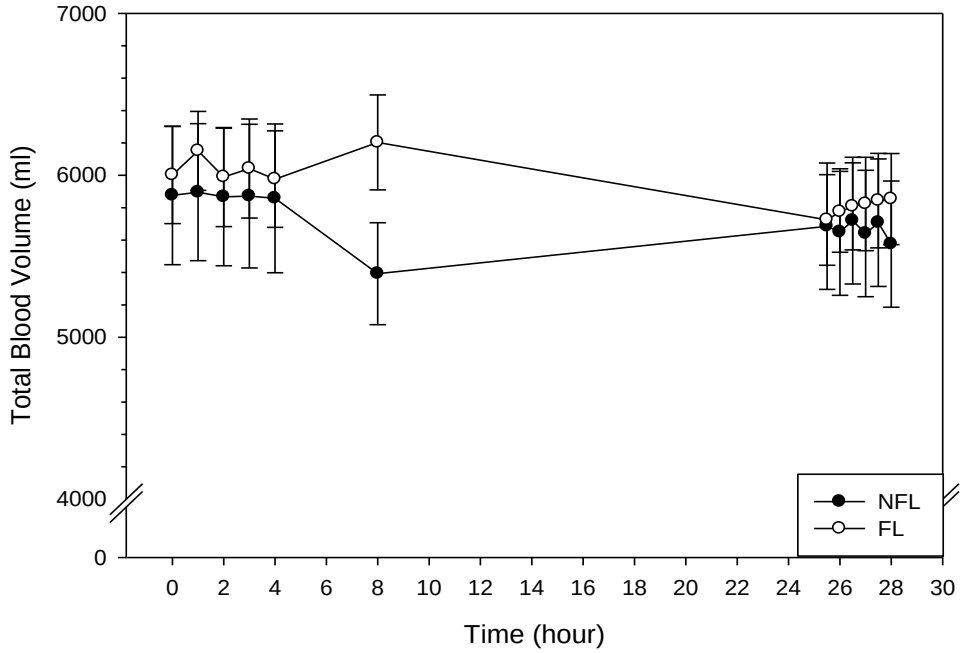


**Figure 5.2-1: Total Blood Volume Changes during 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=6); Post FL/NFL HDBR p=0.29**

However, examination of the fluid loading period showed that fluid loading did affect total blood and plasma volume. The open circles in Figure 5.2-1 illustrate the total blood volume response during the 28h FL HDBR test. It can be seen that after the 25.5h time point (the start of the fluid loading period), total blood volume progressively increased until the final 28h time point. However, total blood volume was not yet restored to its initial pre bed-rest value. If fluid loading protocol had been started earlier, fluid restoration may have been successful.

The difference in total blood volume at the 8h time point between FL and NFL tests is noted, and may be due to unregulated fluid intake between hour 4 and hour 24. The reader is also reminded that subjects who did not finish the fluid loading protocol were removed from this analysis, which accounts for

the differences in 28h NFL blood volume data described in Sections 5.1 and 5.2.

Failure to restore blood volume was also apparent by analysis of hematocrit over the course of 28h FL HDBR testing. Hematocrit was increased by 2.60% ± 0.34 at the completion of the 28h FL HDBR protocol, which was similar to the increase of 3.04% ± 0.68 seen after the 28h NFL HDBR protocol ($p=0.18$). Weight change between pre and post testing also indicated that fluid volume loss was sustained despite fluid loading. Weight loss was 1.1 kg ± 0.3 following 28h FL HDBR, compared with a loss of 1.0 kg ± 0.6 following 28h NFL HDBR ($p=0.98$).

### 5.2.3 Effect of Fluid Loading Protocol on Blood Pressure Response

After 28h FL HDBR all subjects successfully completed the progressive LBNP test except for one subject who experienced a presyncopal drop in blood pressure. This subject had an adverse reaction to the fluid loading protocol, experienced extreme nausea to the point of vomiting, and did not complete the prescribed salt and water intake of the fluid loading protocol. The subject was a strong finisher in each of the other four testing models (and 9 LBNP tests), and it was not his first time participating in orthostatic testing. His presyncopal symptoms may be attributed to vomiting and feeling of malaise following the fluid loading protocol. Data from this subject, as well as the second subject that did not complete the fluid loading protocol, were removed from analysis of the

fluid loading protocol's efficacy; only subjects that completed fluid loading were analyzed (n=7).

Blood pressure during post bed-rest testing was not affected by fluid loading. There were no significant differences between the 28h NFL Post and 28h FL Post tests concerning MAP (p=0.35), SBP (p=0.35), DBP (p=0.43), and PP responses (0.41). Figure 5.2-2 illustrates that MAP and DBP responses remained constant and SBP fell similarly during orthostatic testing after 28h FL HDBR and 28h NFL HDBR testing. Figure 5.2-3 illustrates the PP response.

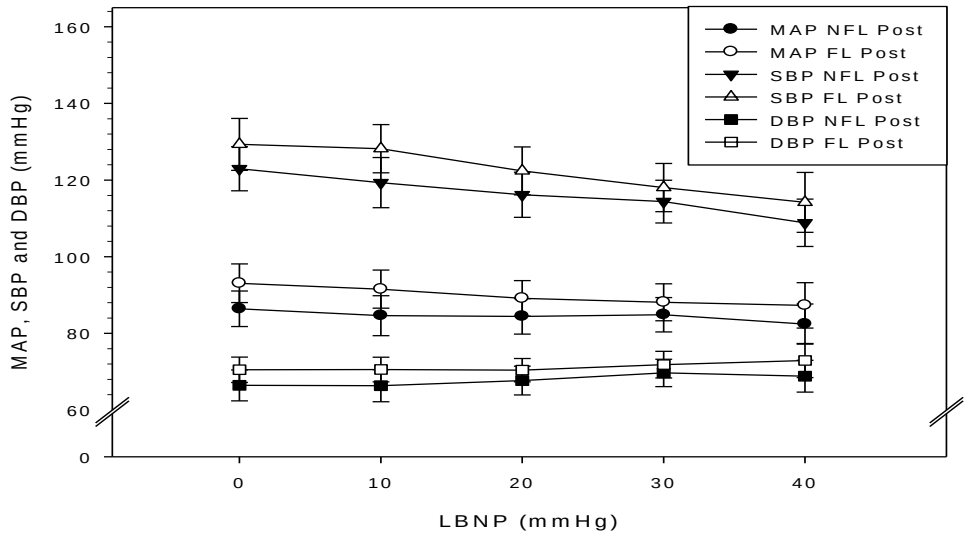


**Figure 5.2-2: MAP, SBP, and DBP Responses during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol** n=7**); MAP Post FL/NFL HDBR p=0.35, SBP Post FL/NFL HDBR p=0.35, DBP Post FL/NFL HDBR p=0.43**

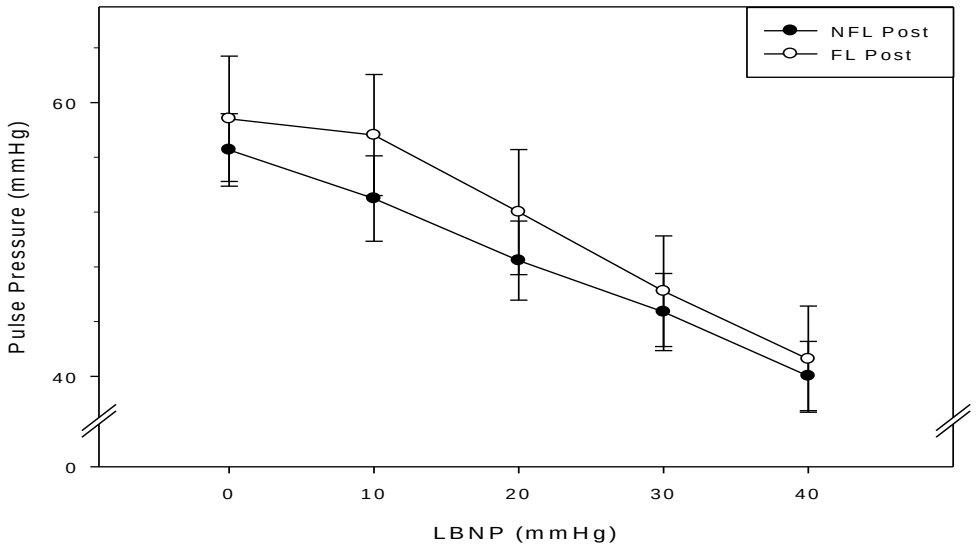


**Figure 5.2-3: Pulse Pressure Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.40**

### 5.2.4 EFFECT OF FLUID LOADING PROTOCOL ON HEART RATE RESPONSE

Analysis of heart rate confirmed that the fluid loading protocol did not prevent cardiovascular deconditioning. Heart rate was increased to a similar extent during LBNP testing following 28h FL HDBR as it was after 28h NFL HDBR ($p=0.34$). Analysis of R-R interval also showed there was no significant difference between the FL and NFL Post responses ($p=0.43$). The heart rate responses during orthostatic testing following 28h FL HDBR and 28h NFL HDBR are illustrated in Figure 5.2-4.

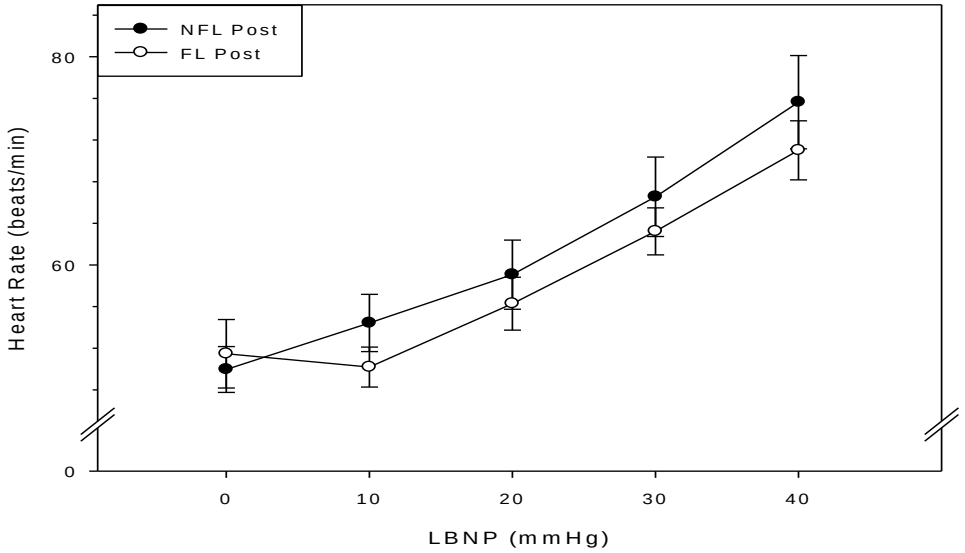


**Figure 5.2-4: Heart Rate Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.34**

### 5.2.5 EFFECT OF FLUID LOADING PROTOCOL ON THE ARTERIAL HEMODYNAMICS RESPONSE

Arterial hemodynamic responses were not affected by fluid loading. Stroke volume tended to be higher following 28h FL HBBR compared to 28h NFL HDBR however this effect was not significant (p=0.07). Although there was an interaction effect of LBNP and the fluid loading treatment (p<0.05), comparison of stroke volume responses in the supine position (p=0.19) and at maximum orthostatic stress (p=0.34) showed no significant differences between FL and NFL post testing. Figure 5.2-5 illustrates the stroke volume response to LBNP following 28h FL HDBR, with the 28h FL HDBR Post response included for comparison.

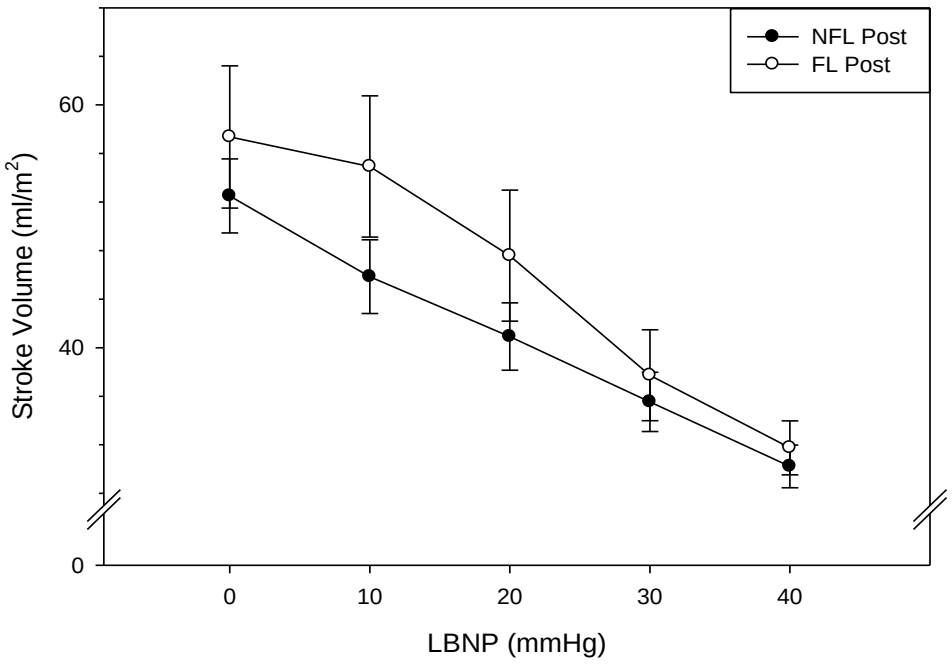


**Figure 5.2-5: Stroke Volume Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.07, Post FL/NFL HDBR-LBNP interaction p<0.05**

Like stroke volume, the cardiac output response following 28h HDBR was not affected by fluid loading. There was no statistical difference between the cardiac output response following 28h FL HDBR and 28h NFL HDBR (p=0.32). Like stroke volume, there was an interaction between LBNP and the fluid loading treatment (p=0.01), however there were no significant differences in the 28h FL HDBR Post and 28h NFL HDBR Post cardiac output responses in the supine position (p=0.13) or during maximum orthostatic stress (p=0.98). The cardiac output responses during post LBNP testing following 28h NFL HDBR and 28h FL HDBR are illustrated in Figure 5.2-6.

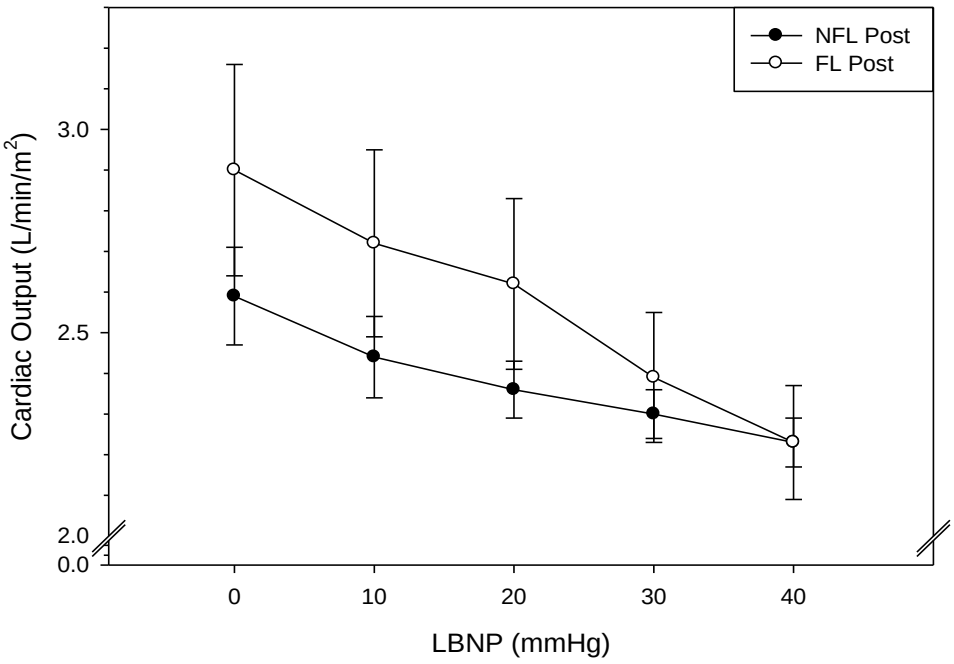


**Figure 5.2-6: Cardiac Output Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.32, Post FL/NFL HDBR-LBNP interaction p<0.05**

Fluid loading did not affect the systemic vasoconstriction response following 28h HDBR. There were no significant differences between the TPR

responses during LBNP testing following 28h FL HDBR and 28h NFL HDBR (p=0.50), as seen in Figure 5.2-7.

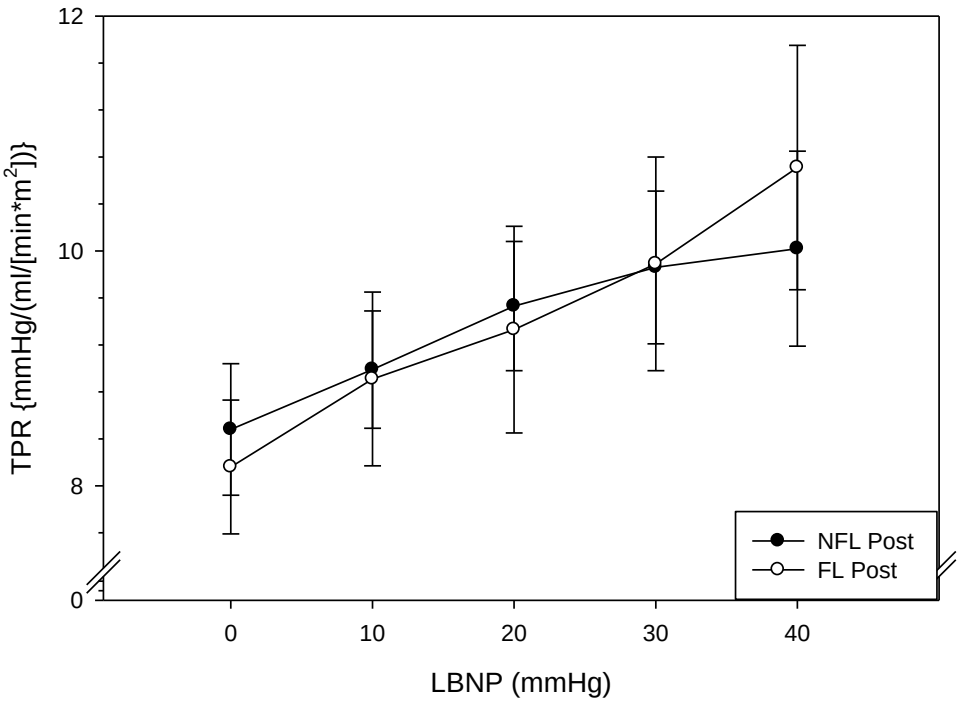


**Figure 5.2-7: Total Peripheral Resistance Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.50**

Arterial compliance was also unaffected by the fluid loading protocol. There was no significant difference between the arterial compliance following 28h FL HDBR and 28h NFL HDBR (p=0.16). Arterial compliance after 28h FL HDBR was 1.48 $(ml/m^2)/mmHg \pm 0.14$, which did not significantly differ from the response following 28h NFL HDBR, which was 1.22 $(ml/m^2)/mmHg \pm 0.18$.

### 5.2.6 EFFECT OF FLUID LOADING PROTOCOL ON VENOUS HEMODYNAMICS

There was no significant difference between the CVP responses following 28h FL HDBR and 28h NFL HDBR (p=0.44), illustrating that blood return to the heart remained diminished following bed-rest despite the countermeasure. However, CVP was increased following 28h FL HDBR compared to the response following 28h NFL HDBR. This result should be considered since the sample size was small (n=5), and since there was a slow increase in total blood volume throughout the fluid loading period. Stroke volume was also elevated (not significantly) after fluid loading, which is consistent with increased CVP. If the fluid loading period was longer, CVP may have been elevated following 28h FL HDBR compared to NFL testing. . The CVP responses during post LBNP testing following 28h NFL HDBR and 28h FL HDBR are illustrated in Figure 5.2-8.

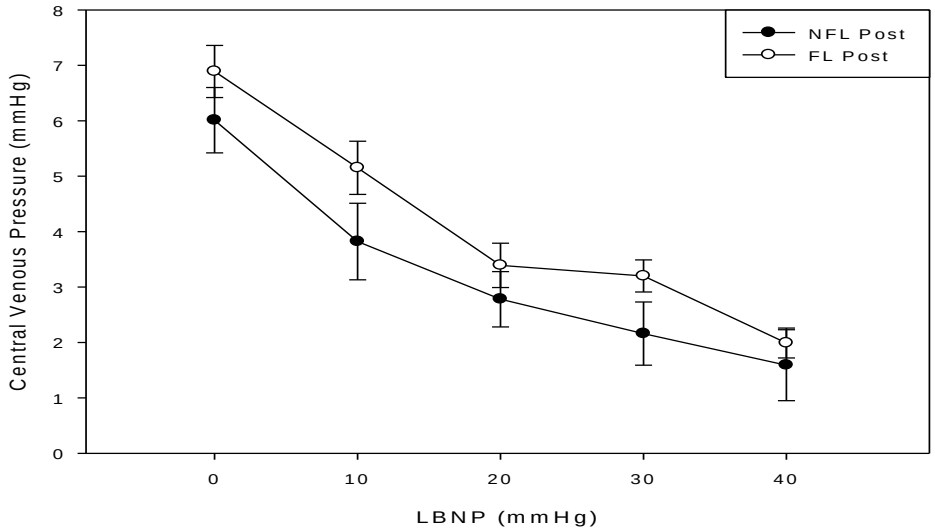


**Figure 5.2-8: Central Venous Pressure Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.44**

Analysis of IVC diameter responses further supported the findings that CVP remained diminished despite fluid loading. There were no significant differences between the 28h FL HDBR and 28h NFL HDBR post responses to LBNP (p=0.50). IVC diameter remained diminished following 28h FL HDBR, as illustrated in Figure 5.2-9.

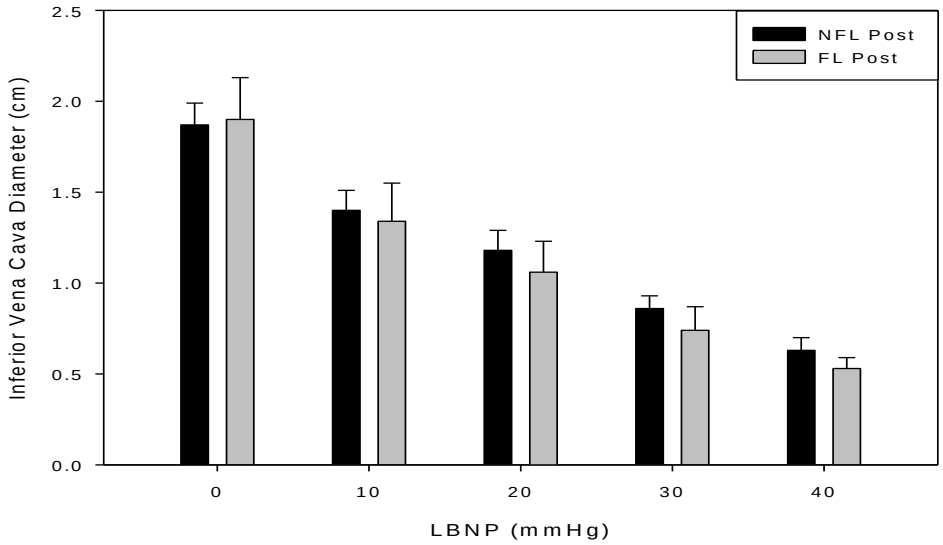


**Figure 5.2-9: Inferior Vena Cava Diameter Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.50**

Venous compliance was also not affected by fluid loading, as there were no differences in the venous compliance response between the 28h FL HDBR and 28h NFL post testing (p=0.81), as illustrated in Figure 5.2-10.

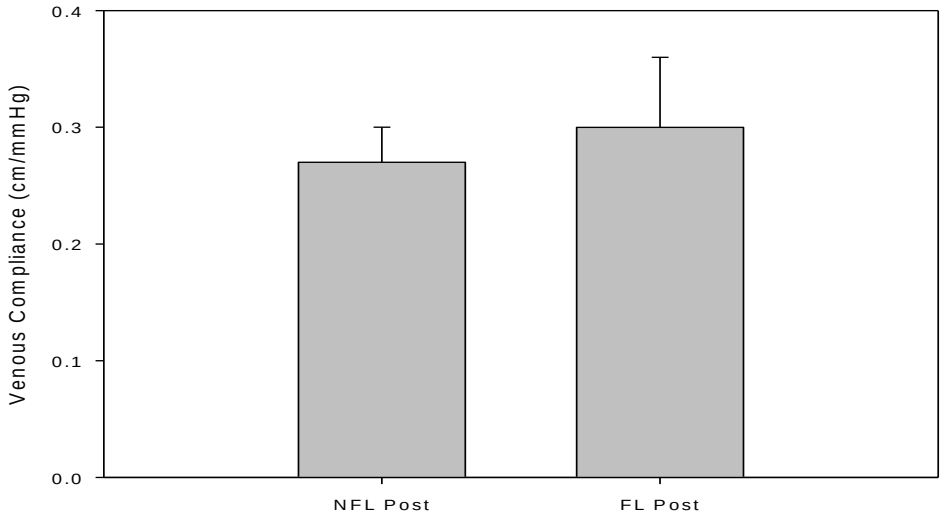


**Figure 5.2-10: Venous Compliance Response following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=6); Post FL/NFL HDBR p=0.81**

### 5.2.7 EFFECT OF FLUID LOADING ON SPLANCHNIC HEMODYNAMICS

Splanchnic hemodynamics were unaffected by fluid loading. Portal vein diameter remained diminished following 28h FL HDBR despite ingested salt and water. There was no significant difference in portal vein diameter between 28 FL HDBR and 28 FL HDBR post tests (p=0.27), as illustrated in 5.2-11.

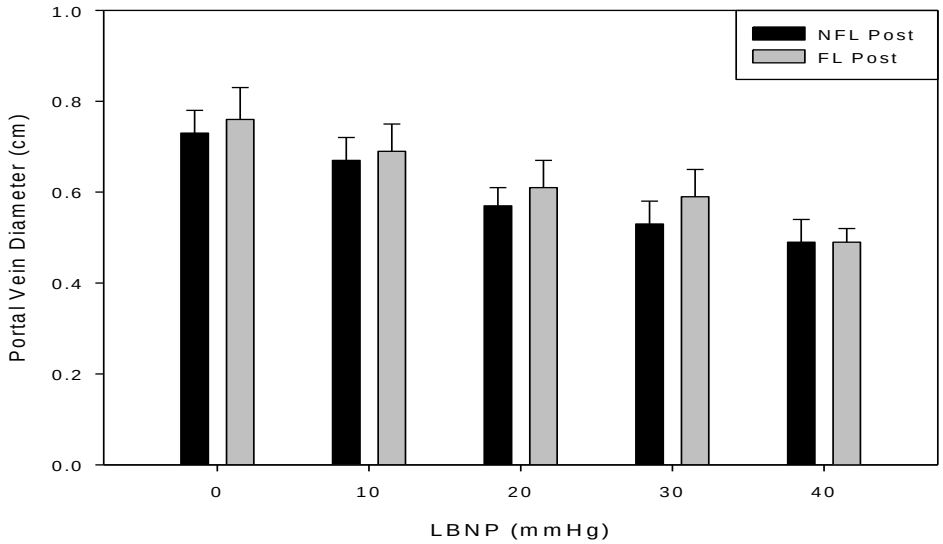


**Figure 5.2-11: Portal Vein Diameter Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.27**

Splanchnic impedance was not significantly altered by the fluid loading protocol. There was no difference in the splanchnic impedance response following 28h FL HDBR and 28h NFL HDBR (p=0.23), as illustrated by Figure 5.2-12.

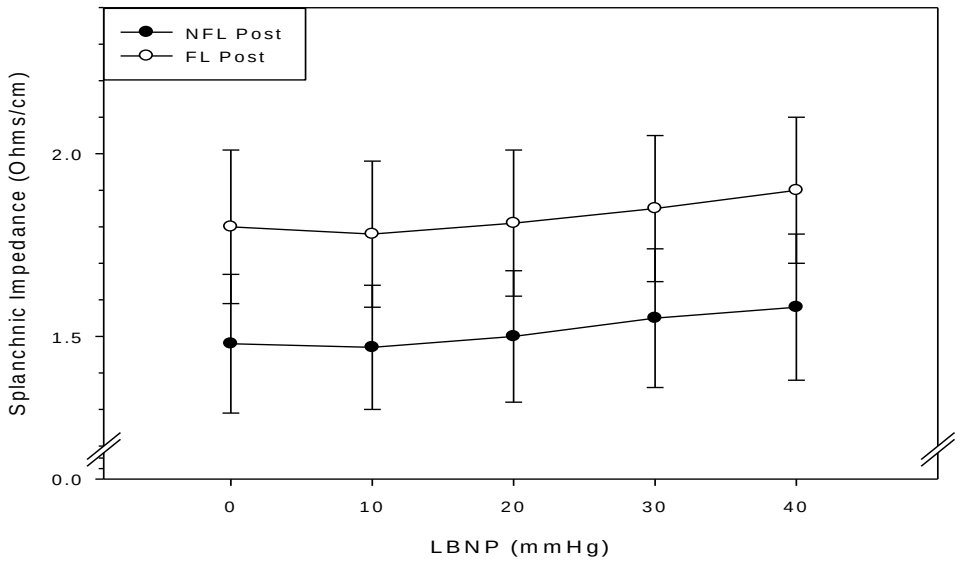


**Figure 5.2-12: Splanchnic Impedance Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=6); Post FL/NFL HDBR p=0.23**

### 5.2.8 EFFECT OF FLUID LOADING ON LEFT VENTRICULAR PERFORMANCE

Left ventricular performance was not affected by 28h NFL HDBR, and this response was unchanged with fluid loading. There was no significant difference in ejection fraction responses between 28h FL HDBR and 28h NFL HDBR post tests (p=0.71), as illustrated in Figure 5.2-13.

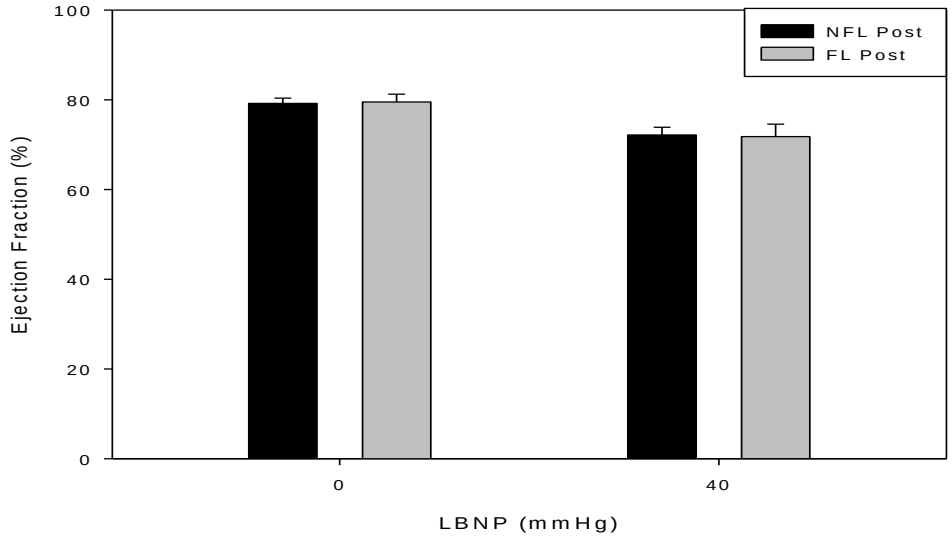


**Figure 5.2-13: Ejection Fraction Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=6); Post FL/NFL HDBR p=0.71**

Similarly, there were no changes in fractional shortening between the 28h FL HDBR and 28h NFL HDBR post tests (p=0.78), as illustrated in Figure 5.2-14.

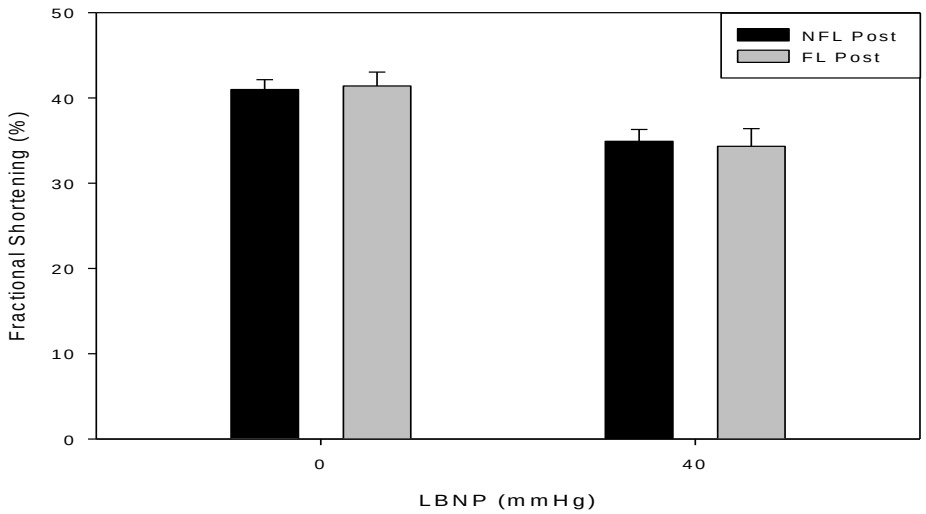


**Figure 5.2-14: Fractional Shortening Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=6); Post FL/NFL HDBR p=0.78**

### 5.2.9 Effect of Fluid Loading on Arterial Baroreflex Response

The arterial baroreflex analysis comparing 28h FL HDBR and 28h NFL HDBR was performed with 5 subjects. Two subjects were removed since they did not complete the fluid loading protocol and two were removed for discontinuities in their SBP signal, as described in Section 5.1.9.

There were no differences in arterial baroreflex response responses between 28h FL HDBR and 28h NFL HDBR. MF gain responses were not statistically different between NFL and FL post orthostatic testing (p=0.13), nor did HF gain responses differ (p=0.94). The MF gain and HF gain responses during post LBNP testing following 28h NFL HDBR and 28h FL HDBR are illustrated in Figures 5.2-15 and 5.2-16.

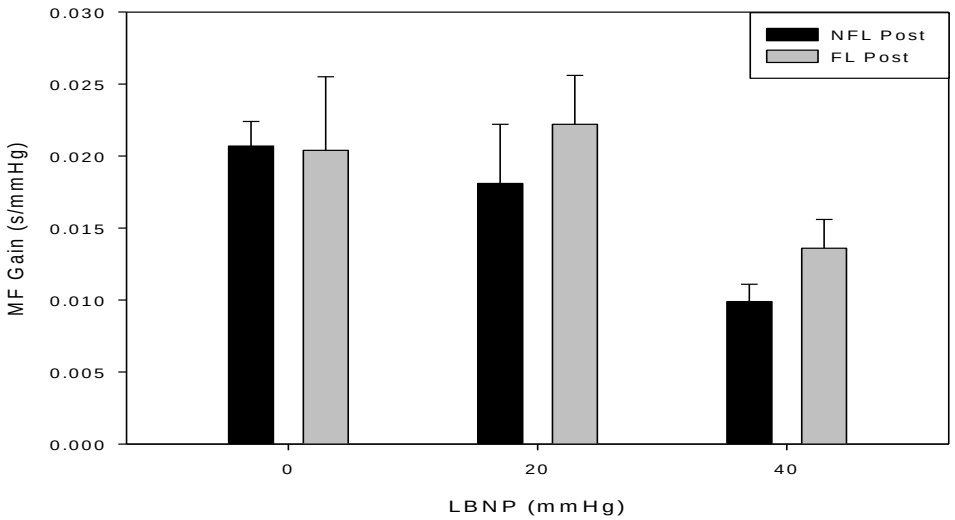


**Figure 5.2-15: MF Gain Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.13**

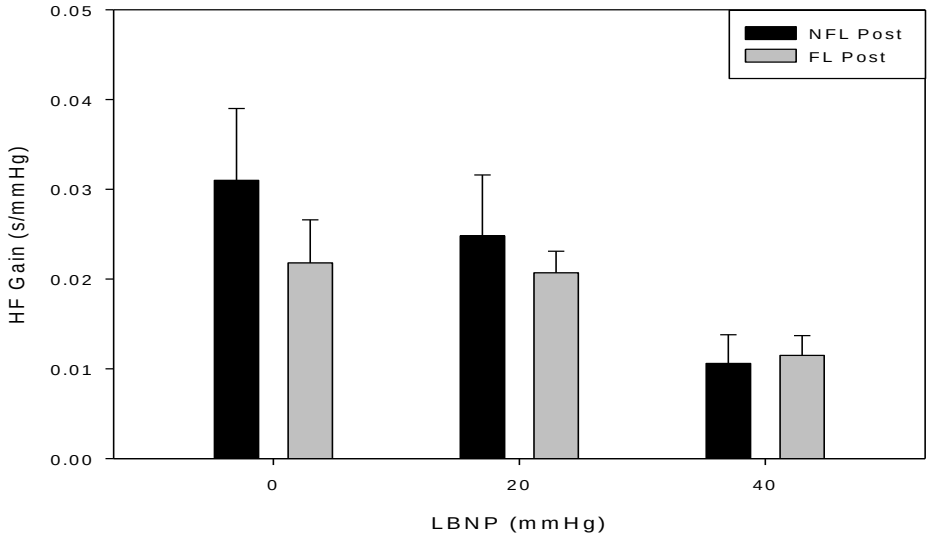


**Figure 5.2-16: HF Gain Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.94**

There were also no significant differences in the time course of the response. MF phase did not differ between 28h NFL HDBR and 28h FL HDBR post orthostatic testing (p=0.97), nor did HF phase differ (p=0.73). The MF phase and HF phase responses during post LBNP testing following 28h NFL HDBR and 28h FL HDBR are illustrated in Figures 5.2-17 and 5.2-18.

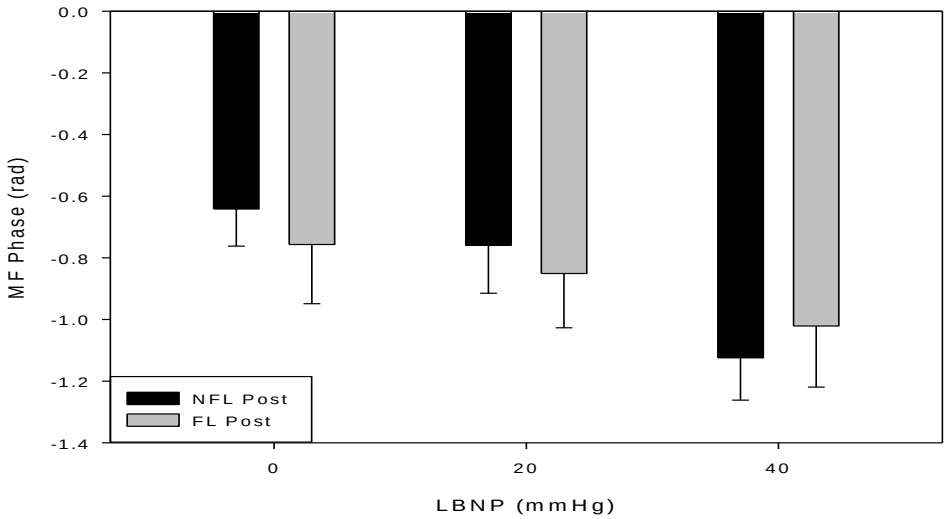


**Figure 5.2-17: MF Phase Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.98**

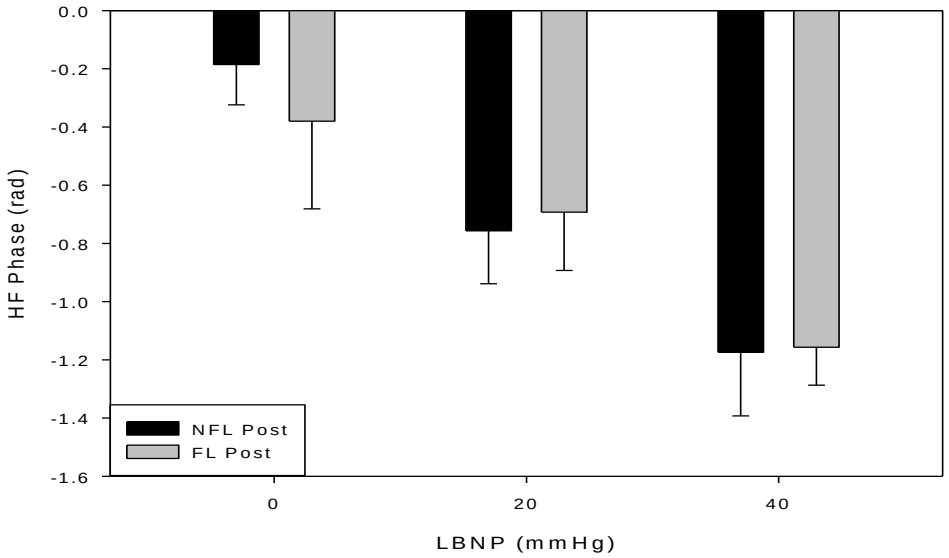


**Figure 5.2-18: Phase Response during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.73**

It must be noted that as seen during 28h NFL HDBR testing, coherence was on average less than 0.5 in the HF region during -20 and -40 mmHg during 28h FL HDBR, perhaps due to a low signal to noise ratio. Coherence was below 0.5 in 2 of the 5 subjects at -20 mmHg, and in 3 of the 5 subjects at -20 mmHg in the 28h FL HDBR test.

### 5.2.10 EFFECT OF FLUID LOADING ON CARDIOPULMONARY BAROREFLEX RESPONSE

Fluid loading, like bed-rest, had no significant impact on cardiopulmonary baroreflex. There were no significant changes in brachial diameter (p=0.66), brachial blood flow velocity (p=0.74), or brachial blood flow (p=0.94) in the supine position following 28h FL HDBR compared to 28h NFL HDBR. This indicates that fluid loading did not alter cardiopulmonary baroreflex function following 28h bed-rest. The brachial blood flow velocity, brachial blood flow, and brachial diameter responses following 28h FL HDBR are illustrated in Figures 5.2-19, 5.2-20, and 5.2-21 respectively.

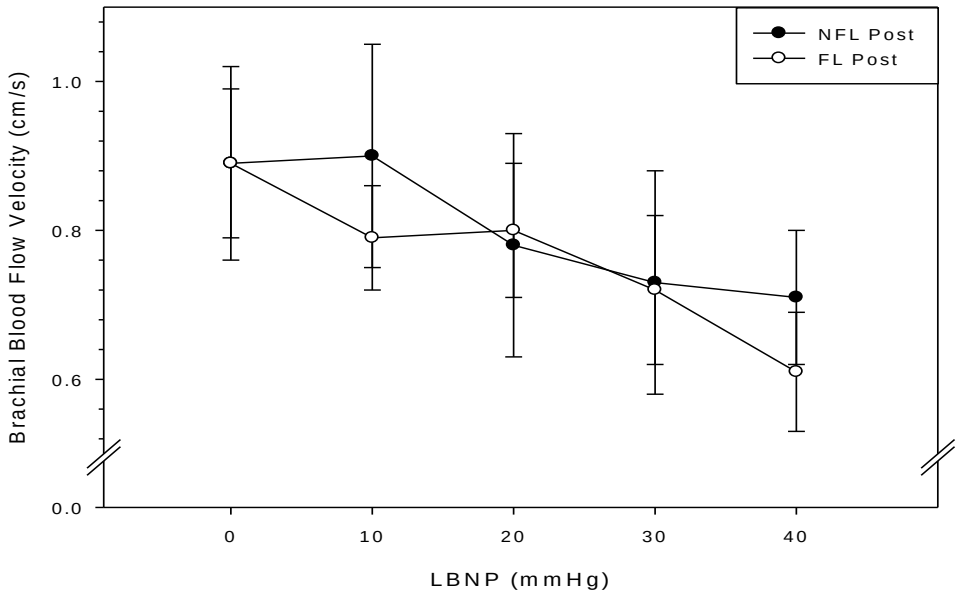


**Figure 5.2-19: Brachial Blood Flow Velocity Response following 28h NFL HDBR and 28h FL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.94**

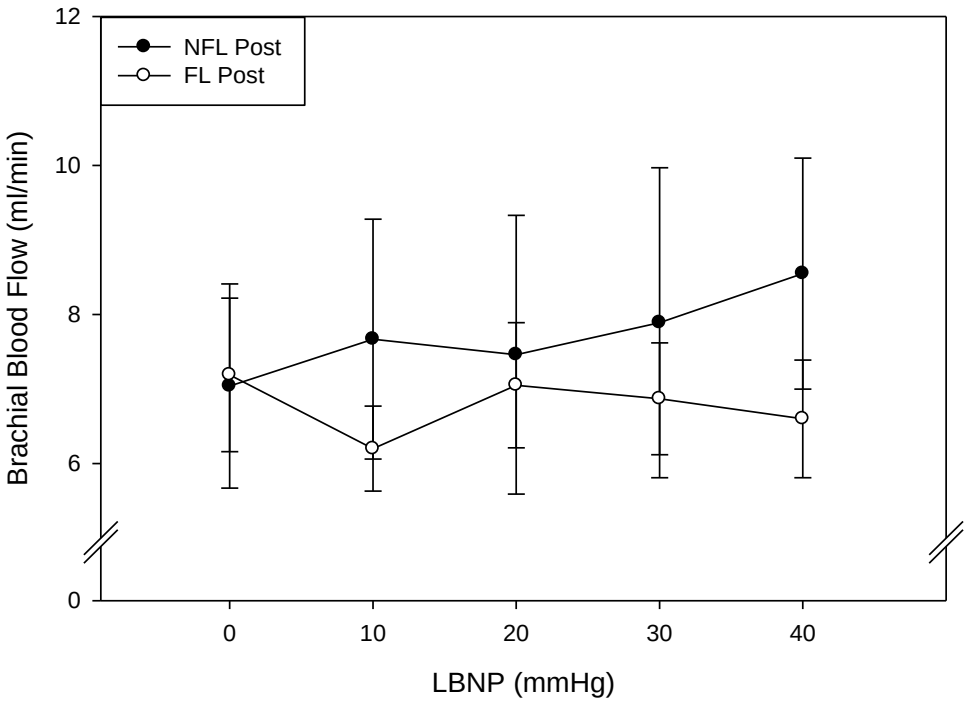


**Figure 5.2-20: Brachial Blood Flow Response following 28h NFL HDBR and 28h FL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=5); Post FL/NFL HDBR p=0.74**

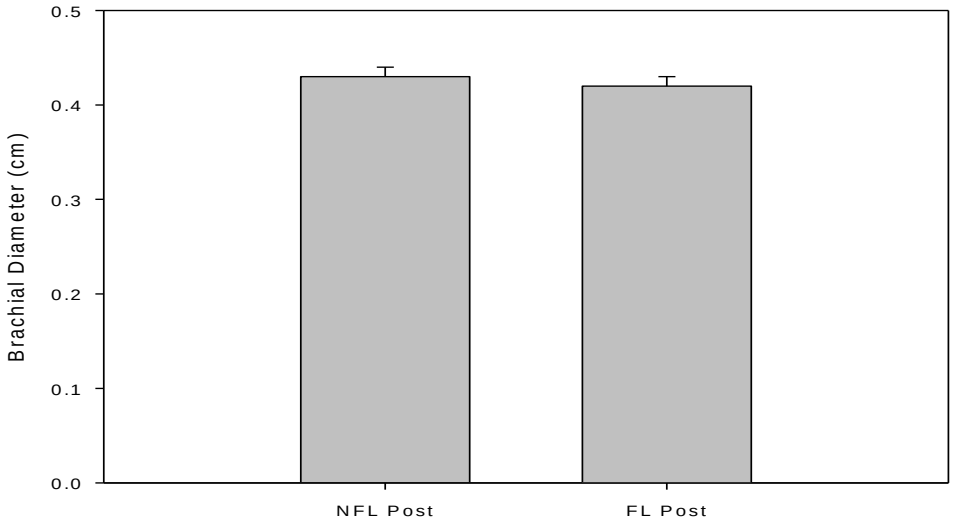


**Figure 5.2-21: Brachial Diameter Response following 28h NFL HDBR and 28h FL HDBR (data points for FL and NFL include only subjects who completed FL protocol, n=7); Post FL/NFL HDBR p=0.66**

### 5.2.11 FLUID REDISTRIBUTION WITH FLUID LOADING PROTOCOL

It is apparent that water ingested during the fluid loading protocol was either not absorbed or did not remain in the vasculature for a substantial period of time since blood volume was not significantly increased or fully restored to its pre 28h HDBR values following the fluid loading protocol. Instead, the fluid may have been absorbed into the extravascular space, excreted by the kidneys, or it may have remained in the stomach for the duration of the bed-rest period. This study was not designed to analyze fluid distribution, however some data may provide a rough indication.

As described earlier, 4 of the 9 subjects became nauseated during the fluid loading protocol. They were lying in the 6° head-down position, so ingested fluid and salt would tend towards the upper part of the stomach by the esophagus. This may have been the source of the nausea. Some subjects had the feeling of nausea up until commencement of orthostatic testing, suggesting that some of the ingested fluid remained in the stomach. This hypothesis is supported by the slow steady increase in total blood volume and plasma volume seen during the fluid loading period. The progressive increase was not fast enough to restore blood volume during the 2.5h fluid loading period, therefore fluid may have remained in the stomach at the end of the fluid loading period.

A comparison of fluid intake and output during 28h NFL HDBR and 28h FL HDBR provides insight into water redistribution. Fluid intake greatly differed between the two bed-rest protocols. During 28h NFL HDBR subjects ingested 3155.44 ml ± 343.65, whereas subjects ingested 4349.43 ml ± 347.54

during the fluid loading protocol ($p<0.05$), the difference due to the prescribed water in the fluid load as well as water ingested at libitum between hour 8 and bed-time during the 28h bed-rest period. Urinary output was not significantly different between the two tests. Urine excretion throughout 28h NFL HDBR was 4139.63 ml ± 611.89, during the 28h FL HDBR test urine excretion was 4668.18 ml ± 406.81 ($p=0.14$). These data suggest that the fluid ingested during the fluid loading protocol was not absorbed by the kidneys, and supports the hypothesis stated above that some subjects experienced delayed absorption of fluid during the fluid loading protocol. The elevated urinary excretion during the 28h FL test, though not significantly different from the 28h NFL urinary excretion, also supports the idea that some subjects experienced increased fluid excretion during 28h FL HDBR. It is possible that the fluid loading protocol was not very effective because ingested fluid was quickly compensated for to keep fluid levels at the new baseline created by HDBR. Table 5.2-2 illustrates fluid intake and urine output data for NFL and FL tests.

**Table 5.2-2: Average Fluid Intake and Urinary Excretion during 28h NFL HDBR and 28h FL HDBR Tests (n=9)**

| Test | Total Fluid Intake including Fluid Load (ml) | Water Intake during Fluid Load (ml) | Urine Output (ml) |
|---|---|---|---|
| 28h NFL HDBR | 3155.44 ± 343.65 | 0 | 4139.63 ± 611.89 |
| 28h FL HDBR | 4349.43 ± 347.65 | 1067.33 ± 73.17 | 4668.18 ± 406.81 |

### 5.2.12 EFFECT OF FLUID AND SALT INTAKE ON NAUSEA AND ORTHOSTATIC TEST COMPLETION

Successful completion of LBNP testing was independent of fluid intake during the 28h HDBR period. Individual fluid intake (excluding prescribed water intake for the fluid loading protocol) ranged from 2010 ml to 5544 ml across each 28h NFL HDBR and 28h FL HDBR test. The variation in subjects' individual intake between FL and NFL tests was due to ad libitum water intake between hour 4 and bed-time. Fluid intake for each subject during both 28h NFL HDBR and 28h FL HDBR testing is summarized in Table 5.2-3. There was only one case of syncope that occurred following 28h FL HDBR, which is indicated by the asterisk in the table. Figure 5.2-22 plots total fluid intake (including prescribed water during fluid loading) versus orthostatic test completion. Test completion does not appear to be affected by fluid intake during the 28 hour bed-rest period as the lone case where presyncopal symptoms caused orthostatic testing to be terminated early was a test where the subject ingested an average amount of water compared to all other tests.

**Table 5.2-3: Fluid Volume Intake during 28h NFL HDBR and 28h FL HDBR Tests (Water Prescribed for Fluid Load in brackets)**

| | Fluid Intake (ml) | |
|---|---|---|
| Subject | 28h NFL HDBR | 28h FL HDBR (+ FL) |
| 1 | 3609 | 4915.5 (+ 1102.5) |
| 2 | 2388 | 1955 (+ 1057.5) |
| 3 | 2525 | 2820 (+ 1084) |
| 4 | 2755 | 2720 (+ 1143) |
| 5 | 3480 | 2735 (+ 1167) |
| 6* | 3006 | 3252 (+ 1125) |
| 7 | 2010 | 3073 (+ 500) |
| 8 | 3082 | 3223 (+ 1225.5) |
| 9 | 5544 | 4845.5 (+ 1201.5) |

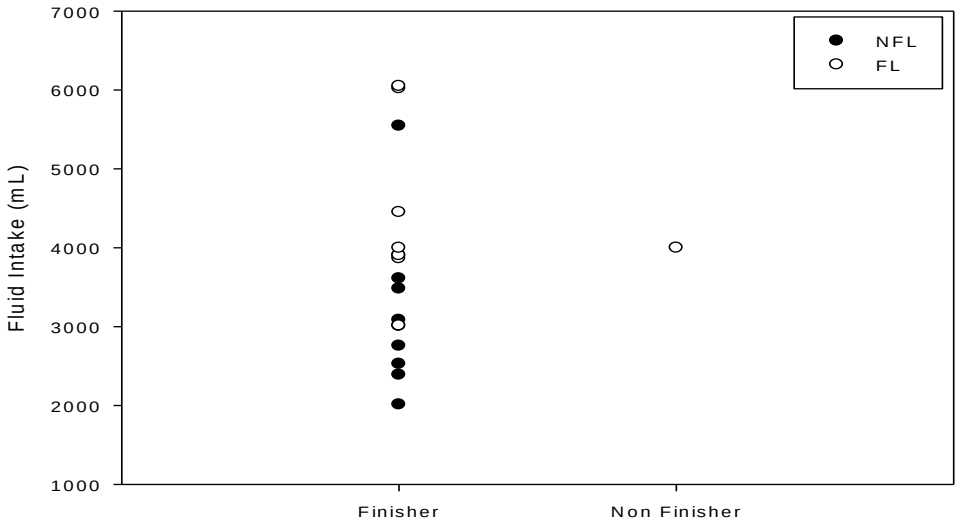


**Figure 5.2-22: Effect of Fluid Volume Intake (including prescribed intake for fluid load) on Onset of Syncope following 28h FL HDBR and 28h NFL HDBR (n=9)**

Fluid intake was not the cause for the onset of nausea and vomiting during 28h FL HDBR. Figure 5.2-23 plots total fluid intake versus nausea/no nausea, and it is apparent that subjects who experienced nausea were not the subjects who ingested high amounts of water, nor were they the subjects who ingested small amounts of water throughout 28h FL HDBR. Rather, nausea only occurred following the fluid loading protocol.

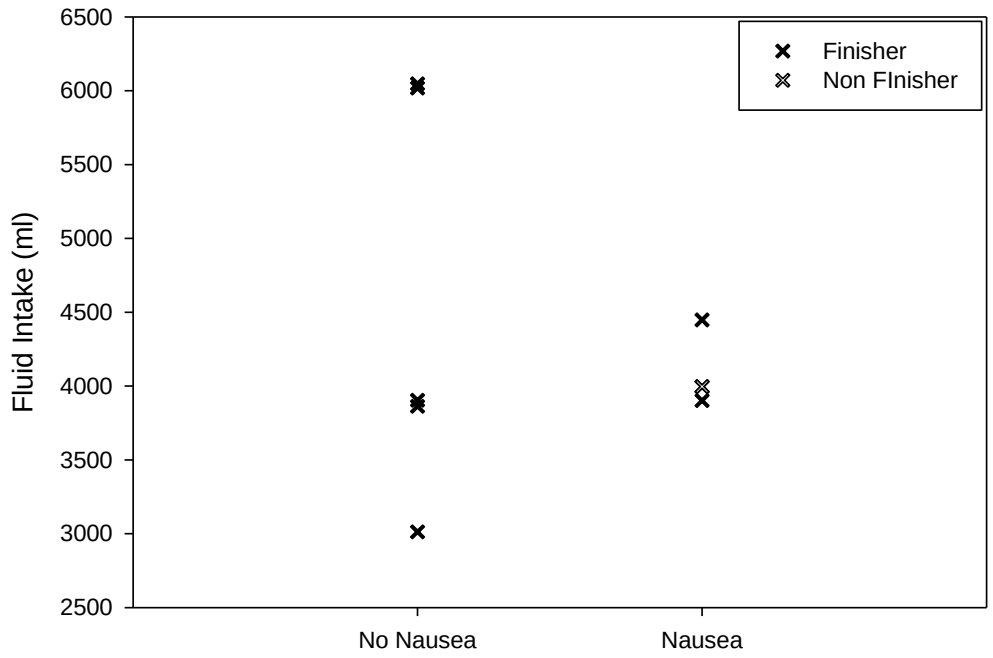


**Figure 5.2-23: Effect of Fluid Intake on Nausea (n=9)**

Onset of nausea following fluid loading seems to be subject specific rather than related to a high prescribed salt intake during the protocol. Figure 5.2-24 plots the prescribed salt intake of each subject during fluid loading versus nausea/no nausea and highlights subjects who did not complete the fluid loading protocol. Subjects who experienced nausea represented a broad spectrum of prescribed salt intake, from the highest (9.8g) to the lowest (7.4g) quantities. Therefore factors other than, or in addition to quantity of salt, played a role in onset of nausea following fluid loading.

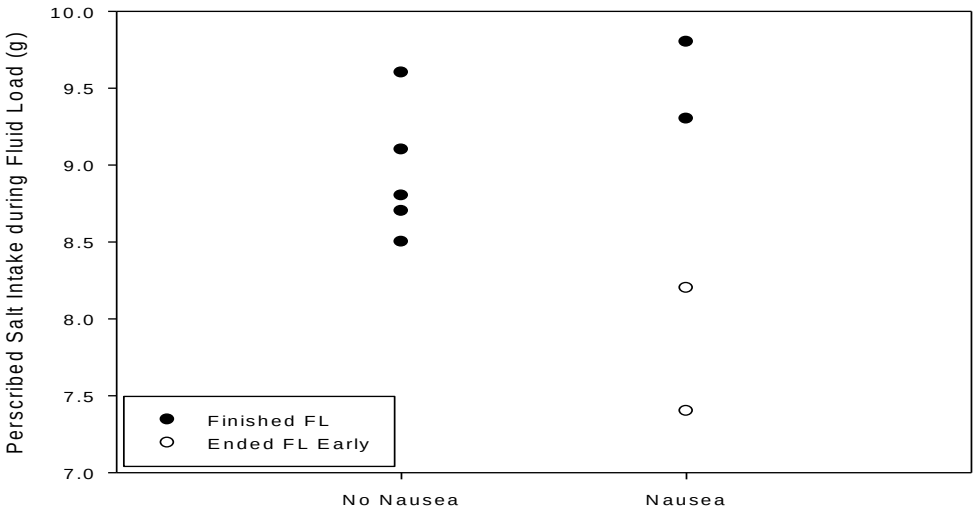


**Figure 5.2-24: Effect of Prescribed Salt Intake during Fluid Loading on Nausea and Protocol Completion (n=9)**

### 5.2.13 SUMMARY OF EFFICACY OF FLUID LOADING PROTOCOL

Contrary to the hypothesis, fluid loading was unsuccessful at restoring blood volume lost during 28h HDBR. Total blood volume, plasma volume and hematocrit were not significantly changed following 28h FL HDBR compared to their responses following 28h NFL HDBR. These data show that total blood volume and plasma volume were not fully restored by the fluid loading protocol after 28h HDBR.

A closer examination of changes in total blood volume during the fluid loading period showed that total blood volume progressively increased, illustrating that the fluid loading protocol had the desired effect, but was given insufficient time to restore normovolemia. Plasma volume increased similarly during the fluid load. The volume of the prescribed fluid was not yet absorbed into the vasculature after 2.5 hours. It was also noted that the volume of excreted urine was larger (though not significantly) during 28h FL HDBR compared to 28h NFL HDBR, indicating that fluid ingested during the fluid loading protocol was compensated for to keep fluid levels at the new baseline established by HDBR. It should also be reiterated that the sample size of the fluid loading analysis was reduced compared to the analysis of 28h NFL pre versus post testing.

Fluid loading was not fully successful at preventing the cardiovascular deconditioning that resulted from 28h HDBR. Heart rate responses remained increased following 28h HDBR with fluid loading, although they were lower than post NFL HDBR values. There were no significant changes in stroke

volume, cardiac output, TPR, or arterial compliance with fluid loading, although stroke volume did show an elevated response. Central venous pressure and inferior vena cava diameter were statistically unchanged compared the NFL Post test; however CVP did exhibit a non-significant elevation following fluid loading. The small sample size could have played a role in the statistical analysis of 28h FL HDBR versus 28h NFL HDBR post tests.

Splanchnic hemodynamics were not altered by fluid loading. Portal vein diameter remained reduced following 28h FL HDBR, indicating the splanchnic resistance remained increased following fluid loading compared to baseline. Splanchnic impedance was also not affected by fluid loading, and was unchanged compared to baseline, indicating that ingested volume may not have been absorbed into the extravascular space and that splanchnic pooling was not occurring.

Fluid loading had no effect on left-ventricular heart performance, arterial baroreflex sensitivity, or cardiopulmonary baroreflex. Contrary to expectations, these were not compromised by exposure to 28h bed-rest, and fluid loading provided no adverse effects.

This study was not designed to investigate fluid balance or the timing of water absorption into the vasculature, but analysis of water intake and urinary output suggests is was not excreted. Due to subjects' nausea and vomiting, it was suggested that some of the ingested water volume remained in the stomach.

One subject experienced presyncopal symptoms during LBNP testing following 28h FL HDBR, which resulted in premature termination of orthostatic testing. This individual's response was likely due to the effects of the fluid load itself rather than bed-rest induced cardiovascular deconditioning as the subject had vomited following the fluid loading protocol, and had previously completed numerous LBNP sessions successfully, even after 28h NFL HDBR. Four subjects experienced nausea and two subjects vomited during the fluid loading protocol, which is more evidence that this countermeasure may be unsuitable.

Contrary to the hypotheses, fluid loading was not effective at restoring lost blood volume resulting from 28h HDBR, nor did it restore any of the cardiovascular responses that experienced cardiovascular deconditioning following 28h HDBR.

## 5.3 CONTROL TESTS

Summaries of the raw data used in the analysis of control tests examining the effects of circadian rhythm, the fluid loading protocol, and HDBR are Appendices A3, A4, and A5 respectively. Summaries of statistical testing results for circadian rhythm, fluid loading protocol, and HDBR are provided in Appendices B3, B4, and B5.

### 5.3.1 EFFECT OF CIRCADIAN RHYTHM

Cardiovascular variables that were significantly altered by 28h NFL HDBR (blood volume, heart rate, CVP, and portal vein diameter) were examined to isolate the effects of circadian rhythm. First, pre and post 4h NFL data were compared to investigate if there were any circadian effects in the seated position. There were no significant differences between pre and post 4h S NFL responses regarding total blood volume, plasma volume, hematocrit, heart rate, central venous pressure, or portal vein diameter, indicating that there were no effects of circadian rhythm in the seated position on these variables.

To isolate the effects of HDBR from circadian rhythm, the 4h S NFL Post and 4h HDBR Post responses were also analyzed. This analysis found that there were significant differences in heart rate ($p<0.05$), central venous pressure ($p=0.02$), and portal vein diameter ($p=0.02$) due to HDBR, isolating the effects of HDBR from circadian rhythm on these variables. Heart rate ($p=0.01$) and portal vein diameter ($p=0.04$) also experienced significant interaction effects between HDBR and LBNP. Blood volume variables were not affected, as after 4 hours of HDBR, blood volume has returned to its baseline value after a transit

increase due to an influx of fluid from the extracellular space in the legs. After 4 hours of HDBR blood volume begins to reduce below baseline. Figures 5.3-1, 5.3-2, and 5.3-3 illustrate the heart rate, central venous pressure, and portal vein diameter responses during LBNP testing following 4h S NFL and 4h HDBR. The pre tests for 4h S NFL and 4h HDBR were also compared, revealing no significant differences in baseline conditions.

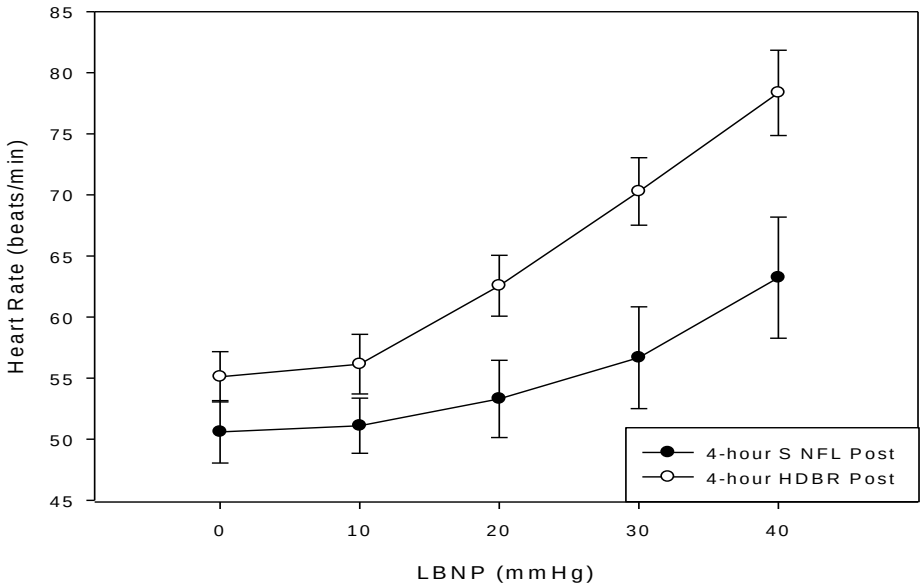


**Figure 5.3-1: Heart Rate Response during LBNP Testing following 4h S NFL and 4h HDBR (n=8); Post 4hSNFL/4hHDBR HDBR $p<0.05$, Post 4hSNFL/4hHDBR LBNP $p<0.05$, Post 4hSNFL/4hHDBR HDBR-LBNP interaction $p<0.05$**

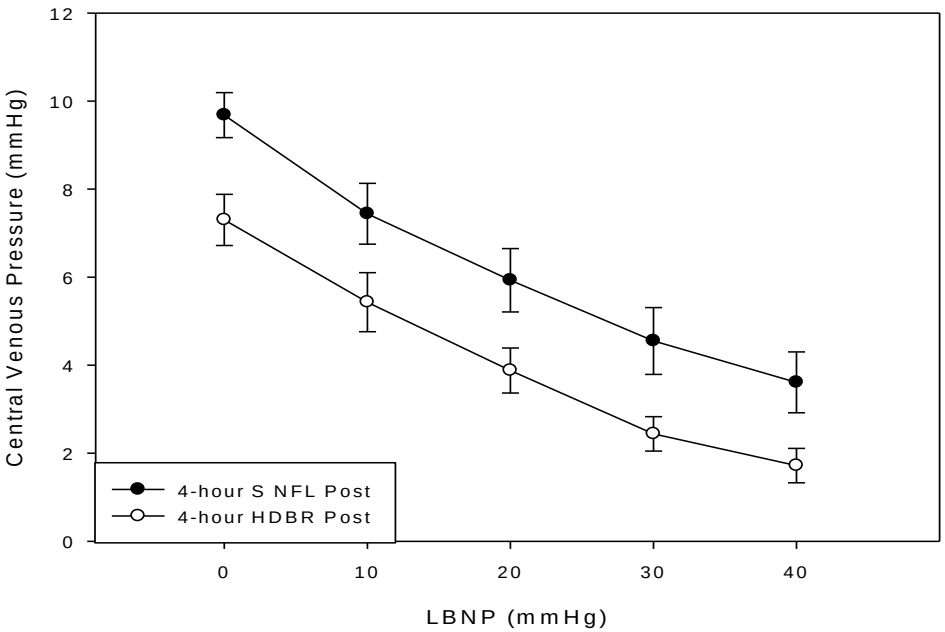


**Figure 5.3-2: Central Venous Pressure Response during LBNP Testing following 4h S NFL and 4h HDBR (n=7); Post 4hSNFL/4hHDBR HDBR p<0.05, Post 4hSNFL/4hHDBR LBNP p<0.05**

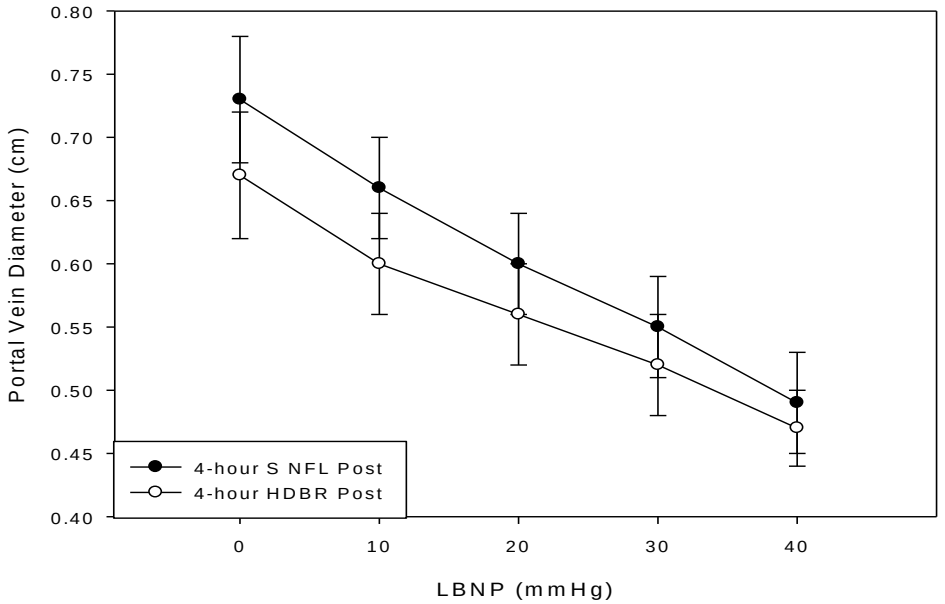


**Figure 5.3-3: Portal Vein Diameter Response during LBNP Testing following 4h S NFL and 4h HDBR (n=9); Post 4hSNFL/4hHDBR HDBR p<0.05, Post 4hSNFL/4hHDBR LBNP p<0.05, Post 4hSNFL/4hHDBR HDBR-LBNP interaction p<0.05**

### 5.3.2 EFFECT OF FLUID LOADING

To isolate the effects of fluid loading, a single 2-way ANOVA was performed comparing 4h S NFL Post and 4h S FL Post test responses (4h NFL Post/4h FL Post, LBNP) on the cardiovascular variables that were affected by 28h NFL HDBR, including total blood and plasma volume, hematocrit, heart rate, CVP, and portal vein diameter. This test concluded that fluid loading protocol did not elevate blood volume variables, or have a significant effect on heart rate, CVP, or portal vein diameter in the absence of HDBR. Responses from the 4h S NFL Post and 4h FL Post tests did not show any statistical differences. Data and statistics are summarized in Appendix A4 and B4. The pre tests for 4h S NFL and 4h S FL were also compared, revealing no significant differences in baseline conditions.

### 5.3.3 EFFECT OF HDBR

To isolate the effects of HDBR, a single 2-way ANOVA was performed comparing 4h S FL Post and 28h FL HDBR Post test responses (4h S FL Post/28h FL HDBR Post, LBNP). This analysis indicated that the changes in total blood volume, plasma volume, hematocrit, heart rate, and central venous pressure seen after 28h FL HDBR were the result of exposure to HDBR. Portal vein diameter was not significantly different between 4h S FL and 28h FL HDBR, indicating that the changes seen following 28h HDBR were not due to bed-rest. Figures 5.3-4 and 5.3-5 show the responses of heart rate and central venous pressure following 4h S FL and 28h FL HDBR. Data and statistics are summarized in Appendix A5 and B5. The pre tests for 4h S FL and 28h FL

HDBR were also compared, revealing no significant differences in baseline conditions.

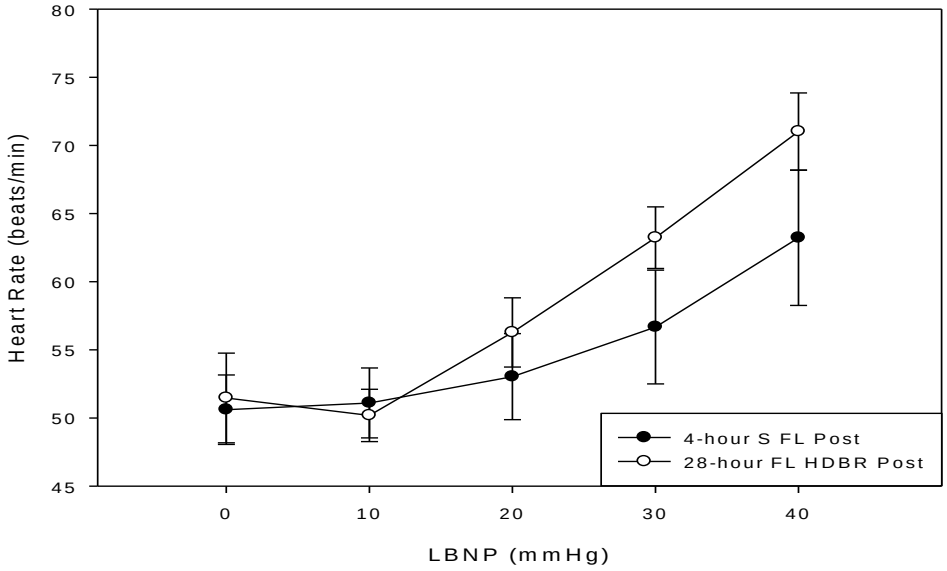


**Figure 5.3-4: Heart Rate Response during LBNP Testing following 4-hour S FL and 28h FL HDBR (n=8); Post 4hSFL/28hFL LBNP $p<0.05$, Post 4hSFL/28hFL HDBR-LBNP interaction $p<0.05$**

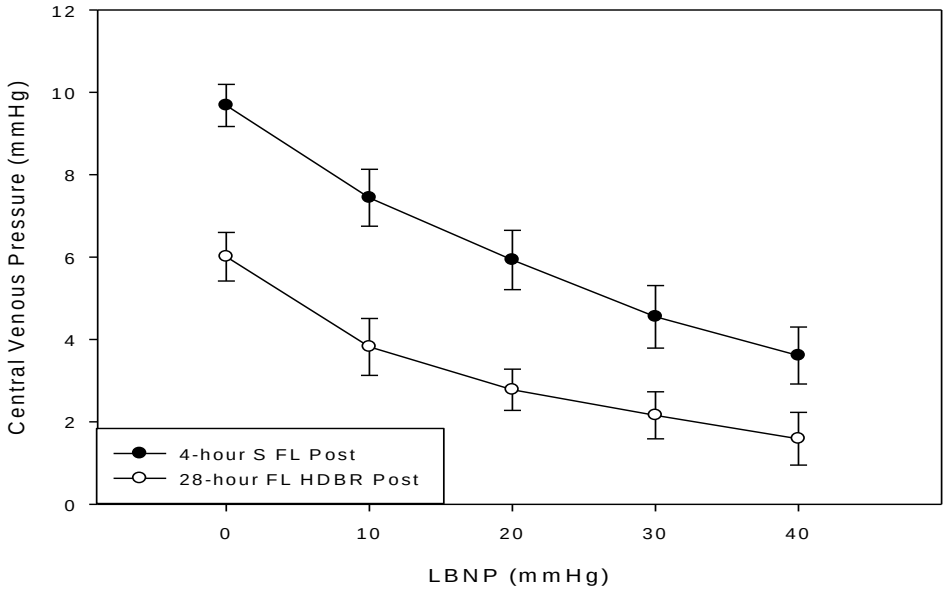


**Figure 5.3-5: Central Venous Pressure Response during LBNP Testing following 4-hour S FL and 28h FL HDBR (n=7); Post 4hSFL/28hFL LBNP $p<0.05$**

# 6 DISCUSSION

This study found that, as hypothesized, the cardiovascular system had begun the deconditioning process following exposure to 28h of bed-rest. Although deconditioning was not as extensive as examples documented after longer durations of bed-rest, evidence of cardiovascular deconditioning after 28h HDBR included significant hypovolemia, elevated heart rate response, and reductions in CVP. Mean arterial pressure was successfully maintained by all subjects during post bed-rest orthostatic testing. Systemic vascular resistance, left-ventricular function as well as arterial and cardiopulmonary baroreflex function were probably not due to hypovolemia as seen with this short-duration HDBR, but might have occurred with the deconditioning associated with longer bed rest studies. There was also no evidence of splanchnic or venous pooling after bed-28h bed-rest.

Contrary to the hypothesized outcome, this study also found that NASA's fluid loading protocol, as employed by Waters et al. (2004), was not fully effective at restoring the lost plasma volume resulting from 28h HDBR and the subsequent deconditioning. Fluid loading also did not fully prevent the elevated heart rate response and reductions in CVP that were seen after 28h NFL HDBR. In addition, the fluid loading protocol resulted in increased malaise and nausea. Two subjects vomited as a result of the nausea, and one of these subjects was unable to complete post bed-rest orthostatic challenge. None of these symptoms occurred after 28h NFL HDBR. It is interesting to note that during the 2.5 hour fluid loading period, total blood and plasma volume did

progressively increase. It is possible that fluid volume restoration may have been successful if the fluid loading protocol had been started earlier.

Analysis of the control models indicated that there was no effect of circadian rhythm on the cardiovascular responses to HDBR, nor was there an effect of fluid loading in the absence of HDBR on the cardiovascular variables that were altered following 28h HDBR (total blood volume, plasma volume, hematocrit, heart rate, central venous pressure, and portal vein diameter). It was also found that the responses seen in these variables following 28h FL HDBR were due to bed-rest itself, and not fluid loading.

## HYPOVOLEMIA

As hypothesized, the circulating blood volume was significantly diminished following exposure to 28h HDBR. This study showed that significant hypovolemia resulted after 28h HDBR, where plasma volume was reduced by 8%. The time course of plasma volume changes throughout the bed-rest period showed an initial slight increase in plasma volume, peaking after 2 to 3 hours of HDBR exposure. The increase was likely due to an influx of extracellular fluid into the vasculature of the lower extremities upon assuming the head-down position. This study showed a small peak increase of 0.9% after 3 hours of bed-rest (Hour 0= 3293.6ml, Hour 3= 3321.7ml). After peaking, plasma volume reduced progressively as the cephlad fluid shift caused removal of perceived 'excess' fluid volume by the kidneys. After 4 hours, plasma volume had returned to its baseline value (Hour 0 = 3293.6 ml, Hour 4=3292.5 ml). This

evidence is supported by the 4-hour bed-rest study by Fischer et al, where total blood and plasma volume were unchanged between pre and post measurements (Fischer et al. 2007). Blood volume progressively reduced after returning to baseline until reaching a steady-state hypovolemic state. After 8 hours, plasma volume was reduced by 8.5%. After 28 hours of bed-rest, plasma volume was reduced by 7.8% compared to baseline. These small variations in plasma volume did not statistically differ from each other and were likely due to measurement error or natural variation. These data illustrate that the onset of hypovolemia occurs quickly following bed-rest exposure and that the reductions in volume level off within the early hours of bed-rest.

Other studies have found similar reductions in plasma volume after longer durations of bed-rest. Waters found an 8% reduction in plasma volume and a 5% reduction in total blood volume after 12 days of HDBR (Waters 2004). Similarly, Vernikos and Convertino found that after 7 days of bed-rest, plasma volume was reduced by 12% (Vernikos and Convertino 1994). After 2 weeks, Iwasaki and colleagues saw a reduction of 12% in plasma volume (Iwasaki et al. 2000). Although Bestle and colleagues did not measure plasma volume directly during their 9-day bed-rest study, they noted that weight significantly decreased by 1.4% (Bestle et al 2001). This is comparable to the reduction in weight seen of 1.2% in this study. Together, this evidence describes an acute reduction in plasma volume in the hours immediately following bed-rest exposure, and the leveling off at a hypovolemic plateau within the first days of exposure.

## ORTHOSTATIC HYPOTENSION

It was hypothesized that instances of presyncope would arise following 28h HDBR. The current study was not designed to test orthostatic tolerance, but it was expected that some individuals might have difficulty maintaining arterial blood pressure during relatively mild orthostatic stress based on observations from previous 4h bed-rest studies (Butler et al. 1991, Fischer et al. 2007). There were no observations of presyncopal responses or orthostatic hypotension during the relatively mild levels of LBNP employed after the 28h NFL HDBR. Subjects were able to maintain arterial blood pressure during a maximum orthostatic stress of -40 mmHg, and there were no reports of dizziness or nausea. Butler and colleagues (1991) observed that 5 of the 8 subjects could not complete a 10 minute tilt test after 4h HDBR, and Fischer and colleagues (2007) observed that 3 of 11 subjects experienced presyncope during LBNP testing following 4h HDBR. In general, susceptibility to orthostatic hypotension appears to increase with time likely because cardiovascular deconditioning increases with time. Bed-rest studies of longer duration had higher incidences of orthostatic hypotension. In Vernikos and Convertino's 7 day HDBR study, 4 of 5 subjects showed presyncopal symptoms. However, Fischer and colleagues saw 3 of 11 subjects experience presyncope following only 4 hours of bed-rest. Susceptibility to orthostatic hypotension is not only dependent on the duration of bed-rest exposure but on the integrated effects of the individual elements that comprise the cardiovascular system.

## CARDIOVASCULAR DECONDITIONING

It was hypothesized that cardiovascular deconditioning would result after 28h HDBR, manifested during orthostatic testing as elevated tachycardia, reduced stroke volume, reduced cardiac output, and reduced central venous pressure, as well as an increased vasoconstriction response and reduction of the vasoconstrictor reserve. As hypothesized, evidence of cardiovascular deconditioning was apparent following 28h bed-rest in the form of an elevated heart rate response during orthostatic stress. However, this was not accompanied by a significant change in stroke volume despite a significant reduction in CVP (although there was a trend towards a reduction of stroke volume). Cardiac output and TPR also remained unchanged after 28h NFL HDBR. This evidence indicates that there are different stages of cardiovascular deconditioning, with heart rate and central venous pressure being the first cardiovascular variables to be affected. Reductions in stroke volume, cardiac output and TPR follow sequentially as deconditioning becomes more severe. Vernikos and Convertino (1994) observed deconditioning of all these responses after 7 days of HDBR.

This study's hypotheses included a statement that vasoconstrictor reserve would be diminished following 28h HDBR. It is not known if vasoconstrictor reserve was affected by 28h HDBR. There was no change in baseline TPR, or in the TPR response to orthostatic stress. However, progressive LBNP testing from 0 mmHg to -40 mmHg was insufficient to

produce a plateau in TPR before and after 28h HDBR, therefore no information on vasoconstrictor reserve was obtained.

In the current study, the small reductions in stroke volume during LBNP were not significant, although the increase in heart rate suggests a cardiovascular compensation. These data can be put in the perspective of an 8% reduction in plasma volume that was similar to several longer duration studies that reported significant changes in stroke volume, cardiac output, and total peripheral resistance (Vernikos and Convertino 1994, Waters 2005, Zhang 2001). From the current study, it appears that HDBR-induced hypovolemia is not the only mechanism for cardiovascular deconditioning, contrary to the hypotheses of many studies (Convertino et al 1990, Iwasaki et al. 2000, Sawka et al. 1999, Steiner et al. 2007, Waters et al. 2005). It is possible that the changes in heart rate and central venous pressure resulting from short duration bed-rest are primarily due to hypovolemia, although there is no conclusive evidence in this study to distinguish between the effects of short term bed-rest and hypovolemia. It is likely that deconditioning of cardiovascular variables such as stroke volume, cardiac output, and systemic vascular resistance is a stronger contributor to onset of syncope after bed-rest than hypovolemia. This conclusion is supported by data from this study, since no instances of syncope arose despite significant hypovolemia after 28h HDBR. It is also supported by Fischer et al, who saw 3 of 11 subjects succumb to syncope while there was no change in plasma volume (Fischer et al. 2007).

## SPLANCHNIC BLOOD FLOW

It was hypothesized that differential vasoconstriction would occur following 28h HDBR, leading to splanchnic pooling and impacting blood pressure maintenance; TPR would be elevated at baseline, whereas splanchnic vasoconstriction would be diminished. Contrary to the results seen by Fischer after four hours of bed-rest (Fischer et al. 2007), this study showed that splanchnic vasoconstriction was increased following 28h NFL HDBR.

Splanchnic vasoconstriction may play an important role in maintaining blood pressure. As the cardiovascular system becomes stressed, sympathetic nervous system activation causes systemic vasoconstriction that can facilitate return of blood to the heart and act with the increased heart rate due primarily to parasympathetic withdrawal to maintain cardiac output. Sympathetic activation may also cause changes in splanchnic vasoconstriction. Splanchnic vasoconstriction increases with orthostatic stress, reducing the blood volume in the splanchnic areas and directing blood to the heart to protect blood pressure. It would be expected that the splanchnic vasoconstriction response would be increased after bed-rest in order to maintain blood pressure and support orthostasis and also due to hypovolemia. However, research has indicated that there can be differential responses in the splanchnic blood flow with orthostasis. Stewart and colleagues found that a subset of patients suffering from postural tachycardia syndrome (POTS) exhibited increased splanchnic blood flow and splanchnic pooling during orthostatic stress in spite of excessive peripheral vasoconstriction (Stewart et al. 2005). Splanchnic hyperemia has also been seen

following bed-rest studies. Fischer and colleagues found evidence of increased splanchnic pooling in the form of increased portal vein diameter and portal vein blood flow following a bed-rest period as short as 4-hours (Fischer et al. 2007). Arbeille and colleagues found evidence of both unchanged and reduced splanchnic vasoconstriction responses following 60 and 85 days of bed-rest, in spite of significant hypovolemia (Arbeille et al 2008, Arbeille et al 1005).

This study yielded no evidence of splanchnic pooling after 28 hours of HDBR. Portal vein diameter was significantly reduced after bed-rest, indicating reduced splanchnic blood flow and providing evidence that the splanchnic vasculature was directing blood to the systemic vasculature where it is needed. Splanchnic impedance was unchanged following 28h bed-rest, providing a further indication that splanchnic pooling was not occurring. It must also be restated that no subjects experienced presyncopal symptoms in their natural response to 28h HDBR. This study's 4 hour HDBR control tests showed that after 4 hours of exposure the subjects had no change in splanchnic blood flow as portal vein diameter and splanchnic impedance were unchanged. These findings contrast those of Fischer's. Despite a shorter duration of exposure to HDBR, Fischer and colleagues noted 3 instances of presyncope, whereas in this study, subjects were exposed to 24 additional hours of bed-rest, but no presyncopal symptoms arose (Fischer et al 2007). It is interesting to note that the two studies were performed under similar laboratory conditions (testing protocol, LBNP stress, food intake and timing) and subject attributes (male, age range, fitness level, LBNP testing experience). This evidence supports previous

conclusions that increased splanchnic blood flow is detrimental to post bed-rest orthostatic testing success. It is expected that within the population, individuals exhibit a wide range of splanchnic vasoconstriction responses to orthostasis. While the subjects in this study demonstrated the ability to reduce their splanchnic blood flow and volume during orthostatic stress, POTS patients represent the other end of the spectrum. Arbeille and colleagues found that the splanchnic vasoconstriction response was a differentiating feature between finishers and nonfinishers of orthostatic testing following 60 days of HDBR. A reduced vasoconstriction response after bed-rest was associated with orthostatic intolerance (Arbeille et al. 2008). When considered with Fischer's data, this study provides further evidence of the different physiologies exhibited in the general population.

Abnormal splanchnic vasoconstriction may be another mechanism of the cardiovascular system that suffers deterioration with progressive exposure to bed-rest. It is possible that the proportional increase in splanchnic blood flow exhibited by subjects after 60 and 85 days of bed-rest is caused by prolonged exposure to reduced cardiovascular stress rather than their innate physiology (Arbeille et al 2005, Arbeille et al 2008). Systemic vasoconstriction is thought to respond to reductions in aortic pulse pressure, whereas splanchnic vascular resistance responds to reductions in right atrial pressure. This indicates that the splanchnic vascular bed may be more sensitive to signaling from the arterial baroreflex than from the cardiopulmonary baroreflex (Rowell et al. 1970). This explains the differential responses of the two vascular beds and that splanchnic

deconditioning likely begins after changes in systemic vasoconstriction since aortic pulse pressure reductions occur later in the deconditioning process than reductions in venous return. It must be noted that in this study pulse pressure and arterial baroreflex were not affected after 28 hours of HDBR, and splanchnic vasoconstriction was significantly increased. It is also possible that splanchnic resistance was increased as a result of bed-rest induced hypovolemia, although this cannot be definitively stated.

## ARTERIAL BAROREFLEX

It was hypothesized that arterial baroreflex sensitivity would be attenuated after 28 hours of HDBR, which would be reflected in reduced gain and increased phase 'lag' results of transfer function analysis performed on SBP and R-R Interval signals (described in Section 4.9.9). However, transfer function analysis revealed no differences in gain or phase responses between pre and post 28h NFL HDBR. This illustrates that arterial baroreflex is not altered during bed-rest sessions as short as 28 hours, meaning that the change in arterial pressure needed to elicit a given change in heart rate is not changed within short durations of bed-rest exposure.

When integrating these results into the existing body of data, it can be concluded that attenuation of arterial baroreflex is a progressive process that increases with longer bed-rest exposure. Evidence of arterial baroreflex attenuation was not apparent after 28h bed-rest, but there is evidence of such deconditioning after longer bed-rest durations. Evidence accumulated from a variety of methods, include neck suction and transfer function analysis,

illustrate that prolonged bed-rest has a negative impact baroreflex function. Exposure to 7 days, 14 days, 28 days, 30 days, 60 and 120 days of bed-rest all exhibited significant arterial baroreflex attenuation (Vernikos and Convertino 1994, Iwasaki et al 2000, Hughson et al. 1994, Convertino et al 1990, Linnarsson et al. 2006). Since many diverse methods have been used to measure these parameters, it is not possible to compare these data sets regarding the relationship between arterial deconditioning and bed-rest duration. However, a study performed by Convertino and colleagues found that arterial baroreflex attenuated progressively during 30 days of bed-rest. Reductions initially became apparent after day 15 and progressively worsened until day 25 (Convertino et al 1990). The results of this study fit in with these data, supporting the hypothesis that arterial baroreflex attenuation is not a result of acute bed-rest, rather the degree of attenuation increases with the duration of exposure.

Hypovolemia has been hypothesized to be the principal cause of arterial baroreflex attenuation that arises following bed-rest exposure. There are extensive data relating altered arterial baroreflex function to acute hypovolemic conditions, resulting from hemorrhage or induced hypovolemia (Triedman 1993, Saitoh et al. 2008, Blake 2000, Sopher et al. 1990, Wang et al. 1990). Since hypovolemia is a known consequence of bed-rest, Iwasaki and colleagues (2000) hypothesized that the attenuation in arterial baroreflex function seen after bed-rest is due to hypovolemia rather than cardiovascular deconditioning. Their study found that arterial baroreflex function reduced similarly after 2

weeks of bed-rest and acute hypovolemia by furosemide. Bed-rest and acute hypovolemia both resulted in reductions in plasma volume of 11-12% providing an indication that hypovolemia may be responsible for baroreflex attenuation (Iwasaki et al 2000). However, the results of this study suggest that hypovolemia is not the principal mechanism in bed-rest related baroreflex attenuation. Although plasma volume was reduced by 8% with 28 hours of bed-rest, there were no significant alterations in the gain and phase results of the transfer function analysis of R-R interval and SBP signals in the MF and HF regions attributed to arterial baroreflex regulation. Therefore, significant reductions of blood volume do not lead to arterial baroreflex attenuation during periods of 28 hour bed-rest. Convertino and colleagues (1990) found that during a 30 day bed-rest study, the changes in plasma volume did not occur in parallel with the attenuation of arterial baroreflex. A 15% reduction in plasma volume stabilized within the first days of bed-rest. However, the arterial baroreflex response only began to decline after 12 days of bed-rest. Taken together, these data indicate that that although plasma volume may play a role in baroreflex attenuation, it is not the sole or primary contributor to the attenuation seen after bed-rest. Instead hypovolemia may be a secondary factor supporting the cardiovascular deconditioning that leads to reduction in arterial baroreflex function. Lastly, it is suggested that hypovolemia resulting from bed-rest and acute hypovolemia resulting from hemorrhage or medication likely represent very different physiological conditions.

The causes of progressive arterial baroreflex attenuation during bed-rest may be due to peripheral resetting of the baroreflex response. Peripheral resetting is defined by Chapleau and colleagues (1989) as a 'shifting of the pressure-baroreceptor activity curve in the direction of the prevailing arterial blood pressure'. This describes the alteration of baroreceptor function due to adaptation of the mechanical properties of the conduit arteries to prolonged exposure to a hypertensive or hypotensive state. Arterial vascular compliance is a principal determinant in the magnitude of arterial deformation experienced during transmission of a pressure pulse, and therefore has profound effect on the degree of baroreflex stimulation (Lanfranchi and Somers 2002). Arterial vascular compliance can be defined as the change in stroke volume over the change in pulse pressure (Chemla et al. 1998). It has been shown that hypertensive subjects generally have a lower arterial compliance than normotensive individuals, and this reduction in compliance occurs concurrently with reductions arterial baroreflex sensitivity (Dart et al. 1993, O'Rourke et al. 1990, Pagani et al. 1988, Somers et al. 1991). Arterial compliance has been increased in both hypertensive and normotensive subjects through moderate exercise training, and arterial baroreflex sensitivity restored, showing that not only are arterial baroreflex and arterial compliance related, but arterial compliance adapts to the level of cardiovascular stress imposed on the body and this in turn affects baroreflex function.

Arterial compliance may play a similar role in bed-rest related arterial baroreflex attenuation since reductions in arterial vascular compliance increase

longer duration exposure. Once bed-rest induced cardiovascular deconditioning has led to reductions in stroke volume, arterial vascular compliance is consequently reduced. This may have the same implications reductions in arterial compliance have on hypertensive individuals and be the cause of arterial baroreflex attenuation by reducing the ability for the baroreceptors to detect reductions in blood pressure. This may explain why arterial baroreflex reductions occur long after hypovolemia has been established. Rather than resulting from hypovolemia, arterial baroreflex occurs when stroke volume becomes diminished. Following 28 hours of bed-rest there were no reductions in stroke volume, nor reductions in arterial vascular compliance, and consequently there was no evidence of baroreflex attenuation. The bed-rest studies previously mentioned provided evidence of deteriorating arterial baroreflex as well as reductions in stroke volume after 7 days, 28 days, 30 days, 60 and 120 days of bed-rest (Iwasaki et al 2000, Hughson et al. 1994, Convertino et al 1990, Linnarsson et al. 2006).

## LEFT VENTRICULAR PERFORMANCE

The results of this study indicate that, contrary to the hypothesized outcome, there were no changes in left-ventricular heart function after 28 hours of HDBR despite hypovolemia and reduced central venous pressure. There were no changes in fractional shortening and ejection fraction when comparing pre and post data.

Previous studies have shown that the heart adapts to changes in loading conditions when exposed to long periods of bed-rest. The acute adaptations to HDBR include hypovolemia, reductions in central venous pressure, and a prominent drop in left ventricular filling. Researchers have also seen evidence of diminished cardiac function after bed-rest. Takenaka and colleagues saw reductions in left ventricular dimensions following 20 days of bed-rest, which led to reductions in stroke volume and cardiac output, and orthostatic hypotension (Takenaka 1997). Levine and colleagues showed that there was evidence of LV mass reduction after only 12 as well as after 18 days of bed-rest (Levine et al. 1997, Levine et al.1999). Following 60 day of HDBR, Dorfman and colleagues noted reductions in left ventricular volumes and masses (Dorfman et al. 2007).

The data from this study indicate that despite reduced cardiac filling, left ventricular function is not affected within bed-rest exposure as short as 28 hours. Rather, it is thought that that cardiac remodeling occurs after significant exposure to bed-rest (at least 12 days) and the corresponding chronic reduction in cardiac filling (Dorfman et al. 2007). Perhonen and colleagues saw significant and progressive reduction in cardiac function in sedentary subjects who underwent 6 and 12 days of bed-rest (Perhonen et al 2001). Reductions in left ventricular end-diastolic volume stabilized after 2 weeks but there was a 5% reduction in left ventricular mass following 2 weeks of bed-rest, 8% reduction after 6 weeks, and 16% reduction after 12 weeks (Perhonen et al. 2001).

The data from this study also support the hypothesis that cardiac atrophy is a progressive process since no changes in left ventricular function were seen during only 28 hours of HDBR despite a significant reduction in plasma volume. The heart is able to function normally under reduced myocardial load resulting from hypovolemia. Instead, chronic hypovolemic results in a sustained reduction in volume loading of the heart, which leads to slow but progressive impairment of cardiac function.

This hypothesis is also supported by data from Dorfman and colleagues who studied women who exercised and women who underwent a natural response during 60 days of bed-rest. Interestingly, the women who exercised did not experience reductions in left ventricular volumes and the length of the major axis was preserved, which contrasted the results of the women who did not exercise (Dorfman et al. 2007). These data indicate that diminished cardiac function can be prevented by adequate cardiovascular stress during bed-rest. Therefore, chronic exposure to hypovolemia and lack of cardiovascular stress is likely the cause of heart dysfunction following prolonged bed-rest.

## CARDIOPULMONARY BAROREFLEX

It was hypothesized that following 28h HDBR, the cardiopulmonary baroreflex would be elevated, illustrated by an increased forearm vascular resistance response throughout orthostatic testing. However, there was no evidence of an altered cardiopulmonary baroreflex response after 28h NFL HDBR. Forearm vascular resistance data was not used for the analysis due to the presence of

measurement error. However there were no changes in supine brachial diameter or brachial blood flow, nor was the brachial blood flow velocity response changed after bed-rest. This indicated that the magnitude and set-point of the cardiopulmonary baroreflex response was unchanged.

These data fit well with the systemic vasoconstriction data discussed earlier, indicating the vasoconstriction response was unchanged by bed-rest, and is a further indication that systemic vasoconstriction is not primarily a result of bed-rest induced hypovolemia. This is contrary to the results of Vernikos and Convertino, who determined that the cardiopulmonary baroreflex was increased following 7 days of bed-rest, attributing the elevation to hypovolemia (Vernikos and Convertino 1994).

## EFFECT OF FLUID LOADING PROTOCOL

This study found that, contrary to the hypothesized outcome, fluid loading was ineffective at both restoring blood volume (its primary purpose) and preventing cardiovascular deconditioning (its secondary purpose). Total blood and plasma volume were not significantly different following bed-rest with fluid load compared to their natural response to bed-rest. After the fluid loading protocol, plasma volume remained reduced by 9.8 %.

Fluid loading did not alter the physiological responses to bed-rest compared to the natural responses to 28h bed-rest. Completion of fluid loading following 28 hours HDBR still resulted in the same evidence of cardiovascular deconditioning as seen following 28h bed-rest: reductions in mean arterial

pressure during post bed-rest orthostatic testing, orthostatic tachycardia, and reduced central venous pressure. Cardiovascular variables that were unchanged by bed-rest remained unchanged following bed-rest with fluid load. These included stroke volume, cardiac output, systemic vasoconstriction, splanchnic hemodynamics, and left ventricular performance. Taken together, this illustrates that the cardiovascular deconditioning resulting from 28h bed-rest was not prevented by the fluid loading protocol. In addition, the onset of nausea in the bed-rest position may be evidence that water remained in the stomach and was not absorbed in to the vasculature.

Fluid volume replacement is a practice commonly used in hospitals to reduce the degree of vasoconstriction, heart rate and renin-angiotensin-aldosterone system responses required to maintain blood pressure. It was adopted by the space program to support blood pressure regulation during re-entry, a period where astronauts must be capable of responding quickly. Two studies have previously investigated the efficacy of NASA's fluid loading protocol, however this is the first study that compares the subjects' natural response following bed-rest to their response following fluid loading, while using NASA's prescription of salt and water.

These findings counter the results of Water's study, which found no evidence of cardiovascular deconditioning following 12 days of bed-rest with fluid loading. However, Waters' results should be viewed with caution since the subject's natural cardiovascular response to orthostatic stress following bed-rest, without the effects of fluid loading, was not measured. Cardiovascular

responses (including blood pressure, stoke volume, cardiac output, CVP, and ejection fraction) were only measured on day 13 following completion of the fluid loading protocol. Therefore, the subjects studied by Waters and colleagues may not have been affected by bed-rest to the degree that cardiovascular deconditioning would be apparent in the variables measured, and fluid loading may not have 'restored' deconditioned variables.

The data presented in this paper compliment the results of Vernikos and Convertino's study in which significant deconditioning was found after 7 days of HDBR. Vernikos and Convertino's data showed a reduction of mean arterial pressure during orthostatic stress following bed-rest. Stroke volume and central venous pressure were reduced, heart rate and vasoconstriction responses were elevated, as well as attenuation of the carotid baroreflex and up-regulation of the cardiopulmonary baroreflex. Blood volume was not restored by the fluid load, and significant evidence of cardiovascular deconditioning was present.

One of the most interesting results was that fluid loading increased the incidence of presyncopal conditions in both the 28h FL test as well as the 4-hour fluid loading control test. Some subjects experienced feelings of nausea and could not complete the fluid loading protocol. Within this group of subjects, a smaller portion experienced vomiting and succumbed to syncope testing. This is the strongest evidence against the use of salt and water as a fluid volume replacement strategy. Not only does the protocol fail to replace volume or support blood pressure regulation, it can also illicit symptoms of malaise and cause presyncopal symptoms in a subject who completed orthostatic testing

after 28 hours of bed-rest successfully. Once again, it must be reiterated that subjects who vomited or did not complete the fluid loading protocol were not included in the statistical analysis investigating fluid loading, however their response to the fluid loading protocol must be highlighted.

In contrast to Water's study (Waters et al. 2004), where blood volume was restored by fluid loading, this study did not result in full restoration of blood volume after the bed-rest period. The protocol used in this study, as well as that of Waters, followed NASA's fluid loading prescription of oral consumption of 1 g salt tablet per 125 ml water with a total volume of 15ml/kg within a 2 hour period prior to orthostatic challenge. However, where Waters fully restored the 8% plasma volume loss after 12 days, our study failed to restore the 12% plasma volume loss that resulted after 28 hours. The reasons for this may include onset of nausea and vomiting experienced in some subjects (such reactions are not mentioned by Waters, although it is important to reiterate here that subjects who did not complete the fluid loading protocol were not included in the statistical analysis of fluid loading efficacy), as well as the time course of salt and water uptake into the vasculature. Urine output was not significantly different between the 28h NFL and 28h FL tests, although there appeared to be more urine excreted during FL testing compared to NFL testing. No information on extracellular fluid volume is known, so filtration into the extravascular space is unknown. However, due to the prevalence of nausea in the head-down position during the fluid loading period, it is suggested that a portion ingested salt and water remained in the stomach and was not yet fully

absorbed into the vasculature. In this study, the fluid loading prescription was separated into increments of one salt-filled capsule of approximately 1 gram and the corresponding water intake cumulating into approximately 10 tablets taken in twelve minute increments across the two hour fluid loading period (with small deviations depending on individual subject's weight). Neither Vernikos and Convertino nor Waters and describe how their fluid loading prescription was delivered throughout the 2 hour period. The timeline for consumption may also play a significant role in the physiological effects of fluid loading in addition to the prescription of salt and water.

## ROLE OF HYPOVOLEMIA IN CARDIOVASCULAR DECONDITIONING

The role of hypovolemia in cardiovascular deconditioning and orthostatic hypotension has been a topic of debate. Researchers (Iwasaki et al. 2000) have hypothesized that hypovolemia resulting from bed-rest is the primary cause of deconditioning, causing reductions in stroke volume, increased vasoconstriction response, affecting left-ventricular function by altered pre-load and ventricular filling. However, data from this study and others (Vernikos and Convertino 1994) support the argument that cardiovascular deconditioning occurs independently from bed-rest induced hypovolemia. For example, cardiovascular variables such as stroke volume, TPR, and ejection fraction were unchanged following 28 hours HDBR, despite a 12% plasma volume loss. Aortic baroreflex was also unaffected by 28h HDBR. Waters and colleagues (2005) data also provide supporting evidence. In their study, the 8% reduction in fluid volume after 12 days was fully restored; however heart rate, systemic

vasoconstriction, stroke volume, cardiac output and central venous pressure responses were significantly impacted. Taken together, it is suggested that hypovolemia is an acute reaction to HDBR, but cardiovascular deconditioning is a progressive process that worsens with increasing bed-rest exposure and is not a direct result of hypovolemia. If in fact cardiovascular deconditioning is independent of bed-rest induced hypovolemia, the rationale of seeking a countermeasure to restore fluid volume in order to prevent orthostatic hypotension should be re-considered.

# 7 LIMITATIONS

This study was limited by several factors. Most importantly, it should be mentioned that the results from this study are directly applicable to men undergoing bed-rest in a terrestrial environment. Research has shown that the responses of astronauts returning from spaceflight may differ from those of bed-rest due to additional neurovestibular problems and other factors. Fluid dynamics differ greatly between terrestrial bed-rest and space, since gravity is still acting on the body during bed-rest. Additionally, women may exhibit different responses due to their menstrual cycle. Therefore additional research is needed to apply these results to women and astronauts.

The subject pool for this study was small. Only nine subjects were tested due to the time demands of performing 5 testing protocols. It was a demanding time commitment as two of the protocols required over 30 consecutive hours of laboratory time, and the remaining three required 8 hours. Test scheduling was limited by subjects´ personal schedules, laboratory and equipment scheduling, as well as maintaining adequate recovery time between testing session to prevent confounding effects. P values are presented wherever possible due to the small subject pool as recommended by Hathaway and colleagues (Hathaway et al. 1997).

It must be made clear that this study was not designed to measure the subjects‘ orthostatic tolerance. Rather, this study was designed to examine the relative changes in cardiovascular responses following each protocol and

quantify if cardiovascular deconditioning had occurred. Tolerance is determined by standardized protocols such as a tilt tests with LBNP as performed in the WISE study (Arbeille et al. 2005). Since this study analyzed each subject's physiological response during a progressive LBNP test, and did not perform a standardized test, conclusions cannot be made defining a subject as orthostatically tolerant or intolerant.

It must also be mentioned that this study focused on testing the efficacy of NASA's fluid loading protocol of water intake coupled with salt tablets. There are other similar methods thought to increase blood volume prior to re-entry, such as ingestion of soup broth which is commonly used by the Russian space program. While similar in principal, bouillon contains additional electrolytes and potentially glucose, which likely result in different digestion dynamics than NASA's protocol. Additionally, the taste and smell of bouillion would likely prevent feelings of nausea exhibited by salt and water ingestion. Therefore, these results may not be applicable to the broad methodology of fluid loading to restore normovolemia following space flight. Additional work must be done to examine other methods.

Another major limitation concerning the presented data is that the study was not designed to focus on only the cardiovascular variables presented in this paper. The data collected were part of a large series of bed-rest studies that were the data source for 3 Masters theses and one PhD thesis. In addition to the cardiovascular variables presented here, data collection included blood analysis, urine, diet analysis, and cardiac impedance. Therefore, the protocol planning

involved compromises to allow for all the desired datasets being reliably collected. For example, arterial baroreflex data required continuous R-R Interval and SBP data sets to be collected, and the quality of analysis was dependant on the length of the data set. At the same time, the accuracy of Finometer measurements of BP and Q was essential, as it was the basis for the collective research. Measurement accuracy required Physiocal, a Finometer calibration signal, to be enabled to periodically measure finger blood vessel diameter. Changes in vessel diameter occur when adapting to orthostatic challenge. However, Physiocal caused the datasets to be non-continuous as blood pressure is not measured during the 3 beats it used to measure vessel diameter. For these reasons Physiocal remained on during the 28h protocol for 1 minute and 15 seconds following an increase in orthostatic stress, and also was turned on 15 seconds prior to a change in LBNP. Arterial baroreflex data were collected in the remaining 3 minutes and 30 seconds at 0, -20, and -40 mmHg. Physiocal remained enabled throughout 4-hour bed-rest orthostatic testing protocols due to the wishes of another researcher using data from those protocols. Also, use of a metronome at 0.15 Hz to regulate breathing had been proposed to maintain a high power at 0.15 Hz and create a defined respiration frequency. Since there was concern this would alter other physiological responses that were the focus of other members of the research group, especially at higher levels of orthostatic stress when most subjects naturally increase their breathing frequency, a metronome was not used.

It must be noted that 6 of the 9 subjects had never participated in a bed-rest study before. Since it was often not possible to subject them to LBNP testing prior to their participation in the study, the order the 5 protocols were performed in was randomized to reduce the physiological stress of a new experience. We were not able to adapt our subjects to a specific circadian rhythm before they participated in the study. Each testing session began at 7 am, which was often not the time the subject would normally wake up at. Therefore their circadian rhythm was disrupted during each test. However, since each subject participated in each protocol, and their natural sleeping rhythm was disrupted similarly during each of the 5 protocols, it is thought that these effects will be similar across all the testing protocols. Due to scheduling limitations, some subjects participated in testing over a period of 6 months. It is a possibility that their body composition changed over that time period, however an examination of their masses did not reveal significant changes, nor did their levels of physical activity change.

Diet would have been ideally controlled in the days leading up to each test in order to know specific salt intakes. However this was not possible since the study lacked the funding needed to provide food to subjects in the days leading up to the test.

Another variable that was considered during testing was ingested water during the bed-rest period. Subjects' fluid intakes were determined from their thirst on their first testing day, with subsequent tests allowing them that amount with a 25% buffer. Ingested water varied greatly between subjects. Subjects

would often experience a different thirst response during subsequent tests and ask for the amount to be reduced or increased, though it was maintained as consistently as possible. It is debatable whether subjects should have been allowed to drink ad libitum, or had their intake regulated for comparable conditions. Nevertheless, analysis illustrated that the variation in water intake across the subject pool was not related to performance during orthostatic testing following bed-rest.

To support fluid volume measurements, fluid intake and urine output, it would have been useful to gauge water output through respiration, perspiration, and defecation. However, due to laboratory constraints, it was not possible to collect these data. Thus we can only provide a rough indication of fluid distribution following fluid loading and cannot be sure how much fluid was absorbed into the extracellular (of particular interest) and intracellular fluid volumes.

Nausea was experienced by several subjects during the fluid loading protocol. This must be taken into consideration when examining their responses to orthostatic stress following bed-rest. Two subjects vomited, which impacted their blood volumes. Therefore, their responses were not included in the analysis of fluid volume efficacy. Feelings of malaise associated with nausea may have contributed to increased heart rate response and reductions in blood pressure.

There was measurement error present in some measurement techniques. Measurements of cardiac output using two different methods were not similar (aortic blood flow velocity by the Doppler ultrasound versus Finometer estimation). Finometer estimation of cardiac output was used in the analyses since it followed trends exhibited by previous bed-rest research and also resulted in statistically similar pre test responses. Doppler ultrasound may have resulted in erroneous aortic blood flow velocity data due to changes in the ascending aorta anatomy during increasing orthostatic stress. Figures D-1, D-2, D-3, and D-4 in Appendix D illustrate the stroke volume, cardiac output, total peripheral resistance and arterial compliance responses pre and post 28h NFL HDBR using Doppler Ultrasound.

As described earlier, portal vein blood flow velocity data did not follow the typical trend recorded in previous studies with LBNP testing. While this may have been physiological, there are also sources of measurement error in the ultrasound technique as probe placement during velocity measurement is not visualized. When examining an individual's response at a specific level, averages were taken of up to 9 ultrasound images of portal blood flow. Often these samples were similar; however there were cases where a subject exhibited a wide range of flows at a specific level of LBNP. The different response can be partly attributed to respiration shifting the vessel out of view during measurement, affecting the overall average during the window. Figure 7-1 provides an image without respiration on the left and with respiration on the right, with portal blood flow recorded for each. These images are taken from the

same subject at the same level of LBNP (-20 mmHg). It was not uncommon, especially at high levels of LBNP, for respiration to affect every measurement due to an increased breathing frequency. For this reason, regions of the image where portal blood flow was affected by respiration were not included in the analysis. There were also cases where the portal flow averaged over the entire window, without respiration, yielded a very different response compared to another sample taken at the same level. Figure 7-2 provides an illustration of this. Measurements of portal blood flow were taken with the probe in the same position used for portal vein diameter on the same subject at the same level of LBNP stress. The blood flow velocity in these two images varied between 26.23 cm/s and 19.54 cm/s. It was not possible to see the placement of the gate in real time while measuring portal blood flow. Therefore, it is possible that the gate was not surrounding the entire blood vessel during measurement, leading to an erroneous result. Without a real-time updated image of probe placement, it is impossible to discern which measurements are erroneous and which provide a reliable result. Figure E-1 and E-2 in the Appendix E illustrate the measured portal blood flow responses following bed-rest and fluid loading. Therefore, portal vein diameter measurements are relied upon to describe the splanchnic vasculature dynamics, as described by Arbeille (Arbeille et al. 2003).

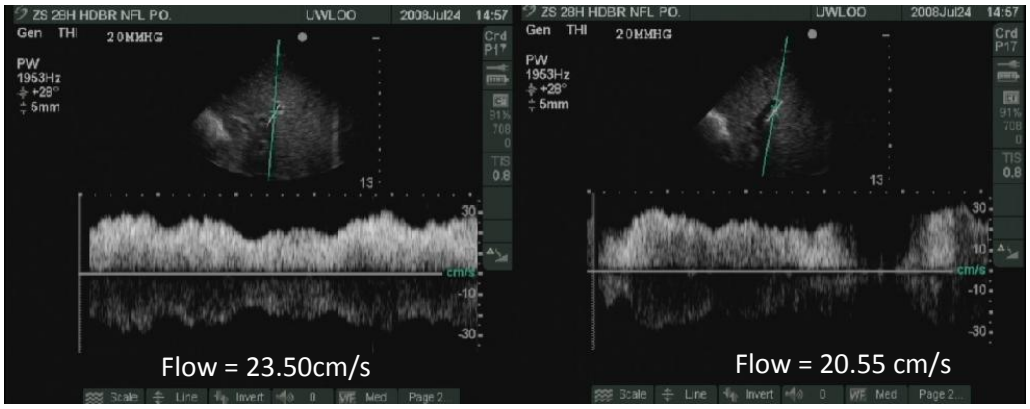


**Figure 7-1: Effect of Respiration on Portal Blood (Subject ID: ZS, LBNP Level: -20 mmHg)**

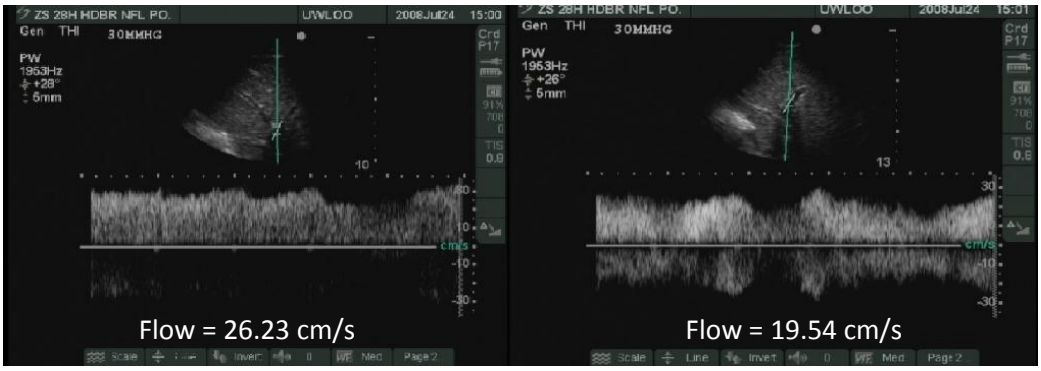


**Figure 7-2: Variation of Portal Blood Flow Velocity with one LNBP Level (Subject ID: ZS, LBNP Level: -30 mmHg)**

Cardiopulmonary baroreflex data was initially planned to be analyzed by investigating the relationship between forearm vascular resistance and LBNP, as described in Section 4.9.10 and by Vernikos and Convertino (Vernikos and Convertino 1994). However the data from this study did not follow the trends published previously. As expected, brachial blood flow velocity diminished with increasing orthostatic stress. However brachial blood flow (which contains brachial blood flow velocity, heart rate, and brachial diameter in its calculation) was constant with increasing orthostatic stress. Brachial blood flow is expected to diminish, corresponding to an increase in

forearm vascular resistance. This unexpected result may be attributable to use of supine brachial artery diameter for subsequent measurements of brachial blood flow during orthostatic testing. Unfortunately it was not possible to measure brachial artery diameter during orthostatic testing due to use of different probes and interference with CVP and brachial blood flow velocity measurements. Graphs of forearm vascular resistance responses to increasing orthostatic stress pre and post 28h NFL HDBR (Figure F-1) and post NFL and FL HDBR (Figure F-2) are available in Appendix F. Since cardiopulmonary baroreflex could not be examined by forearm vascular resistance responses, brachial diameter and brachial blood flow velocity responses were examined to provide an indication on cardiopulmonary baroreflex responses following bed-rest and fluid loading.

Measurements of IVC diameter at high levels of LBNP should be taken with caution as respiration and alterations in anatomy made it difficult to measure diameters in the same location used during low levels of LBNP. It should be mentioned that the standard error in IVC measurements at -40 mmHg was not greater than measurements at lower levels, indicating the reliability of the measurements (and justifying their use inclusion in the analysis). An example of an ultrasound image of IVC diameter at 0 mmHg is provided in Figure 7-3, and examples of images taken at -40 mmHg are illustrated in Figure 7-4 and Figure 7-5 respectively. The images are taken on the same subject during 28h NFL HDBR Post testing. Figure 7-4 provides a good quality image for IVC diameter measurement at -40 mmHg due to clarity and IVC position,

whereas Figure 7-5 was not as desirable image since the IVC was viewed in a different position compared to the image taken at 0 mmHg. When available, images such as Figure 7-4 were used in analysis of IVC diameter. Otherwise, as many measurements of IVC diameter as possible were measured from available images to increase accuracy. Analysis showed that the measured IVC diameter was clearly reduced with each level of increasing stress despite measurement difficulties at higher levels of LBNP.

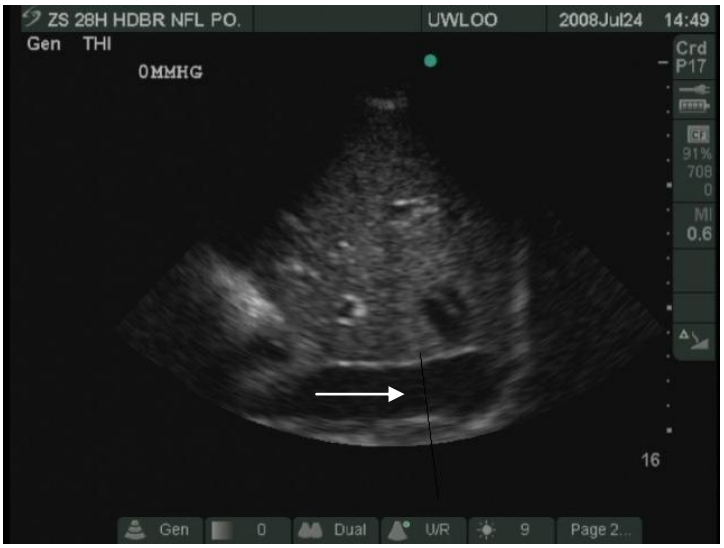


**Figure 7-3: IVC diameter at 0 mmHg during 28h NFL HDBR Testing for Subject 9**

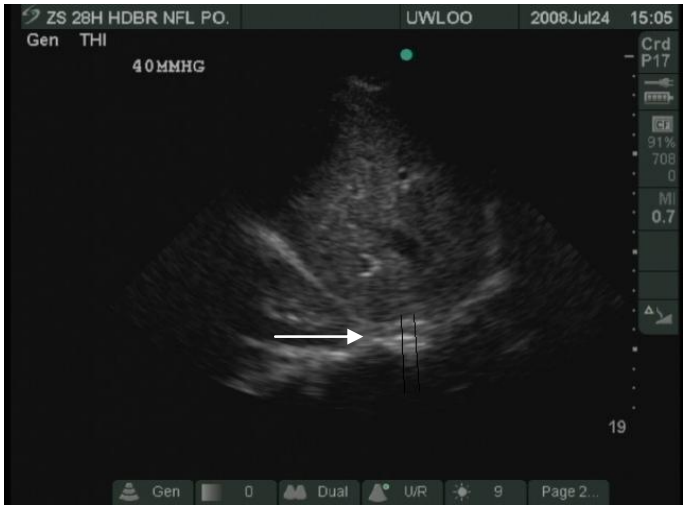


**Figure 7-4: IVC Diameter at -40 mmHg during 28h NFL HDBR Testing for Subject 9 (IVC in same position as 0 mmHg image)**

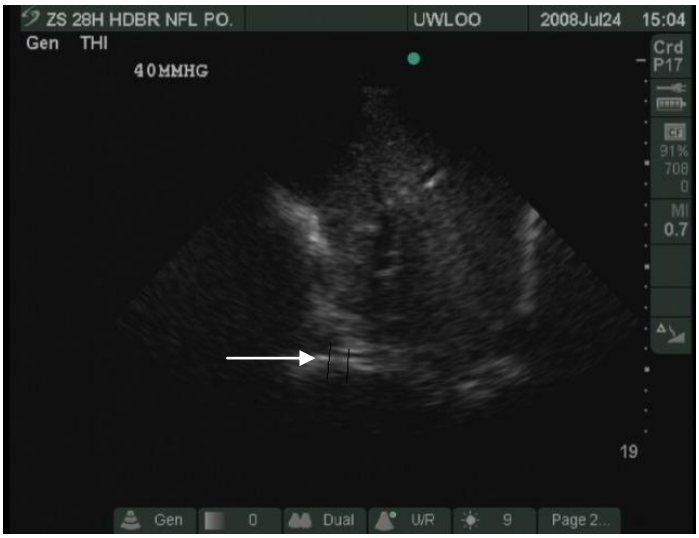


**Figure 7-5: IVC Diameter at -40 mmHg during 28h NFL HDBR Testing for Subject 9 (IVC in different position from 0 mmHg image)**

As a final note, it is recognized that there were datasets within this study in which significant differences were found, but where this significance was not obvious from the graphed data. These datasets illustrated a wide range of responses within the subject pool, but with each individual response following a similar trend. For example, the overall change in plasma volume between pre and post 28h NFL HDBR was less than 10%, but the standard deviation

exceeded 10%. Analysis of splanchnic impedance provides a second example where the difference between pre and post HDBR testing was not significant; however there was a significant LBNP effect. From the graphed data, this LBNP effect would not have been obvious due to the range of subjects' response, but was uncovered through statistical analysis.

# 8 CONCLUSION

This study examined the hypotheses that 1) 28h HDBR would result in significant hypovolemia and cardiovascular deconditioning, and that 2) NASA's fluid loading protocol (15 ml/kg water with a 1g NaCl for every 125ml of water consumed) would restore normovolemia and prevent cardiovascular deconditioning resulting from 28h HDBR. Nine healthy young men were tested in 5 testing scenarios, with a progressive LBNP protocol performed before and after the scenario to measure the subjects' cardiovascular responses. Subjects were tested in two 28h HDBR conditions, without fluid loading (NFL) and with fluid loading (FL), as well as in three 4h control conditions to isolate the effects of circadian rhythm, HDBR, and fluid loading.

This study found that the cardiovascular system had begun the process of deconditioning after exposure to only 28 hours of bed-rest. Affects of this short-term bed-rest exposure included significant hypovolemia, reductions in central venous pressure, and increased heart rate responses during orthostatic stress. Deconditioning did not include reductions in stroke volume or cardiac output, increased systemic vascular resistance, diminished cardiac performance or attenuation of the arterial and cardiopulmonary baroreflex, all of which have resulted from longer duration bed-rest exposure. There was also no evidence of venous or splanchnic pooling. From these results it can be concluded that hypovolemia is not a principal contributor to the cardiovascular deconditioning that accompanies bed-rest. Rather, prolonged inactivity in the supine position

may be responsible, with progressive cardiovascular deterioration occurring with longer durations of exposure.

This study also indicated that, in contrast to the findings by Waters and colleagues, attempts to restore lost blood volume by ingestion of NASA's fluid loading protocol of salt and water was ineffective (Waters et al. 2005). In addition, subjects performed better during orthostatic challenge when the fluid loading protocol was not performed, as fluid loading caused symptoms of nausea and vomiting in 4 of the 9 subjects, and caused presyncopal symptoms during LBNP testing in one subject (subjects who did not complete the fluid loading protocol or vomited were not included in the overall statistical analysis studying the efficacy of fluid loading). Therefore other methods of fluid volume replacement should be explored if fluid volume replacement is to be pursued as a countermeasure to orthostatic hypotension following bed-rest and spaceflight. However, since this study provided evidence that hypovolemia is not the root cause of orthostatic hypotension following bed-rest, it is recommended that other methodologies, such as exercise, artificial gravity with a short-arm centrifuge, or application of lower body negative pressure (like the Russian Chibis suit), be pursued.

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# APPENDIX A: NUMERICAL SUMMARY OF DATA

## APPENDIX A1: 28H NFL HDBR DATA

**Table A1-1: Mean Values of Blood Volume Variables Pre and Post 28h NFL HDBR (Mean ± Standard Error, † indicates HDBR effect (p<0.05))**

| Variable | 28h NFL HDBR Protocol Results | | |
|---|---|---|---|
| | Hour 0 (Pre) | Hour 28 (Post) | Difference |
| Total Blood Volume (ml)† | 5641.6 ± 425.2 | 5246.5 ± 378.6 | 395.1 ± 46.6 |
| Plasma Volume (ml) † | 3431.9 ± 271.7 | 3036.8 ± 225.1 | 395.1 ± 46.6 |
| Hematocrit (%) † | 39.61 ± 0.74 | 42.31 ± 0.63 | -2.7 ± 0.11 |
| Weight (kg) † | 73.7 ± 1.9 | 72.5 ± 1.9 | -1.2 ± 0.5 |

**Table A-2: Mean Values of Cardiovascular Pulmonary Baroreflex Responses during LBNP Testing Pre and Post 28h HDBR (Mean ± Standard Error, † HDBR Effect (p<0.05))**

| Variable | 28h NFL HDBR Pre Test | | | | | 28h NFL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Brachial Diameter (cm) | 0.42 ± 0.01 | NA | NA | NA | NA | 0.43 ± 0.01 | NA | NA | NA | NA |
| Brachial Blood Flow Velocity † (cm/s) | 0.88 ± 0.15 | 0.85 ± 0.15 | 0.75 ± 0.09 | 0.72 ± 0.09 | 0.70 ± 0.10 | 1.13 ± 0.23 | 1.06 ± 0.18 | 0.93 ±0.16 | 0.86 ± 0.15 | 0.85 ± 0.11 |
| Brachial Blood Flow (ml/min) | 7.96 ± 1.82 | 7.83 ± 1.86 | 7.21 ± 1.18 | 7.66 ± 1.18 | 8.10 ± 1.37 | 11.78 ± 4.15 | 11.24 ± 3.16 | 11.17 ± 3.08 | 11.09 ± 2.79 | 12.32 ± 2.78 |

**Table A-3: Mean Values of Arterial Baroreflex Responses during LBNP Testing Pre and Post 28h NFL HDBR (Mean ± Standard Error, † HDBR Effect (p<0.05))**

| Variable | 28h NFL HDBR Pre | | | 28h NFL HDBR Post | | |
|---|---|---|---|---|---|---|
| | 0 mmHg | -20 mmHg | -40 mmHg | 0 mmHg | -20 mmHg | -40 mmHg |
| MF Gain † (s/mmHg) | 0.0177 ± 0.0024 | 0.0183 ± 0.0020 | 0.0077 ± 0.00144 | 0.0178 ± 0.0023 | 0.0161 ± 0.0031 | 0.0087 ± 0.0012 |
| HF Gain † (s/mmHg) | 0.0220 ± 0.0034 | 0.0185 ± 0.0052 | 0.0073 ± 0.0031 | 0.0263 ± 0.0064 | 0.0206 ± 0.0054 | 0.0093 ± 0.0023 |
| MF Phase (rad) | -0.6746 ± 0.1629 | -0.8679 ± 0.0563 | -0.8164 ± 0.1409 | -0.6667 ± 0.1214 | -0.8230 ± 0.1164 | -1.0467 ± 0.1295 |
| HF Phase † (rad) | -0.2004 ± 0.1214 | -0.3325 ± 0.1099 | -0.7782 ± 0.2218 | -0.1446 ± 0.0995 | -0.8214 ± 0.1324 | -1.3149 ± 0.1842 |
| MF Coherence (no units) | 0.6019 ± 0.0552 | 0.5963 ± 0.0234 | 0.5903 ± 0.0637 | 0.5764 ± 0.0558 | 0.6725 ± 0.0376 | 0.6844 ± 0.0373 |
| HF Coherence (no units) | 0.5676 ± 0.0378 | 0.4814 ± 0.0642 | 0.3206 ± 0.0549 | 0.5692 ± 0.0329 | 0.4489 ± 0.0353 | 0.4647 ± 0.0346 |

**Table A1-4: Mean Values of Cardiovascular Blood Pressure Responses during LBNP Testing Pre and Post 28h HDBR (*LBNP Effect, † HDBR Effect, ‡ LBNP-HDBR Interaction Effect (p<0.05))**

| | 28h NFL HDBR Pre Test | | | | | 28h NFL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| MAP (mmHg) | 87.6 ± 1.6 | 86.5 ± 1.9 | 85.7 ± 2.0 | 85.5 ± 2.3 | 87.0 ± 4.6 | 88.1 ± 3.8 | 87.0 ± 4.3 | 86.0 ± 3.9 | 86.0 ± 3.6 | 84.1 ± 4.2 |
| SBP † (mmHg) | 124.7 ± 3.8 | 124.3 ± 4.1 | 120.5 ± 4.0 | 116.5 ± 4.1 | 116.5 ± 6.4 | 126.7 ± 5.1 | 123.8 ± 6.0 | 119.2 ± 5.2 | 116.9 ± 4.7 | 112.0 ± 5.3 |
| DBP (mmHg) | 68.1 ± 1.1 | 68.0 ± 1.3 | 68.6 ± 1.6 | 69.7 ± 1.8 | 72.1 ± 4.0 | 67.7 ± 3.2 | 68.2 ± 3.4 | 68.8 ± 3.1 | 70.4 ± 2.9 | 69.8 ± 3.4 |
| PP † (mmHg) | 56.6 ± 3.7 | 56.3 ± 3.8 | 51.9 ± 3.7 | 46.8 ± 3.6 | 44.3 ± 3.7 | 59.0 ± 2.7 | 55.6 ± 3.1 | 50.4 ± 2.6 | 46.5 ± 2.5 | 42.2 ± 2.4 |
| HR † ‡ (beats/min) | 53.8 ± 2.5 | 54.9 ± 3.32 | 58.4 ± 3.2 | 65.6 ± 3.6 | 71.9 ± 4.0 | 53.0 ± 2.6 | 57.6 ± 2.9 | 62.6 ± 3.5 | 69.7 ± 3.6 | 79.3 ± 4.4 |
| R-R Interval † (s) | 1.14 ± 0.06 | 1.13 ± 0.07 | 1.06 ± 0.06 | 0.95 ± 0.06 | 0.87 ± 0.05 | 1.16 ± 0.06 | 1.08 ± 0.06 | 0.99 ± 0.06 | 0.89 ± 0.06 | 0.78 ± 0.05 |
| SV † ‡ ($ml/m^2$) | 52.2 ± 3.2 | 50.2 ± 3.4 | 45.0 ± 3.1 | 39.2 ± 3.3 | 34.6 ± 3.3 | 54.2 ± 2.7 | 47.4 ± 2.8 | 42.0 ± 2.3 | 36.3 ± 1.9 | 31.7 ± 1.7 |
| Q † ($L/min/m^2$) | 2.77 ± 0.16 | 2.70 ± 0.19 | 2.56 ± 0.15 | 2.49 ± 0.14 | 2.41 ± 0.18 | 2.87 ± 0.22 | 2.70 ± 0.19 | 2.60 ± 0.16 | 2.49 ± 0.14 | 2.49 ± 0.20 |
| TPR † (mmHg/{ml/ [min*$m^2$]}) | 30.15 ± 1.15 | 31.84 ±1.41 | 33.33 ±1.61 | 34.54 ±1.78 | 38.54 ± 4.63 | 29.54 ± 2.00 | 31.58 ± 1.86 | 33.45± 2.12 | 35.67 ± 2.19 | 35.50 ± 2.84 |
| Arterial † Compliance ([ml/$m^2$]/mmHg) | 1.72 ± 0.25 | | | | | 1.22 ± 0.18 | | | | |
| CVP *†‡ (mmHg) | 8.7 ± 0.5 | 6.1 ± 0.4 | 4.2 ± 0.4 | 2.7 ± 0.5 | 1.8 ± 0.5 | 5.8 ± 0.5 | 3.6 ± 0.6 | 2.5 ± 0.5 | 1.8 ± 0.5 | 1.3 ± 0.5 |
| IVC diameter † (cm) | 2.19 ± 0.17 | 1.69 ± 0.14 | 1.24 ± 0.09 | 0.93 ± 0.10 | 0.67 ± 0.09 | 1.89 ± 0.14 | 1.46 ± 0.15 | 1.23 ± 0.14 | 0.92 ± 0.09 | 0.64 ± 0.06 |
| Venous Compliance (cm/mmHg) | 0.22 ± 0.03 | | | | | 0.25 ±0.03 | | | | |
| Portal Diameter *†‡ (cm) | 0.75 ± 0.05 | 0.68 ± 0.05 | 0.65 ± 0.04 | 0.60 ± 0.04 | 0.55 ± 0.04 | 0.70 ± 0.05 | 0.64 ± 0.05 | 0.55 ± 0.04 | 0.52 ±0.04 | 0.47 ± 0.04 |
| Portal Flow * (cm/s) | 19.63 ± 1.66 | 21.39 ± 1.78 | 21.36 ± 1.50 | 21.36 ± 1.65 | 20.08 ± 2.27 | 16.77 ± 1.22 | 16.12 ± 1.35 | 18.30 ± 1.32 | 17.91 ± 1.30 | 18.14 ± 1.49 |
| Splanchnic Impedance † (Ω/cm) | 1.73 ± 0.31 | 1.78 ± 0.31 | 1.80 ± 0.31 | 1.84 ± 0.32 | 1.88 ± 0.32 | 1.52 ± 0.16 | 1.50 ± 0.15 | 1.52 ± 0.15 | 1.56 ± 0.17 | 1.60 ± 0.17 |
| Ejection Fraction † (%) | 82.3 ± 1.7 | NA | NA | NA | 74.3 ± 1.9 | 79.5 ± 1.1 | NA | NA | NA | 72.2 ± 1.4 |
| Fractional Shortening † (%) | 44.3 ± 1.7 | NA | NA | NA | 36.8 ± 1.6 | 41.2 ± 1.0 | NA | NA | NA | 34.9 ± 1.2 |

# APPENDIX A2: 28H FL AND NFL POST DATA

**Table A.2-1: Mean Values of Blood Volume Variables Pre and Post 28h NFL and FL HDBR (Mean ± Standard Error)**

| Variable | 28h NFL HBDR | | | 28h FL HDBR | | |
|---|---|---|---|---|---|---|
| | Hour 0 | Hour 28 | Difference | Hour 0 | Hour 28 | Difference |
| Total Blood Volume (ml) | 5706.9 ± 531.1 | 5315.6 ± 475.3 | -391.3 ± 66.8 | 6170.8 ± 310.8 | 5790.7 ± 310.6 | -380.1 ± 74.3 |
| Plasma Volume (ml) | 3440.5 ± 319.6 | 3049.2 ± 268.2 | -391.6 ± 66.9 | 3784.9 ± 223.0 | 3404.9 ± 222.3 | -380.1 ± 74.3 |
| Hematocrit (%) | 40.29 ± 0.75 | 42.89 ± 0.59 | -2.60 ± 0.34 | 38.16 ± 1.03 | 41.21 ± 0.89 | -3.05 ± 0.68 |
| Weight (kg) | 75.0 ± 1.5 | 74.0 ± 1.5 | 1.0 ± 0.6 | 76.0 ± 1.6 | 74.9 ± 1.5 | 1.1 ± 0.3 |

**Table A2-2: Comparison Mean Values of Arterial Baroreflex Responses during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (Mean ± Standard Error, † HDBR Effect ($p<0.05$))**

| Variable | 28h NFL HDBR Post | | | 28h FL HDBR Post | | |
|---|---|---|---|---|---|---|
| | 0 mmHg | -20 mmHg | -40 mmHg | 0 mmHg | -20 mmHg | -40 mmHg |
| MF Gain † (s/mmHg) | 0.0207 ± 0.0017 | 0.0181 ± 0.0041 | 0.0099 ± 0.0012 | 0.0204 ± 0.0051 | 0.0222 ± 0.0034 | 0.0136 ± 0.0020 |
| HF Gain † (s/mmHg) | 0.0310 ± 0.0080 | 0.0248 ± 0.0068 | 0.0106 ± 0.0032 | 0.0218 ± 0.0048 | 0.0207 ± 0.0024 | 0.0115 ± 0.0022 |
| MF Phase (rad) | -0.6418 ± 0.1205 | -0.7592 ± 0.1559 | -1.1247 ± 0.1371 | -0.7571 ± 0.1917 | -0.8508 ± 0.1763 | -1.0213 ± 0.1982 |
| HF Phase † (rad) | -0.1853 ± 0.1388 | -0.7566 ± 0.1820 | -1.1740 ± 0.2186 | -0.3803 ± 0.3011 | -0.6929 ± 0.2001 | -1.1570 ± 0.1303 |
| MF Coherence | 0.58 ± 0.06 | 0.67 ± 0.04 | 0.68 ± 0.04 | 0.54 ± 0.05 | 0.63 ± 0.04 | 0.59 ± 0.08 |
| HF Coherence | 0.57 ± 0.03 | 0.45 ± 0.04 | 0.46 ± 0.03 | 0.57 ± 0.05 | 0.48 ± 0.06 | 0.41 ± 0.05 |

**Table A2-3: Comparison Mean Values of Cardiopulmonary Baroreflex Responses during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (Mean ± Standard Error, † HDBR Effect ($p<0.05$))**

| Variable | 28h NFL HDBR Post Test | | | | | 28h FL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Brachial Diameter (cm) | 0.43 ± 0.01 | NA | NA | NA | NA | 0.42 ± 0.01 | NA | NA | NA | NA |
| Brachial Blood Flow Velocity (cm/s) † | 0.89 ± 0.13 | 0.90 ± 0.15 | 0.78 ± 0.15 | 0.73 ± 0.15 | 0.71 ± 0.09 | 0.89 ± 0.1 | 0.79 ± 0.07 | 0.80 ± 0.09 | 0.72 ± 0.10 | 0.61 ± 0.08 |
| Brachial Blood Flow (ml/min) | 7.04 ± 1.37 | 7.67 ± 1.61 | 7.46 ± 1.87 | 7.89 ± 2.08 | 8.55 ± 1.55 | 7.19 ± 1.03 | 6.20 ± 0.57 | 7.05 ± 0.84 | 6.87 ± 0.75 | 6.60 ± 0.79 |

**Table A2-4: Mean Values of Cardiovascular Blood Pressure Responses during LBNP Testing following 28h FL HDBR and 28h NFL HDBR (Mean ± Standard Error, *LBNP Effect, † HDBR Effect, ‡ LBNP-HDBR Interaction Effect (p<0.05))**

| | 28h NFL HDBR Pre Test | | | | | 28h NFL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| MAP (mmHg) | 86.41 ± 4.64 | 84.62 ± 5.21 | 84.44 ± 4.62 | 84.86 ± 4.50 | 82.43 ± 5.14 | 92.08 ± 5.03 | 91.54 ± 4.96 | 89.13 ± 4.63 | 88.10 ± 4.82 | 87.30 ± 5.92 |
| SBP † (mmHg) | 122.97 ± 5.75 | 119.33 ± 6.56 | 116.16 ± 5.89 | 114.38 ± 5.57 | 108.85 ± 6.18 | 129.31 ± 6.78 | 128.18 ± 6.30 | 122.39 ± 6.27 | 118.05 ± 6.29 | 114.18 ± 7.81 |
| DBP (mmHg) | 66.43 ± 4.02 | 66.34 ± 4.18 | 67.69 ± 3.75 | 69.68 ± 3.57 | 68.83 ± 4.15 | 70.49 ± 3.32 | 70.56 ± 3.22 | 70.39 ± 3.05 | 71.83 ± 3.49 | 72.92 ± 4.45 |
| PP † (mmHg) | 56.54 ± 2.65 | 52.99 ± 3.12 | 48.46 ± 2.86 | 44.70 ± 2.82 | 40.02 ± 2.53 | 58.82 ± 4.58 | 57.63 ± 4.42 | 52.00 ± 4.57 | 46.22 ± 4.04 | 41.26 ± 3.88 |
| HR † ‡ (beats/min) | 49.96 ± 2.20 | 54.43 ± 2.75 | 59.07 ± 3.32 | 66.55 ± 3.81 | 75.64 ± 4.48 | 51.47 ± 3.29 | 50.19 ± 1.92 | 56.28 ± 2.54 | 63.23 ± 2.26 | 71.01 ± 2.83 |
| R-R Interval † (s) | 1.22 ± 0.06 | 1.13 ± 0.07 | 1.04 ± 0.07 | 0.93 ± 0.07 | 0.82 ± 0.06 | 1.20 ± 0.07 | 1.21± 0.06 | 1.09 ± 0.06 | 0.96 ± 0.04 | 0.86 ± 0.03 |
| SV † ‡ ($ml/m^2$) | 52.50 ± 3.05 | 45.85 ± 3.04 | 40.92 ± 2.78 | 35.54 ± 2.44 | 30.22 ± 1.76 | 57.36 ± 5.86 | 54.93 ± 5.82 | 47.58 ± 5.40 | 37.73 ± 3.75 | 31. 75 ± 2.22 |
| Q † ($L/min/m^2$) | 2.59 ± 0.12 | 2.44 ± 0.10 | 2.36 ± 0.07 | 2.30 ± 0.06 | 2.23 ± 0.06 | 2.90 ± 0.26 | 2.72 ± 0.23 | 2.62 ± 0.21 | 2.39 ± 0.16 | 2.23 ± 0.14 |
| TPR † ($mmHg/\{ml/ [min*m^2]\}$) | 8.48 ± 0.56 | 8.99 ± 0.50 | 9.53 ± 0.55 | 9.86 ± 0.65 | 10.02 ± 0.83 | 8.16 ± 0.57 | 8.91 ± 0.74 | 9.33 ± 0.88 | 9.89 ± 0.91 | 10.71 ± 1.04 |
| Arterial Compliance † ($[ml/m^2]/mmHg$) | 1.22 ± 0.18 | | | | | 1.48 ± 0.14 | | | | |
| CVP *†‡ (mmHg) | 2.16 ± 0.57 | 1.59 ± 0.64 | 6.89 ± 0.47 | 5.15 ± 0.48 | 3.39 ± 0.40 | 3.20 ± 0.29 | 1.99 ± 0.27 | | | |
| IVC diameter † (cm) | 0.86 ± 0.07 | 0.63 ± 0.07 | 1.90 ± 0.23 | 1.34 ± 0.21 | 1.06 ± 0.17 | 0.74 ± 0.13 | 0.53 ± 0.06 | | | |
| Venous Compliance (cm/mmHg) | 0.27 ± 0.03 | | | | | 0.30 ± 0.06 | | | | |
| Portal Diameter *†‡ (cm) | 0.73 ± 0.05 | 0.67 ± 0.05 | 0.57 ± 0.04 | 0.53 ± 0.05 | 0.49 ± 0.05 | 0.76 ± 0.07 | 0.69 ± 0.06 | 0.61 ± 0.06 | 0.59 ± 0.06 | 0.49 ± 0.03 |
| Splanchnic Impedance † (Ω/cm) | 1.48 ± 0.19 | 1.47 ± 0.17 | 1.50 ± 0.18 | 1.55 ± 0.19 | 1.58 ± 0.20 | 1.80 ± 0.21 | 1.78 ± 0.20 | 1.81 ± 0.20 | 1.85 ± 0.20 | 1.90 ± 0.20 |
| Ejection Fraction † (%) | 79.19 ± 1.20 | NA | NA | NA | 72.17 ± 1.71 | 79.51 ± 1.73 | NA | NA | NA | 71.08 ± 2.75 |

# APPENDIX A3: CIRCADIAN RHYTHM CONTROL DATA

**Table A3-1: Mean Values of Cardiovascular Variables during LBNP Testing Pre and Post 4h S NFL (Mean ± Standard Error, † HDBR Effect (p<0.05))**

| | 4h S NFL Pre | | | | | 4h S NFL Post | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Total Blood Volume (ml) | 5391.4 ± 384.4 | NA | NA | NA | NA | 5374.7 ± 342.8 | NA | NA | NA | NA |
| Plasma Volume (ml) | 3239.5 ± 265.5 | NA | NA | NA | NA | 3222.9 ± 220.3 | NA | NA | NA | NA |
| Hematocrit (%) | 40.19 | NA | NA | NA | NA | 40.11 | NA | NA | NA | NA |
| Heart Rate † (beats/min) | 51.20 ± 2.10 | 51.45 ± 2.10 | 53.86 ± 2.44 | 56.52 ± 2.98 | 60.91 ± 3.48 | 50.61 ± 2.55 | 51.10 ± 2.56 | 53.03 ± 3.16 | 56.67 ± 4.17 | 63.22 ± 4.96 |
| Central Venous Pressure † (mmHg) | 10.64 ± 0.56 | 8.46 ± 0.56 | 6.18 ± 0.62 | 4.74 ± 0.65 | 3.93 ± 0.65 | 9.68 ± 0.51 | 7.44 ± 0.69 | 5.93 ± 0.72 | 4.55 ± 0.76 | 3.61 ± 0.69 |
| Portal Vein † Diameter (cm) | 0.75 ± 0.05 | 0.67 ± 0.04 | 0.60 ± 0.04 | 0.56 ± 0.04 | 0.50 ± 0.03 | 0.73 ± 0.05 | 0.66 ± 0.04 | 0.60 ± 0.04 | 0.55 ± 0.04 | 0.49 ± 0.04 |

**Table A3-2: Mean Values of Cardiovascular Variables during LBNP Testing Post 4h S NFL and Post 4h HDBR (Mean ± Standard Error, *LBNP Effect, † HDBR Effect, ‡ LBNP-HDBR Interaction Effect (p<0.05))**

| | 4h S NFL Post | | | | | 4h HDBR Post | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Δ Total Blood Volume (ml) | 16.7 ± 64.2 | NA | NA | NA | NA | -27.1 ± 58.0 | NA | NA | NA | NA |
| Δ Plasma Volume (ml) | 16.7 ± 64. 2 | NA | NA | NA | NA | -27.1 ± 58.0 | NA | NA | NA | NA |
| Δ Hematocrit (%) | -0.07 ± 0.44 | NA | NA | NA | NA | 0.57 ± 0.54 | NA | NA | NA | NA |
| Heart Rate *†‡ (beats/min) | 50.61 ± 2.55 | 51.10 ± 2.56 | 53.03 ± 3.16 | 56.67 ± 4.17 | 63.22 ± 4.96 | 55.11 ± 2.06 | 56.14 ± 2.44 | 62.56 ± 2.49 | 70.28 ± 2.76 | 78.35 ± 3.49 |
| Central * † Venous Pressure (mmHg) | 9.68 ± 0.51 | 7.44 ± 0.69 | 5.93 ± 0.72 | 4.55 ± 0.76 | 3.61 ± 0.69 | 7.30 ± 0.58 | 5.43 ± 0.67 | 3.88 ± 0.51 | 2.44 ± 0.39 | 1.72 ± 0.39 |
| Portal Vein*†‡ Diameter (cm) | 0.73 ± 0.05 | 0.66 ± 0.04 | 0.60 ± 0.04 | 0.55 ± 0.04 | 0.49 ± 0.04 | 0.67 ± 0.05 | 0.60 ± 0.04 | 0.56 ± 0.04 | 0.52 ± 0.04 | 0.47 ± 0.03 |

# APPENDIX A4: FLUID LOADING CONTROL DATA

**Table A4-1: Mean Values of Cardiovascular Variables during LBNP Testing Post 4-hour S NFL and Post 4-hour S FL (Mean ± Standard Error, † HDBR Effect (p<0.05))**

| | 4-hour S NFL Post | | | | | 4-hour S FL Post | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Δ Total Blood Volume (ml) | 16.7 ± 64.2 | NA | NA | NA | NA | -232.0 ± 98.6 | NA | NA | NA | NA |
| Δ Plasma Volume (ml) | 16.7 ± 64.2 | NA | NA | NA | NA | -232.0 ± 98.6 | NA | NA | NA | NA |
| Δ Hematocrit (%) | -0.07 ± 0.44 | NA | NA | NA | NA | 1.82 ± 0.80 | NA | NA | NA | NA |
| Heart Rate † (beats/min) | 50.61 ± 2.55 | 51.10 ± 2.56 | 53.03 ± 3.16 | 56.67 ± 4.17 | 63.22 ± 4.96 | 52.59 ± 3.27 | 53.90 ± 3.19 | 58.10 ± 5.23 | 61.50 ± 5.53 | 62.22 ± 4.53 |
| Central † Venous Pressure (mmHg) | 9.68 ± 0.51 | 7.44 ± 0.69 | 5.93 ± 0.72 | 4.55 ± 0.76 | 3.61 ± 0.69 | 8.53 ± 0.52 | 6.20 ± 0.47 | 4.05 ± 0.44 | 2.78 ± 0.52 | 2.10 ± 0.49 |
| Portal Vein † Diameter (cm) | 0.73 ± 0.05 | 0.66 ± 0.04 | 0.60 ± 0.04 | 0.55 ± 0.04 | 0.49 ± 0.04 | 0.72 ± 0.08 | 0.66 ± 0.08 | 0.64 ± 0.08 | 0.59 ± 0.07 | 0.57 ± 0.08 |

# APPENDIX A5: HDBR CONTROL DATA

**Table A5-1: Mean Values of Cardiovascular Variables during LBNP Testing Post 4-hour S FL and Post 28h FL HDBR (Mean ± Standard Error, *LBNP Effect, † HDBR Effect, ‡ LBNP-HDBR Interaction Effect (p<0.05))**

| | 4-hour S FL Post | | | | | 28h FL HDBR Post | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Δ Total Blood Volume * (ml) | 232.0 ± 98.5 | NA | NA | NA | NA | -409.9 ± 163.8 | NA | NA | NA | NA |
| Δ Plasma Volume * (ml) | 232.0 ± 98.5 | NA | NA | NA | NA | -409.9 ± 163.8 | NA | NA | NA | NA |
| Δ Hematocrit* (%) | -1.82 ± 0.80 | NA | NA | NA | NA | 2.88 ± 0.53 | NA | NA | NA | NA |
| Heart Rate † ‡ (beats/min) | 50.61 ± 2.55 | 51.10 ± 2.56 | 53.03 ± 3.16 | 56.67 ± 4.17 | 63.22 ± 4.96 | 51.47 ± 3.29 | 50.19 ± 1.92 | 56.28 ± 2.54 | 63.23 ± 2.26 | 71.01 ± 2.83 |
| CVP † ‡ (mmHg) | 9.68 ± 0.51 | 7.44 ± 0.69 | 5.93 ± 0.72 | 4.55 ± 0.76 | 3.61 ± 0.69 | 6.01 ± 0.59 | 3.82 ± 0.69 | 2.78 ± 0.50 | 2.16 ± 0.57 | 1.59 ± 0.64 |
| Portal Vein † Diameter (cm) | 0.73 ± 0.05 | 0.66 ± 0.04 | 0.60 ± 0.04 | 0.55 ± 0.04 | 0.49 ± 0.04 | 0.76 ± 0.07 | 0.69 ± 0.06 | 0.61 ± 0.06 | 0.59 ± 0.06 | 0.49 ± 0.03 |

# APPENDIX B: STATISTICS TABLES

# APPENDIX B1: 28H NFL HDBR STATISTICS SUMMARY

**Table B1-1: Results of 2-way Repeated Measures ANOVA (Pre/Post, LBNP) for Analysis of 28h NFL HDBR**

| | P (*P<0.05) | | |
|---|---|---|---|
| Variable | Pre/Post Effect | LBNP Effect | Pre/Post vs. LBNP Effect |
| Total Blood Volume | 0.0002* | NA | NA |
| Plasma Volume | 0.0002* | NA | NA |
| Hematocrit | < 0.0001* | NA | NA |
| Weight | 0.0419* | NA | NA |
| Heart Rate | 0.2260 | < 0.0001* | 0.0124* |
| R-R Interval | 0.3154 | < 0.0001* | 0.0853 |
| Mean Arterial Pressure | 0.9533 | 0.5039 | 0.4483 |
| Systolic Blood Pressure | 0.8816 | < 0.0001* | 0.1236 |
| Diastolic Blood Pressure | 0.9352 | 0.1662 | 0.6350 |
| Pulse Pressure | 0.8584 | <0.0001* | 0.0307* |
| Stroke Volume | 0.2947 | <0.0001* | 0.0165* |
| Cardiac Output | 0.6732 | 0.0007* | 0.7862 |
| Total Peripheral Resistance | 0.8739 | 0.0014* | 0.3582 |
| Arterial Compliance | 0.2449 | NA | NA |
| Venous Compliance | 0.3100 | NA | NA |
| Central Venous Pressure | 0.0107* | <0.0001* | 0.0070* |
| IVC Diameter | 0.1944 | <0.0001* | 0.0600 |
| Portal Vein Diameter | 0.0077* | <0.0001* | 0.0354* |
| Portal Vein Blood Flow | 0.0291* | 0.2838 | 0.5117 |
| Splanchnic Impedance | 0.3708 | <0.0001* | 0.3628 |
| Ejection Fraction | 0.1948 | 0.0041* | 0.9180 |
| Fractional Shortening | 0.1664 | 0.0033* | 0.4939 |
| MF Gain | 0.8772 | 0.0008* | 0.4911 |
| HF Gain | 0.3735 | 0.0041* | 0.9241 |
| MF Phase | 0.4498 | 0.1232 | 0.6152 |
| HF Phase | 0.0862 | 0.0010* | 0.0614 |
| Brachial Blood Flow Velocity | 0.1418 | 0.0021* | 0.6222 |
| Brachial Blood Flow | 0.0897 | 0.6487 | 0.9787 |
| Brachial Diameter | 0.7152 | NA | NA |

**Table B1-2: Preplanned Comparisons for Analysis of 28h NFL HDBR**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | Pre 0 mmHg vs. Post 0 mmHg | Pre -40 mmHg vs. Post -40 mmHg | Post 0 mmHg vs. Post -40 mmHg |
| Mean Arterial Pressure | 0.8748 | 0.6306 | 0.0412* |
| Heart Rate | 0.6400 | 0.0500* | <0.0001* |
| R-R Interval | 0.6496 | 0.0479* | <0.0001* |
| Stroke Volume | 0.2263 | 0.0855 | <0.0001* |
| Cardiac Output | 0.4387 | 0.5700 | 0.0825 |
| Total Peripheral Resistance | 0.8480 | 0.6279 | 0.0583 |
| Arterial Compliance | 0.7535 | 0.4539 | 0.4539 |
| Central Venous Pressure | 0.0231* | 0.0470* | <0.0001* |
| IVC Diameter | 0.0470* | 0.6132 | <0.0001* |
| Portal Vein Diameter | 0.0669 | 0.0201* | <0.0001* |
| Portal Vein Blood Flow | 0.0968 | 0.4050 | 0.2944 |
| Splanchnic Impedance | 0.4274 | 0.3360 | 0.0105* |
| Ejection Fraction | 0.1877 | 0.2254 | 0.0014* |
| Fractional Shortening | 0.8663 | 0.9326 | 0.0015* |
| Brachial Blood Flow Velocity | 0.1060 | 0.1326 | 0.0999 |
| Brachial Blood Flow | 0.1592 | 0.0692 | 0.7976 |

# APPENDIX B2: 28H FL AND NFL POST STATISTICS SUMMARY

**Table B2-1: Results of 2-way Repeated Measures ANOVA Comparing 28h FL HDBR and 28h NFL HDBR Post Results (FL/NFL, LBNP)**

| Variable | P (*P < 0.05) | | |
|---|---|---|---|
| | NFL/FL Effect | LBNP Effect | NFL/FL vs. LBNP Effect |
| Total Blood Volume | 0.2908 | NA | NA |
| Plasma Volume | 0.2190 | NA | NA |
| Hematocrit | 0.1848 | NA | NA |
| Weight Change | 0.9763 | NA | NA |
| Heart Rate | 0.3427 | <0.0001* | 0.2528 |
| R-R Interval | 0.4257 | <0.0001* | 0.2235 |
| Mean Arterial Pressure | 0.3545 | 0.0368* | 0.3413 |
| Systolic Blood Pressure | 0.3503 | <0.0001 | 0.3890 |
| Diastolic Blood Pressure | 0.4295 | 0.1485 | 0.4431 |
| Pulse Pressure | 0.4046 | <0.0001* | 0.1982 |
| Stroke Volume | 0.0667 | <0.0001* | <0.0087* |
| Cardiac Output | 0.3201 | 0.0002* | 0.0125* |
| Total Peripheral Resistance | 0.4970 | 0.0004* | 0.0824 |
| Arterial Compliance | 0.1586 | NA | NA |
| Central Venous Pressure | 0.4425 | <0.0001* | 0.7071 |
| IVC diameter | 0.4967 | <0.0001* | 0.7358 |
| Venous Compliance | 0.8100 | NA | NA |
| Portal Vein Diameter | 0.2710 | <0.0001* | 0.5612 |
| Splanchnic Impedance | 0.2252 | <0.0001* | 0.9590 |
| Ejection Fraction | 0.7121 | 0.0139* | 0.5184 |
| Fractional Shortening | 0.7814 | 0.0100* | 0.7882 |
| MF Gain | 0.1277 | 0.0031* | 0.5131 |
| HF Gain | 0.9367 | 0.0009* | 0.7680 |
| MF Phase | 0.9762 | 0.3473 | 0.4524 |
| HF Phase | 0.7323 | 0.0023 | 0.7936 |
| Brachial Blood Flow Velocity | 0.9409 | <0.0001* | 0.2031 |
| Brachial Blood Flow | 0.7388 | 0.4600 | 0.0828 |
| Brachial Diameter | 0.6645 | NA | NA |

**Table B2-2: Preplanned 1-way Repeated Measures ANOVA Comparisons between 28h FL HDBR Post and 28h NFL HDBR Post Tests**

| Variable | P (*P<0.05) | |
|---|---|---|
| | NFL Post 0 mmHg vs. FL Post 0 mmHg | NFL Post -40 mmHg vs. FL Post -40 mmHg |
| Heart Rate | 0.6062 | 0.3328 |
| R-R Interval | 0.6524 | 0.8600 |
| Mean Arterial Pressure | 0.3004 | 0.4663 |
| Systolic Blood Pressure | 0.3321 | 0.5327 |
| Diastolic Blood Pressure | 0.3680 | 0.4352 |
| Pulse Pressure | 0.5223 | 0.7470 |
| Stroke Volume | 0.1874 | 0.3371 |
| Cardiac Output | 0.1330 | 0.9805 |
| Total Peripheral Resistance | 0.7662 | 0.2990 |
| Arterial Compliance | 0.3281 | 0.4885 |
| Central Venous Pressure | 0.5070 | 0.6734 |
| IVC Diameter | 0.8468 | 0.2210 |
| Portal Vein Diameter | 0.4611 | 0.8588 |
| Splanchnic Impedance | 0.2329 | 0.2127 |
| Ejection Fraction | 0.8097 | 0.6396 |
| Fractional Shortening | 0.8356 | 0.7280 |
| Brachial Blood Flow Velocity | 0.7360 | 0.6383 |
| Brachial Blood Flow | 0.7748 | 0.4546 |

**Table B2-3: Results of 2-way Repeated Measures ANOVA Comparing 28h NFL HDBR and 28h FL HDBR Pre Results (NFL/FL, LBNP)**

| Variable | P (*P < 0.05) | | |
|---|---|---|---|
| | NFL/FL Effect | LBNP Effect | NFL/FL vs. LBNP Effect |
| Total Blood Volume | 0.8962 | NA | NA |
| Plasma Volume | 0.5764 | NA | NA |
| Hematocrit | 0.2993 | NA | NA |
| Heart Rate | 0.2211 | <0.0001* | 0.7142 |
| R-R Interval | 0.1737 | <0.0001* | 0.6178 |
| Mean Arterial Pressure | 0.3835 | 0.7721 | 0.6406 |
| Systolic Blood Pressure | 0.2077 | <0.0001* | 0.3736 |
| Diastolic Blood Pressure | 0.8763 | 0.0262* | 0.8950 |
| Pulse Pressure | 0.3682 | <0.0001* | 0.7231 |
| Stroke Volume | 0.0510 | <0.0001* | 0.8700 |
| Cardiac Output | 0.0567 | 0.0005* | 0.9223 |
| Total Peripheral Resistance | 0.1752 | 0.0005* | 0.5386 |
| Arterial Compliance | 0.0873 | <0.0001* | 0.5437 |
| Central Venous Pressure | 0.1154 | <0.0001* | 0.7499 |
| IVC diameter | 0.1663 | <0.0001* | 0.0771 |
| Venous Compliance | 0.9812 | NA | NA |
| Portal Vein Diameter | 0.5097 | <0.0001* | 0.1615 |
| Splanchnic Impedance | 0.3666 | <0.0001* | 0.9864 |
| Ejection Fraction | 0.9455 | 0.0016* | 0.2490 |
| Fractional Shortening | 0.8700 | 0.0013* | 0.1924 |
| MF Gain | 0.1745 | <0.0001* | 0.9968 |
| HF Gain | 0.1944 | 0.0023* | 0.7702 |
| MF Phase | 0.5789 | 0.1451 | 0.6829 |
| HF Phase | 0.8660 | 0.0005* | 0.6625 |
| Brachial Blood Flow Velocity | 0.6939 | 0.0030* | 0.4125 |
| Brachial Blood Flow | 0.4562 | 0.0817 | 0.4977 |
| Brachial Diameter | 0.6354 | NA | NA |

# APPENDIX B3: CIRCADIAN RHYTHM CONTROL STATISTICS SUMMARY

**Table B3-1: Results of 2-way Repeated Measures ANOVA (Pre/Post, LBNP) for Analysis of 4-hour S NFL**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | Pre/Post Effect | LBNP Effect | Pre/Post vs. LBNP Effect |
| Total Blood Volume | 0.8017 | NA | NA |
| Plasma Volume | 0.8017 | NA | NA |
| Hematocrit | 0.8705 | NA | NA |
| Heart Rate | 0.9217 | <0.0001* | 0.4765 |
| Central Venous Pressure | 0.2004 | <0.0001* | 0.0933 |
| Portal Vein Diameter | 0.3859 | <0.0001* | 0.9504 |

**Table B3-2: Results of 2-way Repeated Measures ANOVA comparing 4-hour S NFL Post and 4-hour HDBR Post Responses (4 S NFL Post/4 HDBR Post, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4 S NFL Post/4 HDBR Post Effect | LBNP Effect | SNFL/HDBR vs. LBNP Effect |
| Δ Total Blood Volume | 0.5568 | NA | NA |
| Δ Plasma Volume | 0.9195 | NA | NA |
| Hematocrit | 0.4552 | NA | NA |
| Heart Rate | 0.0009* | <0.0001* | 0.0100* |
| Central Venous Pressure | 0.0150* | <0.0001* | 0.9102 |
| Portal Vein Diameter | 0.0178* | <0.0001* | 0.0462* |

**Table B3-3: Results of 2-way Repeated Measures ANOVA comparing 4-hour S NFL Pre and 4-hour HDBR Pre Responses (4 S NFL Pre/4 HDBR Pre, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4 S NFL Pre/4 HDBR Pre Effect | LBNP Effect | SNFL/HDBR vs. LBNP Effect |
| Heart Rate | 0.1342 | <0.0001* | 0.1543 |
| Central Venous Pressure | 0.1328 | <0.0001* | 0.2578 |
| Portal Vein Diameter | 0.0963 | <0.0001* | 0.4931 |

# APPENDIX B4: FLUID LOADING CONTROL STATISTICS SUMMARY

**Table B4-1: Results of 2-way Repeated Measures ANOVA comparing 4-hour S NFL Post and 4-hour S FL Post Responses (4 S NFL Post/4 S FL Post, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4 S NFL Post/4 S FL Post Effect | LBNP Effect | S NFL/S FL vs. LBNP Effect |
| Δ Total Blood Volume | 0.8017 | NA | NA |
| Δ Plasma Volume | 0.8017 | NA | NA |
| Δ Hematocrit | 0.8705 | NA | NA |
| Heart Rate | 0.2063 | <0.0001* | 0.1752 |
| Central Venous Pressure | 0.7658 | <0.0001* | 0.3072 |
| Portal Vein Diameter | 0.5706 | <0.0001* | 0.8525 |

**Table B4-2: Results of 2-way Repeated Measures ANOVA comparing 4-hour S NFL Pre and 4-hour S FL Pre Responses (4 S NFL Pre/4 S FL Pre, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4 S NFL Pre/4 S FL Pre Effect | LBNP Effect | S NFL/S FL vs. LBNP Effect |
| Heart Rate | 0.3227 | <0.0001* | 0.1930 |
| Central Venous Pressure | 0.0793 | <0.0001* | 0.4784 |
| Portal Vein Diameter | 0.9744 | <0.0001* | 0.8530 |

# APPENDIX B5: HDBR CONTROL STATISTICS SUMMARY

**Table B5-1: Results of 2-way Repeated Measures ANOVA comparing 4-hour S FL Post and 28h FL HDBR Post Responses (4h S FL Post /28h FL HDBR Post, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4h S FL Post/28h FL Post HDBR Effect | LBNP Effect | Interaction Effect |
| Δ Total Blood Volume | 0.0022 | NA | NA |
| Δ Plasma Volume | 0.0022 | NA | NA |
| Δ Hematocrit | 0.0013 | NA | NA |
| Heart Rate | 0.1817 | <0.0001* | 0.0101* |
| Central Venous Pressure | 0.6495 | <0.0001* | 0.0353* |
| Portal Vein Diameter | 0.3725 | <0.0001* | 0.5215 |

**Table B5-2: Results of 2-way Repeated Measures ANOVA comparing 4-hour S FL Pre and 28h FL HDBR Pre Responses (4h S FL Pre /28h FL HDBR Pre, LBNP)**

| Variable | P (*P<0.05) | | |
|---|---|---|---|
| | 4h S FL Pre/28h FL HDBR Pre Effect | LBNP Effect | Interaction Effect |
| Heart Rate | 0.9520 | <0.0001* | 0.8018 |
| Central Venous Pressure | 0.2911 | <0.0001* | 0.9811 |
| Portal Vein Diameter | 0.2185 | <0.0001* | 0.7289 |

# APPENDIX C: SAMPLE SIZES

**Table C-1: Sample Sizes for 28h HDBR Pre versus Post Comparison Data**

| | 28h NFL HDBR Pre Test | | | | | 28h NFL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Total Blood Volume | | | 8 | | | | | 8 | | |
| Plasma Volume | | | 8 | | | | | 8 | | |
| Hematocrit | | | 9 | | | | | 9 | | |
| Weight | | | 9 | | | | | 9 | | |
| Heart Rate | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| R-R Interval | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Mean Arterial Pressure | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Systolic Blood Pressure | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Diastolic Blood Pressure | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Pulse Pressure | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Stroke Volume | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Cardiac Output | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Total Peripheral Resistance | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Arterial Compliance | | | 8 | | | | | 8 | | |
| Central Venous Pressure | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| IVC Diameter | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Venous Compliance | | | 6 | | | | | 6 | | |
| Portal Vein Diameter | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Portal Flow | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Splanchnic Impedance | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Ejection Fraction | 8 | NA | NA | NA | 8 | 8 | NA | NA | NA | 7 |
| Fractional Shortening | 7 | NA | NA | NA | 7 | 7 | NA | NA | NA | 7 |
| MF Gain | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| HF Gain | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| MF Phase | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| MF Phase | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| MF Coherence | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| HF Coherence | 7 | NA | 7 | NA | 7 | 7 | NA | 7 | NA | 7 |
| Brachial Diameter | | | 9 | | | | | 9 | | |
| Brachial Blood Flow Velocity | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Brachial Blood Flow | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

## Table C-2: Sample Sizes for 28h FL HDBR Post verses 28h NFL HDBR Post Comparison Data

| | 28h NFL HDBR Post Test | | | | | 28h FL HDBR Post Test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | -10 | -20 | -30 | -40 | 0 | -10 | -20 | -30 | -40 |
| Total Blood Volume | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Plasma Volume | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Hematocrit | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Weight | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Heart Rate | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| R-R Interval | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Mean Arterial Pressure | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Systolic Blood Pressure | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Diastolic Blood Pressure | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Pulse Pressure | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Stroke Volume | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Cardiac Output | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Total Peripheral Resistance | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Arterial Compliance | 8 | | | | | 8 | | | | |
| Central Venous Pressure | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| IVC Diameter | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Venous Compliance | 6 | | | | | 6 | | | | |
| Portal Vein Diameter | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Portal Flow | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Splanchnic Impedance | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Ejection Fraction | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Fractional Shortening | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| MF Gain | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| HF Gain | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| MF Phase | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| HF Phase | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| MF Coherence | 5 | NA | 5 | NA | 5 | 5 | NA | 5 | NA | 5 |
| HF Coherence | 5 | NA | 5 | NA | 5 | 5 | NA | 5 | NA | 5 |
| Brachial Diameter | 7 | NA | NA | NA | NA | 7 | NA | NA | NA | NA |
| Brachial Blood Flow Velocity | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Brachial Blood Flow | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

# APPENDIX D: AORTIC DOPPLER ULTRASOUND DATA

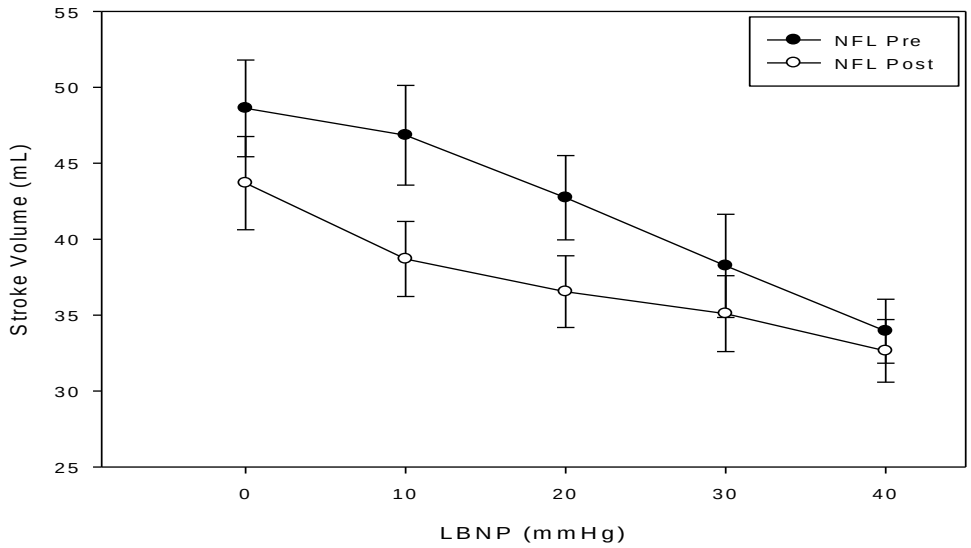


**Figure D-1: Stroke Volume Response during Pre and Post 28h NFL HDBR Testing (measured by Doppler Ultrasound)**

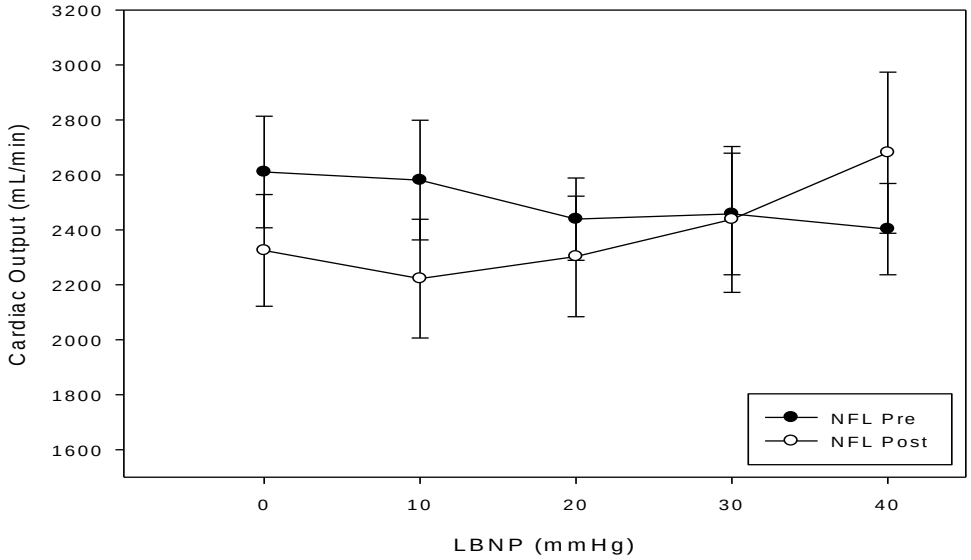


**Figure D-2: Cardiac Output during Pre and Post 28h NFL HDBR Testing (measured by Doppler Ultrasound)**

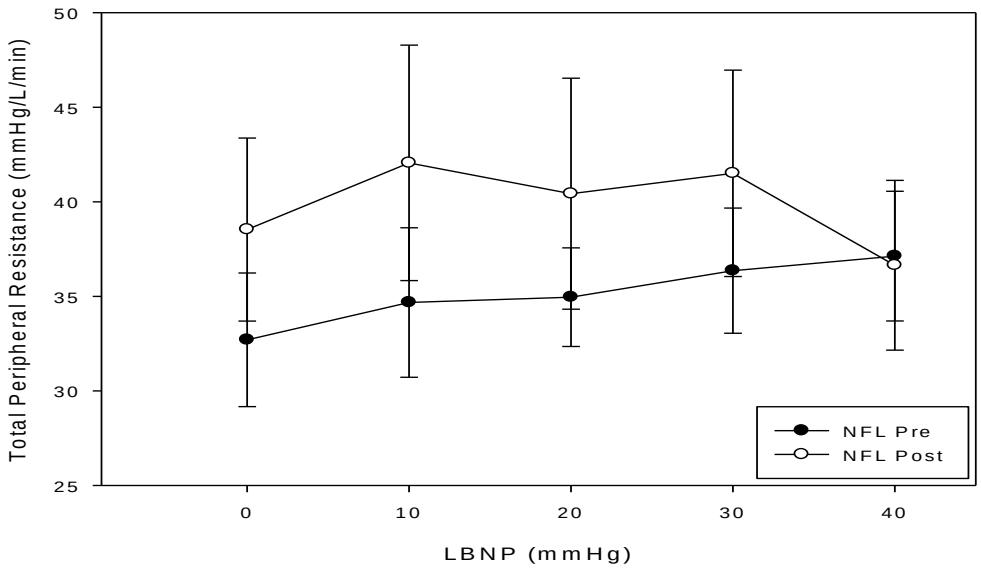


**Figure D-3: Total Peripheral Resistance Response during Pre and Post 28h NFL HDBR Testing (measured by Doppler Ultrasound)**

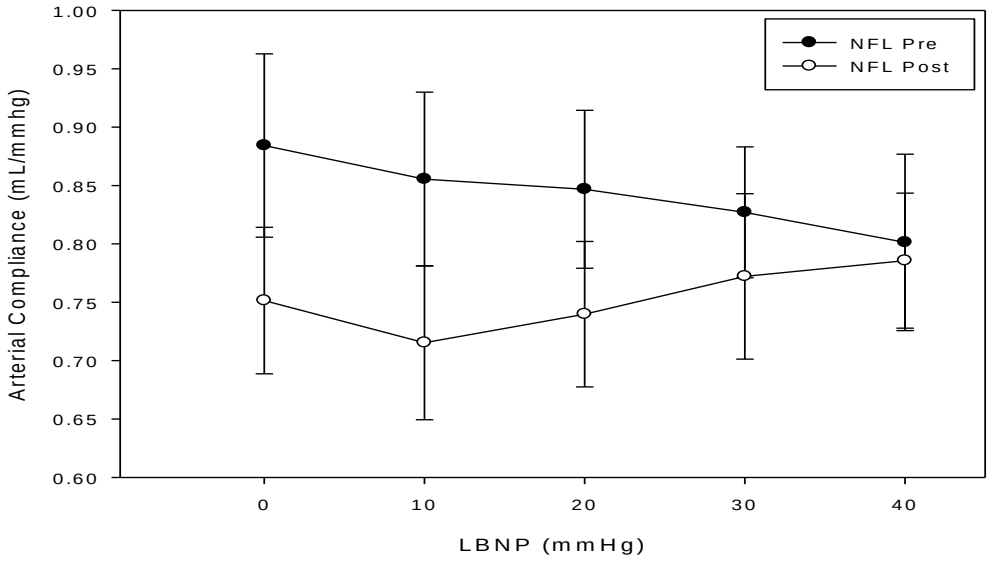


**Figure D-4: Arterial Compliance Response during Pre and Post 28h NFL HDBR Testing (measured by Doppler Ultrasound)**

# APPENDIX E: PORTAL VEIN BLOOD FLOW DATA

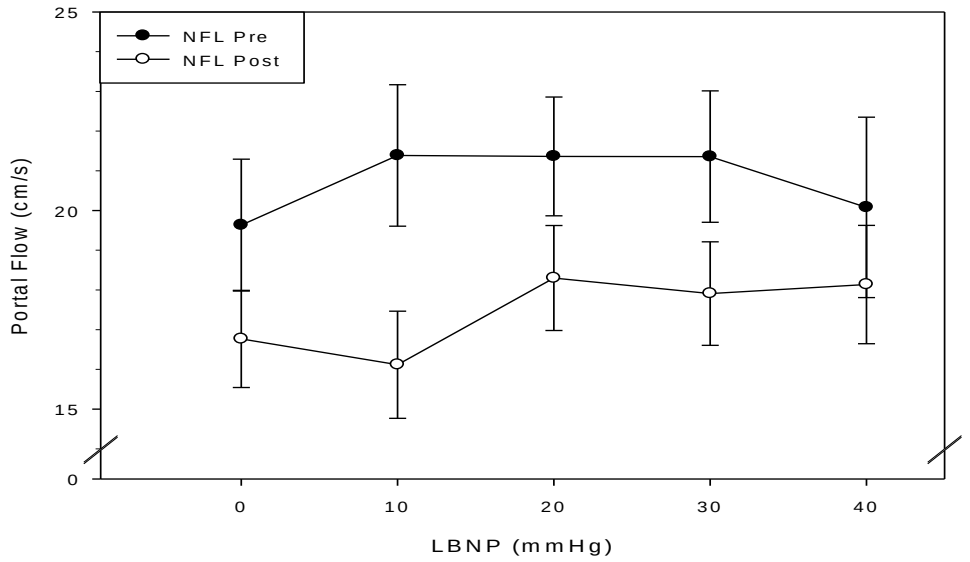


**Figure E-1: Portal Vein Blood Flow Response during Pre and Post 28h NFL HDBR Testing**

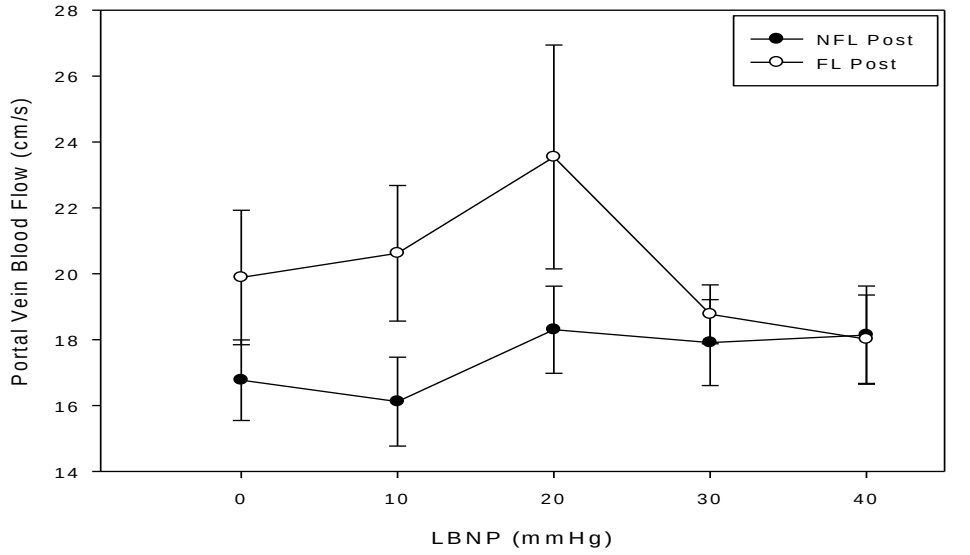


**Figure E-2: Portal Vein Blood Flow Response following 28h NFL HDBR and 28h FL HDBR Response**

## APPENDIX F: CARDIOPULMONARY BAROREFLEX DATA

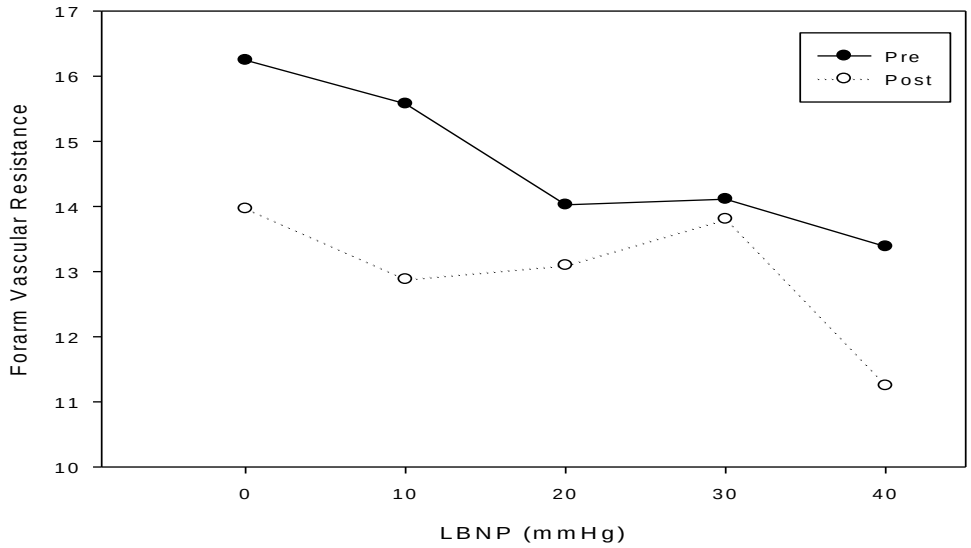


**Figure F-1: Cardiopulmonary Baroreflex Response (Forearm Vascular Resistance vs. Orthostatic Stress) during Pre and Post 28h NFL HDBR Testing**

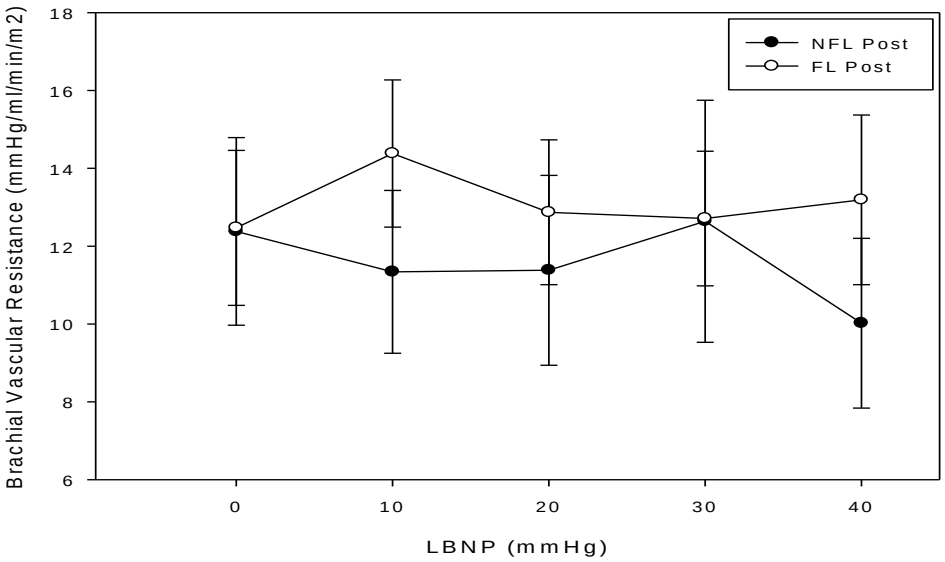


**Figure F-2: Cardiopulmonary Baroreflex Response (Forearm Vascular Resistance vs. Orthostatic Stress) following 28h NFL HDBR and 28h FL HDBR Response**