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CALF HEMODYNAMICS DURING VENOUS OCCLUSION AND HEAD-UP TILT

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ABSTRACT OF THESIS

CALF HEMODYNAMICS DURING VENOUS OCCLUSION AND HEAD-UP TILT

The potential role of lower limb blood pooling in reducing venous return to the heart during orthostasis and elevated venous pressure is investigated. This study compares lower limb capacitance, microvascular filtration, and peripheral resistance between a group of highly trained endurance athletes and a group of their sedentary peers. Seven endurance trained males were selected between the ages of 23-33 [(29.1 ± 4.1 yr), mean ± SD]. The subjects’ weekly cycling mileage ranged from 80 to 150 miles per week with an average of 125 ± 8.5 miles/week. Nine healthy, age-matched sedentary subjects (25.8 ± 4.8 yr.) were selected for the control group, based upon their reporting they had not participated in repeated lower-body or cardiovascular exercise in the months prior to their study. Results show that both subject groups had similar calf venous capacitances, rates of capillary fluid filtration, and local flow shunting (vascular resistance change) in response to venous thigh occlusion and 70° head-up tilt (HUT). The only significant difference found between groups was the cyclist group’s smaller rise in heart rate in response to HUT. The findings of this study suggest that cyclists are not predisposed to orthostatic intolerance due to any changes in lower limb function.

KEYWORDS: Orthostatic Intolerance, Calf Hemodynamics, Venous Occlusion, Head-Up Tilt, Endurance Training

Peter J Kilfoil II
Feb. 17, 2007

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CALF HEMODYNAMICS DURING VENOUS OCCLUSION AND HEAD-UP TILT

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Feb. 17, 2007
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Peter Joseph Kilfoil II

The Graduate School
University of Kentucky
2007
CALF HEMODYNAMICS DURING VENOUS OCCLUSION AND HEAD-UP TILT

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master’s of Science

By

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The Graduate School

University of Kentucky

Director: Dr. Abhijit Patwardhan, Professor of Biomedical Engineering

Lexington, Kentucky

2007

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ............................................................................................................. iii
LIST OF FIGURES ......................................................................................................................... v
LIST OF FILES ............................................................................................................................... vii
Chapter 1: INTRODUCTION ........................................................................................................... 1
Chapter 2: BACKGROUND ............................................................................................................... 4
  Effects of Gravity on the Cardiovascular System: ................................................................. 4
  Lower body venous pooling .................................................................................................. 6
  Venoarteriolar Reflex ............................................................................................................. 7
  Myogenic Reflex ..................................................................................................................... 7
  Spaceflight deconditioning ..................................................................................................... 7
Chapter 3: METHODS .................................................................................................................... 11
  Subjects .................................................................................................................................. 11
  Protocol ................................................................................................................................. 11
  Measurements ....................................................................................................................... 14
    Calf Circumference ............................................................................................................ 14
    Arterial Pressure ................................................................................................................ 14
    Skin Perfusion ..................................................................................................................... 14
    Heart Rate .......................................................................................................................... 15
    Segmental Impedance ........................................................................................................ 15
    Venous Collecting Cuff Pressure ..................................................................................... 15
    Tilt Angle ............................................................................................................................ 16
  Data Acquisition .................................................................................................................... 16
  Data Analysis .......................................................................................................................... 16
    Mean Value Analysis ......................................................................................................... 16
    Spectral Power Analysis ..................................................................................................... 18
    Calf Circumference Rate of Change ................................................................................. 18
  Statistics .................................................................................................................................. 18
Chapter 4: RESULTS ...................................................................................................................... 21
Chapter 5: DISCUSSION .................................................................................................................. 37
Chapter 6: CONCLUSION .............................................................................................................. 43
APPENDIX ...................................................................................................................................... 44
REFERENCES ................................................................................................................................. 48
Vita .................................................................................................................................................. 52
Figure 3.1: One typical subject’s data record showing (from top to bottom) venous occlusion pressure, tilt angle, calf circumference change, calf resistance, and calf skin perfusion.

Figure 3.2: Method used for calculating arterial inflow in response to 90 mmHg venous occlusion. Upper figure (a) shows CC response to progressively larger venous occlusion pressures, lower figure (b) depicts the portion of the CC curve used to calculate arterial inflow slope.

Figure 4.1: Mean heart rates during control, head-up tilt, and recovery for sedentary and cyclist groups (mean +/- sem).

Figure 4.2: Mean heart rate rise in response to head-up tilt for sedentary and cyclist groups (mean +/- sem).

Figure 4.3: Mean stroke volume calculated using Kubicek’s method (9, 44) during control (pretilt), head-up tilt, and recovery, for sedentary and cyclist groups (mean +/- sem).

Figure 4.4: Mean stroke volume calculated using Sramek’s (9, 44) method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups (mean +/- sem).

Figure 4.5: Mean cardiac output calculated using Kubicek’s method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups.

Figure 4.6: Mean cardiac output calculated using Sramek’s method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups.

Figure 4.7: Percent change in cardiac output from control during head-up tilt, calculated using Sramek’s method (mean +/- sem).

Figure 4.8: Mean total peripheral resistance (TPR) for sedentary and cyclist groups during control (pretilt), tilt, and recovery, calculated using Kubicek’s method.

Figure 4.9: Mean total peripheral resistance (TPR) for sedentary and cyclist groups during control (pretilt), tilt, and recovery, calculated using Sramek’s method.

Figure 4.10: Mean arterial pressure measured at heart level for sedentary and cyclist groups during control (pretilt), tilt, and recovery periods (mean +/- sem).

Figure 4.11: Percent change in calf resistance as measured using impedance plethysmography from control (pretill) values and successive thigh occlusions of increasing pressure and after 10 minutes HUT for both groups (mean +/- sem).

Figure 4.12: Percent change in calf circumference as measured using strain gauge plethysmography from control (pretill) values and successive thigh occlusions of increasing pressure and after 10 minutes HUT for both groups (mean +/- sem).

Figure 4.13: Percent change in skin blood flow (anterior calf) measured using laser-Doppler from control (pretill) values and successive thigh occlusions of increasing pressure, and after 10 minutes HUT for both groups (mean +/- sem).

Figure 4.14: Mean calf arterial inflow rates (ml / 100ml tissue / min) calculated from the rate of change of the calf circumference response to increasing venous occlusion pressures for both groups (mean +/- sem).
Figure 4.15: Correlation between skin perfusion and arterial inflow values for all
subjects ($R^2 = 0.96$). ................................................................. 32
Figure 4.16: Very low frequency (0.01-0.02 Hz) spectral power of calf skin
perfusion signal during venous occlusions and HUT for both groups (mean +/-
SEM). ........................................................................................................... 32
Figure 4.17: Low frequency (0.03-0.05 Hz) spectral power of calf skin perfusion
during venous occlusions and HUT for both groups (mean +/- SEM). ........... 33
Figure 4.18: Middle frequency (0.08-0.12 Hz) spectral power of calf skin
perfusion during venous occlusions and HUT for both groups (mean +/- SEM). ........... 33
Figure 4.19: Fluid filtration rates (ml / 100ml tissue /min) during venous
occlusions of 30, 35, 40, 45, and 50mm Hg for both groups (mean +/- SEM). .......... 34
Figure 4.20: Mean capillary filtration capacity (capillary filtration coefficient),
$K_f$ for both groups (mean +/- SEM). ............................................................ 34
Figure 4.21: Calculation of isovolumetric venous pressure, $P_{v,i}$ for one typical
subject (mean +/- SEM). .............................................................................. 35
Figure 4.22: Mean isovolumetric venous pressures calculated for both groups
(mean +/- SEM). .............................................................................................. 35
Figure 4.23: Illustration of methods used to calculate venous outflow rates
immediately following cuff deflation for one typical subject. .......................... 36
Figure 4.24: Mean venous emptying rates, primary and secondary slopes,
calculated for both groups (mean +/- SEM). .................................................. 36
LIST OF FILES

pkthesis.pdf  1.2MB
Chapter 1: INTRODUCTION

The main goal of this study was to investigate whether endurance trained athletes have a tendency to pool more fluid in their lower limbs during periods of orthostatic stress as compared to their untrained counterparts, and, if so, to uncover the mechanisms that may contribute to this phenomenon.

Previous research has been inconclusive in determining how endurance training affects orthostatic tolerance; results from different investigations have shown no influence, a negative effect, or a positive effect (16, 28). This lack of clarity may potentially cause a conflict with current research investigating the effects of artificial gravity (AG) training to increase orthostatic tolerance. Current research in our laboratory focuses on Human Powered Centrifuge (HPC) training in which subjects perform bicycle ergometry while being exposed to a $G_z$ (head-to-foot) gravity gradient. In these studies performed at NASA Ames two subjects rode on the short-arm HPC, with one of the subjects pedaling to provide the centripetal acceleration used to simulate gravity. A hypothesis of this HPC research is that the combination of exercise with AG training may provide the best countermeasure for the cardiovascular deconditioning that results in orthostatic intolerance following exposure to microgravity. The foundation of this argument is based upon both the positive effects of the cardiovascular remodeling associated with exercise training (increased plasma volume and cardiac output), as well as the shown tendency for short bouts of 1 $G_z$ gravity exposure to maintain tolerance in bed rest subjects (49). Results from the studies conducted by investigators in our laboratory demonstrated improved orthostatic tolerance after HPC training in ambulatory subjects as well (11).

The effects of exercise training during microgravity to successfully combat the reduction in plasma volume and muscle mass associated with space flight are well documented. Decreased plasma volume has the ability to markedly affect cerebral perfusion pressure during post flight orthostasis. During microgravity exposure, blood is redistributed towards the body’s core, as the downward pull of gravity that causes blood to remain in the lower body is removed. Upon return to Earth’s gravity, the tendency for blood to pool in the lower body returns, amplifying the effects of decreased plasma volume on venous return, and thus stroke volume and ultimately
cardiac output. Maintenance of plasma volume therefore appears to be key in preventing post-flight orthostatic tolerance.

Another beneficial effect of lower body exercise during space flight is maintenance of leg musculature, which is of importance due to the contribution of the muscle pump on venous return. The muscle pump is a mechanism in which small, rhythmic contractions of leg muscles push blood back towards the heart from the lower venous vasculature. Loss of muscle mass has the ability to attenuate the effects of this “second heart,” and thus ultimately diminish ventricular filling pressure. Unfortunately, NASA’s current cardiovascular deconditioning countermeasure, riding on a stationary bicycle, has not had any appreciable effects in preventing syncope upon return to earth (6, 38).

Vernikos et al. demonstrated that walking or standing for as little as two hours per day, in 15 minute bouts, one per hour, prevented decreased orthostatic tolerance during four days of $6^\circ$ head-down bed rest (HDBR) (49). Head-down bed rest has been shown to simulate microgravity, with HDBR eliciting orthostatic intolerance similar to that seen in astronauts returning from space travel (41). In the study by Vernikos et al., it was found that passive standing and activity (walking) under $1 \text{G}_z$ exposure helped subjects to maintain plasma volume as compared to a group who remained in the HDBR position for the duration of the investigation. Their results showed that intermittent gravity exposure successfully attenuated the orthostatic intolerance caused by bed rest.

Despite the apparent benefits that exercise, specifically when combined with $a + G_z$ force, would logically have on orthostatic tolerance, the paradox that highly trained individuals tend to exhibit decreased tolerance remains (48). Possible explanations have included an increased sensitivity to decreased preload due to cardiac muscle remodeling (48), increased lower body capillary filtration (4), and increased venous compliance (50). Several studies have shown that decreased orthostatic tolerance fails to correlate with increased venous compliance (3, 18, 22, 44, 50, 52), as is associated with highly trained individuals. Nevertheless, the mechanisms that dictate the amount of blood that is trapped in the lower body upon standing are of interest and are worthy of investigation, as they may explain the causes for the decreased venous return in these subjects.

Lower body blood pooling is generally believed to be dictated by venous compliance, that is, the elasticity of the venous vasculature. The higher the compliance of a vascular chamber, the
greater the amount of blood it will accommodate under a given fluid pressure. Thus, as athletes have an elevated venous compliance, more blood should be stored in their venous system during orthostasis (i.e. they have greater venous capacitance). In addition, there is evidence that athletes have the tendency to filter more plasma across their microvasculature than the non-trained subjects, which has been proposed as a potential contributor to their decreased tolerance to standing (16).

Data collected from astronauts immediately following space flight have shown that those astronauts with the most marked orthostatic intolerance were those who had the smallest increases in total peripheral resistance (TPR) in response to standing (53). By increasing systemic resistance, subjects divert blood away from the extremities, reducing the amount of blood that flows to their legs. This shunting of arterial blood flow from the legs thus limits the amount of blood available to pool in the venous vasculature during orthostatic stress.

The aim of the present study is to compare the function of the above stated mechanisms (lower limb capacitance, microvascular filtration, peripheral resistance) between a group of highly endurance trained athletes and a group of their sedentary peers, in a noninvasive manner. Avid cyclist (124 ± 9 mile/week, mean ± sem) were chosen to represent the athlete group. This level of exercise was similar to that performed by subjects on the HPC. Each subject’s calf capacitance was measured using both strain gauge and impedance plethysmographs, their cardiovascular indices compared using impedance cardiography, filtration assessed from strain gauge information, and local resistance mechanisms investigated using information derived from skin perfusion and strain gauge signals. All procedures used were in accordance with similar investigations found in the literature.

Head-up tilt (HUT) and venous occlusion were used to elicit a footward fluid shift and lower leg fluid retention. We believe that comparing the filtration and vascular resistance changes caused by these two stresses can lend insight into the relative contribution that local and systemic mechanisms play in determining fluid sequestration in the lower limbs in the two subject groups.
Effects of Gravity on the Cardiovascular System:

The human cardiovascular system is continually subjected to the forces of Earth’s gravitational field, which causes blood to be distributed towards the lower extremities during upright posture. Orthostasis (which literally means “standing upright”) increases the arterial and venous pressures in the legs, as compared to a reference in the supine posture. The venous vasculature, through which blood is returned to the heart, has a much higher compliance than that in the arterial circulation, causing large increases in venous volume in response to small increases in transmural pressure. Accordingly, during orthostasis, approximately 75% of the blood volume is found in compliant veins. In addition, about 70-75% of the total blood volume is located below the right atrium, and thus must be driven back to the heart against a hydrostatic gradient (30).

In addition to the heart, an important actuator of venous return from the extremities is the skeletal muscle pump system (34), with a lesser degree of venous return modulation coming as a result of central nervous system (CNS) mediated active venuconstriction (13, 19). Veins, particularly those in the extremities, contain a series of one-way valves that only allow blood to flow in the direction of the heart. Much of this venous vasculature is surrounded by skeletal muscle; when this muscle contracts, a force is exerted on the outside of the vein, increasing local venous pressure, opening the ‘downstream’ valve, and closing the ‘upstream’ valve. This pumping action forces blood toward the heart during muscular contractions and keeps it from flowing retrograde to circulation when the veins are fully distended. The function of this system is crucial in the maintenance of ventricular filling pressure because at normal levels of venous smooth muscle sympathetic tone, blood volume is too small to fill the vascular container in the upright posture (30). This muscle pump is particularly active during the rhythmic muscular contractions associated with walking, though the small rhythmic contraction of antigravity muscles during quiet standing produces a significant pumping effect, even without conscious leg movement.

Venoconstriction, the constriction of the smooth muscle in the outer venous wall, can also serve to increase the volume of blood returned to the heart. In response to sympathetic discharge, adrenaline, and/or angiotensin II, venous diameter decreases, reducing the volume of
blood that can ‘pool’ in the venous circulation (2, 10, 29). However, research has shown little evidence for active venoconstriction in the limbs in response to orthostasis (45), though neurovascular and humoral signals do cause significant venoconstriction in splanchnic tissues (17).

The respiratory pump also significantly affects venous return to the heart. Expansion of the chest during inspiration causes negative pressure in the thoracic cavity, creating a suction effect which increases the pressure gradient between the venous vasculature and right atrium, thus increasing end diastolic volume (EDV).

All enhancements in venous return elevate EDV, which results in an increase in stroke volume (SV) due to an intrinsic property of the cardiac tissue, the Frank-Starling Mechanism, which increases the force of contraction during systole in response to an increase in the stretch of cardiac muscle fibers during diastole.

The head-up tilt (HUT) test is a common procedure used to illicit passive and active responses to postural stress. As the body is shifted from supine to upright posture, a host of physiological changes occur. The hydrostatic pressure difference this creates between the heart and specialized stretch-sensitive tissues known as baroreceptors, located at the carotid sinus and aortic arch, detect drops in arterial blood pressure associated with standing. The signals sent by the baroreceptors to the CNS cause a shift in parasympathetic (within ~1 sec) and/or sympathetic (within ~10 sec) discharge, causing a reflex rise in heart rate and cardiac contractility. The increased heart rate acts to maintain cardiac output in the face of the decreased preload (and thus reduced stroke volume), caused by the pooling of blood in the lower limbs and splanchnic region. A reserve volume of blood located in the pulmonary circulation acts as a short term buffer, maintaining left ventricular EDV for several heart beats as the activated leg muscle pump begins to drive blood upward.

Sympathetic discharge triggered by baroreceptor unloading also acts to increase total peripheral resistance (TPR) in the arterial circulation through fast (neural) and slower (humoral) mechanisms acting primarily on arterioles. This vasoconstriction acts to maintain arterial pressure, despite a reduction in cardiac output, as well as shunting blood flow from peripheral circulation, such as to the lower limbs. It is well documented that the regions with the greatest vascular conductance undergo the most dramatic resistance changes: a 40% reduction in splanchnic blood flow during 70° HUT (54) and a 32% reduction in renal flow during 60° HUT.
Blood flow to cutaneous tissue has also been shown to be under sympathetic arteriolar control (40).

**Lower body venous pooling:**

The hydrostatic pressure increase in the legs’ veins caused during standing results in pooling of blood in these dependent veins. Blood pooling refers not to a total sequestration of blood to the lower limb vasculature, but rather to the increased transit time of blood through veins. This hydrostatically induced pressure rise increases venous transmural pressure, which due to the highly compliant nature of the venous wall, causes large increases in venous cross-sectional area, and thus venous volume. The increase in lower limb venous volume and reduction in flow rate cause the phenomenon commonly known as “pooling,” which reduces cardiac preload and the amount of blood available to the central circulation. In pathological individuals who lack one or more of the natural compensatory cardiovascular reflex responses to upright posture (increased TPR, vеноconstriction, muscle pump activity, vagal and sympathetic heart rate responses etc.), lower limb pooling puts a severe stress on the circulatory system. When the cardiovascular system is stressed to the point where cerebral perfusion pressure cannot be maintained, the body’s protective reflex is to initiate fainting, causing a fall to the recumbent position, providing the conditions for increased venous return to the right atrium.

Venous compliance, the change in calf capacitance per venous occlusion pressure, has been shown to be reduced in the elderly (37) and in endurance trained athletes as compared to their sedentary peers (31). Increases in lower limb compliance are associated with increased pooling, though Louisy’s research showed that endurance training increased venous dispensability without affecting venous emptying and venous return from the legs.

A great deal of research has been focused on examining the relationship between lower-limb venous compliance and orthostatic intolerance. Almost all results have demonstrated no direct relationship between the two: increased venous compliance does not result in decreased tolerance to orthostatic stress (3, 44, 50).
**Venoarteriolar Reflex:**

The venoarteriolar reflex (VAR) is a local axonal reflex causing enhanced arterial vasoconstriction in response to increased transmural pressure in the venous circulation. In an axonal reflex, action potentials in an afferent neuron move peripherally, that is, without conduction up the axon in the direction of the CNS. Stretch receptors, believed to be located in the small veins, cause an increase in upstream arteriolar tone via an unknown neural pathway, which is non-α-adrenergic in nature (8). This response, first described by Henriksen et al.(20), is invoked when the venous pressure in a limb exceeds about 25mm Hg, resulting from either venous stasis or a hydrostatic gradient caused by a lowering of the limb below heart level. Activation of the VAR acts upon local cutaneous, subcutaneous, and muscle tissues, resulting in a decrease in blood flow of about ~40% (20, 42, 43). It has been estimated that up to 45 % of the total peripheral resistance response to orthostatic stress is due to the VAR, with the remaining 55 % resulting from centrally mediated adrenergic vasoconstriction brought about by arterial and cardiopulmonary baroreceptor unloading (21).

**Myogenic Reflex:**

Another closely related mechanism regulating regional blood flow is the myogenic reflex, which describes the ability of the afferent arteriole to either constrict or dilate in response to changes in intraluminal pressure. Myogenic influences on blood flow are most prominently displayed in the lower limbs during standing, due to the hydrostatically-induced rise in arterial pressure. Evidence shows that this mechanism is local in nature: leg vascular resistance increases in response to HUT in subjects with synaptic blockade and CNS denervation (5,9). In addition, leg vascular resistance is elevated 2.5 times that in the arm during HUT, despite similar changes in muscle sympathetic nerve activity (MSNA) (24).

**Spaceflight deconditioning:**

As previously mentioned, human exposure to microgravity (by both prolonged bed rest and space flight) results in “deconditioning” of the cardiovascular, manifested as tachycardia (excessively high heart rate), reduced exercise capacity, and orthostatic intolerance (3) upon return to normal gravity conditions.
When the human body moves from earth to microgravity, the removal of the downward pulling gravity vector causes a redistribution of blood away from the lower extremities towards the thorax. This elevation in core plasma volume is detected by arterial baroreceptors, ultimately reducing plasma volume by reducing thirst. This decrease in plasma volume has not proven to be problematic during space flight, as the CV system adjusts to this lower operating point. Increased lower limb vascular resistance during microgravity exposure (46) may be one mechanism by which the body responds to this change, aiding in the maintenance of arterial pressure despite a reduction in cardiac output (50).

The lower limbs are greatly affected by microgravity due to muscular disuse of supporting leg muscles, reduced blood flow, and lower intraluminal pressures. Skeletal muscle atrophy seems to be the main determiner of calf compliance changes during short-term microgravity (< 20 days) (32, 33), with increasing compliance correlating with decreased calf muscle volume (6). Studies examining longer term microgravity exposure reveal that after this initial period of ~20 days, calf compliance begins to decrease (32) while calf skeletal muscle volume loss reaches a plateau. It is reasoned that muscle atrophy dominates whole calf compliance, while changes in the compliance of the venous vasculature are not detectable until the atrophy has subsided (3).

One of the countermeasures currently in place by NASA to combat generalized cardiovascular deconditioning is bicycle ergometry, which is successful in maintaining plasma volume and minimizing muscle loss. Nevertheless, in-flight ergometry has proven to have no positive affect on post-flight orthostatic intolerance. Researchers have hypothesized that the in-flight ergometry has no affect on orthostatic tolerance due to the lack of a downward pulling G-force on the body during the exercise. The notion that exposure to short bouts of $G_z$-gravity aid in the maintenance of orthostatic tolerance became the topic of research after it was found that bedridden subjects retained a significant degree of their orthostatic tolerance by merely standing upright for 2 hours/ day (49). This idea later became one of the driving forces behind artificial gravity training, in which astronauts potentially will be exposed to repeated bouts of artificial gravity, produced through whole body centrifugation, while in microgravity.

Taking into account the space limitations of the currently available Shuttle fleet, NASA designed the Human Powered Centrifuge (HPC), which is a short arm centrifuge of 1.9 m radius,
on which two subjects ride simultaneously. The HPC is designed such that one or both riders provide the power for the centrifuge to rotate by way of a pedal system.

Our research team is concurrently investigating the effects of HPC training on orthostatic tolerance. In this protocol, ambulatory subjects were exposed to three weeks (5x/week) of $G_z$ acceleration training on NASA-Ames’ (Moffet Field, CA) human-powered centrifuge. Subjects trained supine (head near the center of rotation) and in pairs; one subject rode passively while the other provided power to operate the 1.9-m centrifuge. The acceleration profile consisted of 7 min at 1 $G_z$ before alternating between 1 and 2.5 $G_z$ at 2-min intervals for 28 min. Each subject’s presyncopal orthostatic tolerance limit (to a combination of 70° head-up tilt and increasing lower body negative pressure) was determined before and after training (11). Results from this study show that artificial gravity (AG) training did increase orthostatic tolerance by 17 ± 10% (p<0.05), though no difference was found between the active and passive protocols.

Mechanisms that were associated with increased tolerance in the above study included increased cardiac output and stroke volume, and decreased arterial pressure and total peripheral resistance. The relationship between increased cardiac output with decreased peripheral resistance led to the conclusion that venous return had too been improved. These results spurned this study, one of the goals of which was to develop a procedure for assessing the venous vascular mechanics of the lower leg.

One significant concern that arises is whether endurance bicycle training may actually have detrimental effect on orthostatic tolerance. While improving the aerobic capacity of moderately fit individuals though endurance training has been shown to increase plasma volume, which is usually associated with increased orthostatic tolerance (48), highly trained individuals often exhibit diminished orthostatic tolerance when compared to their untrained counterparts. It is widely accepted that “trained men can run, but cannot stand,” although the mechanisms causing this phenomenon are unknown. Evidence points to possible carotid baroreceptor (CBR) attenuation, and to the fact that endurance-trained subjects’ hearts are remodeled to operate on a steeper slope of the left cardiac pressure-stroke volume curve (36). Increasing cardiac muscle contractility has a positive inotropic effect, causing an increase in stroke volume (SV) in response to a given pre-load. This allows for athletes to have a higher cardiac output as compared to sedentary subjects, which is especially useful during strenuous exercise. On the other hand, this makes their CO more
susceptible to a fall in venous filling pressure, which accompanies central volume depletion during orthostasis.

In the present study, we chose to examine the potential role of the lower limbs in reducing venous return to the heart by pooling blood during orthostasis and elevated venous pressure. The investigation should determine whether or not endurance cycling promotes the pooling of blood in the lower limbs in response to +Gz.
Chapter 3: METHODS

Subjects
Cyclists were recruited from a local cycling club (Bluegrass Cycling Club), and were selected for participation in this study according to their age, sex, and cycling routine. Seven males were selected between the ages of 23-33 [ (29.1 ± 4.1 yr), mean ± SD], of weight (89.3 ± 12.6 kg), and of height (183.2 ± 8.5 cm). The subjects’ weekly cycling mileage ranged from 80 to 150 miles per week with an average of 125 ± 8.5 miles/week.

Nine healthy, age-matched sedentary men (25.8 ± 4.8 yr.) were selected to participate in the control group, based upon their reporting that they had not participated in repeated lower-body or cardiovascular exercise in the months prior to their study. This control group’s weight (80.8 ± 6.9 kg) and height (178 ± 6 cm) were statistically similar to that of the cyclist group. All subject information can be found in Appendix A. Only men were chosen to participate in this pilot experiment as it was believed that the effects of estrogen on smooth muscles would add to the complexity of women’s data. It was decided that any future studies would contain female subjects, but for this initial investigatory study, only men would be used to minimize variables such as those related to hormonal interactions which could affect the results of the testing.

Each subject was informed of the purpose and risks of this institutionally approved study (IRB# 97-00034) before providing written consent. The consent form was approved by the Institutional Review Board at the University of Kentucky.

Protocol
All data collection took place in Room 13 at the Wenner-Gren Research Laboratory, University of Kentucky between 3/24/05 and 5/11/05. All subjects were studied at least two hours post-prandial and were asked to refrain from alcohol and caffeine on the day of the experiment. During the initial briefing period prior to instrumentation, participants were also asked to empty their bladders before commencing the approximately 90 minute study. Subjects were also asked to wear loose shorts and a T-shirt to provide accessibility during the instrumentation process.

Following the subjects’ briefing, during which they were acquainted with the instrumentation and experimental procedures, subjects were instructed to rest supine on the tilt
table. A bicycle seat, connected to sturdy PVC tubing supported by the foot rest of the tilt table, was adjusted according to the subjects’ height such that upon 70° head-up tilt (HUT), the bicycle seat would bear most or all of the subject’s weight, allowing the legs to be dependant. Once comfortable on the tilt table, the subject’s left foot was placed on a foam block approximately 15 cm above the level of the table, where it remained elevated for the duration of data collection. After being fixed to the tilt table by straps at the chest and pelvis, the tilt table was rotated to 70° for approximately 10 seconds to ensure that the seat was providing proper support, that neither leg was bearing significant weight, and that the subjects could make any requests regarding comfort in anticipation of the 10 minute HUT that would follow.

The experimental procedure consisted of two distinct phases: a thigh-cuff venous occlusion phase followed by 10 minutes of HUT. The venous occlusion phase began with an assessment of resting venous pressure in the elevated calf, which was performed by slowly ramping up the venous occlusion pressure from 0mm Hg to approximately 15mm Hg, and by detecting the occlusion pressure at which calf circumference first began to rise. Three assessments of resting venous pressure were performed, with at least 30 seconds between measurements, which was more than enough time for calf circumference to return to its pre-occlusion value.

Next, an assessment of arterial inflow was made by rapidly inflating the occlusion cuff to 90mm Hg in order to calculating the resultant rate of change in calf circumference. Supine arterial inflow assessments were made three times, with one minute separating the inflations.

Following the arterial inflow assessments, venous congestion pressure was raised in a series of 5mm Hg steps, starting at 20mm Hg and continuing to 50mm Hg. Each occlusion level was held for three minutes, at the conclusion of which, the congestion pressure was briefly raised to 90mm Hg and held for less than 10 seconds to assess arterial inflow. Cuff pressure was returned to 0mm Hg for one minute before increasing the venous congestion pressure by 5mm Hg from the previous occlusion pressure. Following the completion of 50mm Hg venous congestion stage, the subject was asked to remain resting on the tilt table while preparations were made for the HUT.

Prior to the HUT, subjects were resecured, and checked for tightness to the tilt table by straps placed across their pelvis and chest, taking care not to disturb the instrumentation present at these locations. In addition, shoulder slings were placed on the subjects’ left arms so that they
would be supported when the table rotated, keeping the hand in the same position (resting above the sternum) without requiring voluntary subject involvement.

Following approximately 10-15 minutes of rest in the supine position, the tilt table was rotated to and held at 70° for approximately 10 minutes. Care was taken to ensure that the instrumented leg remained dependent and lightly rested upon the foam foot rest. If a subject’s opposing foot came in contact with the tilt table’s foot rest, they were instructed that they could use it to provide a little balance, but not to support weight upon it. Approximately 10 minutes into the tilt, the venous congestion cuff pressure was raised from 0mm Hg to 90mm Hg for less than 10 seconds to make the final arterial inflow assessment. Immediately following cuff deflation, the tilt table was returned to the supine position, where the subject remained for at least five minutes before the experiment was completed.

During all tilts, a certified team member continuously monitored the subjects’ hemodynamic variables and general alertness. If a subject developed any syncopal symptoms (precursors to fainting) during the tilt, they were immediately returned to the supine position, where they were held for 15 minutes to ensure full recovery.

Labeled photographs of the experimental setup are provided in Appendix B; including one instrumented subject during HUT and supine recovery.
Measurements:

Calf Circumference:
Calf circumference was measured using a Hokanson EC-4 Plethysmograph (Hokanson, Bellvue, Washington) and a mercury-in-rubber strain gauge placed around the calf at the point of maximal circumference.

Arterial Pressure:
Beat-to-beat non-invasive continuous blood pressure was measured and referenced at heart level using a Finapres (Finapres Medical Systems, The Netherlands), with its finger cuff placed on either the subjects’ left index, middle or ring finger, depending on the integrity of the signals produced in each.

Manual readings of brachial arterial blood pressure were made using an automated blood pressure cuff at the onset of data collection and prior to the HUT.

Skin Perfusion:
Laser-Doppler probes (Perimed, Stockholm, Sweden) were placed on the lateral tibia at the level of the rubber strain gauge and on the medial malleolus of the left foot to measure superficial red-cell flux, which was measured in arbitrary perfusion units (p.u.). These sites were shaven and cleaned with an alcohol prep pad if any body hair was present. The initial skin red-cell flux signals were noted, and probes were removed and placed over a directly adjacent position if the initial site did not provide a sustained perfusion signal, which was chosen to be at about 15 arbitrary perfusion units, read from the device’s digital display.

Due to the nature of laser-Doppler flowmetry, perfusion in only a minute area of skin is measured, and exact placement is thus irreproducible between subjects. Because of the potential for large variations in skin perfusion between anatomically adjacent sites, relative changes in the red-cell flux were examined, rather than raw perfusion units.
Heart Rate:
A continuous recording of heart rate was made using a three-lead ECG (UFI, Morrow Bay, CA), the output of which was post-processed to provide heart rate and r-r interval. Real-time beat-to-beat estimates of heart rate were also displayed on the Finapres, which was monitored during HUT to detect presyncopal symptoms.

Segmental Impedance:
Impedance plethysmography was used to determine segmental volume shifts in three body segments: the calf, splanchnic area, and thorax. Impedance plethysmography is based on the principle that when the volume of fluid residing in a tissue segment increases, the electrical impedance in that segment decreases proportionally. A four-channel tetrapolar high-resolution impedance meter, or THRIM (UFI, Morrow Bay, CA), was used to pass a 51.2 kHz, 1mA excitation signal through the body, with current insertion electrodes placed on the forehead and on the top of the foot on the instrumented leg. Three pairs of detection electrodes were used to define the body segments (details of electrode placements are provided in Appendix C).

From each segmental resistance value, \( R_o \), the THRIM used A.C. coupling and amplification to calculate the pulsatile components of impedance, \( \Delta R_o \) and \( dR_o/dt \), which are associated with beat-to-beat changes in blood flow resulting from the pumping of the heart. \( \Delta R_o \) is an amplification of the \( R_o \) signal following A.C. coupling, which removes the static (D.C.) components of the input signal, leaving only the components of the signal that vary with time. \( dR_o/dt \) is the time-derivative of the \( \Delta R_o \) signal, calculated internally by the THRIM. The thoracic pulsatile impedance signals were later used to make estimates of stroke volume, cardiac output, and total peripheral resistance, using a program developed by Dr. Andre Deidrich (Vanderbilt University, Nashville, TN).

Venous Collecting Cuff Pressure:
A Parks MODEL 1105 (Parks Medical, Spokane, WA) venous occlusion plethysmographic system was used to impede venous outflow from the lower limb and thus cause venous pooling in the calf. While our device provides a digital readout of occlusion cuff pressure, it does not provide this data through an analog output compatible with data acquisition system. Therefore, a pressure transducer (CyQ, Nicholasville, KY) was placed inline between
the Parks pneumatic output and the thigh cuff. Transducer output was fed to a CyQ 103 Blood Pressure Signal Conditioner, which allowed for easy calibration and balancing, and for interfacing with our data acquisition system.

Tilt Angle:
An accelerometer mounted on the underside of the tilt table measured the HUT angle.

Figure 3.1 depicts the calf circumference, resistance, and skin blood flow responses to the occlusion and tilting protocol.

Data Acquisition
All variables were acquired at a sampling rate of 500Hz using a 32-channel Dataq DI-720-USB (Dataq Instruments Inc., Akron, OH) analog-to-digital converter, with a total of 15 channels being recorded to a Dell Inspiron 4100 laptop PC running WinDaq/Pro+ waveform recording software. These data files were then transferred to the Dell Dimension 8400 desktop PC, on which data analysis was performed.

The WinDaq data files were then converted to a binary file format (.bin) compatible with MATLAB 6.5 using software developed by Dr. David Brown from the Department of Biomedical Engineering, University of Kentucky. During this conversion process, the recorded signals were visually inspected for anomalous content, which was removed before conversion to the final binary format.

All channels except heart rate and the pulsatile resistances ($\Delta R_0$ and $dR_0/dt$) were down-sampled to 10 Hz using MATLAB prior to being saved in a workspace file (.mat) used during data analysis.

Data Analysis
Data analysis was performed using MATLAB, Microsoft Excel, Dr. Brown’s (University of Kentucky, Department of Physiology) converter and browser programs, and P-WAVE, which ran Dr. Deidrich’s (Vanderbilt University, Autonomic Dysfunction Center) software.

Mean Value Analysis:
Using MATLAB, skin red-cell flux mean values were calculated for the following segments of the signal: the 60 seconds prior to each level of occlusion, the last 30 seconds of
each level of venous occlusion, the 60 seconds immediately preceding tilt, and the 60 seconds preceding the inflation of the venous collection cuff at the end of tilt. Skin-cell flux values during the occlusion and tilt were normalized against their corresponding control values, and are expressed as a percentage change.

Mean values of heart rate and finger arterial blood pressure were calculated for a period of at least 5 minutes immediately preceding HUT (control), during tilt (beginning 60 seconds after the table reached 70°, ending immediately prior to the lowering of the table), and for an approximately 5 min recovery period (starting 60 seconds after the table returned to 0°). In any case where blood pressure and/or heart rate displayed the marked drops characteristic of pre-syncope, the mean values of these variables were calculated for only the periods antecedent to the onset of these symptoms, which was determined through visual inspection of the signals. The mean values of blood pressure during HUT were corrected for the hydrostatic pressure difference caused by the finger’s change in position relative to the heart. MATLAB was used to calculate the mean values of blood pressure during these periods, and Dr. Deidrich’s P-WAVE heart rate output was analyzed using Excel to calculate mean heart rate values for the corresponding segments.

The P-WAVE program made beat-to-beat calculations of stroke volume using the Kubicek (KUB) and Sramek (SRA) equations from the thoracic impedance signals (9, 47) Cardiac output was calculated as the product of stroke volume and heart rate for each heart beat. Total peripheral resistance was calculated by dividing mean arterial pressure by cardiac output. Mean values of stroke volume, cardiac output, and total peripheral resistance were computed for the control, tilt, and recovery periods described above.

The change in calf resistance, $R_o$, elicited by venous occlusion and tilt were calculated as the difference between the value immediately preceding the perturbation and the minimum resistance value reached. This change was normalized by the subject’s initial resistance reading during the corresponding control period, and expressed as a percent change in calf fluid volume. The change in calf circumference, $CC$, was calculated in a similar manner, with it being calibrated against an internally generated voltage square-wave corresponding to a 1 % change in calf circumference, and measured after 3 and 10 min HUT.
Spectral Power Analysis:
Matlab was used to estimate the power spectral density of cutaneous red-cell flux prior and during tilt. Power spectral density was also estimated for each 3 min occlusion level, and compared to that of the 5 min before the start of the HUT protocol. A 1024-point Fast Fourier Transform (FFT) was performed using MATLAB’s PSD command, which used a 512-point Hanning Window and a 256-point overlap to minimize power spectrum leakage, and linearly detrended the signal.

Calf Circumference Rate of Change:
The slope of the calf circumference signal was calculated during venous occlusion and HUT for the points shown in Figure 3.2, which correspond to microvascular filtration (point a) and arterial inflow (point b). A linear regression line was calculated through the points in question using the MATLAB function POLYFIT. A 3-point moving average (MA) filter (0.3 s) was used to slightly smooth the calf circumference signal prior to slope calculations.

Statistics:
Differences within and between the two groups (sedentary and cyclist) during supine control, the seven levels of venous occlusion, 70° HUT, and recovery were tested for significance using a two factor, repeated measure, analysis of variance (ANOVA) using SAS statistical software. The within factor was used to indicate occlusion and tilt effects among the groups. The between factor was used to indicate differences between the groups. Fischer’s least significant differences method was used, and as such, a p-value ≤ 0.05 was considered to indicate statistical significance. Results were expressed as mean and standard error.
Figure 3.1: One typical subject’s data record showing (from top to bottom) venous occlusion pressure, tilt angle, calf circumference change, calf resistance, and calf skin perfusion.
Figure 3.2: Method used for calculating arterial inflow in response to 90 mmHg venous occlusion. Upper figure (a) shows CC response to progressively larger venous occlusion pressures, lower figure (b) depicts the portion of the CC curve used to calculate arterial inflow slope.
Chapter 4: RESULTS

Two subjects, one from each subject group, displayed presyncopal symptoms during the final minutes of HUT. Testing was therefore abruptly stopped, and the tilt table was lowered immediately. Subjects were given ample time for their cardiovascular system to recover before being allowed off of the lowered table. Because one subject from each group displayed this orthostatic intolerance, no conclusion could be drawn as to whether it could be a result of endurance training.

At rest, the difference in HR between groups is not statistically significant, although there is a strong trend for the cyclist group to have reduced resting HR (p<0.08) (Fig. 4.1). During HUT, the difference in the HR increase between groups became statistically significant (p<0.02), as did the HR difference during the recovery period (p<0.04). Figure 4.2 shows the mean rise in HR in response to HUT for each group, with the sedentary group mean HR increasing over 17 beats/min, in comparison to the 8 beats/min rise in the cyclists (p<0.03).

Stroke volume decreases in response to head-up tilt, as filling volume is diminished by reduced venous return, and was demonstrated in all subjects. Mean stroke volumes (SV) during control, HUT, and recovery were not statistically different between the sedentary and control groups. Figure 4.3 depicts the mean SV calculated- using Kubicek’s method; the SV calculated using Sramek’s method is provided in Figure 4.4. The Sramek calculated SV difference between groups during recovery did near statistical significance (p < 0.08), and, therefore, the general trend for SV to decrease during orthostatic challenge is shown.

There were no significant differences in cardiac output (CO) between the two groups during supine rest or HUT, as calculated using the Kubicek (Fig. 4.5) and Sramek (Fig. 4.6) methods. Interestingly, CO showed a trend to be slightly higher in the sedentary group as compared to the cyclist group. During tilt, CO tended to increase approximately 14% for the sedentary subjects, whereas cyclist CO tended to decrease about 8 % (Fig. 4.7). This is likely due in large part to the sedentary group’s tendency to significantly increase HR more than in the cyclists (Fig. 4.2) in response to orthostatic stress, while SV tended to decrease about the same in each group.
The total peripheral resistance (TPR) differences between the two groups were not significantly different, nor were the responses to postural change (Fig. 4.8, 4.9).

Cycling apparently had an effect on resting mean arterial pressure (MAP), as the sedentary group’s MAP was significantly (p<0.05) higher (Fig. 4.10). Although HUT did not have a significant effect on MAP, the sedentary group showed a trend towards maintaining their blood pressure slightly better than the cyclists during orthostatic challenge, whose blood pressure fell approximately 6%.

The percent change in calf electrical resistance, a direct indicator of calf fluid volume, was not statistically different between groups at any venous congestion pressure or during tilt (Fig. 4.11) other than at 20mm Hg (p < 0.05). On the other hand, during every stress level, calf fluid volume change was lower in the cyclists than in the sedentary subjects, which is a noteworthy trend despite statistical insignificance. A nearly linear relationship of increased lower limb fluid volume with increased venous occlusion pressure was observed for both groups. The fact that at 20mm Hg occlusion pressure (a venous pressure almost certainly so low as to fail to cause microvascular filtration in all subjects) calf volume rose more in the sedentary group than in the cyclists is evidence that this volume change may be attributed entirely to an increased venous vascular expansion, and that the sedentary group may have more compliant calves.

The calf circumference response to venous occlusion seen in Figure 4.12 closely mirrors the change in calf resistance (Fig. 4.11), although the percentage change in calf circumference appeared to be slightly greater. Our results show that calf circumference over-estimated the percent change in calf volume by approximately 50%, as compared to the percent change calculated using impedance plethysmography, at most occlusion pressures. Possible causes and implications of the differences reported by these two methods will be discussed later. The results show that the percent rise in calf circumference appeared greater in cyclists after 10 min HUT (cyclists = 4.83 ± 0.39 %, sedentary = 3.89 ± 0.25 %) (p=0.07), while there were no differences at any other stress level. This observation was in direct contrast to the results obtained through impedance plethysmography, raising the question of which method provides a more reliable measure of segmental volume, to be discussed later. One interesting trend in calf circumference was that the changes in response to three minutes at 50 mm Hg venous congestion and three minutes of HUT were similar in sedentary subjects, while nearing statistical difference (p< .08 ) in cyclists, cyclist being larger. This comparison of calf circumference change after three
minutes of HUT and 50mm Hg venous occlusion was made because a previous study (15) found that calf venous pressure rose to 51.0 ± 2.8 mm Hg during 60° HUT.

The pressure-volume relationship between congestion pressure and calf circumference was examined to give possible insight into the stretching characteristics of the lower limb vasculature. A PV curve was generated using data from three typical subjects, and is shown in Appendix B. Due to the small number of pressure data points collected (5mm Hg increments from 20-50mm Hg), it appears the oscillations in the PV relationships do not show the true nature of the expanding venous vasculature, but rather can be attributed to scatter.

Calf cutaneous red cell flux (perfusion) was reduced for all subjects with increasing occlusion pressure (p < 0.01, Figure 4.13). The magnitude of skin perfusion reduction was marginally greater in the sedentary group at each occlusion pressure, though there was no statistical significance between groups. It may also be worth noting that the sedentary group’s skin perfusion stopped decreasing after 40mm Hg venous occlusion, whereas the cyclists’ skin perfusion continued its linear decrease throughout 50mm Hg venous congestion and HUT.

Arterial inflow decreased steadily with increased venous occlusion pressure for both subject groups (Fig. 4.14). At all occlusion pressures, arterial inflow was lower in the cyclists than in the sedentary group, with statistical difference throughout. During HUT, sedentary arterial inflow (1.02 ml / 100 ml tissue min⁻¹) tended to exceed cyclist inflow (0.65 ml / 100 ml tissue min⁻¹) [not shown], which neared significance (p < 0.08).

Skin perfusion and arterial inflow were strongly correlated (R² = 0.96, p < 0.0001) as shown in Figure 4.15, as cutaneous red cell flux appears to decrease linearly with reduced arterial inflow, as may be expected.

Spectral analysis of the calf skin perfusion signal showed no clear effect of venous occlusion on very low (0.01-0.02 Hz), low (0.03-0.05 Hz) or medium (0.08–0.12 Hz) frequency oscillations. However, some interesting trends were observed between groups and in response to orthostatic stress in the very-low frequency (VLF) region and the medium frequency region (MF).

Oscillations around 0.01 Hz were not different between subject groups during rest or HUT, but tended to increase in both groups in response to tilt (Fig. 4.16). Oscillation in this VLF range is considered to be modulated by the vascular endothelium, and is an indicator of the endothelial vasodilator properties intrinsic to vascular smooth muscles cells (27).
The spectral amplitude in the next frequency range we examined (LF, thought to be neurogenic in origin) show no response to tilt, nor a difference between groups at any stress level (Figure 4.17).

Oscillations with peak amplitude at around 0.1 Hz were detected by the third frequency range specified, MF (0.08-0.12 Hz). Several researchers have suggested that oscillations at this frequency reflect the intrinsic myogenic activity of the smooth muscle cells in the resistance vasculature, primarily arterioles (23, 26, 35). Our results show a trend for higher spectral amplitude at this frequency for our cyclists as compared to the sedentary group (p<0.15), whereas Kvernmo et al. described lower relative amplitudes at 0.1 Hz. More striking is the reduction in the magnitude of oscillation at this frequency in response to HUT, which was reduced in both groups, significantly in the cyclists (p < 0.05, Fig. 4.18).

The calculated fluid filtration rates ($J_v$) are displayed in Figure 4.19 for occlusion pressures of 30-50mm Hg, as pressures below 30mm Hg were inconsistent in eliciting the linear calf expansion phase associated with transcapillary filtration. There appears to be no significant statistical difference between cyclist and sedentary $J_v$ levels, although mean values were consistently lower in the cyclists as compared to their sedentary counterparts.

For each subject, $J_v$ was plotted against occlusion pressure, with the slope of the relationship equaling the subject’s capillary filtration coefficient ($k_i$). Figure 4.20 shows that the means for each group were nearly identical. The method for calculating each subject’s isovolumetric venous pressure ($P_{v,i}$) from the regression of $J_v$ values is depicted for one typical subject in Figure 4.21. Mean $P_{v,i}$ values were the same for both groups (Fig. 4.22), and the values calculated are in good agreement with the literature (15).

Venous emptying rates (VER) were calculated as the derivative of the venous outflow curve at 0.5 s and 5 s after rapid deflation of the venous occlusion cuff from 50mm Hg. Figure 4.23 depicts how the slopes of interest were calculated using linear regression analysis. In all subjects following deflation, calf circumference fell steeply for ~2 s, at which point a “shoulder” was seen, during which calf circumference momentarily rose or decreased only very slightly. Following this shoulder, calf circumference resumed its decrease, albeit at a much reduced rate, at which time the secondary slope was calculated. Our results showed nearly identical outflow rates between groups for both slopes (Figure 4.24).
Results Figures

Figure 4.1: Mean heart rates during control, head-up tilt, and recovery for sedentary and cyclist groups (mean +/- sem).

Figure 4.2: Mean heart rate rise in response to head-up tilt for sedentary and cyclist groups (mean +/- sem).
Figure 4.3: Mean stroke volume calculated using Kubicek's method (9, 44) during control (pretilt), head-up tilt, and recovery, for sedentary and cyclist groups (mean +/- sem).

Figure 4.4: Mean stroke volume calculated using Sramek's (9, 44) method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups (mean +/- sem).
Figure 4.5: Mean cardiac output calculated using Kubicek's method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups.

Figure 4.6: Mean cardiac output calculated using Sramek's method during control (pretilt), head-up tilt, and recovery for sedentary and cyclist groups.
Figure 4.7: Percent change in cardiac output from control during head-up tilt, calculated using Sramek’s method (mean +/- sem).

Figure 4.8: Mean total peripheral resistance (TPR) for sedentary and cyclist groups during control (pretilt), tilt, and recovery, calculated using Kubicek’s method (mean +/- sem).
Figure 4.9: Mean total peripheral resistance (TPR) for sedentary and cyclist groups during control (pretilt), tilt, and recovery, calculated using Sramek’s method (mean +/- sem).

Figure 4.10: Mean arterial pressure measured at heart level for sedentary and cyclist groups during control (pretilt), tilt, and recovery periods (mean +/- sem).
Figure 4.11: Percent change in calf resistance as measured using impedance plethysmography from control (pretilt) values and successive thigh occlusions of increasing pressure and after 10 minutes HUT for both groups (mean +/- sem).

Figure 4.12: Percent change in calf circumference as measured using strain gauge plethysmography from control (pretilt) values and successive thigh occlusions of increasing pressure and after 10 minutes HUT for both groups (mean +/- sem).
Figure 4.13: Percent change in skin blood flow (anterior calf) measured using laser-Doppler from control (pretilt) values and successive thigh occlusions of increasing pressure, and after 10 minutes HUT for both groups (mean +/- sem).

Figure 4.14: Mean calf arterial inflow rates (ml / 100ml tissue / min) calculated from the rate of change of the calf circumference response to increasing venous occlusion pressures for both groups (mean +/- sem).
**Figure 4.15:** Correlation between skin perfusion and arterial inflow values for all subjects ($R^2 \approx 0.96$).

**Figure 4.16:** Very low frequency (0.01-0.02 Hz) spectral power of calf skin perfusion signal during venous occlusions and HUT for both groups (mean +/- sem).
Figure 4.17: Low frequency (0.03-0.05 Hz) spectral power of calf skin perfusion during venous occlusions and HUT for both groups (mean +/- sem).

Figure 4.18: Middle frequency (0.08-0.12 Hz) spectral power of calf skin perfusion during venous occlusions and HUT for both groups (mean +/- sem).
Figure 4.19: Fluid filtration rates (ml / 100ml tissue /min) during venous occlusions of 30, 35, 40, 45, and 50mm Hg for both groups (mean +/- sem).

Figure 4.20: Mean capillary filtration capacity (capillary filtration coefficient), Kf, for both groups (mean +/- sem).
Figure 4.21: Calculation of isovolumetric venous pressure, $P_{v,i}$, for one typical subject (mean +/- sem).

Figure 4.22: Mean isovolumetric venous pressures calculated for both groups (mean +/- sem).
Figure 4.23: Illustration of methods used to calculate venous outflow rates immediately following cuff deflation for one typical subject.

Figure 4.24: Mean venous emptying rates, primary and secondary slopes, calculated for both groups (mean +/- sem).
Chapter 5: DISCUSSION

The purpose of this discussion is to answer the questions we initially asked: Are cyclists more prone to pool fluids in their lower body, and was our experimental protocol able to detect the mechanisms which may regulate this pooling? The results of all aspects of the investigation will be discussed, as will the role that they appear to play in the greater picture, that is, how they may contribute to the widely held notion that endurance training promotes orthostatic intolerance.

The cardiac impedance results were, for the most part, inconclusive in distinguishing between the two subject groups. Resting levels of stroke volume (SV) in the supine position showed a trend to be slightly elevated in the cyclist group, though not to the level of statistical significance (Figure 4.3, 4.4). Mean cardiac output was estimated to be slightly higher in cyclists (Figure 4.5, 4.6), though again with no significant difference between groups. It was expected that cyclists would display significantly greater stroke volumes, which is a known effect of endurance training (55). As in many cases in this investigation, lack of statistical significance can probably be attributed to the small sample size studied.

The change in cardiac output in response to HUT did however differ (p<0.01) between groups (Figure 4.7), which is mainly due to the large differences in heart rate response to HUT (Figure 4.2). Heart rate rose significantly more in the sedentary group (18.3 ± 3.0 bpm) than in cyclists (8.4 ± 2.4 bpm) during HUT (p < 0.03). This significant difference in cardiac pacing may be indicative of attenuated baroreflex regulation, which has been described in the literature (12, 48) to occur in highly trained individuals. Resting heart rate levels were significantly lower in the cyclist group as well (Figure 4.1), attributable to their higher levels of cardiovascular conditioning.

Mean arterial pressure (MAP) was reduced in the cyclists as compared to the sedentary group (Figure 4.10), which during supine control was approximately 10mm Hg lower in the cyclists. Neither groups’ MAP reacted significantly to HUT, although there was a trend for cyclists’ MAP to fall about 5mm Hg as compared to the sedentary groups’ 0.5mm Hg fall. Mean values of total peripheral resistance (TPR) did not show any significant difference either (Figures 4.8, 4.9), though the cyclist group’s TPR appears to have been maintained slightly better during HUT.
Calf fluid volume changes were measured using impedance plethysmography, and are expressed as a percent change in calf resistance (Figure 4.11). It was found that at all venous occlusion levels, as well as after 10 min HUT, calf fluid volume expanded more in the sedentary than cyclist group, although the differences were not significantly different between groups. The fact that both groups of subjects appeared to undergo similar calf fluid expansion provides strong evidence that cyclists were not at an increased risk of pooling excessive fluid in their lower limbs.

Calf circumference (CC) has been long used as an estimator of calf volume expansion. Our results show that the sedentary and cycling groups had similar CC changes during all levels of venous congestion, with the trend of slightly higher calf expansion in the sedentary group (Figure 4.12), similar to that found using the impedance procedure (Figure 4.11). During HUT, however, the mean change in CC in the cyclist group was significantly greater than in the sedentary group, which conflicts with the results using impedance, which showed similar calf fluid expansion during HUT, and if anything, slightly greater expansion in sedentary subjects. Since comparing calf fluid volume increases during orthostatic stress between our groups was a major goal of our study, we asked the question: Which of these two techniques is providing a true indication of fluid accumulation in this body segment?

It is our opinion that the impedance results provide a more reliable measure of fluid expansion in the calf during HUT, due to some drawbacks associated with our strain gauge plethysmograph procedure. Firstly, we believe that since the strain gauge only measured changes at the point of maximal circumference of the calf, the measured change may not be representative of the calf as a whole. Had a system of multiple strain gauges been used to measure circumferential changes at several points in the calf, the CC change recorded may have been fully representative of the calf as a whole. In addition, we suspect that the initial imposition of gravity as the tilt table was rotated could have contributed an artifact to the CC signal, as superficial calf tissue was pulled downward by gravity during initial tilting, which may have contributed to the group difference based on the two groups’ significantly different calf anatomical properties. Lastly, the fact that impedance plethysmography makes a measure of the resistance, and thus fluid content, of a body segment, rather than the gross changes elicited by the said fluid expansion, we believe that it provides a reliable indicator of blood accumulation in the calf. Therefore, we interpret our results to show that the cyclist group had no greater
tendency to pool blood in their lower limbs during orthostatic stress, as compared to their sedentary counterparts.

Skin perfusion and arterial inflow measurements were used to indirectly measure the activity of local restrictor mechanisms which act to shunt flow away from the lower limbs during both venous occlusion and orthostatic stress. Previous research has used cutaneous perfusion as an indicator of the venoarteriolar reflex (VAR), which has been described as a feedback loop, causing arteriolar vasoconstriction in response to stretching of the venous wall (7, 8, 18). Venous occlusion was used to cause venous stretching without activating the centrally mediated vasoconstrictive mechanisms that accompany HUT. We made the assumption that venous pressure in the veins immediately upstream was equal to congestion cuff pressure, based upon previous research (5) that showed that $P_{\text{cuff}}$ and $P_v$ were not significantly different over a range of 10 – 70mm Hg, which they verified using invasive venous pressure catheters. During HUT, skin perfusion changes would also encompass the effects of the local myogenic response, as well as vasoconstriction adrenergic in origin, initiated by baroreflexes. We had hypothesized that, during strenuous exercise, increased blood flow to the lower limbs would tend to cause greater oscillations in arterial and venous diameter, thus attenuating the detection of wall stretch caused by increased arterial and venous pressures. Had these local reflexes been attenuated, the cyclists may have had a reduced ability to shunt blood flow away from their lower bodies during venous congestion and HUT.

Our results showed that, during venous occlusion, skin perfusion was reduced in both subject groups with increasing venous pressure, evidence that their VAR was functioning well. No significant differences were displayed between groups in the percent reduction of skin blood flow for a given venous congestion pressure, though there was a trend for sedentary skin flow to be reduced slightly more across the occlusions (Figure 4.13). Interestingly, skin blood flow at 50mm Hg was not significantly different than that after 10 min HUT, indicating the strong contribution of the VAR alone to cause substantial reduction in cutaneous blood flow. Crandall et al. demonstrated that cutaneous blood flow is not under adrenergic mediation (36), so we assumed that vasoconstriction, myogenic in origin, would contribute during HUT, although flow decreased only slightly more during HUT, as compared to 50mm Hg venous occlusion.

Arterial inflow was measured in accordance with the literature (15, 44), as the slope of the calf circumference signal following 90mm Hg venous occlusion. In contrast to these studies,
where the slope of the CC curve was calculated within the first three heart beats following inflation, we calculated the slope after approximately five beats. The reasoning behind doing this was that our thigh occluder did not inflate the occlusion cuff as rapidly as those used in theses other studies. In addition, when we programmed our device to occlude to 90mm Hg, it would occlude past this pressure to approximately 120mm Hg for a very short period (~1 sec), and then return to the value programmed in (see cuff pressure signal in Figure 3.1). We believe that the combination of the relatively slow inflation and this pressure artifact may have compromised the CC signal during this period. Further evidence was provided by the fact that every 90mm Hg occlusion for each subject (> 8 measurements, for all 16 subjects) displayed a biphasic CC response (see Figure 3.2), in which a secondary slope of lesser magnitude followed the initial upslope. Arterial inflow was calculated, through linear regression, as the slope of this secondary rise. It was found that arterial inflow decreased with increasing venous occlusion pressure for all subjects, and was significantly smaller in cyclists than in the sedentary group (Figure 4.14). Figure 4.15 shows that calf skin blood flow and arterial inflow measurements were strongly correlated (p<0.001), indicating that both measurements were detecting a reduction in arterial flow during occlusion and HUT. From these results we conclude that the local vasoconstrictive mechanisms in the cyclist group did not appear to be compromised.

No differences in skin perfusion spectral power were displayed in the regions 0.01-0.02 Hz or 0.03-0.05 Hz, which correspond to endothelial and neurogenically mediated mechanisms, respectively. Kvernmo et al. found that, at rest, oscillations around 0.01 Hz were greater in amplitude in exercise-trained subjects than in a non-exercising control group, indicating an enhanced endothelial vasodilator property among athletes (26), though our results show no difference between our cyclist and sedentary groups during control.

Oscillations with a peak at around 0.04 Hz have been shown to be neurogenic in origin, due to the disappearance of these oscillations after local and ganglionic nerve blockade and after sympathectomy (25-27). Although some literature shows enhanced spectral amplitude at this frequency in athletes, our results show no difference between groups (Fig. 4.17). Interestingly, HUT had no effect on oscillations in this frequency, despite the increased sympathetic discharge expected during orthostatic challenge.

Spectral power in the 0.08-0.12 Hz region, which corresponds to myogenic activity of smooth muscle cells in the arterial vasculature (26) was significantly greater in the cyclist group during
venous occlusions (Figure 4.18). During HUT, both groups’ oscillations in this frequency range were significantly diminished, as compared to supine control. We interpret these results to mean that, during HUT, the myogenic reflex activity in the arteriolar smooth muscle was heightened. As maximal myogenic control (sustained reduction in arteriolar diameter) was being exhibited, it removed the normally oscillatory nature of this mechanism. A possible explanation of this phenomenon may be that under normal conditions, myogenic modulation of the arteriolar resistance vessels is robust, with large oscillations of the diameter of small arterioles occurring periodically. Research has shown that arterial vasculature has the intrinsic ability to change diameter in response to slight variations in local arterial pressure, as well as spontaneously (14). We believe that the reduced oscillation at this frequency represents a myogenic response in which arteriolar diameter is significantly reduced, with this reduced diameter being maintained over the course of the orthostatic stress in response to raised local arterial pressure, and may therefore be an indicator of the myogenic reflex known to occur in response to orthostasis (24). The fact that spectral power in this frequency range was greater in cyclists during control and equal during HUT indicates that the cyclists’ myogenic reflex may be more sensitive than that in the sedentary group.

Fluid filtration rates ($J_B \nu$) show that the cyclist group did not display an increased ability to filter fluid into the extravascular space (Figure 4.19) during venous congestion. The microvascular filtration coefficient ($k_f$), the relationship between $J_B \nu$ and congestion pressure, was likewise similar between groups (Figure 4.20). Isovolumetric venous pressure, the pressure required to induce a positive fluid flux out of the microvasculature, was calculated to be nearly identical between the two groups (Figure 4.21), which indicates that cyclists were not prone to filter more plasma out of the vasculature at a lower venous pressure. The combination of these findings support the claim that endurance training does not lead to increased microvascular filtration, and thus fluid accumulation in the lower limbs.

Venous outflow after release of congestion pressure was calculated as the derivative of the CC slope approximately 0.5 and 5 seconds following cuff deflation. Previous investigations have proposed that the first phase of venous emptying (primary slope) is mainly dependent upon the elastic recoil properties of the venous tissue, whereas later emptying (secondary slope) is indicative of a vеноconstrictive mechanism (51). Regression slopes were calculated as shown in Figure 4.22 after release of 50mm Hg venous congestion pressure; nearly all subjects displayed a
biphasic reduction in calf circumference following cuff deflation. Mean regression slopes calculated for each emptying phase, shown in Figure 4.23, and were found to be nearly identical between subject groups. We therefore believe that the elastic and venoconstrictive properties of the venous vasculature to be similar in cyclists and their sedentary counterparts.

It should be noted that because of the small sample size used in this experiment, the potential for Type II error in the results is very plausible. Type II error refers to the situation when an opportunity to reject the null hypothesis correctly was lost, due to wider confidence intervals caused by small sample size. Prior to the study, the study power was calculated to minimize type II error, but as certain data records for some subjects became compromised or unusable, sample size decreased and the potential for type II error rose.

The results of this investigation lead us to believe that cyclists do not show the propensity to pool more fluid in their lower limbs than non-exercising subjects. We also believe that the parameters measured prove to be useful in showing that, despite gross lower limb anatomical differences, cyclists maintain similar local hemodynamic function as non-exercisers.
Chapter 6: CONCLUSION

The present study utilized noninvasively acquired variables mainly to compare the lower limb hemodynamic properties between two distinct groups of subjects: endurance trained cyclists and sedentary subjects. The initial hypothesis was that the two groups would display different properties due to the anatomical and physiological changes brought about by long-term rigorous lower-body exercise.

Our plethysmography results show that cyclists had no greater tendency to pool blood in their calves than their sedentary counterparts during both thigh venous occlusion and head-up tilt. Skin perfusion reductions during venous congestion and head-up tilt due to the venoarteriolar reflex were similar between the groups. Calf arterial inflow tended to be slightly greater in sedentary subjects (not significant) at low occlusion pressures, which suggests that the athletes may have an attenuated local resistance mechanism. Fluid filtration rates were also found to be similar for both groups. Major statistical significance was only found between groups for baseline heart rate and heart rate response to head-up tilt.

Our results suggest that cyclists are not predisposed to orthostatic intolerance due to any changes in lower limb function.
## APPENDIX

### Appendix A

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**mean** 25.78 177.0 80.8 "

**SD** 4.82 6.0 6.9 "

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**mean** 29.14 183.2 89.3 125.00

**SD** 4.06 8.5 12.6 8.45
Appendix B

Pressure-Volume relationship for three typical subjects
REFERENCES


Vita

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Education
1997-2001                  Vanderbilt University                          Nashville, TN
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Professional
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Teaching Assistant
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Conference
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2004 Gill-Heart Cardiovascular Research Symposium, Lexington, KY

Scholastic Honors
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Research Fellow in University of Colorado Department of Mechanical Engineering