# Učinci stupnjevanog negativnog tlaka donjeg tijela na mikrovaskulaturu muškaraca i žena

**Vladić, Nikola**

**Master's thesis / Diplomski rad**

**2023**

*Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj:* **University of Rijeka, Faculty of Medicine / Sveučilište u Rijeci, Medicinski fakultet**

*Permanent link / Trajna poveznica:* https://urn.nsk.hr/urn:nbn:hr:184:576498

Diplomarbeit

# Effects of Graded Lower Body Negative Pressure on the Microvasculature in Males and Females


eingereicht von

**Nikola Vladić**


zur Erlangung des akademischen Grades

**Doktor der gesamten Heilkunde**

**(Dr. med. univ.)**


an der

**Medizinischen Universität Graz – Medizinischen Universität Rijeka**

ausgeführt am

**Lehrstuhl für Physiologie & Pathophysiologie**

unter der Anleitung von

**Prof.dr.sc. Jagoda Ravlić-Gulan dr.med.**

**Assoz. Prof. Priv.-Doz. Dr.med. MMedSci PhD Nandu Goswami**

**Sen. Lecturer Dr.scient.med. BSc MSc Bianca Steuber**


Graz, am 31. Juli 2022.

# Eidesstattliche Erklärung

*Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.*

Graz, am 02. Mai 2023.

Nikola Vladić eh.

# Acknowledgment

First of all, I would like to express my gratitude to Prof. Dr. Nandu Goswami, it was an immense honor to be given the opportunity of writing a thesis under his supervision, for this I will always be thankful. I also have to express my utmost gratitude to Dr. Bianca Steuber, without her constant guidance, consults and countless emails I would have never finished my thesis.

Secondly, I want to say, thank you to Prof. Dr. Jagoda Ravlić Gulan, who supported me during my writing as a mentor from my home University in Rijeka, Croatia.

Thirdly, I want to thank all the great people that I met and with whom I had the great opportunity to work with at the Division of Physiology & Pathophysiology in Graz, with very special thanks to Mrs. Karin Schmid-Zalaudek and Mr. Adam Salon for their immense and unselfish help as well as all the kind people who enabled me to visit the Medical University in Graz, without all of you, none of this would have ever been possible.

Last, but none the least, for the never-ending support, for always believing in me through my journey in medical school and during my thesis I say thank you to my parents, my brothers, my sister, Iris and Sara, these people made me who I am today. Thank you all!

I would like to end this acknowledgment with a quote from Prof. Dr. Abraham Verghese: “I think we learn from medicine everywhere that it is, at its heart, a human endeavor, requiring good science, but also a limitless curiosity and interest in your fellow human being.”

Yours truly,
Nikola Vladić

## Table of Contents

## Abbreviations

| | |
|---|---|
| PVS | peripheral vascular system |
| SVR | systemic vascular resistance |
| MAP | mean arterial pressure |
| CO | cardiac output |
| HR | heart rate |
| SV | stroke volume |
| PL | preload |
| RAAS | renin angiotensin aldosterone system |
| ACE | angiotensin converting enzyme |
| ICP | intracranial pressure |
| FMD | flow mediated dilatation |
| LBNP | lower body negative pressure |
| LVED | left ventricular end-diastolic volume |
| FVC | forced vital capacity |
| FEV1 | forced expiratory volume in one second |
| V/Q matching | ventilation-perfusion matching |
| CO2 | carbon dioxide |
| CRAE | central retinal artery equivalent |
| CRVE | central retinal vein equivalent |
| AVR | artery to vein ratio |
| OCTA | optical coherence tomography angiography |
| FFA | fundus fluorescein angiography |
| CVP | central venous pressure |

# List of figures and tables

## List of Figures



## List of tables

## Zusammenfassung

**Hintergrund**: Es gibt starke Hinweise auf geschlechtsspezifische Unterschiede in Bezug auf hämodynamische Reaktionen während einer Flüssigkeitsverlagerung oder zentralen Hypovolämie, die durch die Verwendung von „lower body negative pressure“ (LBNP) simuliert werden kann. Es gibt jedoch nur begrenzte Forschungsergebnisse zu der mikrozirkulatorischen Antwort auf die zentrale Hypovolämie. In dieser Arbeit soll, mit einer gründlichen Untersuchung der retinalen Mikrozirkulation eine Lücke in der Forschung zu Geschlechtsunterschieden in der orthostatischen Toleranz geschlossen werden.

**Ziele**: Die Ziele dieser Arbeit sind (1) zu beurteilen, ob die Auswirkungen des „lower body negative pressure“ als Veränderungen in der Mikrozirkulation der Netzhaut sichtbar gemacht werden können, und (2) Unterschiede in den mikrozirkulatorischen Reaktionen bei Männern und Frauen zu bewerten.

**Methoden**: Es wurden 42 Teilnehmer rekrutiert, 20 Männer und 22 Frauen. LBNP wurde verwendet, um eine Flüssigkeitsverlagerung zu induzieren. Ein 6-Phasen-Protokoll wurde mit einer Basismessungsphase, vier zunehmenden LBNP-Stufen (von -10 mmHg LBNP bis -40 mmHg LBNP) und einer Erholungsphase verwendet. Während der gesamten Zeit wurden hämodynamische Messungen durchgeführt. Retinabilder wurden aufgenommen und später ausgewertet, um nach Änderungen im Gefäßdurchmesser zu suchen. Der Gefäßdurchmesser wurde als zentraler Netzhautarterienäquivalent (CRAE), zentraler Netzhautvenenäquivalent (CRVE) und Arteriovenöser Ratio (AVR) ausgedrückt**.**

**Ergebnisse:** Es wurden keine signifikanten Änderungen in den Mikrovaskulatur-Gefäßdurchmessern (CRAE und CRVE) oder ihrer Ratio (AVR) während der LBNP-Phasen gefunden. Das Geschlecht scheint auch keine signifikante Rolle als Faktor während der LBNP-Phasen und Gefäßdurchmesseränderungen zu spielen. Weitere Forschung auf diesem Gebiet ist gerechtfertigt, um Licht in das komplexe Zusammenspiel von mikrovaskulärer Perfusionsregulation und geschlechtsspezifischen Unterschieden zu bringen.

## Abstract

**Background:** There is strong evidence for sex differences in hemodynamic responses during fluid shifts or central hypovolemia, which can be simulated using “lower body negative pressure” (LBNP). However, there is limited research on the microcirculatory response during central hypovolemia. This work aims to fill a gap in research on sex differences in orthostatic tolerance with a thorough investigation of retinal microcirculation.

**Aims:** The aims of this thesis are (1) to assess if the effects of lower body negative pressure can be visualized as changes in the microcirculation of the retina and (2) to evaluate differences in microcirculatory responses in males and females.

**Methodology:** 42 participants were recruited, 20 men and 22 women. LBNP was used to induce fluid shifts. A 6-phase protocol was used with a baseline measurement phase, four increasing LBNP levels (from -10 mmHg LBNP to -40 mmHg LBNP), and a recovery phase. Hemodynamic measurements were taken throughout. Retinal images were acquired and later analyzed to look for changes in vessel diameter. Vessel diameter was expressed as central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), and arteriovenous ratio (AVR).

**Results:** No significant changes in microvasculature vessel diameters (CRAE and CRVE) or their ratio (AVR) were found during the LBNP phases. Sex also does not appear to play a significant role as a factor during LBNP phases and vessel diameter changes. Further research in this area is warranted to shed light on the complex interplay between microvascular perfusion regulation and sex differences.

# 1. Introduction

To fully understand the complex subject of: “the differences in the microcirculation between the sexes”, let us first take a look at the fundamentals of the cardiovascular system: it’s anatomy, histology and physiology.

## 1.1. The Cardiovascular system

### 1.1.1. The heart

In the center of the cardiovascular system lies the heart, just behind the breastbone in a region called the mediastinum. The heart is a hollow muscular organ the size of a closed fist and weighs around 300 grams (1). It deviates normally to the left so that it does not lie perfectly in the middle of the chest. It is divided into four distinct chambers into which and out of which the great blood vessels arise. The heart is the muscular generator, which pumps blood through our vessels that then run to every single part of our body. The left heart pumps blood to the peripheral organs and tissues of the body through the systemic circulation, while the right heart pumps blood to the lungs via the pulmonary circulation. Both the left and the right heart pump can be described as a two-chamber pump-system since they are both composed of an atrium (a weaker primer chamber), which then opens into the ventricle (a stronger, muscular chamber), which does the most, if not all of the pumping work. The left and the right heart pump are divided by a wall called the septum (an interatrial and interventricular septum respectively) while the atrium-ventricle unit is connected via a set of opening and closing heart valves. A schematic insight into the heart’s anatomy is provided in figure 1. Although the right and the left heart pumps, work somewhat independently of each other they still pump blood simultaneously. While they pump blood for all of the peripheral tissue’s they themselves are the only organ in the body that gets its own unique perfusion system, which is perfused in the diastole. That system is the coronary artery system (2,3).

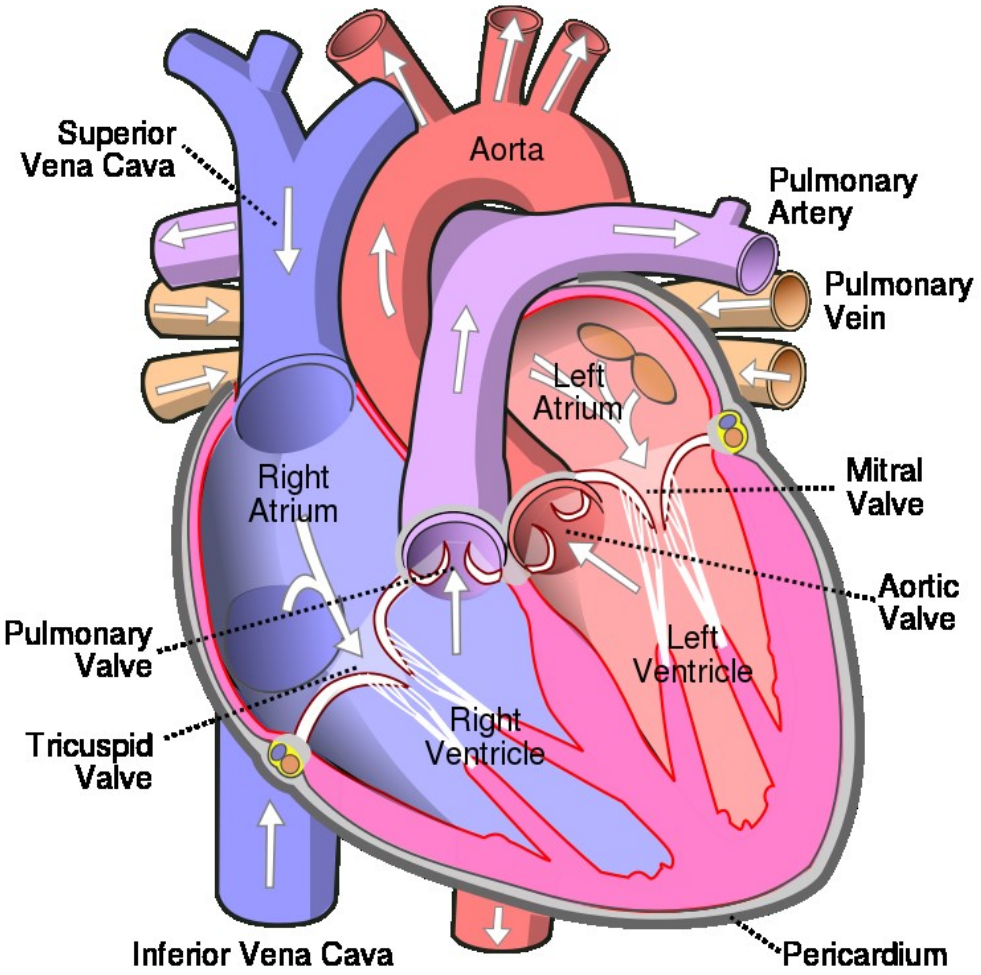


**Figure 1: The anatomy of the heart**
*Obtained under CC BY from:*
*https://en.wikipedia.org/wiki/Atrium_(heart)#/media/File:Diagram_of_the_human_heart_(cropped).svg* (4)

### 1.1.2. The vessels

The blood vessels comprise the peripheral vascular system (PVS), which is essentially a set of tubes or pipes, which run throughout the body and deliver blood to every single tissue with, of course, the help of the heart, which as previously stated acts as the pump. Comparing blood vessels to pipes is a train of thought used to simplify the intricacies of the PVS but, there are different types of blood vessels with different compositions and functions, they are not just simple connecting tubes but live organs with their own biology and regulatory systems. Although each type of blood vessel has a unique histological structure there are three common layers found in most blood vessels: the adventitia (the outer layer and structural support), the tunica media (composed of varying levels of smooth muscle cells and elastic fibers) and the tunica intima (the inner endothelial lining).

The PVS can be roughly divided into:

- Arteries
- Arterioles
- Capillaries
- Venules
- Veins

Arteries are blood vessels, which branch out of the heart into the periphery. A common misconception is that: arteries carry oxygen-rich blood, which is not true, since the pulmonary arteries carry oxygen-poor blood out of the heart and towards the lungs to be oxygenated.

Arteries are muscular blood vessels, which have to withstand high and pulsatile blood pressures. They can be further subdivided into elastic arteries and muscular arteries. Elastic arteries include primarily the aorta, but also the pulmonary arteries, they have a high amount of elastic fibers in their tunica media, which allows for a greater elasticity. It is this elasticity that is responsible for a relatively constant pressure gradient in spite of the pulsatile pumping action of the heart through the *"Windkessel effect"*. All other arteries fall under the category of muscular arteries, named after their high amount of smooth muscle cells in the tunica media.

Arterioles are smaller, muscular blood vessels, which play a very important role in systemic vascular resistance (SVR), and in the control of the blood flow in regards to the peripheral tissue oxygen/nutrient needs. Arterioles "smoothen" the pulsatile pressures of the heart and therefore there is a single perfusion pressure at the arterioles and distally to the arterioles as opposed to the arteries, which have pulsatile, diastolic and systolic pressures generated by the heart's pumping nature.

Capillaries are comprised of a single endothelial layer, these very thin-walled vessels allow for a great diffusion membrane through, which oxygen and other nutrients can freely exchange.

Venules are the smallest veins in the body, they arise from the distal ends of the capillaries. Veins flow from the periphery back to the pump/heart. The veins are less

muscular and elastic than their arterial counterparts but they have a great compliance and as such serve as a tank or blood volume storage, which can be mobilized if needed. Nearly three fourths of the total blood volume can be stored in the venous system. The venous system is a low-pressure system and as such can be influenced by various external forces, such as breathing and gravity (2,3,5).

## 1.2. Cardiovascular physiology

### 1.2.1. Blood pressure

Per definition blood pressure is the force exerted by the blood against any unit area of the vessel wall (2). It is usually measured in millimeters of mercury (mmHg) since historically the first blood pressure measurements were performed using a mercury manometer. When one says that: “the pressure is 100 mmHg” this translates to an exerted force that is sufficient to push a column of mercury up to the level of 100 millimeters high, while working against gravity. When blood pressure is measured, it is usually measured during heart contraction (systole) and during heart relaxation (diastole) so that it results in two measurements: the systolic and diastolic blood pressure, however, the mean arterial pressure (MAP) is commonly used to explain basic blood pressure principles. MAP is defined as the average arterial pressure throughout one cardiac cycle, which of course includes systole and diastole. MAP is under the influence of the cardiac output (CO) and the SVR, which also have their own influencing factors (6). The relations of MAP, CO and SVR are best demonstrated in the “*MAP equation*”.

$$MAP = CO \times SVR$$

Therefore, an increase in CO and/or SVR leads to an increase in MAP and vice versa. The cardiac output represents the amount of blood that is pumped by each ventricle in one minute. This results in about five liters per minute on average. The cardiac output is the product of the heart rate (HR) and stroke volume (SV). The heart rate is measured in beats per minute and represents the amount of heart contractions in one minute, while the SV is defined as the volume of blood ejected by each ventricle during one heart contraction. The SV on the other hand is the product of the hearts contractility and preload (PL), where contractility is defined as

the strength of the heart contraction and PL as the amount of blood in the ventricles just before contraction (the end-diastolic ventricular volume). The Frank-Starlings law of the heart describes that with a greater end-diastolic ventricular volume the heart muscle stretches out more and with that stretching the contractility of the heart increases. The Frank-Starlings law is often simplified to “what goes into the heart must come out of the heart”. In summary any change in: PL, contractility, SV, and/or HR will lead to a change in CO and per the “MAP equation” also to a change in the MAP (2,7).

The other component of the MAP equation is the SVR. Resistance is the force that opposes blood flow and is best described with “*Poiseuille’s law”*, which boils down to two major components: the radius of the vessel and the viscosity of the blood. The viscosity of the blood plays a minor role and is only relevant in states such as: anemia, polycythemia and other viscosity altering states. With higher blood viscosity (for example in polycythemia) the resistance increases and vice versa. The radius of the vessel plays a major role since the resistance is inversely proportional to the fourth power of the radius, so with an increase in the radius of a vessel (vasodilatation) the resistance falls drastically, and with a decrease in the radius (vasoconstriction) the resistance increases rapidly. Resistance is inversely proportional to blood flow. When talking about the whole body, the term SVR is used. The SVR can be related to the radius (or diameter) of all the bodies arterioles since it’s the muscular arterioles that can significantly change their radius via vasoconstriction or vasodilatation. In a state of systemic arteriolar vasoconstriction there is a decrease in the arteriolar diameter (and radius), which relates to a rapid increase in resistance and decreases blood flow distal to the arterioles, this can be easily clinically assessed by feeling for the temperature of the patients extremities where a poor blood flow relates to a cold extremity (8). Per definition an increased resistance to total blood flow is therefore an increase in SVR which will raise the MAP and vice versa (2,6,9).

### 1.2.2. Blood pressure regulation

Changes in MAP are sensed by various peripheral sensory organs, which then feed into compensatory mechanisms, which ultimately regulate MAP by increasing and

or decreasing CO and/or SVR. The two main mechanisms contributing to MAP regulation are: the autonomic nervous system, primarily acting on acute MAP regulation, and the long-term regulatory system of the kidneys also known as the: renin-angiotensin aldosterone-system or RAAS.

The autonomic nervous system is composed of a complex web of sympathetic and parasympathetic fibers, which help regulate the MAP acutely. The center of this regulatory system is located in the brainstem, mainly in the medulla oblongata and the pons, this is where the vasomotor center is located. Out of this center the efferent fibers innervate three main structures: the heart, the arterioles and the veins. Through sympathetic Beta-one receptors, which can be found in the conducting and myocardial tissue of the heart, the sympathetic nervous system can increase the HR, and increase the hearts strength of contraction. By increasing the contractility, the SV increases, with an increased SV and HR the CO increases, which in turn can increase the MAP in states where the MAP is low. The M2 parasympathetic receptors can be only found on the conducting heart tissue and as such parasympathetic activation only works on decreasing the HR and has little to no impact on the heart's contractility, none the less a decrease in HR will of course decrease the CO and MAP. Alpha-one sympathetic receptors found on arterioles cause vasoconstriction and therefore increase resistance, lower blood flow and raise the SVR. A raise in the SVR, as known from the "*MAP equation"* will raise the MAP. It is known that sympathetic activation also causes constriction of the venous system, which in turn increases the PL of the heart and therefore also raises the CO and ultimately the MAP. The efferent or sensing fibers of this autonomic system come from baroreceptors, which are stress sensitive receptors found in the carotid and aortic sinuses. In a low MAP state, the baroreceptors are not stretched and therefore no input is sent to the vasomotor center, this means that the sympathetic system is uninhibited and will cause an increase in HR, contractility and, arteriolar and venous tone all in hopes to recover the MAP. In a state of high MAP, the baroreceptors are stretched and send inhibitory impulses to the vasomotor center, vasoconstriction of arterioles and veins is therefore inhibited, as well as the beta-one heart stimulation. Additionally, the parasympathetic nervous system activates and lowers the HR through M2 heart receptors. The described mechanism is known as the "Baroreflex". This baroreflex is a rapid reflex, which manages the MAP

acutely, changes of MAP are sensed in seconds and the efferent fibers fire almost instantly and as such the baroreflex provides a powerful moment-to-moment regulation of our blood pressure (2,10).

The long-term blood pressure regulatory system is mainly controlled by the RAAS system.

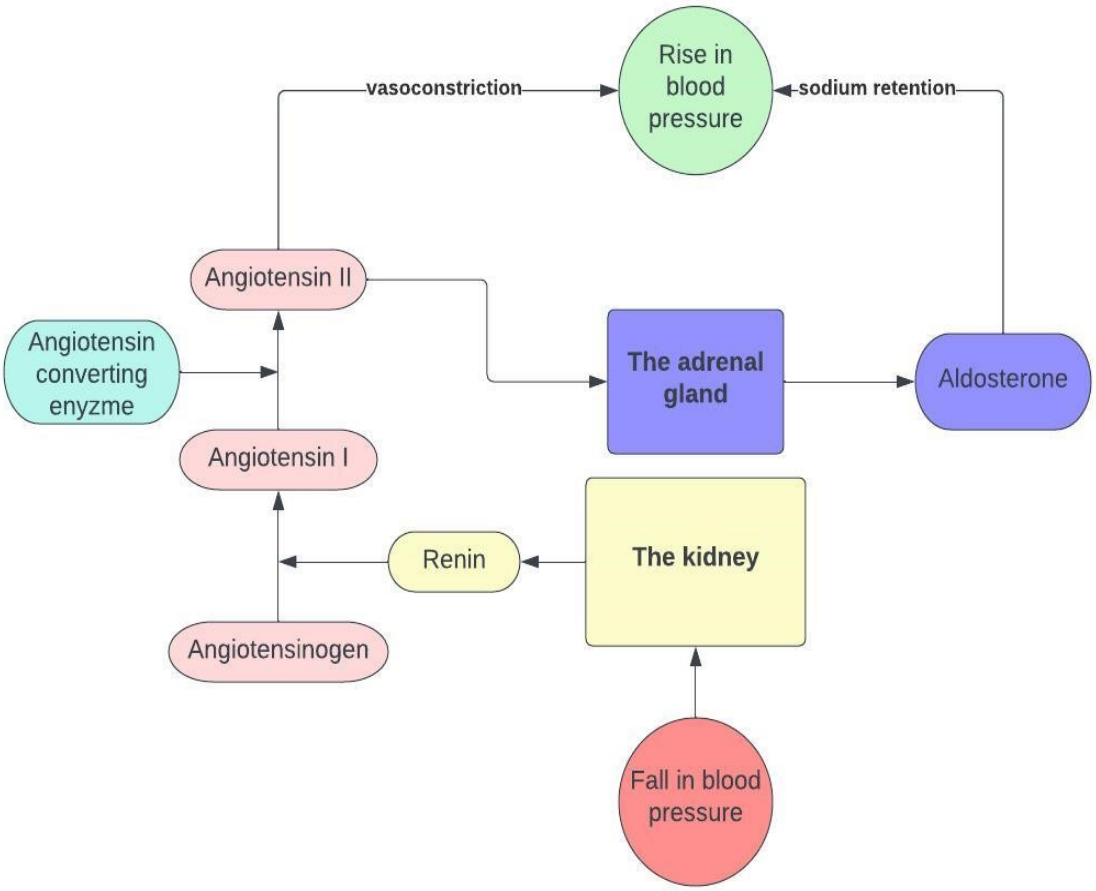


**Figure 2: The RAAS system**
*Illustration by the author using information from: "MSD manuals: Regulating blood pressure: the renin-angiotensin-aldosterone system"*(11)

As can be seen from the depiction in "Figure 2", the afferent arm of the RAAS system is found in the kidney, specifically in the juxtaglomerular cells of the kidney, which are sensitive to any decrease in blood flow through the kidney. The kidney interprets a reduction in its own blood flow as a decrease in the bodies MAP and starts converting prorenin (a precursor hormone which is present in the blood) into its active form: renin. Renin then functions as an enzyme and converts angiotensinogen into angiotensin I. The last metabolic step takes part mostly in the

lung through the enzyme angiotensin-converting-enzyme (ACE) which metabolises angiotensin I into angiotensin II, this is the final product of the RAAS system. The role of angiotensin II is double: firstly, it binds to arterioles onto AT1 receptors. which cause vasoconstriction just like the activation of alpha-one receptors. Its second role is to promote aldosterone release from the adrenal glands. Aldosterone then promotes absorption of sodium and water in the kidneys, which leads to an increase in blood volume and PL. Summarised once the RAAS system gets activated it promotes peripheral vasoconstriction, an increase in SVR, an increase in sodium and water retention, which leads to an increase in PL and per the Frank-Starlings law an increase in CO. Both mechanisms ultimately try to increase the MAP. Unlike the fast-acting baroreflex the RAAS needs hours before it starts showing any significant effects, but has a longer lasting impact on the MAP then the quick-acting baroreflex (2,12).

Although the regulation through the baroreflex and the RAAS are commonly thought of as the two primary ways of blood pressure regulation, "*Cushing reflex"* or "*Cushing response"* is another important and specific physiological system response, which activates in situations where the intracranial pressure (ICP) is increased. The Cushing reflex is defined as a clinical triad: bradycardia, systolic hypertension and irregular decreased respirations. A response with systolic hypertension and bradycardia may seem paradoxical at first, but makes sense when the mechanism behind it is understood. An increased intracranial pressure would lead to the collapse of intracranial arteries if it exceeded the MAP. To counter this, a sympathetic response is activated when the ICP is increased, which leads to: vasoconstriction, increased contractility and HR in the first stage of the reflex. This sympathetic response stretches the baroreceptors, which then activate strong parasympathetic responses, which as previously stated work mainly on the hearts conduction system causing a lower HR. It seems that this combination of high sympathetic and high parasympathetic output leads to the combination of high CO and systolic blood pressure while also inducing bradycardia at the same time (13,14).

### 1.2.3. Orthostasis

Understanding the etymology of the word “orthostasis” gives away the answer to its definition. From the Greek “*Orthos”* meaning upright and “*histanai”* to stand, one can conclude that orthostasis represents a movement from a horizontal to a vertical position, as defined by Chopra et al. (15). When the posture of the human body changes from a horizontal to a vertical plane, or simply said: “when one stands up”, there is a fluid shift of blood in the body due to gravity. While lying down, gravity acts equally upon the whole body so the blood reserves, which are filling up the distensible veins are spread out equally in the upper and lower body. When one stands up there is an acute change in the gravitational force, which pushes the blood reserves from the veins of the upper body to the veins of the lower body. As much as 500 to 800 ml of blood volume can redistribute. This acute shift of blood volume reduces the PL of the heart, which of course leads to a decrease in CO and MAP (16).

To combat these changes and to allow the human species to stand up and walk upright without constant drops in the CO and MAP, the human body has developed compensatory mechanisms, which activate and keep the CO and MAP ideally unchanged. Out of these mechanisms two are thought to be the primary ways our body fights against gravity: autonomic-baroreflexes and the use of muscle pumps. The concepts of baroreflexes are discussed above, so in summary the acute change in blood pressure is sensed by the baroreceptors, which then cause a switch from parasympathetic to sympathetic output, which will cause vasoconstriction, increased heart contractility and increased HR all of which will lead to an increase in the MAP so that the MAP will remain constant during standing up. The skeletal muscles, specifically those of the leg muscles make up the “muscle pump” which by contraction empty the distensible veins of the legs, which in turn will lead to an increase in right heart PL and will maintain a constant MAP, all while venous valves prevent blood backflow, a schematic of this system can be seen in figure 3. Even gentle contractions of the skeletal leg muscles can effectively nullify the effect of gravity (3).

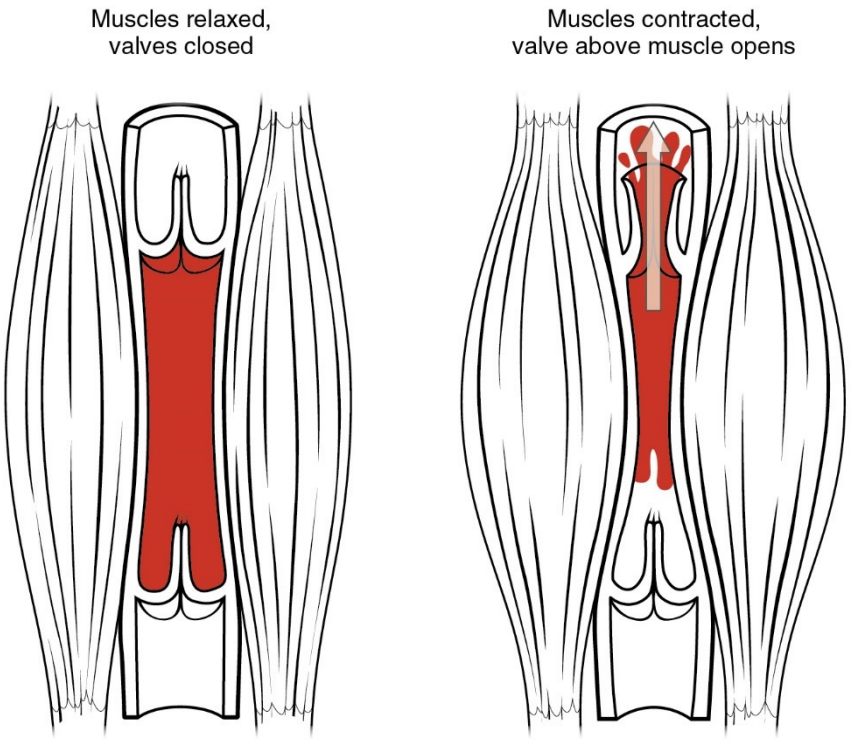


**Figure 3: The leg muscle pump**
*Obtained under CC BY from:*
*https://en.wikipedia.org/wiki/Skeletal_muscle_pump#/media/File:2114_Skeletal_Muscle_Vein_Pump.jpg* (17)

It is when these compensatory mechanisms fail that one talks about “orthostatic intolerance”. Orthostatic intolerance can be defined as a group of symptoms all caused by the decreased CO during orthostasis and the consequent hypoperfusion of peripheral organs (mostly the brain). These symptoms can be divided into three distinct clinical entities or syndromes: vasovagal syncope, orthostatic hypotension and postural tachycardia syndrome (18).

Syncope is defined as “complete loss of consciousness and postural tone due to transient global cerebral hypoperfusion, characterized by: rapid onset, short duration and spontaneous complete recovery”(19). Usually caused by failure of compensatory mechanisms to keep the MAP high enough to secure good cerebral perfusion. Symptoms of cerebral hypoperfusion usually occur, such as: disorientation, headache, visual changes and feeling lightheaded just before the syncope occurs and usually also after the patient recovers. This “postdrome” state is characteristic of vasovagal syncope (18).

Orthostatic hypotension is defined as: “a drop of 20 millimeters of mercury (mmHg) in systolic blood pressure within two to five minutes of standing, or a drop of ten

mmHg in diastolic blood pressure”(20). In orthostatic hypotension there is a failure of the compensatory mechanisms to keep a normal MAP during orthostasis, but the failure is not as severe as to provoke a syncope. It is also commonly accompanied by symptoms of cerebral hypoperfusion including: visual changes, disturbed speech, confusion and impaired cognition (18).

Postural tachycardia syndrome is on the other hand defined as an increase in the HR by 30 to 40 beats per minute without the presence of orthostatic hypotension. It seems that in these individuals despite the increased HR there are symptoms of cerebral hypoperfusion, but there is no drastic change in the blood pressure (18).

The clinical usefulness of orthostasis intolerance assessment is great when used in the appropriate clinical situations. Postural vital signs should be obtained after the patient has been supine for at least two minutes and the patient should be then assessed for one minute in an upright position. Supine vital signs should be compared to vital signs while standing. This can help with the diagnosis of hypovolemic states. “Postural dizziness (severe enough to stop the test) or an increase in heart rate of at least 30 beats per minute has a sensitivity of 97% and specificity of 96% for blood loss greater than 640 ml.” (21,22).

## 1.3. Microcirculation

Microcirculation refers to the circulation of blood in the smallest of blood vessels. These include: arterioles, capillaries and venules. Its primary job is to secure an adequate blood flow, which meets the metabolic requirements of the supplying tissue, and it is rare for any functional cell in the body to be more than 20-30 micrometers away from a capillary. Blood flow is regulated through vasoconstriction and vasodilatation, primarily through the muscular arterioles, which have the ability to drastically change their diameter. Vasodilatation of course increases blood flow while vasoconstriction decreases blood flow, which is reasonable if one remembers that resistance to flow is inversely proportional to the fourth of the blood vessel’s radius.

The capillaries with their single-layer highly permeable endothelial cells are suited for interchange of vital cellular nutrients such as: oxygen, glucose, amino acids and fatty acids; the removal of $CO_2$ and hydrogen ions as well as the maintenance of proper ion concentrations and the transport of various hormones. The capillaries then drain into the venules, which have a much weaker muscular coat, but they also have to endure lower pressures than arterioles.

The degree of blood flow through a tissue is primarily regulated by the tissue itself, or in other words by the tissue's metabolic needs. This is called the metabolic control of local blood flow. For example: kidneys, which are highly metabolically active but relatively small organs have a large blood flow of around 1100 ml/min, while inactive muscles have a relatively low blood flow of only 750 ml/min, even though they constitute between 30 and 40 percent of the total body mass. The flow through the muscles can increase drastically when physically active (2). This autoregulation of local blood flow is thought to be under the influence of different physiologic mechanisms, most importantly the metabolic control of blood flow. It is known that tissue oxygen deficiency leads to vasodilatation and an increase in blood flow. Many different vasodilatory substances are known to be released in low oxygen states, such as: adenosine, lactic acid, CO2, histamine, potassium and hydrogen ions. All of these substances promote vasodilatation as a response to oxygen deficiency.

Another theory in regards to the low oxygen level response states that: for the smooth muscle contraction itself, oxygen is needed, therefore it is reasonable to believe that in the absence of oxygen blood vessels would relax and dilate. One important exception to this rule is the "*Euler-Liljestrand mechanism"* in which pulmonary vessels contract in hypoxic states to ensure a better ventilation-perfusion match.

$CO_2$ levels play an important role in cerebral blood flow, as $CO_2$ seems to be a strong factor in determining the level of tone of the cerebral vessels. A low level of $CO_2$ also known as hypocapnia causes profound vasoconstriction, while hypercapnia causes vasodilatation.
Two examples of metabolic control of local blood flow are reactive and active hyperemia. Reactive hyperemia occurs after a short time blockage of tissue blood

supply, which is then unblocked. This results in an immediate increase in the blood flow as to repay for the tissue oxygen deficit that occurred in the period of blockage. Active hyperemia refers to the increase in blood flow in a tissue that has become highly active, such as in muscles that are actively used, but also blood flow to the brain in increased mental activity.

It seems that not only metabolic pathways play a role in local blood flow regulation, physical changes to the blood vessels itself such as sudden stretching, arterial wall shear stress, and flow mediated dilatation (FMD) are all mechanisms that play an important role. A sudden stretch of a small blood vessel causes the smooth musculature of the vessel to contract, this sudden stretch can for example be caused by an increase in MAP. This mechanism is important to keep the blood flow in normal ranges, and the same applies for situations of low or no stretching, this then promotes smooth muscle relaxation and also maintains a normal blood flow in states such as a decrease in MAP (2).

Shear stress is the tangential force of flowing blood on the endothelial surface of the blood vessel. Low or changing shear stress such as in turbulent blood flow has been found to promote endothelial apoptosis, proliferation, and secretion of vasoconstrictor and procoagulant substances. While in high shear stress states such as in laminar blood flow, vasodilatory substances are released and endothelial cell survival is promoted (23).

FMD refers to a vasodilatory effect through the release of NO by endothelial cells when blood flow increases in an artery. FMD is used as a noninvasive measure of endothelial dysfunction(24,25). Low FMD is also a strong predictor for future cardiovascular events in patients with existing cardiovascular disease (26). NO is an endothelial-derived lipophilic gas, that gets released in response to a variety of stimuli. As previously mentioned, shear stress exerted on the endothelial cells because of the viscous drag of blood against the vascular walls contorts the endothelial cells and causes a release of NO, which then dilates the vessel. Damage to endothelial cells by atherosclerosis or hypertension causes impaired NO synthesis and contributes to excessive vasoconstriction and further endothelial damage.

In contrast to NO, endothelin is a powerful vasoconstrictor that is also of endothelial origin. It gets released primarily by damaged endothelial cells like in traumatic injury, and its primary function is to prevent major bleeding and hemorrhagic shock.

All of the aforementioned mechanisms are short term blood flow regulation mechanisms, the main long-term regulation is done by changes in tissue vascularity. Increased metabolic needs of a tissue leads to angiogenesis and an increase in capillaries while on the other hand in blood vessel blockage, collateral vessel formation and collateral perfusion is the bodies physiologic response.

The last kind of blood flow control is the humoral control of circulation, which relies on vasoconstrictor agents such as adrenaline and noradrenaline (both powerful vasoconstrictor hormones), angiotensin II from the RAAS and vasopressin (also called antidiuretic hormone) and vasodilatory agents such as: bradykinin and histamine (2).

There have been many papers that found a link between orthostasis and a dysfunction of microcirculation. Specifically postural tachycardia syndrome, is linked with a endothelial dysfunction, and lower blood flow due to an impaired NO effect (27–29).

## 1.4. The retina and microcirculation

The retina comprises the inner most layer of the ocular bulbus. It is a highly sophisticated tissue, which is responsible for the sensation of vision. It can be divided into two parts separated by the "*Ora serrata"*: the dorsal "*Pars optica"* and the ventral "*Pars caeca"*. The "*Pars caeca"* is comprised only of pigmented epithelial cells and does not take part in the sensation of vision. The "*Pars optica"* consists of multiple layers and is light sensitive. The light sensing cells can be found in this area, of which there are two types: rods and cones. In these cells light that strikes the retina initiates a cascade of events, which will trigger an impulse. This impulse will be sent through the optic nerve to the visual cortex in the brain resulting in the sensation of vision (30,31).

The retina is available for inspection via fundoscopy, which is a method to inspect the fundus of the eye. The fundus is the inside, back surface of the eye. One can examine the retina, the optic disc, the macula lutea with the fovea centralis, and the microvasculature. The optic disc is the small circular bright structure as seen on figure 4, it represents the entry point of the optic nerve and is a blind spot since it does not take part in the sensation of vision. The macula lutea with the fovea centralis is the darker oval area as seen on figure 4, it represents the point of sharpest vision. Out of the optic disc the retinal vasculature protrudes (30). The arterial portion arises from the *Arteria centralis retinae,* while the venous portion drains into the *Vena centralis retinae*. The venous vessels are darker and thicker in appearance than their arterial counterparts. These vessels spread out from the optic disc throughout the whole fundus leaving only the macula lutea as an avascular area. Generally, an artery does not cross another artery and the same goes for the veins while artery-vein crossings are normal. Commonly there is an alternation of the order of “artery then vein” repeating around the entire optic disc. From the *Arteria centralis retinae* rises the radial-peripapillary-capillary-plexus and the superficial-vascular-plexus as well as the intermediate and deep-capillary-plexus all perfusing the retina (32). Fundoscopy gives us an unique direct look into the usually non accessible small vessels of the cardiovascular circuit (33). A look into the microcirculation can give us an idea about the state of the cardiovascular system as a whole.

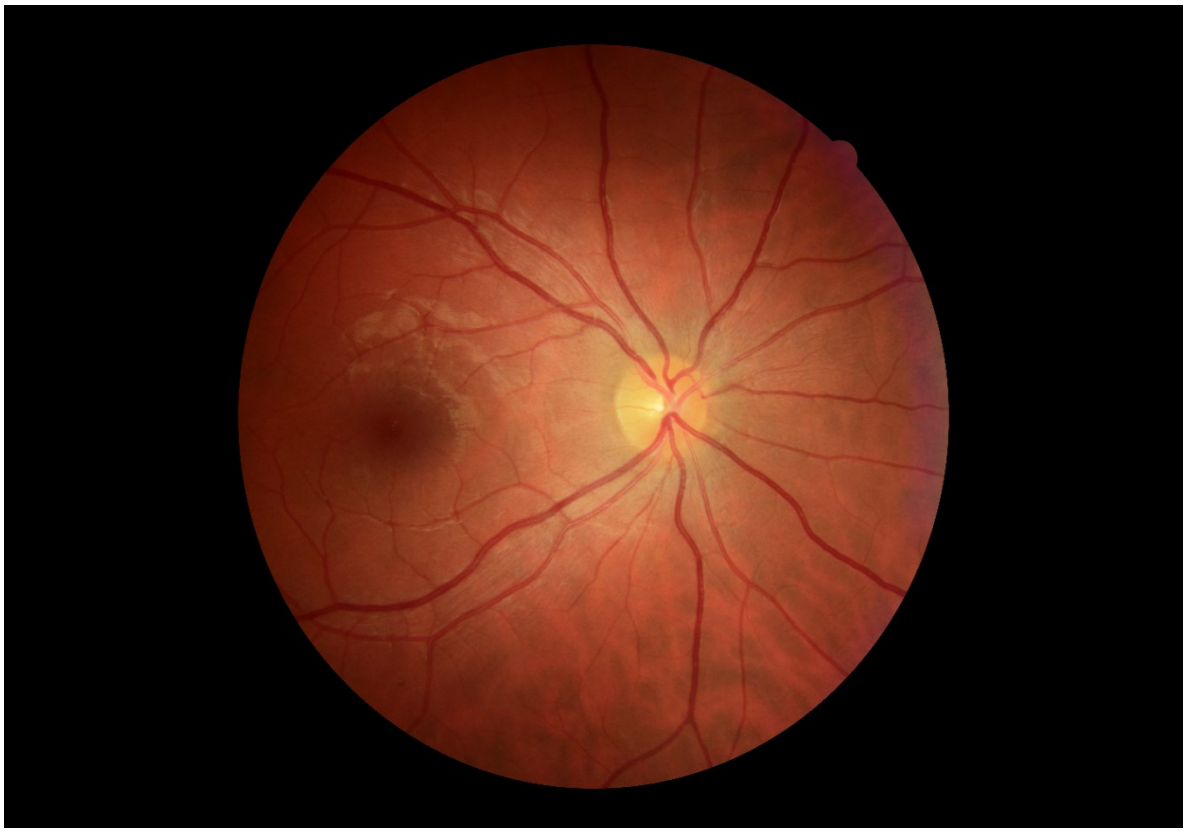

**Figure 4: The fundus of the right eye**
*This retinal image was obtained via a non-mydriatic digital retinal camera "Canon CR-2" (Canon Medical Systems Europe B.V., Netherlands), from one of the participants.*

The retinal vessels can provide important information on the morphological changes in cardiovascular disease such as: hypertension and diabetes, as well as giving us insight into the function of the microcirculation (34) with findings suggesting that photographs of the retinal vessels and their caliber provide information about the association between the microvasculature and microvascular disease (35).

## 1.5. Lower body negative pressure

Lower body negative pressure (LBNP) is an elegant method of inducing a blood shift from the upper body compartments to the lower body compartments, where the upper body compartments are defined as being above the iliac crest, eliminating effects such as full orthostatic weight loading on the lower extremities or altered stimulus to the otolithic receptors. The method is as follows: the examinees legs are enclosed in a chamber or in trousers. The legs are then exposed to negative pressures, which in turn cause a fluid shift to the lower parts of the body. Negative pressures of -40 to -50 mmHg while supine are consistent with the fluid shifts induced by the gravity in an upright position (36–38). LBNP provides a noninvasive method, which is capable of producing a reduction in central blood volume, this state

can then be used to analyze a whole plethora of different physiologic and pathophysiologic mechanisms. It has been found to have multiple applications such as: being used as an assessment tool for the autonomic system function, resistance training, a screening test for pilots, in the Russian space program it has been used to partially reverse the cephalad fluid shifts that occur in weightlessness, investigating acute hemorrhagic shock, as a method to reduce orthostatic intolerance, to study the effects of reduced gravity environments, as a tool to study hormonal blood pressure regulation mechanisms and many more.

LBNP has multiple effects on different physiologic systems, most importantly on the cardiovascular system and it's autoregulatory mechanisms. As the lower body chamber decompresses, the negative pressure causes blood to pool in the lower body compartment. This volume is then "lost" to the lower body compartment and does not return to the right heart, which lowers the right heart PL, in turn decreasing left heart PL and left ventricular end-diastolic volume (LVED) and per the Frank-Starling mechanism: the reduction of heart filling, will reduce the heart output, meaning SV will be reduced and ultimately CO.

A reduced CO then induces a decrease in the MAP, which will activate autoregulatory mechanisms. The first mechanism to activate is the baroreflex through the baroreceptors in the carotids and the aortic arch. A withdrawal of the parasympathetic tonus in addition with the activation of the sympathetic system induces an increase in cardiac HR and contractility through the activation of beta-one receptors. The beta-one sympathetic pathway seems to play a major role since patients on beta-adrenergic blockade are known to have a decreased tolerance to central hypovolemia (39,40). Besides the beta-one activation, the alpha-one receptors are also activated, which in turn constrict not only the arterioles leading to a higher SVR but also venous vasculature, which produces an increased PL. Both mechanisms serve to increase MAP as to provide an adequate perfusion pressure (38).

The RAAS system also gets activated through the juxtaglomerular efferent center, which senses a change in renal perfusion during the state of central hypovolemia and responds with elevated plasma renin that ultimately leads to an increase in

angiotensin II, which promotes the release of aldosterone and also promotes vasoconstriction. The role of the RAAS system is to increase blood volume and thusly preload. It accomplishes this effect with aldosterone, which promotes sodium and water reabsorption within the kidney, while angiotensin II promotes an increase in SVR with vasoconstriction, with an effect similar to that of activation of alpha-one receptors (38).

With changes in cardiovascular physiology one can also expect changes in cerebral blood flow. With reduced CO and MAP the cerebral blood flow also suffers, which can be measured by the blood velocity in the middle cerebral artery. What is interesting is that there is evidence that the changes in blood flow are not homogenous, the cortical gray matter suffers more (about a 25% reduction) than the white matter or basal ganglia regions during LBNP (41). Another interesting point is that the cerebral tissue has the possibility to extract more oxygen out of the blood while in a state of hypoperfusion, thus increasing the brains ability to withstand hypoperfusion (42). None the less eventually symptoms of cerebral hypoperfusion are inevitable with presyncope symptoms such as dizziness, sudden heart rate decrease and impaired cognition (38).

The respiratory system is also affected by application of LBNP. The negative pressure in the lower body compartment drives the diaphragm caudally, which is associated with: an increased functional residual capacity, increased forced vital capacity (FVC) and increased forced expiratory volume in one second (FEV1) along with the effect of loss of blood volume, which causes pulmonary hypoperfusion. The circulating blood volume in the pulmonary vascular system causes impedance to air flow in the lungs. Evidence for these changes (lower pulmonary blood volumes and increases in FVC and FEV1) are supported by greater increases in FVC and FEV1 in participants with larger lung volumes who also therefore have increased pulmonary blood volumes (43). Diffusion or gas exchange is one of the main functions of the respiratory system. Diffusion can be described with Fick’s law of diffusion, which states: that the rate of diffusion of any given gas across a permeable membrane such as the alveolar-capillary membrane is determined by the surface area of the membrane, the difference in partial pressures across the membrane, the thickness of the membrane and the diffusion coefficient of that particular gas. The surface area of the membrane is also under the influence of ventilatory and

perfusion matching (V/Q matching), which states that any change in pulmonary ventilation, be it an increase or decrease as well as any change in blood flow through the pulmonary capillary system be it again, an increase or decrease will result in poor V/Q matching, and therefore a decreased surface area of the membrane or a so called “diffusion problem”. With the decrease in pulmonary blood volume a decrease in blood flow is also expected and this results in a poor V/Q match, which ultimately results in a “diffusion problem” across the alveolar-capillary membranes (2). These changes are supported by evidence of linear decreases of diffusion when measured by the carbon monoxide technique (44,45). The so called “respiratory pump” also plays a vital role in elevating venous return and PL to the right heart during deeper inspirations. During an inspiration the generated loss in intrathoracic pressure “sucks” not only air into the lungs but also venous blood from the great venous vessels into the right heart, thereby increasing venous return and PL. This mechanism helps to maintain a good amount of PL and CO during the application of LBNP, during which, tidal volumes increase. Another potential compensatory mechanism during LBNP application is an increased respiratory drive, despite of low end-tidal $CO_2$ , leading to the conclusion that it is the central hypovolemia that drives this mechanism, which can potentially increase the delivery of oxygen in the state of central hypovolemia induced by LBNP (38).

LBNP with its ability to imitate states of low volume such as hemorrhage from trauma, has been used to assess for changes in the coagulation system. LBNP causes hemoconcentration and increases in blood viscosity and plasma proteins all of which promote a prothrombotic state, which could be potentially vital mechanisms in a state of hemorrhage (38).

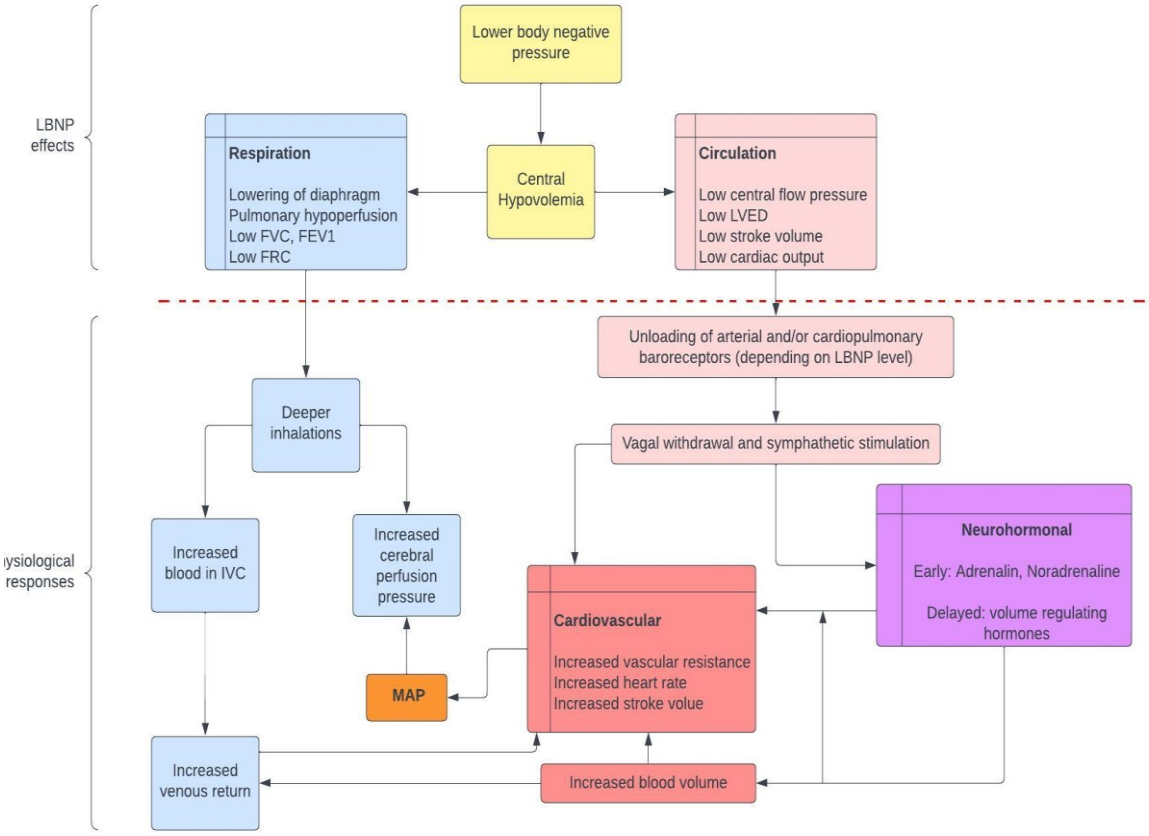


**Figure 5: Overview of mechanical effects and physiological responses of lower body negative pressure**

*Illustration by the author using information from: "Nandu Goswami et al. LOWER BODY NEGATIVE PRESSURE: PHYSIOLOGICAL EFFECTS, APPLICATIONS, AND IMPLEMENTATION. Physiol Rev 12. 2018."* (38)

Figure five provides a succinct summary of all the main physiologic changes one can expect with the application of LBNP. LBNP induces a state of central hypovolemia, which has severe impacts on the cardiovascular system and respiratory system. The loss of CO induces the activation of compensatory mechanisms such as the baroreflex and the RAAS, which in turn tend to increase the CO by increasing the total blood volume, HR, SV and SVR. The caudal shift of the diaphragm together with pulmonary hypoperfusion causes an increased respiratory drive, which helps in increasing venous return and PL. All of these changes represent a compensatory response with the goal of improving the MAP and thus preventing a loss of cerebral perfusion pressure (38).

## 1.6. Differences between the sexes

The literature is filled with examples of differences between the sexes in regards to cardiovascular regulation and orthostatic tolerance. Women display a lower ability to tolerate central hypovolemia as discussed by Goswami et al. (38). Although data regarding to the lower tolerance in women is established (46–50), data regarding to the underlying causing mechanisms are somewhat contradictory and as such they are not fully understood.

Taken generally, women display on average a lower SV, with a higher ejection fraction than men (51). One of the reasons are structural-anatomical differences, after adjusting for height and body surface area women still have a smaller left ventricular mass when compared to men (52,53) while in addition having lower end-diastolic dimensions. Total blood volume is also lower even after adjusting for body weight (50,54). It is not difficult to imagine how these differences could lead to a lower level of tolerance of central hypovolemia. Another anatomical factor that is often cited as a source of lower orthostatic tolerance in women (54,55) is that women seem to have an additional blood pooling plexus, which is missing in men. That is the plexus of blood vessels that supply the uterus, ovaries and the vagina. However the importance of this additional blood pool has been controversial as authors as such as Halliwill et al. (56) have reported a negligible effect on hemodynamics.

A widely reported phenomenon is the characteristic difference in response to central hypovolemia between the sexes. Women typically respond with parasympathetic withdrawal, which does not seem to be as effective as a sympathetic activation that is common in men, in maintaining a normal MAP. Sympathetic alpha-one receptor activation in men, with an increase in SVR seems to play an important role in orthostatic tolerance. SVR changes in women are not as high and their primary response is an increase in HR (57). Hachiya et al. reported in his study using LBNP that women have a reduced vasoconstrictor response to a given accumulation of calf blood volume (58). As per the MAP equation, both an increase in SVR and HR should lead to an increase in MAP but since the main hemodynamic issue in central hypovolemia is a decreased venous return with low PL it makes sense that increasing the PL through an increase in SVR is more effective than just an increase

in HR (59). This would explain the larger decrease in CO and MAP in women. The fact that women have a stronger tachycardic response while men have a stronger vasoconstrictor response to central hypovolemia has been reported in various studies (54,55,60). Not only can this difference be reproduced when looking at “male vs female” performance, but it has also been found that in women who are highly tolerant to orthostatic stress the response is the same (primarily peripheral vasoconstriction) while low tolerant women also respond with tachycardia primarily as shown by Wenner et al. (61). A very similar observation was made in astronauts who are returning from space. Astronauts with post spaceflight induced orthostatic intolerance display the same pattern of increased HR with a relatively low increase in SVR. Female astronauts were more affected than their male counterparts (62–65).

Carbon dioxide (CO2) has a profound effect on cerebral blood flow. Hypercapnia causes cerebral vasodilatation with an increased cerebral blood flow while hypocapnia causes cerebral vasoconstriction and therefore a decreased cerebral blood flow, this has as well been proposed as a mechanism of orthostatic tolerance since women have a higher minute ventilation and as such, intrinsically lower $CO_2$ levels they might be at a disadvantage, ultimately having lower reserves once hyperventilation occurs in central hypovolemia. It has been found that inspiring five percent $CO_2$ prolongs the time until presyncope is reached in both sexes confirming the important role of $CO_2$ (66).

When comparing differences between the sexes one cannot exclude hormonal differences. Estradiol while both found in males and females, plays a much more important role in female physiology. The cyclic secretion of estrogens and progesterone in the menstrual cycle has an immense impact on female physiology and therefore its effects have been studied also in regard to orthostasis.

Oestradiol is known to attenuate noradrenaline induced vasoconstriction (67), and in lower levels of oestrogen an increased peripheral vasoconstriction is seen (68). Endothelial function seems to be under the influence of oestradiol as well, by altering the mechanical properties of arteries and arterioles, vasodilatation is promoted by an increased nitric oxide (NO) availability (69). Beta-two adrenergic receptor

sensitivity is enhanced by oestradiol as well, causing a weakened alpha-one receptor response as a consequence (63,70). Higher sensitivity to oestradiol has been found in women who show low orthostatic tolerance by Wenner et al. due to their blunted vasoconstriction response (61).
Catecholamine differences are observed as well, in men a larger presyncopal increase in plasma norepinephrine was observed (50). Lower plasma adrenomedullin levels in females in general are also found, and could also play an important role (71).

Although differences between the sexes can be appreciated as already stated studies often show contradictory findings; some studies show no differences in cardiovascular response and tolerance to LBNP between the sexes (72,73). Data relating to the menstrual cycle are contradictory (73,74). Goswami et al. did not observe any sex differences in endothelial function upon orthostatic challenge nor was there any difference under the influence of the menstrual cycle or usage of oral contraception (75). It almost seems that for every published paper on sex differences one can find similar papers with contradictory results. Additional studies with bigger sample sizes should be performed to further investigate this issue.

# 2. Aims and Objectives

Although there have been studies performed on the physiology of orthostasis, not many studies have touched on the specific effects on the microcirculation and sex differences in the microcirculatory response. There is a general consensus that there is a difference in orthostatic tolerance between the sexes, which is supported by various epidemiological data, which shows a greater prevalence of orthostatic issues in the female population, (62,70,75,76) but no studies have been conducted on the effect of orthostasis (as induced by LBNP) on the microcirculation which leaves a gap for additional research to be performed.

Particularly large studies with a higher participant number, including both male and female participants and with an ability to track the microcirculatory changes, are in need to shed more light on this particular topic.

<u>In this thesis, the two main hypotheses were:</u>

(1) There will be a significant and measurable difference in the retinal vessel diameters expressed as CRAE, CRVE and their ratio (AVR) during our six-phase LBNP protocol.

(2) There will be a significant and measurable difference in the retinal vessel diameters (CRAE, CRVE and AVR) between the sexes during our six-phase LBNP protocol.

# 3. Methodology

Before starting the study, an approval was obtained from the “Ethics Committee of the Medical University of Graz” as well as written and informed consent from all participants (EK 25-551 ex 12/13). Every participant received detailed information about the study protocol. The study as well as the data analysis were performed at the “Medical University of Graz”. All of the data collection was performed in accordance with good clinical practices as per the WMA Declaration of Helsinki (2013.).

## 3.1. Participants

In order to avoid confounding variables such as athletic training, height and age; on orthostatic tolerance the following inclusion criteria were chosen: healthy male and female participants aged between 18-45 years. Exclusion criteria were: cardiovascular disease, known orthostatic intolerance, participants with or family history of thrombosis, taking any medication that affects the coagulation system, taking any medication affecting cardiac parameters (such as beta blockers), pregnancy, smokers and endurance athletes. Women taking oral contraception were not excluded however the type of medication and the first day of the last period were recorded.

In order to further avoid any confounders, the participants were not allowed any exercise or stressful activity two days before the test as well abstinence from coffee and other stimulants 24 hours before the tests. The investigations were carried out in a room maintained at 23-25°C with a humidity between 50-55%, the room was quiet and dimly lit.

## 3.2. LBNP protocol

LBNP was applied with the participant’s lying supine on a table, with their legs up to the level of the iliac crest in an enclosed chamber. A picture of a participant lying on the table can be seen in figure six. The protocol was divided into six phases: a 30-minute rest phase to acquire baseline measurements, and then four, consecutive, five-minute phases in which the LBNP was gradually increased in ten mmHg intervals, from -10 mmHg all the way to -40 mmHg, ending with a 30-minute recovery phase. LBNP of -40 mmHg corresponds to the fluid shifts of standing upright, but without the use of the “muscle pump” (77). The protocol would be terminated immediately if at any point the participants reported any presyncopal symptoms

(such as dizziness, visual changes, headache or nausea) or if any presyncopal signs (such as a systolic blood pressure below 80 mmHg) were identified, which happened to six participants in this study. Tables (table one and two) are provided for further clarification of the protocol. Hemodynamic parameters were monitored during the whole protocol.

**Figure 6: The LBNP table**
*This is a picture of the LBNP table used in the study, one of the participants can be seen lying supine with the lower extremities in the negative pressure chamber.*

**Table 1: LBNP protocol**

| Phase | LBNP in mmHg | Description |
|---|---|---|
| **Baseline measurements** | 0 | 30 minutes of no LBNP while supine |
| **LBNP10** | -10 | 5 minutes of -10 mmHg LBNP while supine |
| **LBNP20** | -20 | 5 minutes of -20 mmHg LBNP while supine |
| **LBNP30** | -30 | 5 minutes of -30 mmHg LBNP while supine |
| **LBNP40** | -40 | 5 minutes of -40 mmHg LBNP while supine |

| | | |
|---|---|---|
| **Recovery** | 0 | 30 minutes of no LBNP while supine |

**Table 2: LBNP protocol timeline**

| Baseline measurements | LBNP10 | LBNP20 | LBNP30 | LBNP40 | Recovery |
|---|---|---|---|---|---|
| 30 minutes | 5 minutes | 5 minutes | 5 minutes | 5 minutes | 30 minutes |

## 3.3. Retinal measurements

The retinal images from the participants were obtained via a non-mydriatic digital retinal camera “Canon CR-2” (Canon Medical Systems Europe B.V., Netherlands). Images of the left eye were obtained at every stage of the LBNP protocol for every participant. From the images, the dimensions of the retinal blood vessels were analyzed with the help of “MONA REVA” software, developed by VITO (Belgium). The analysis was conducted by an expert at the Medical University of Graz who was blinded to the participants characteristics. In the analysis six of the largest retinal arteries and six of the largest retinal veins per picture would be identified and then using the formulas described by Knudtson et al. (35) the process would result in three parameters obtained from each participant: the central retinal artery equivalent (CRAE), the central retinal vein equivalent (CRVE), and the artery to vein ratio (AV Ratio). Vessel diameters expressed as CRAE and CRVE were measured in micrometers.

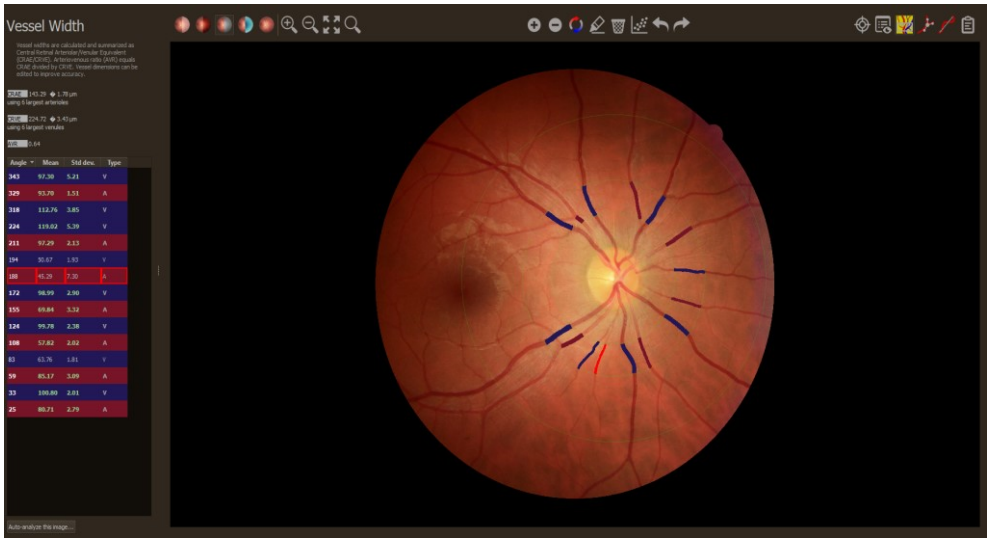


**Figure 7: Example of a picture that is processed by the MONA REVA software**
*An example of how the software is used to measure vessel diameters, an example from one of our participants.*

## 3.4. Statistical analysis

Repeated measures ANOVAs, including the sex as between-subjects factor and the steps of the protocol (baseline, LBNP-10, LBNP-20, LBNP-30, LBNP-40 and recovery) as within-subjects factor, were performed for each variable of interest (CRAE, CRVE, AVR). All data was checked for normality of the distribution by Shapiro-Wilks test and cases largely deviating from the respective group mean (± 3SD) were excluded from further analyses. Statistical analyses were performed by SPSS (IBM SPSS Statistics for Windows, Version 27.0., Armonk, NY: IBM Corp).

Due to the low number of complete data sets, statistical analyses of changes in microvasculature, as measured by retina camera, included only the measurement of: baseline, LBNP-40 and recovery. However, for the purpose of information, means at each step of the protocol are displayed in graphs and tables.

To test our hypothesis (vessel diameter will change over LBNP phases, and sex differences will be present) repeated measures ANOVA was performed to look for significant changes in CRAE, CRVE and AVR between the baseline measurements, LBNP40 and recovery, as well as for significant differences between the male and female participants.

# 4. Results

## 4.1. Participants

In total, 42 participants (22 females) with a mean age of 24.9 (± 5.9SD) were enrolled in the study, of whom 14.3% (six participants) did not finish the entire protocol. Mean age, weight, height and BMI can be seen in table three. Further participant characteristics by sex (female and male) can be seen in tables four and five. Retina pictures were taken in 37 participants during LBNP, though for statistical analyses of the changes from *baseline* to *LBNP-40* and to *recovery* complete data sets were available for a total of n = 27 participants. All further results were gained through analysis of those 27 participants with complete data sets.

**Table 3: Participant characteristics**

| | **Participants** |
|---|---|
| **N** | 42 |
| **Male** | 20 |
| **Female** | 22 |
| **Age [y]** | 24,9 ± 5,9 |
| **Weight [kg]** | 66,9 ± 11,7 |
| **Height [cm]** | 174,5 ± 8,9 |
| **BMI [kg/m$^2$]** | 21,8 ± 2,5 |

*Values are expressed as mean ± standard deviation*

**Table 4: Female Participant characteristics**

| | **Female Participants** |
|---|---|
| **N** | 22 |
| **Age [y]** | 22,0 ± 2,5 |
| **Weight [kg]** | 58,3 ± 6,8 |
| **Height [cm]** | 168,3 ± 5,7 |
| **BMI [kg/m$^2$]** | 20,6 ± 2,0 |

*Values are expressed as mean ± standard deviation*

**Table 5: Male Participant characteristics**

| | Male Participants |
|---|---|
| **N** | 20 |
| **Age [y]** | 27,6 ± 6,8 |
| **Weight [kg]** | 75,1 ± 9,1 |
| **Height [cm]** | 180,5 ± 7,2 |
| **BMI [kg/m$^2$]** | 23,0 ± 2,3 |

*Values are expressed as mean ± standard deviation*

## 4.2 Central retinal artery equivalent (CRAE)

The results of the repeated measures ANOVA indicate that no significant changes in CRAE were found between baseline, LBNP-40 and recovery (F (2,50) = 0,579, p = 0,564). Although women showed throughout higher CRAEs, the effect was not significant (F (1,25) = 2,231, p = 0,148, see table six, and figure nine). The interaction between LBNP and the sex was also found to be non-significant (F (2,50) = 0,088, p = 0,916).

**Table 6: CRAE during LBNP**

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 146,90 | 146,96 | 144,91 | 146,65 | 149,74 | 147,41 |
| | 3,09 | 2,56 | 3,05 | 3,05 | 3,15 | 3,31 |
| Male | 140,81 | 140,53 | 138,48 | 140,68 | 142,18 | 141,34 |
| | 3,72 | 3,09 | 3,68 | 3,67 | 3,80 | 4,00 |

*Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysi*

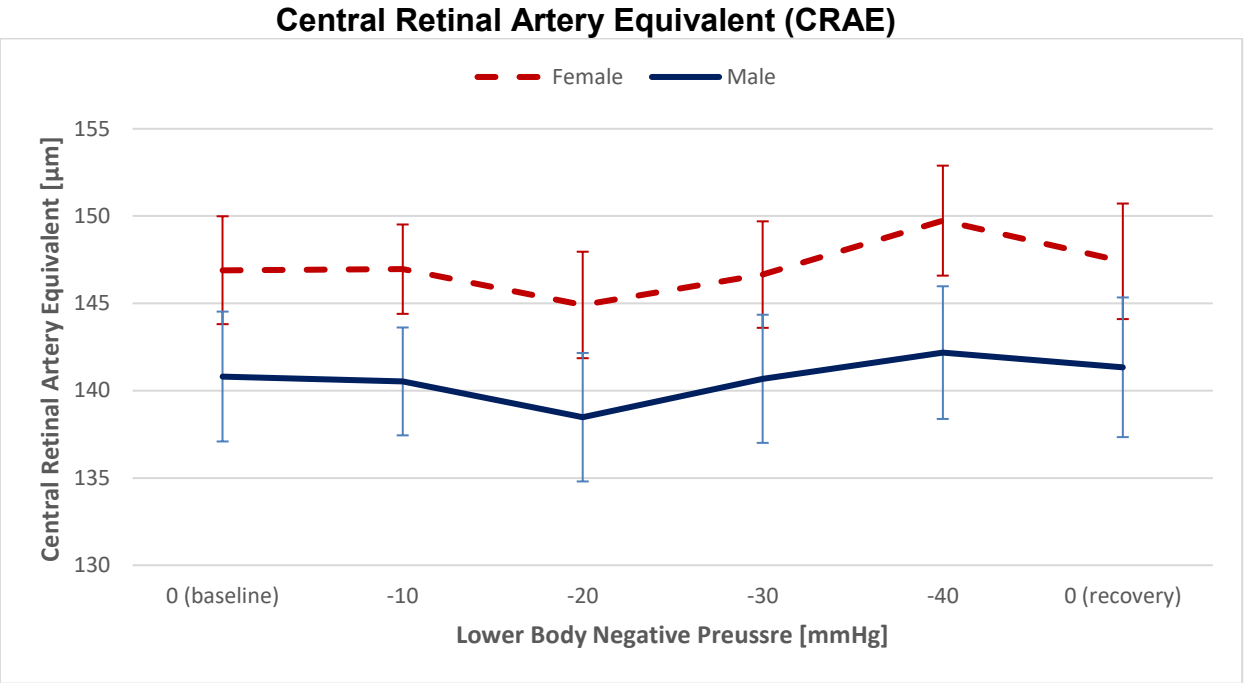


**Figure 8: CRAE during LBNP**

*Note: the LBNP axis indicates the different phases as per protocol*

## 4.3 Central retinal vein equivalent (CRVE)

Similarly, the repeated measures ANOVA also showed that the changes in CRVE were not significantly different ($F_{(2,50)}$ = 0,317, p = 0,730) under the different conditions (recovery, LBNP40, baseline), or between the two sexes (F (1,25) = 0,485, p = 0,492). Also, no interaction was found between the two factors ($F_{(2,50)}$ = 0,076, p = 0,927, see table seven and figure ten).

**Table 7: CRVE during LBNP**

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 210,13 | 207,35 | 206,84 | 209,29 | 210,45 | 211,40 |
| | 5,52 | 4,30 | 4,50 | 4,31 | 4,81 | 4,97 |
| Male | 203,97 | 208,58 | 204,90 | 206,65 | 205,44 | 207,77 |
| | 6,65 | 5,19 | 5,43 | 5,20 | 5,80 | 6,00 |

*Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysis.*

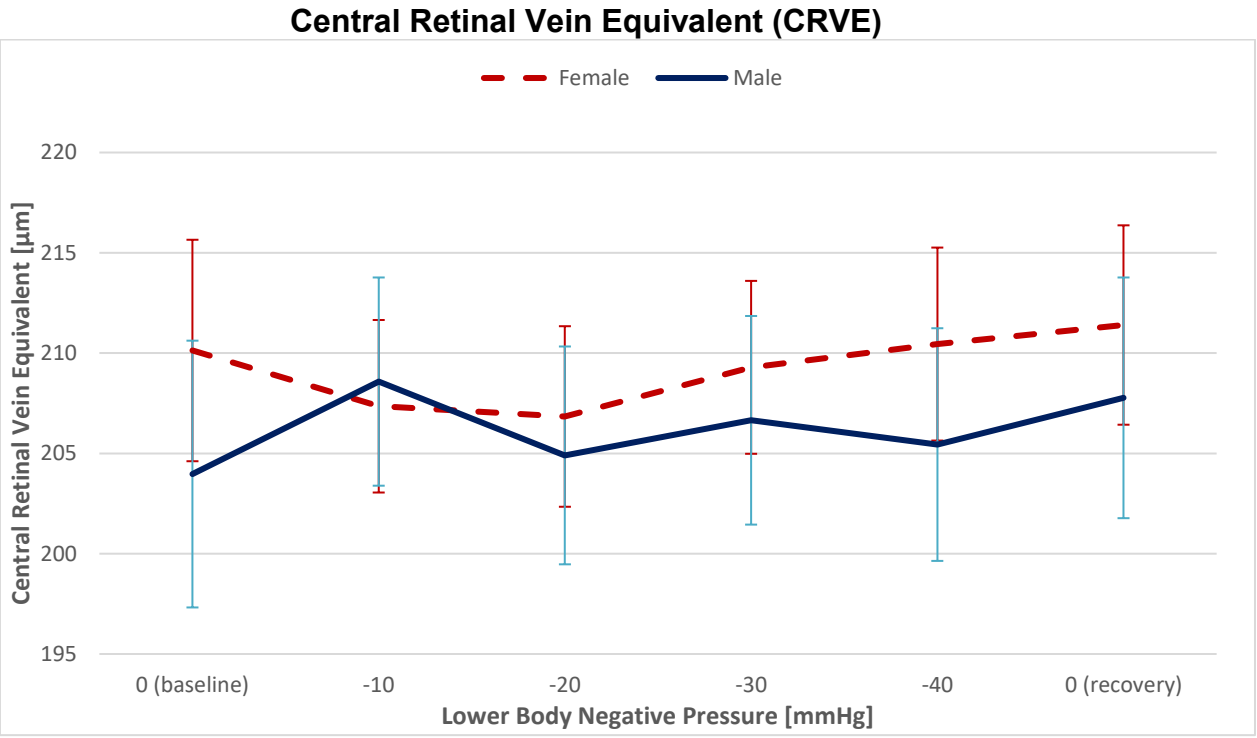


**Figure 9: CRVE during LBNP**
*Note: the LBNP axis indicates the different phases as per protocol*

## 4.4 Artery to Vein ratio (AVR)

Repeated measures ANOVA for the changes in the AVR correspondingly showed no significant effect of the intervention (F (2,50) = 0,570, p = 0,569), nor an interaction (F (2,50) = 0,052, p =0,730) or effect of the sexes (F (1,25) = 0,531, p = 0,473 see table eight and figure eleven).

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 0,70 | 0,71 | 0,71 | 0,70 | 0,71 | 0,70 |
| | 0,02 | 0,02 | 0,02 | 0,02 | 0,01 | 0,02 |
| Male | 0,69 | 0,68 | 0,68 | 0,68 | 0,70 | 0,68 |
| | 0,02 | 0,02 | 0,02 | 0,02 | 0,02 | 0,02 |

**Table 8: Artery to Vein ratio during LBNP**
*Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysis.*

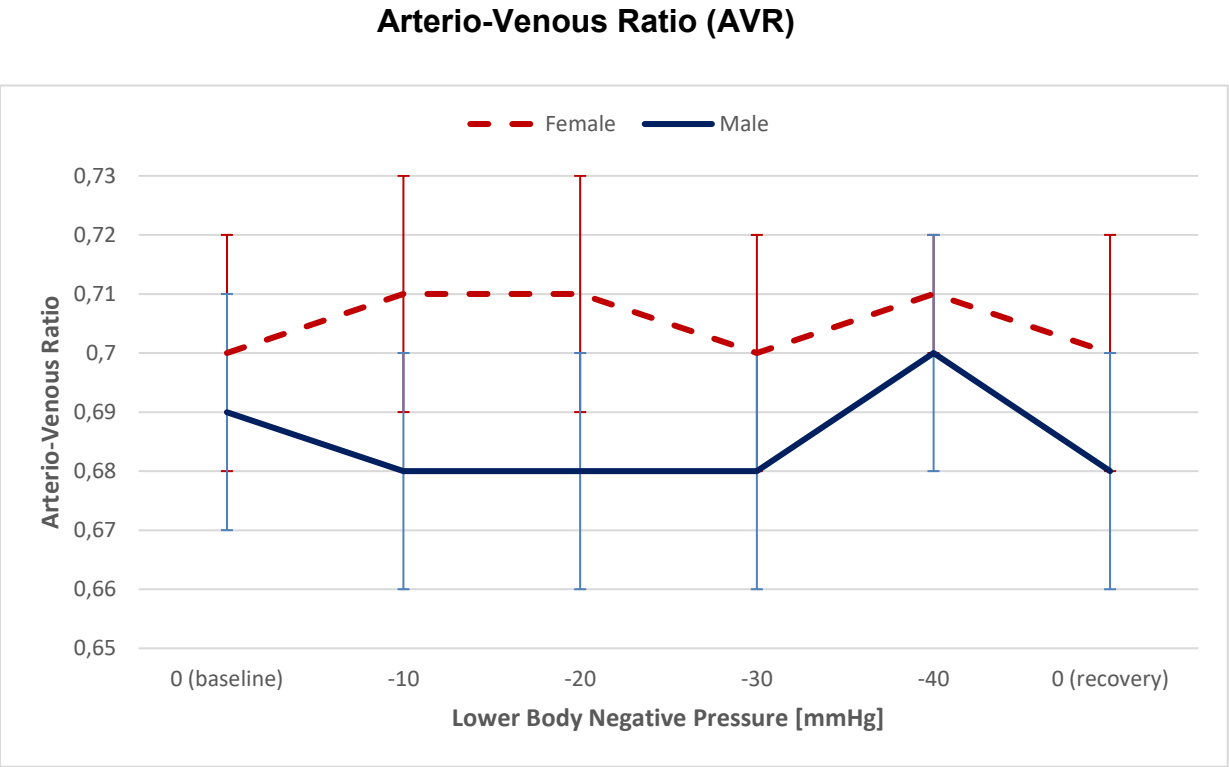


**Figure 10: Artery to Vein ratio during LBNP**
*Note: the LBNP axis indicates the different phases as per protocol*

In summary all of the results gained by the repeated measures ANOVA showed no significant changes in CRAE, CRVE nor AVR between the different LBNP phases (only baseline, LBNP40 and recovery were considered) nor have any significant differences been found between the female and male participants.

# 5. Discussion

The results show no significant differences in the retinal arteriolar and venular diameters (expressed as CRAE and CRVE) nor in their ratio (expressed as arteriolar-venular ratio) during the entire lower body negative pressure (LBNP) protocol. There was also no significant difference between the male and female participants microvascular diameters during the LBNP protocol.

## 5.1. LBNP and the microcirculation

The hypothesis of this study is that the effects of fluid shifting (displacement of blood to the lower extremities) under the influence of lower body negative pressure will induce a change in the microcirculation of the retina, which could then be measured and assessed via retinal imaging. As discussed, in chapters 1.2.2 and 1.3, the expected change in a state of central hypovolemia such as one induced by the LBNP is a narrowing in the diameter of blood vessels as to increase the systemic vascular resistance (SVR) and in that way preserve a normal mean arterial pressure (MAP). Therefore, in a state of hypovolemia, a decrease in the diameter of arterioles and venules through the reflex mechanism of vasoconstriction is expected. This effect has been reproduced successfully in numerous studies such as in the study of Y Miyagatani et al., which demonstrated an increased vascular tone via measuring the volume elastic tone in hypovolemic states (78) and has been implemented in many different ways in daily clinical medicine such as in assessing the volume status in patients via ultrasound imaging of the inferior vena cava and it's diameter, which is a good indicator of hypovolemia as per B.D. Johnson et al. (79). However, in our study we did not observe significant changes in the diameters of microvasculature in the retina. There could be several reasons associated with this observation. In this study young (18 to 45 year old) and healthy participants were recruited, Fabrizio Ricci et al. report that the prevalence of orthostatic hypotension is age dependent ranging from five percent in patients more than 50 years of age and to 30% in those less than 70 years of age (85), it is not unreasonable to assume that if microcirculatory dysfunction plays a significant role in orthostatic intolerance one would more easily see these dysfunctions in the affected population rather than in a healthy population. A point could be made as well that a younger and healthier

population might not need to activate the protective vasoconstrictor mechanisms as much as older individuals whose inotropic and chronotropic heart reserve might be much lower and who therefore might rely more on the vasoconstrictor effect to preserve the MAP, as Xuming Dai et al. report that with increased age the LV wall stiffness increases leading to a lower cardiac reserve among other cardiovascular changes. These factors could explain the lack of significant changes in the vessel diameter in our participants. Furthermore, retinal vessel analysis has been used to assess for various pathological states and risk factors in various studies, such as for the assessment of cardiovascular risk (80), assessment of the level of impairment of neurovascular coupling after subarachnoid hemorrhages(81), the relationship between CRAE and cerebral vessel diameter (82) and many more, no studies could be found in the specific context of retinal vessel assessment during LBNP. In this regard, our results are novel and highly important, as we used microvasculature assessment during states of central hypovolemia. Our results advance the literature in the field of shock, specifically in the understanding of the pathophysiological mechanisms that underline shock and its treatment. There seems to be conflicting data when it comes to retinal microcirculatory changes in shock states. During states of shock a physiological defense mechanism activates, called: blood flow centralization, also known as the brain sparing effect, in which peripheral circulatory systems are “shut down” via vasoconstriction in order to reroute the blood to the vital organs, most importantly to the central nervous system. These changes can be best demonstrated via Doppler ultrasound imaging of the fetal cerebral blood flow in severely growth restricted fetuses as reported by E. Hernandez-Andrade et al. (90) were blood flow centralization can be severe. One could presume that it is this defensive mechanism that prevents severe vasoconstriction in the central nervous system and in extension in the retina so that even in hypovolemic states the blood flow rate does not drop as severely in the central nervous system as it may do in the peripheral organs, such as, for example the skin, causing the characteristic mottling and peripheral cyanosis seen in shock states. Although we, of course, did not induce a shock or shock like state in our participants, similar changes should be seen in hypovolemia (which was induced by LBNP), this proposed mechanism could potentially explain the increases in HR with a concomitant blood pressure drop in our participants during LBNP, while no significant changes in the retinal vessel diameters could be identified.

While no papers on retinal microcirculatory changes in LBNP induced central hypovolemia can be found, there are some papers that report on: changes in the retinal microcirculation in septic patients, which promote the regular use of “retinal monitoring” as an easily accessible organ that could represent the microcirculatory changes in the whole body as a “canary of the body”. However, one must be cautious when making such statements because similar statements were made with sublingual microcirculation perfusion, which then turned out to be flawed, especially is states that are characterized by a heterogeneity of blood flow within and between organs such as in sepsis and septic shock. This was presented by E Christiaan Boerma et al. in a study that looked at the relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis (97). This would imply that although the need for an easily accessible way to monitor microcirculation is high and would provide benefit in clinical medicine, studies have shown that microcirculation monitoring (retinal and sublingual) while readily available, seem to be too unreliable for use in hemodynamically altered states since the values they generate seem to be non-conclusive and even contradicting between various studies. As well as studies on septic shock, some studies can be found on hemorrhagic shock models such as the ovine hemorrhagic shock study conducted by Jenia Kouchek Zadeh et al. Changes in the microcirculation were found to correlate with changes in the systemic hemodynamic parameters (a decrease in MAP, heart rate and cardiac index) when assessing the flow density of the superficial vascular plexus (a vascular plexus derived from the central retinal artery) via OCTA. A decrease from 44.7 % baseline to 34.5% under shock recovered to 46.9% after fluid resuscitation. Similar changes were found in the conjunctival microcirculation (86). In a rat hemorrhagic shock model, which also used optical coherence tomography angiography (OCTA), performed by C E Riva et al, the choroidal blood flow dropped in proportion to MAP but retinal blood flow as assed by OCTA showed no significant change (99). These studies again show the unfortunately heterogenic findings in studies of retinal microcirculation, here under hemorrhagic shock, a state that cannot be directly compared to a fluid shift caused by LBNP, none the less a state that comes close to the pathophysiology of the central hypovolemia induced by LBNP and also probably closer than septic shock states. These studies might imply that more invasive microcirculatory monitoring such as OCTA, as well as inducing more violent fluid changes (such as in septic or

hemorrhagic shock) might lead to more conclusive results. None the less the changes induced by our LBNP protocol provide a valuable insight as they indicate that in healthy and young individuals a LBNP of -40 mmHg is not enough to induce measurable changes in the microcirculation, as measured by retinal imaging.

## 5.2. CRAE, CRVE and the AVR

The CRAE showed no significant changes between the baseline, LBNP40 and recovery after analyzation. Based on these results the null hypothesis that the fluid shifts induced by LBNP will not significantly change the CRAE cannot be rejected, since the measured parameters failed to show the expected fall in arteriolar diameter (fall in CRAE). Our results show an expected fall in CRAE from the baseline to LBNP10 and from LBNP10 to LBNP20 but then show a paradoxical increase in CRAE from LBNP20 to LBNP30 reaching a maximum CRAE in LBNP40, which then decreased again in the recovery phase usually reaching measurements, which are close to the baseline measurements, however all of these changes were not significant. None the less the increased CRAE in LBNP40 (maximal negative pressure) is an unexpected result since in a hypovolemic state one would expect a reflex vasoconstriction and decrease in CRAE. Since the effect of venule contraction is less well established than arteriolar contraction during hypovolemic states these changes are not as unexpected as the changes in CRAE. However, an expected normal response in a person with no cardiovascular conditions would be an increased venous tone as to increase the venous return and therefore the preload (PL). This effect does seem to be mostly relevant in the splanchnic venous system, which serves as a blood pool, which can be recruited during states of hypovolemia (83,84) and therefore the veins of the retina might not play a significant role in maintaining a higher venous return.

One possible answer to our non-significant changes in microcirculatory blood vessel diameter could be the complex nature of microcirculation within the central nervous system and the retina. A Bill and his colleagues reviewed the control of retinal blood flow, which mirrors the cerebral perfusion in healthy individuals, with the highest density of microvascular pericytes being found in the retinal microcirculation, however retinal circulation lacks autonomic innervation and is wholly dependent on

local vasogenic factors, such as endothelin-one acting as a vasoconstrictor. Just like the blood brain barrier, the blood retina barrier protects the cells of the retina from changes in the peripheral blood composition(90,91). This could suggest that no correlation between changes in the peripheral circulation (higher SVR, higher HR, lower blood pressure) and the diameter of the retinal vessels is seen because they are under the control of different regulation systems, the peripheral system being primarily under the regulation of the autonomic nervous system while the retinal vessels are primarily under the influence of local vasogenic factors. According to a paper by Jodie L. Koep et al. (94), there are significant differences in the regulation of cerebral and peripheral circulation. The authors suggest that peripheral sympathetic nerve activity, which regulates blood flow in the peripheral circulation, cannot be used to extrapolate regulation of the cerebral vasculature. In contrast, cerebral sympathetic nerve activity appears to act in the opposite manner to that of peripheral circulation and is mediated by changes in intracranial pressure and cerebral blood volume. Moreover, the distribution and types of adrenoreceptors in the cerebral vessels vary across different regions, highlighting the region-specific autonomic regulation of cerebral blood flow. This complexity in cerebral blood flow regulation stands in contrast to the relatively straightforward regulation of peripheral blood flow, where sympathetic nerve activity generally leads to arterial and venous vasoconstriction and a reduction in blood flow. In the cerebral circulation, however, sympathetic activity can result in both vasoconstriction and vasodilatation, depending on the density and distribution of receptors within each vessel as well as the presence of other vasoactive compounds and neurotransmitter release. The heterogeneity in the distribution of $Ca^{2+}$ and adrenoreceptors in cerebral vessels further supports the notion that the reactivity of cerebral vessels is highly region-dependent (94). This would again support the idea that the circulation regulation of the cerebral vascular system and in extension the retinal vascular system behaves differently than that of the peripheral circulation, and could potentially explain the paradoxical increase in CRAE. The theory that the CNS and therefore also the retina is protected from drastic changes in the peripheral circulation is also supported by the relatively few and occasional case reports of visual impairment and retinal involvement in shock states like the report of Wei Gui et al. (95) in which a 65 year old Caucasian woman with respiratory distress syndrome and septic shock is found to have bilateral central artery occlusion and bilateral anterior and posterior ischemic

optic neuropathy, which also implies that retinal involvement is not common in shock like states. However, one can also find studies that imply that retinal hypoperfusion might be underappreciated in shock and shock like states, which does not support the theory of preservation of the retinal microcirculation. Jurate Simkiene et al. report of changes in the retinal microcirculation in patients with sepsis or septic shock (n=40) in comparison to healthy individuals (n=20) using a hand-held digital fundus camera the CRAE, CRVE and AVR were determined, and the results showed a significantly higher median CRAE in patients with sepsis or septic shock compared to the healthy controls, while the median CRVE and AVR did not significantly differ between the two groups (94). This paper comes very close to the methods used in our LBNP study and it implies that changes in the CRAE can be seen and do correlate with altered hemodynamic states such as in septic shock, and that they can be monitored with fundoscopic imaging. However, the hemodynamic changes in septic shock while similar to changes in central hypovolemia are not equal. The changes seen in septic shock are vastly more severe than those induced by a LBNP of -40 mmHg. Decreased vascular tone being the primary mechanism in septic shock while decreased intravascular volume accompanied with increased vascular tone being the primary mechanism in hypovolemia. Kristo Erikson et al. performed retinal fluorescein angiography in 31 patients with sepsis with the results being that more than half of the observed patients had pathological retinal changes which were associated with a slowdown of arterial retinal blood flow and included vitreous as well as retinal hemorrhages and fluorescein-leaking micro-aneurysms. In 75% of the affected cases these changes were bilateral (96). This would again imply that changes in the retinal microcirculation can be seen in altered hemodynamic states such as sepsis but may be better visualized with fluorescein angiography rather than measuring the retinal vessel diameters. A point has to again be made that in this study a shock like state was not induced in the participants but rather a state of mild central hypovolemia, but again these studies on septic shock come the closest to the pathophysiology of central hypovolemia.

## 1.1. Differences in males vs females

The sex was not a significant factor between male and female participants during the LBNP phases when looking at the CRAE, CRVE, nor AVR. Therefore, the sex did not influence the retinal vessel diameters in our protocol, which goes against our hypothesis that the sex will influence the vessel diameters in response to LBNP. Female participants had an overall higher CRAE during all of the LBNP phases, although this difference was not significant. The changes in CRAE were identical in males and females during the LBNP phases. One could hypothesize that the increased arteriolar tone (lower CRAE) in males compared to females could play a role in better orthostatic tolerance and higher vasoconstrictor effect in males compared to females that are reported in numerous studies such as the Huxley et. al study which argued that females respond with primarily an increase in HR rather than an increase in SVR as opposed to men who respond with the opposite (57). However, since in our study the difference in CRAE between the sexes is not significant this particular study cannot confirm this effect. Female participants had an overall higher CRVE during all LBNP phases except the LBNP10 phase were the male CRVE was higher than the female CRVE, although these changes were not significant. One could again argue that with more dilated venules the venous return is decreased leading to a lower PL and a decreased orthostatic tolerance, which is more common in females, but since this study did not show a significant difference in the CRVE one cannot argue for this hypothesis at least not at an LBNP of -40 mmHg. Overall, the AVR ratio was higher in the female participants probably due to the higher overall CRAE in females compared to males however these differences were not significant. Although no studies linking retinal AVR to changes in orthostatic tolerance could be found, generally a lower AVR correlates to higher blood pressures, various cardiovascular risk factors (such as blood pressure, BMI, the sex and age) being usually lower in males, and in the older population (96–98). The question that rationally arises, is why didn't our protocol show significant changes in the retinal vessel diameters between the sexes when many studies report a higher vasoconstrictor effect in males compared to females? The consensus on the underlying mechanisms by which orthostasis is more prevalent in females when compared to males is not uniform. It seems that for every published paper on sex differences one can find similar papers with contradictory results, many examples of which were already listed in the chapter 1.6. Similar arguments could

be used to explain why there was no significant difference between male and female participants in the study. It is likely that due to our limitations the protocol was not optimised enough to induce a significant fluid shift in our participants which could have yielded significant differences between the sexes.

## 1.2. Limitations

This study had several limitations, which of course need to be acknowledged. 42 participants (20 male and 22 female) were enrolled to the study, of which six participants did not manage to tolerate the whole LBNP protocol and were therefore excluded from further analysis. Retina pictures were taken in 37 participants during LBNP, though due to the difficult nature of getting retinal images good enough for analyzation through the MONA REVA software, complete data sets (baseline, LBNP40 and recovery) were available only for a total of n = 27 participants. Data gathering has therefore proved to be not without its issues and has caused some data to be unusable for evaluation. The fundus photography as well as the MONA REVA software analyzation has a subjective nature to it. Differentiating between arteries and veins relies primarily on the color and diameter of the blood vessels, which may be an unreliable way to assess retinal microcirculation as mentioned in a review by Maliheh Miri et al. (86). The MONA REVA software selects arteries and veins based on the program's algorithm which in it of itself is not perfect since in most of the cases the software needs manual readjustment of the selected retinal vessels from which CRAE and CRVE are measured and AVR is calculated. This is of course another source of potential error and could also have affected our results leading to significant vessel diameter changes. Assessing the retinal microcirculation has proven to be difficult not only via fundus imaging but also via laser doppler velocimetry, which proved unpractical in clinical application since it requires good fixation by the participant for up to 45 minutes, eye movements also alters the doppler angle causing artifacts (87). Optical coherence tomography angiography (OCTA) and fundus fluorescein angiography (FFA) have also shown drawbacks: OCTA also suffering from eye movement and blinking artifacts (100) while FFA requires intra venous fluorescein application, which is known to cause allergic reactions and bad results if the fluorescein leaks out of the retinal vessels (89). The difficulty in assessing the retinal microcirculation with even more invasive and precise methods than fundus imaging represents a problem in the validity of the results found in retinal microcirculation studies. Confounders such as the menstrual

cycle and oral hormonal contraception could play a significant role when looking for differences between the sexes, even though the study assessed for menstrual cycle stage (late vs early) and contraceptive use, the number of females participants would be too small to asses for changes between the groups of early vs late menstrual cycle or contraception use vs no contraception use. Lastly a LBNP rate of maximally -40 mmHg corresponds to the effect of gravity while standing up (38), many LBNP studies are performed with the maximum rate of -50 mmHg (105,106), some going for even more negative values (107), one could argue that with these more exaggerated negative pressures a change in the microvascular diameter might have been more drastic and could have led to a significant changes which might have yielded more similar results as previous studies done on hemorrhagic shock models and septic shock patients.

## 1.3. Conclusions

This study investigated the retinal microvascular response to central hypovolemia induced by LBNP as a well-established method to simulate central hypovolemia in a participant group of 42 healthy young adults (18–45-year-old), however due to limitations, values of 27 participants were analyzed. The findings of this study are novel and therefore valuable. They indicate that there is no significant difference in the retinal arteriolar and venular diameters (expressed as central retinal artery equivalent and central retinal vein equivalent) nor their ratio (expressed as arteriolar-venular ratio) during a six-phase LBNP protocol with a maximum negative pressure of -40mmHg. There is also no significant difference between the male and female participants microvascular diameters during the LBNP protocol. These results suggest that retinal microvascular response may not be influenced by orthostatic stress and that the sex plays no role in the retinal microvascular response during orthostatic stress (of -40mmHg) or that they are difficult to detect with the methods used in this study. Furthermore, blood perfusion heterogenicity between different organs as well as the complex nature of microvascular regulation in the central nervous system and the retina compared to the peripheral circulation as well as the relatively low negative pressure used in the LBNP protocol could be the explanation of these non-significant results. Further studies should be conducted to fully understand the microvascular response to orthostasis and central hypovolemia as well as the role that the sex plays. This could be achieved by investigating individuals with known orthostatic intolerance, and/or conducting studies with a larger participant number including participants of different age groups, and health status, with the use of greater negative pressures than used in this particular study

The potential clinical applications of retinal microcirculation assessment, such as using it to screen for orthostatic intolerance or as a continuous monitoring method for critically ill patients with altered hemodynamic states such as hemorrhagic or septic shock, make further research in this area warranted.

## 1.4. Perspectives

The possibility of exploring changes in the retinal microvasculature during orthostasis and hypovolemia, as an easily accessible and assessable area, calls for future research in this field. Further research could help uncover potential changes in the retinal microvasculature, which were not detected by the protocol used in this study, and deepen the understanding of the pathophysiology of orthostatic intolerance, the sex-dependent prevalence of orthostatic intolerance, and microvascular dysfunction.

This newfound knowledge could have significant clinical applications, such as using retinal microcirculation to screen for orthostatic intolerance, or as a continuous monitoring method for critically ill patients with altered hemodynamic states such as septic or hemorrhagic shock. The retinal microvasculature offers an opportunity to gain insights into our body's inner workings and could lead to improved diagnostic and therapeutic strategies.

# 2. References


1. Molina DK, DiMaio VJM. Normal Organ Weights in Men: Part I—The Heart. Am J Forensic Med Pathol. 2012 Dec;33(4):362–7.
2. Hall JE. Guyton and Hall textbook of medical physiology. 13th edition. Philadelphia, PA: Elsevier; 2016. 1145 p.
3. Eric P. Widmaier, Hershel Raff, Kevin T. Strang. Vander et al's Human Physiology: The Mechanisms of Body Function. 9th edition. The McGraw-Hill Companies; 2003.
4. Atrium (heart). In: Wikipedia [Internet]. 2023 [cited 2023 Mar 26]. Available from: https://en.wikipedia.org/w/index.php?title=Atrium_(heart)&oldid=1144631357
5. Tucker WD, Arora Y, Mahajan K. Anatomy, Blood Vessels. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Aug 6]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK470401/
6. DeMers D, Wachs D. Physiology, Mean Arterial Pressure. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Aug 8]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK538226/
7. King J, Lowery DR. Physiology, Cardiac Output. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Aug 8]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK470455/
8. Yeong Brigitte WF, Childs C. A systematic review on the role of extremity skin temperature as a non-invasive marker for hypoperfusion in critically ill adults in the intensive care setting: JBI Libr Syst Rev. 2012;10(26):1504–48.
9. Magder S. The meaning of blood pressure. Crit Care. 2018 Oct 11;22(1):257.
10. Di Rienzo M, Parati G, Radaelli A, Castiglioni P. Baroreflex contribution to blood pressure and heart rate oscillations: time scales, time-variant characteristics and nonlinearities. Philos Transact A Math Phys Eng Sci. 2009 Apr 13;367(1892):1301–18.
11. Figure: Regulating Blood Pressure: The Renin-Angiotensin-Aldosterone System [Internet]. MSD Manual Consumer Version. [cited 2023 Mar 26]. Available from: https://www.msdmanuals.com/home/multimedia/figure/regulating-blood-pressure-the-renin-angiotensin-aldosterone-system
12. Ames MK, Atkins CE, Pitt B. The renin-angiotensin-aldosterone system and its suppression. J Vet Intern Med. 2019;33(2):363–82.
13. Ayling J. Managing head injuries. Emerg Med Serv. 2002 Aug;31(8):42.
14. Fodstad H, Kelly PJ, Buchfelder M. HISTORY OF THE CUSHING REFLEX. Neurosurgery. 2006 Nov;59(5):1132–7.
15. Chopra S, Baby C, Jacob JJ. Neuro-endocrine regulation of blood pressure. Indian J Endocrinol Metab. 2011 Oct;15(Suppl4):S281–8.
16. Campos Munoz A, Vohra S, Gupta M. Orthostasis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Aug 13]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK532938/
17. Skeletal muscle pump. In: Wikipedia [Internet]. 2023 [cited 2023 Mar 26]. Available from: https://en.wikipedia.org/w/index.php?title=Skeletal_muscle_pump&oldid=1144889302
18. Stewart JM. Common Syndromes of Orthostatic Intolerance. Pediatrics. 2013 May;131(5):968–80.
19. Lewis T. A Lecture On Vasovagal Syncope And The Carotid Sinus Mechanism: With Comments On Gowers's And Nothnagel's Syndrome. Br Med J. 1932;1(3723):873–6.
20. Shen WK, Sheldon RS, Benditt DG, Cohen MI, Forman DE, Goldberger ZD, et al. 2017 ACC/AHA/HRS guideline for the evaluation and management of patients with syncope. Heart Rhythm. 2017 Aug;14(8):e155–217.
21. Steven McGee. Evidence-Based Physical Diagnosis. 4th Edition. Elsevier; 2016.
22. Miachel S. Wagner, Peter D. Sullivan, Salvatore Mangione. Physical diagnosis secrets. third edition. Elsevier; 2020.
23. Paszkowiak JJ, Dardik A. Arterial wall shear stress: observations from the bench to

the bedside. Vasc Endovascular Surg. 2003 Feb;37(1):47–57.
24. Tremblay JC, Pyke KE. Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans? Am J Physiol-Heart Circ Physiol. 2018 Mar;314(3):H508–20.
25. Kelm M. Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects. Am J Physiol-Heart Circ Physiol. 2002 Jan;282(1):H1–5.
26. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. Int J Cardiol. 2013 Sep 20;168(1):344–51.
27. Medow MS, Minson CT, Stewart JM. Decreased microvascular nitric oxide-dependent vasodilation in postural tachycardia syndrome. Circulation. 2005 Oct 25;112(17):2611–8.
28. Chopoorian AH, Wahba A, Celedonio J, Nwazue V, Smith EC, Garland EM, et al. Impaired Endothelial Function in Patients With Postural Tachycardia Syndrome. Hypertension. 2021 Mar 3;77(3):1001–9.
29. Gamboa A. Assessment of Vascular Endothelial Function in Postural Tachycardia Syndrome (POTS) [Internet]. clinicaltrials.gov; 2017 Jan [cited 2022 Oct 6]. Report No.: NCT01308099. Available from: https://clinicaltrials.gov/ct2/show/NCT01308099
30. Pradeep T, Mehra D, Le PH. Histology, Eye. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Oct 9]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK544343/
31. Kiel JW. Anatomy [Internet]. The Ocular Circulation. Morgan & Claypool Life Sciences; 2010 [cited 2022 Oct 9]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53329/
32. Campbell JP, Zhang M, Hwang TS, Bailey ST, Wilson DJ, Jia Y, et al. Detailed Vascular Anatomy of the Human Retina by Projection-Resolved Optical Coherence Tomography Angiography. Sci Rep. 2017 Feb 10;7:42201.
33. Fundus [Internet]. American Academy of Ophthalmology. 2020 [cited 2022 Oct 9]. Available from: https://www.aao.org/eye-health/anatomy/fundus
34. Cristescu IE, Zagrean L, Balta F, Branisteanu DC. RETINAL MICROCIRCULATION INVESTIGATION IN TYPE I AND II DIABETIC PATIENTS WITHOUT RETINOPATHY USING AN ADAPTIVE OPTICS RETINAL CAMERA. Acta Endocrinol Buchar. 2019;15(4):417–22.
35. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res. 2003 Jan;27(3):143–9.
36. Gotshall RW. Gender differences in tolerance to lower body negative pressure. Aviat Space Environ Med. 2000 Nov;71(11):1104–10.
37. Clément G, Pavy-Le Traon A. Centrifugation as a countermeasure during actual and simulated microgravity: a review. Eur J Appl Physiol. 2004 Jul;92(3):235–48.
38. Goswami N, Blaber AP, Hinghofer-Szalkay H, Convertino VA. Lower Body Negative Pressure: Physiological Effects, Applications, and Implementation. Physiol Rev. 2019 Jan 1;99(1):807–51.
39. Convertino VA, Sather TM. Effects of cholinergic and beta-adrenergic blockade on orthostatic tolerance in healthy subjects. Clin Auton Res Off J Clin Auton Res Soc. 2000 Dec;10(6):327–36.
40. Convertino VA. Neurohumoral mechanisms associated with orthostasis: reaffirmation of the significant contribution of the heart rate response. Front Physiol [Internet]. 2014 [cited 2022 Aug 14];5. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2014.00236
41. Reflex-Mediated Reduction in Human Cerebral Blood Volume - Timothy D Wilson, J Kevin Shoemaker, R Kozak, T-Y Lee, Adrian W Gelb, 2005 [Internet]. [cited 2022 Aug 15]. Available from: https://journals.sagepub.com/doi/10.1038/sj.jcbfm.9600015
42. Impact of hypocapnia and cerebral perfusion on orthostatic tolerance - Lewis - 2014 - The Journal of Physiology - Wiley Online Library [Internet]. [cited 2022 Aug 15]. Available from:

https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2014.280586
43. Gotshall RW, Davrath LR. Pulmonary capillary blood volume during lower body negative pressure: effect of gender. Aviat Space Environ Med. 1998 Mar;69(3):277–81.
44. Coast JR, O'kroy JA, Akers FMI, Dahl T. Effects of lower body pressure changes on pulmonary function. Med Sci Sports Exerc. 1998 Jul;30(7):1035–40.
45. Zechman FW, Musgrave FS, Mains RC, Cohn JE. Respiratory mechanics and pulmonary diffusing capacity with lower body negative pressure. J Appl Physiol. 1967 Feb;22(2):247–50.
46. Carter III R, Hinojosa-Laborde C, Convertino VA. Sex comparisons in muscle sympathetic nerve activity and arterial pressure oscillations during progressive central hypovolemia. Physiol Rep. 2015;3(6):e12420.
47. Goswami N, Evans J, Schneider S, Wiesche M von der, Mulder E, Rössler A, et al. Effects of Individualized Centrifugation Training on Orthostatic Tolerance in Men and Women. PLOS ONE. 2015 May 28;10(5):e0125780.
48. White DD, Gotshall RW, Tucker A. Women have lower tolerance to lower body negative pressure than men. J Appl Physiol. 1996 Apr;80(4):1138–43.
49. White DD, Montgomery LD. Pelvic blood pooling of men and women during lower body negative pressure. Aviat Space Environ Med. 1996 Jun;67(6):555–9.
50. Convertino VA. Gender differences in autonomic functions associated with blood pressure regulation. Am J Physiol. 1998 Dec;275(6):R1909-1920.
51. Chung AK, Das SR, Leonard D, Peshock RM, Kazi F, Abdullah SM, et al. Women have higher left ventricular ejection fractions than men independent of differences in left ventricular volume: the Dallas Heart Study. Circulation. 2006 Mar 28;113(12):1597–604.
52. Salton CJ, Chuang ML, O'Donnell CJ, Kupka MJ, Larson MG, Kissinger KV, et al. Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study Offspring cohort. J Am Coll Cardiol. 2002 Mar 20;39(6):1055–60.
53. de Simone G, Devereux RB, Roman MJ, Ganau A, Chien S, Alderman MH, et al. Gender differences in left ventricular anatomy, blood viscosity and volume regulatory hormones in normal adults. Am J Cardiol. 1991 Dec 15;68(17):1704–8.
54. Fu Q, Arbab-Zadeh A, Perhonen MA, Zhang R, Zuckerman JH, Levine BD. Hemodynamics of orthostatic intolerance: implications for gender differences. Am J Physiol Heart Circ Physiol. 2004 Jan;286(1):H449-457.
55. Fu Q, Witkowski S, Okazaki K, Levine BD. Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. Am J Physiol Regul Integr Comp Physiol. 2005 Jul;289(1):R109-116.
56. Halliwill JR, Lawler LA, Eickhoff TJ, Joyner MJ, Mulvagh SL. Reflex responses to regional venous pooling during lower body negative pressure in humans. J Appl Physiol Bethesda Md 1985. 1998 Feb;84(2):454–8.
57. Huxley VH. Sex and the cardiovascular system: the intriguing tale of how women and men regulate cardiovascular function differently. Adv Physiol Educ. 2007 Mar;31(1):17–22.
58. Hachiya T, Hashimoto I, Saito M, Blaber AP. Peripheral Vascular Responses of Men and Women to LBNP. Aviat Space Environ Med. 2012 Feb 1;83(2):118–24.
59. Robert M. Anderson, M.D. The Gross Physiology of the Cardiovascular System. Second Edition. 2012.
60. Olatunji LA, Aaron AO, Michael OS, Oyeyipo IP. Water ingestion affects orthostatic challenge-induced blood pressure and heart rate responses in young healthy subjects: gender implications. Niger J Physiol Sci [Internet]. 2011 [cited 2022 Oct 8];26(1). Available from: https://www.ajol.info/index.php/njps/article/view/84885
61. Wenner MM, Haddadin AS, Taylor HS, Stachenfeld NS. Mechanisms contributing to low orthostatic tolerance in women: the influence of oestradiol. J Physiol. 2013 May 1;591(Pt 9):2345–55.

62. Harm DL, Jennings RT, Meck JV, Powell MR, Putcha L, Sams CP, et al. Invited review: gender issues related to spaceflight: a NASA perspective. J Appl Physiol Bethesda Md 1985. 2001 Nov;91(5):2374–83.
63. Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J, Joyner MJ. Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors. J Physiol. 2011 Nov 1;589(Pt 21):5285–97.
64. Arzeno NM, Stenger MB, Lee SMC, Ploutz-Snyder R, Platts SH. Sex differences in blood pressure control during 6° head-down tilt bed rest. Am J Physiol Heart Circ Physiol. 2013 Apr 15;304(8):H1114-1123.
65. Fritsch-Yelle JM, Whitson PA, Bondar RL, Brown TE. Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight. J Appl Physiol Bethesda Md 1985. 1996 Nov;81(5):2134–41.
66. Howden R, Lightfoot JT, Brown SJ, Swaine IL. The effects of breathing 5% CO2 on human cardiovascular responses and tolerance to orthostatic stress. Exp Physiol. 2004;89(4):465–71.
67. Sudhir K, Elser MD, Jennings GL, Komesaroff PA. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. Hypertens Dallas Tex 1979. 1997 Dec;30(6):1538–43.
68. Choi HM, Stebbins CL, Nho H, Kim MS, Chang MJ, Kim JK. Effects of Ovarian Cycle on Hemodynamic Responses during Dynamic Exercise in Sedentary Women. Korean J Physiol Pharmacol Off J Korean Physiol Soc Korean Soc Pharmacol. 2013 Dec;17(6):499–503.
69. Adkisson EJ, Casey DP, Beck DT, Gurovich AN, Martin JS, Braith RW. Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. Exp Biol Med Maywood NJ. 2010 Jan;235(1):111–8.
70. Kneale BJ, Chowienczyk PJ, Brett SE, Coltart DJ, Ritter JM. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. J Am Coll Cardiol. 2000 Oct;36(4):1233–8.
71. Marinoni E, Di Iorio R, Letizia C, Lucchini C, Alò P, Cosmi EV. Changes in plasma adrenomedullin levels during the menstrual cycle. Regul Pept. 2000 Feb 8;87(1):15–8.
72. Hernandez JP, Franke WD. Age- and fitness-related differences in limb venous compliance do not affect tolerance to maximal lower body negative pressure in men and women. J Appl Physiol. 2004 Sep;97(3):925–9.
73. Kelly D, Scroop G, Tonkin A, Thornton A. Cardiovascular responses to orthostatic and other stressors in men and women are independent of sex. Clin Exp Pharmacol Physiol. 2004;31(1–2):50–6.
74. Phase of the menstrual cycle does not affect orthostatic tolerance in healthy women | SpringerLink [Internet]. [cited 2022 Oct 8]. Available from: https://link.springer.com/article/10.1007/s10286-006-0330-y
75. Goswami N, Gorur P, Pilsl U, Anyaehie B, Green DA, Bondarenko AI, et al. Effect of Orthostasis on Endothelial Function: A Gender Comparative Study. PLOS ONE. 2013 Aug 2;8(8):e71655.
76. Cheng YC, Vyas A, Hymen E, Perlmuter LC. Gender differences in orthostatic hypotension. Am J Med Sci. 2011 Sep;342(3):221–5.
77. Goswami N, Loeppky JA, Hinghofer-Szalkay H. LBNP: Past Protocols and Technical Considerations for Experimental Design. Aviat Space Environ Med. 2008 May 1;79(5):459–71.
78. Miyagatani Y, Yukioka T, Ohta S, Ohta S, Matsuda H, Shimazu H, et al. Vascular tone in patients with hemorrhagic shock. J Trauma. 1999 Aug;47(2):282–7.
79. Johnson BD, Schlader ZJ, Schaake MW, O'Leary MC, Hostler D, Lin H, et al. Inferior Vena Cava Diameter is an Early Marker of Central Hypovolemia during Simulated Blood Loss. Prehospital Emerg Care Off J Natl Assoc EMS Physicians Natl Assoc State EMS Dir. 2021;25(3):341–6.

80. Ricci F, De Caterina R, Fedorowski A. Orthostatic Hypotension: Epidemiology, Prognosis, and Treatment. J Am Coll Cardiol. 2015 Aug 18;66(7):848–60.
81. Drobnjak D, Munch IC, Glümer C, Faerch K, Kessel L, Larsen M, et al. Retinal Vessel Diameters and Their Relationship with Cardiovascular Risk and All-Cause Mortality in the Inter99 Eye Study: A 15-Year Follow-Up. J Ophthalmol. 2016;2016:6138659.
82. Conzen C, Albanna W, Weiss M, Kuerten D, Vilser W, Kotliar K, et al. Vasoconstriction and Impairment of Neurovascular Coupling after Subarachnoid Hemorrhage: a Descriptive Analysis of Retinal Changes. Transl Stroke Res. 2018 Jun 1;9.
83. Zhou M, Imai K, Hamaguchi M, Okuma A, Mori K, Yata K, et al. The relationship between central retinal artery equivalent and cerebral artery diameters. Invest Ophthalmol Vis Sci. 2017 Jun 23;58(8):4821.
84. Hernandez-Andrade E, Figueroa-Diesel H, Jansson T, Rangel-Nava H, Gratacos E. Changes in regional fetal cerebral blood flow perfusion in relation to hemodynamic deterioration in severely growth-restricted fetuses. Ultrasound Obstet Gynecol. 2008;32(1):71–6.
85. Boerma EC, van der Voort PHJ, Spronk PE, Ince C. Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis*: Crit Care Med. 2007 Apr;35(4):1055–60.
86. Park JR, Kim Y, Park T, Oh WY, Yune H, Lee JH, et al. 1423: MICROCIRCULATORY ALTERATIONS IN HEMORRHAGIC SHOCK AND SEPSIS WITH OPTICAL COHERENCE TOMOGRAPHY. Crit Care Med. 2016 Dec;44(12):431.
87. Zadeh JK, Ruemmler R, Hartmann EK, Ziebart A, Ludwig M, Patzak A, et al. Responses of retinal arterioles and ciliary arteries in pigs with acute respiratory distress syndrome (ARDS). Exp Eye Res. 2019 Jul;184:152–61.
88. Aneman A, Pettersson A, Eisenhofer G, Friberg P, Holm M, von Bothmer C, et al. Sympathetic and renin-angiotensin activation during graded hypovolemia in pigs: impact on mesenteric perfusion and duodenal mucosal function. Shock Augusta Ga. 1997 Nov;8(5):378–84.
89. Toung T, Reilly PM, Fuh KC, Ferris R, Bulkley GB. Mesenteric vasoconstriction in response to hemorrhagic shock. Shock Augusta Ga. 2000;13(4):267–73.
90. Bill A, Sperber GO. Control of retinal and choroidal blood flow. Eye. 1990 Mar;4(2):319–25.
91. Luo X, Shen YM, Jiang MN, Lou XF, Shen Y. Ocular Blood Flow Autoregulation Mechanisms and Methods. J Ophthalmol. 2015;2015:864871.
92. Koep JL, Taylor CE, Coombes JS, Bond B, Ainslie PN, Bailey TG. Autonomic control of cerebral blood flow: fundamental comparisons between peripheral and cerebrovascular circulations in humans. J Physiol. 2022 Jan;600(1):15–39.
93. Gui W, Friesen ET, Bonelli L, Wang YE, Arnold AC. Severe bilateral optic nerve and retinal hypoperfusion in a patient with acute respiratory distress syndrome and septic shock. Am J Ophthalmol Case Rep. 2017 Mar 24;6:84–7.
94. Simkiene J, Pranskuniene Z, Patasius M, Trumpaitis J, Boerma EC, Pranskunas A. Alterations of retinal vessels in patients with sepsis. J Clin Monit Comput. 2020 Oct;34(5):937–42.
95. Erikson K, Liisanantti JH, Hautala N, Koskenkari J, Kamakura R, Herzig KH, et al. Retinal arterial blood flow and retinal changes in patients with sepsis: preliminary study using fluorescein angiography. Crit Care Lond Engl. 2017 Apr 10;21(1):86.
96. Ikram MK, de Jong FJ, Vingerling JR, Witteman JCM, Hofman A, Breteler MMB, et al. Are Retinal Arteriolar or Venular Diameters Associated with Markers for Cardiovascular Disorders? The Rotterdam Study. Invest Ophthalmol Vis Sci. 2004 Jul 1;45(7):2129–34.
97. Willikens S, Zitron E, Scholz E, Scherer D, Seyler C, Waegelein M, et al. Retinal Arterio-Venule-Ratio (AVR) in the cardiovascular risk management of hypertension. Eur Heart J. 2013 Aug 1;34(suppl_1):P5002.
98. Wong TY, Klein R, Klein B, Meuer S, Hubbard L. Retinal Vessel Diameters and Their

Associations with Age and Blood Pressure. Invest Ophthalmol Vis Sci. 2003 Nov 1;44:4644–50.
99. Riva CE, Grunwald JE, Sinclair SH, Petrig BL. Blood velocity and volumetric flow rate in human retinal vessels. Invest Ophthalmol Vis Sci. 1985 Aug;26(8):1124–32.
100. Tan ACS, Tan GS, Denniston AK, Keane PA, Ang M, Milea D, et al. An overview of the clinical applications of optical coherence tomography angiography. Eye. 2018 Feb;32(2):262–86.
101. Spaide RF, Klancnik JM, Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. JAMA Ophthalmol. 2015 Jan;133(1):45–50.
102. van Hoeyweghen R, Hanson J, Stewart MJ, Dethune L, Davies I, Little RA, et al. Cardiovascular response to graded lower body negative pressure in young and elderly man. Exp Physiol. 2001 May;86(3):427–35.
103. Butler GC, Yamamoto Y, Xing HC, Northey DR, Hughson RL. Probing heart rate and blood pressure control mechanisms during graded levels of lower body negative pressure (LBNP). Microgravity Q MGQ. 1992 Apr;2(2):133–40.
104. Watenpaugh DE, Ballard RE, Stout MS, Murthy G, Whalen RT, Hargens AR. Dynamic leg exercise improves tolerance to lower body negative pressure. Aviat Space Environ Med. 1994 May;65(5):412–8.

# 3. Supplement

## 3.1. Tables

**Table 1: LBNP protocol**

| Phase | LBNP in mmHg | Description |
|---|---|---|
| **Baseline measurements** | 0 | 30 minutes of no LBNP while supine |
| **LBNP10** | -10 | 5 minutes of -10 mmHg LBNP while supine |
| **LBNP20** | -20 | 5 minutes of -20 mmHg LBNP while supine |
| **LBNP30** | -30 | 5 minutes of -30 mmHg LBNP while supine |
| **LBNP40** | -40 | 5 minutes of -40 mmHg LBNP while supine |
| **Recovery** | 0 | 10 minutes of no LBNP while supine |

**Table 2: LBNP protocol timeline**

| Baseline measurements | LBNP10 | LBNP20 | LBNP30 | LBNP40 | Recovery |
|---|---|---|---|---|---|
| 30 minutes | 5 minutes | 5 minutes | 5 minutes | 5 minutes | 30 minutes |

**Table 3: Participant characteristics**

| | Participants |
|---|---|
| **n** | 42 |
| **Male** | 20 |
| **Female** | 22 |
| **Age [y]** | 24,9 ± 5,9 |
| **Weight [kg]** | 66,9 ± 11,7 |
| **Height [cm]** | 174,5 ± 8,9 |
| **BMI [kg/m$^2$]** | 21,8 ± 2,5 |

*Values are expressed as mean ± standard deviation*

**Table 4: Female Participant characteristics**

| | Female Participants |
|---|---|
| **n** | 22 |
| **Age [y]** | 22,0 ± 2,5 |
| **Weight [kg]** | 58,3 ± 6,8 |
| **Height [cm]** | 168,3 ± 5,7 |
| **BMI [kg/m$^2$]** | 20,6 ± 2,0 |

*Values are expressed as mean ± standard deviation*

**Table 5: Male Participant characteristics**

| | Male Participants |
|---|---|
| **n** | 20 |
| **Age [y]** | 27,6 ± 6,8 |
| **Weight [kg]** | 75,1 ± 9,1 |
| **Height [cm]** | 180,5 ± 7,2 |
| **BMI [kg/m$^2$]** | 23,0 ± 2,3 |

*Values are expressed as mean ± standard deviation*

**Table 6: CRAE during LBNP**

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 146,90 | 146,96 | 144,91 | 146,65 | 149,74 | 147,41 |
| | 3,09 | 2,56 | 3,05 | 3,05 | 3,15 | 3,31 |
| Male | 140,81 | 140,53 | 138,48 | 140,68 | 142,18 | 141,34 |
| | 3,72 | 3,09 | 3,68 | 3,67 | 3,80 | 4,00 |

*Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysis*

**Table 7: CRVE during LBNP**

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 210,13 | 207,35 | 206,84 | 209,29 | 210,45 | 211,40 |
| | 5,52 | 4,30 | 4,50 | 4,31 | 4,81 | 4,97 |
| Male | 203,97 | 208,58 | 204,90 | 206,65 | 205,44 | 207,77 |
| | 6,65 | 5,19 | 5,43 | 5,20 | 5,80 | 6,00 |

*Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysis.*

| LBNP | **baseline** | LBNP-10 | LBNP-20 | LBNP-30 | **LBNP**-40 | **recovery** |
|---|---|---|---|---|---|---|
| Female | 0,70 | 0,71 | 0,71 | 0,70 | 0,71 | 0,70 |
| | 0,02 | 0,02 | 0,02 | 0,02 | 0,01 | 0,02 |
| Male | 0,69 | 0,68 | 0,68 | 0,68 | 0,70 | 0,68 |
| | 0,02 | 0,02 | 0,02 | 0,02 | 0,02 | 0,02 |

**Table 8: Artery to Vein ratio during LBNP**
Note: mean ± SE; mind that only baseline, LBNP-40 and recovery were included in the statistical analysis.

## 7.2 Figures

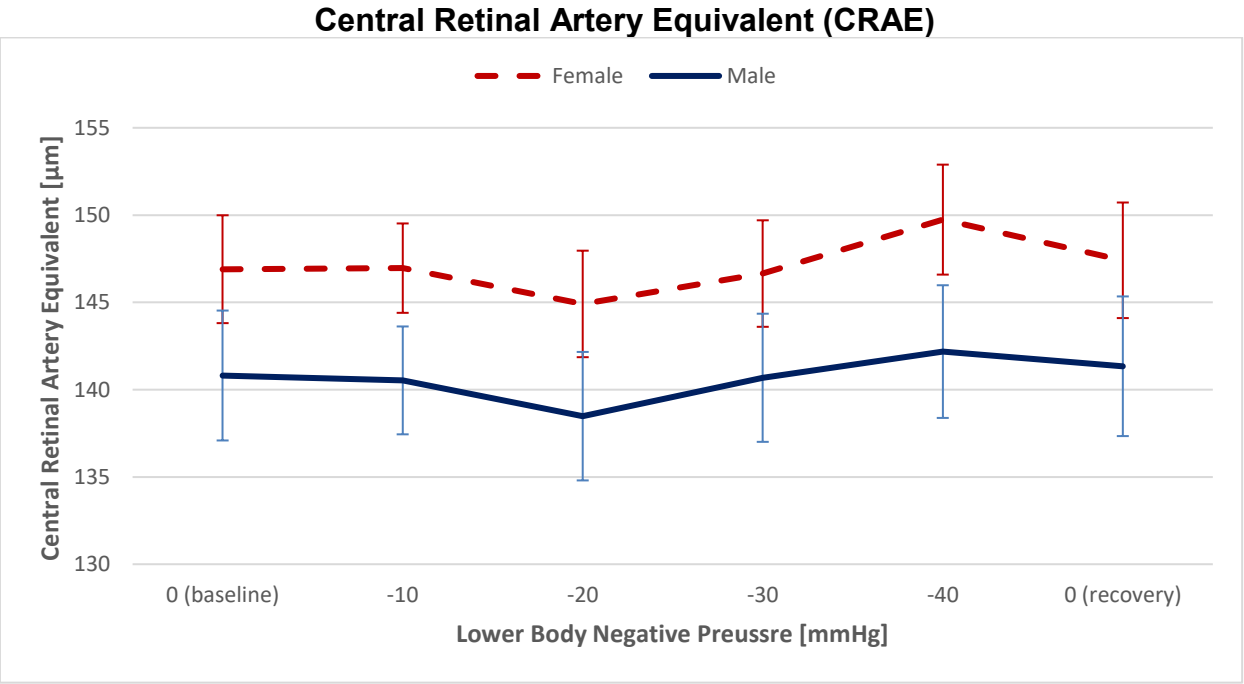


**Figure 8: CRAE during LBNP**
*Note: the LBNP axis indicates the different phases as per protocol*

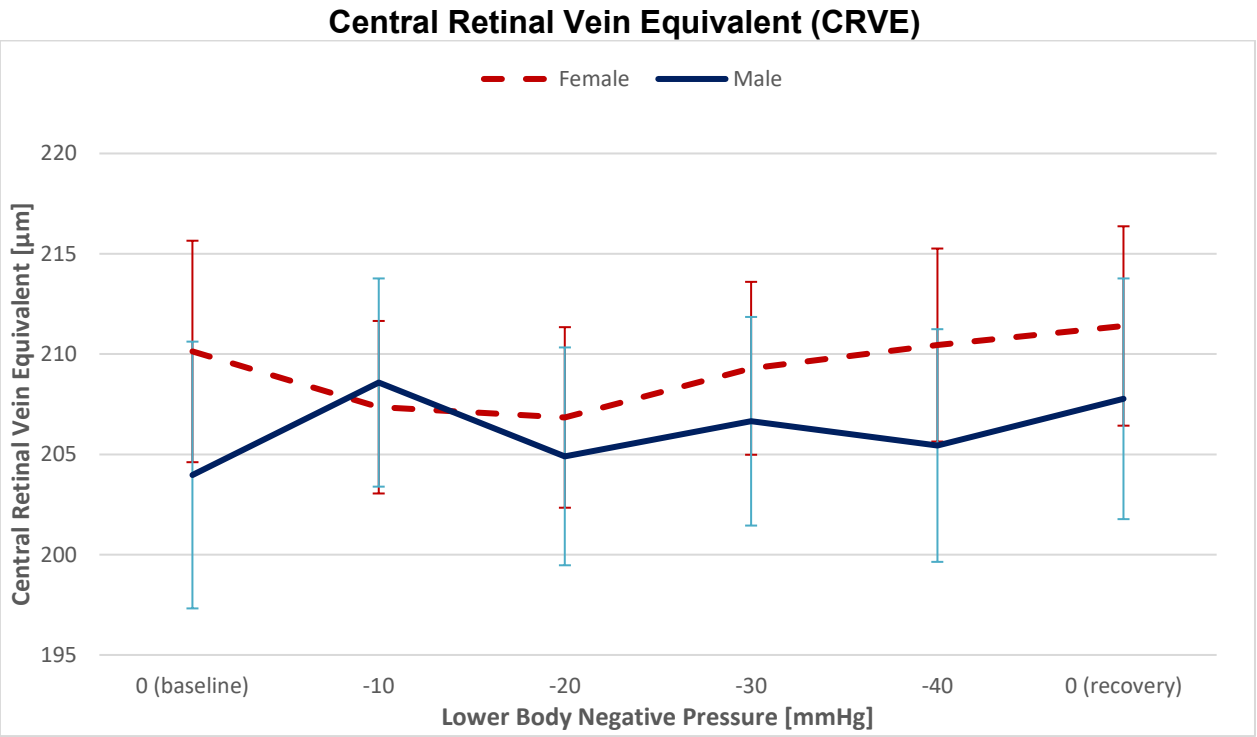


**Figure 9: CRVE during LBNP**
*Note: the LBNP axis indicates the different phases as per protocol*

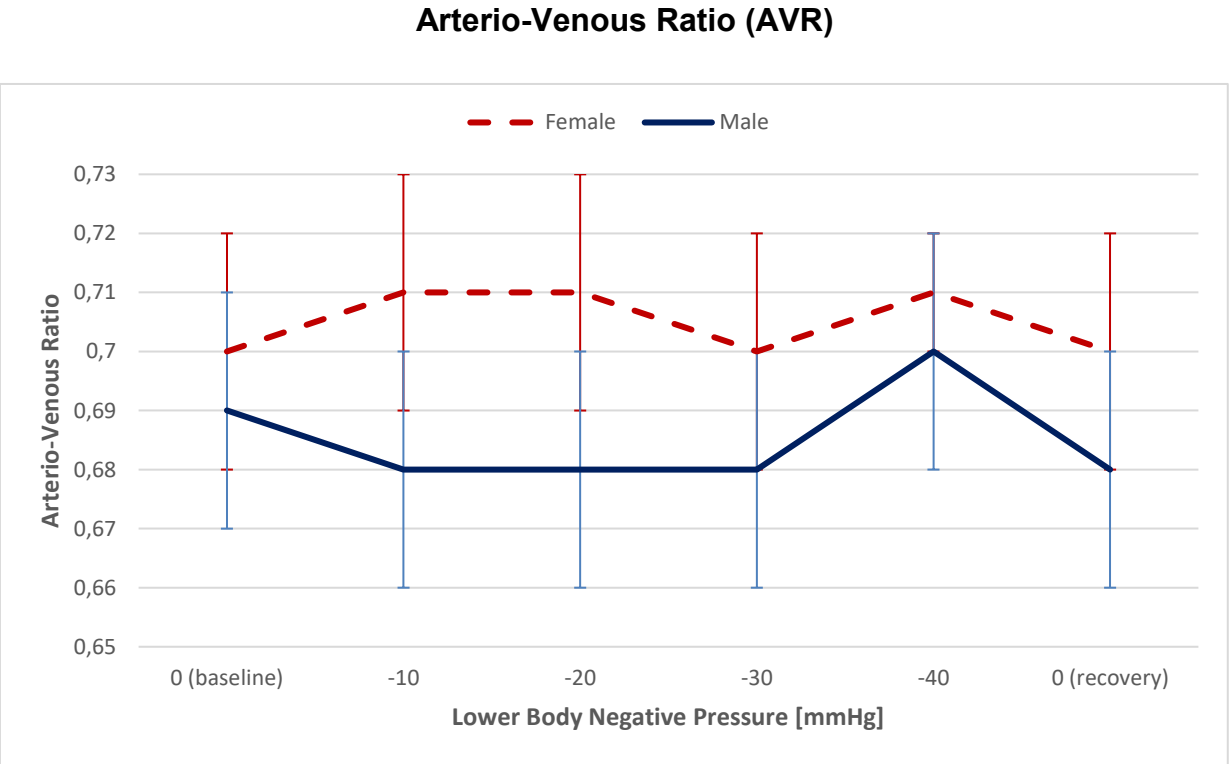


**Figure 10: Artery to Vein ratio during LBNP**
*Note: the LBNP axis indicates the different phases as per protocol*

Medizinische Universität Graz
Ethikkommission

Auenbruggerplatz 2, A-8036 Graz
ethikkommission@medunigraz.at
Tel.: +43 / 316 / 385-13928, Fax: -14348

# FOLGEVOTUM

**gültig bis 30.09.2023**

| | |
|---|---|
| **EK-Nummer:** | 25-551 ex 12/13 |
| **Studientitel:** | Vascular stimulus response in health and disease: a longitudinal study |
| **Prüfer:** | Ass.-Prof. Priv.-Doz. DDr. Thomas Gattringer<br>Universitätsklinik für Neurologie Graz, Abteilung für Allgemeine Neurologie |
| **Sponsor:** | - |
| **CRO:** | - |
| **Antragsteller:** | Inst. für Physiologie |
| Ansprechpartner: | Dr. Nandu Goswami, 8010 Graz, Harrachgasse 21/5 |

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 22.08.2013 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

**Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.**

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben:
Doz.Dr.Christian Fazekas

**Zur Beurteilung vorliegende Dokumente:**

| | | |
|---|---|---|
| **Dokumente eingegangen am 31.07.2013, begutachtet im 'expedited Review' am 22.08.2013** | | |
| | Antragsformular | 29.07.2013 |
| | Informed Consent Form 01 | 29.07.2013 |
| | Case Report Form 01/2013 | |
| | Werbematerial Aushang undatiert | |
| **Dokumente eingegangen am 04.09.2013** | | |
| ✓ | Cover Letter | 04.09.2013 |
| | Antragsformular | 02.09.2013 |
| ✓ | Originalprotokoll 02 | 02.09.2013 |
| | Informed Consent Form 02 | 02.09.2013 |
| ✓ | Case Report Form 01/2013 | |
| ✓ | Werbematerial Aushang 01 | 02.09.2013 |
| **Dokumente eingegangen am 16.09.2013** | | |
| ✓ | Cover Letter | 11.09.2013 |
| ✓ | Antragsformular undatiert | |
| **Dokumente eingegangen am 23.09.2013, begutachtet im 'expedited Review' am 30.09.2013** | | |
| ✓ | Informed Consent Form 03 | 23.09.2013 |
| **Dokumente eingegangen am 04.03.2014, begutachtet im 'expedited Review' am 14.03.2014** | | |
| ✓ | Originalprotokoll 03 | 14.02.2014 |
| ✓ | Informed Consent Form 04 | 14.02.2014 |
| ✓ | Sonstiges: EK-Meldeformular | 28.02.2014 |

| | |
|---|---|
| **Dokumente eingegangen am 02.09.2014, begutachtet im 'expedited Review' am 05.09.2014** | |
| ✓ Zwischenbericht | 02.09.2014 |
| **Dokumente eingegangen am 16.12.2014 (in der nächsten Begutachtung mitbegutachtet)** | |
| Originalprotokoll 03 | 15.12.2014 |
| Informed Consent Form 04 | 15.12.2014 |
| ✓ Sonstiges: EK Meldeformular | 15.12.2014 |
| **Dokumente eingegangen am 05.01.2015, begutachtet im 'expedited Review' am 08.01.2015** | |
| ✓ Originalprotokoll 04 | 05.01.2015 |
| ✓ Informed Consent Form 05 | 05.01.2015 |
| **Dokumente eingegangen am 19.01.2015, begutachtet im 'expedited Review' am 29.01.2015** | |
| ✓ Werbematerial Aushang 02 | 19.01.2015 |
| ✓ Sonstiges: EK Meldeformular | 19.01.2015 |
| **Dokumente eingegangen am 01.07.2015, begutachtet im 'expedited Review' am 21.07.2015** | |
| ✓ Amendment (Originalprotokoll) 05 | 30.06.2015 |
| ✓ Informed Consent Form 06 | 30.06.2015 |
| ✓ Sonstiges: EK-Meldeformular | |
| **Dokumente eingegangen am 02.09.2015, begutachtet im 'expedited Review' am 11.09.2015** | |
| ✓ Zwischenbericht | 28.08.2015 |
| **Dokumente eingegangen am 14.04.2016, begutachtet im 'expedited Review' am 27.04.2016** | |
| ✓ Sonstiges: Anhang zum Amendment undatiert | |
| ✓ Sonstiges: EK-Meldeformular | 14.04.2016 |
| **Dokumente eingegangen am 16.09.2016, begutachtet im 'expedited Review' am 23.09.2016** | |
| ✓ Zwischenbericht | 16.09.2016 |
| **Dokumente eingegangen am 29.09.2017, begutachtet im 'expedited Review' am 05.10.2017** | |
| ✓ Zwischenbericht | 29.09.2017 |
| **Dokumente eingegangen am 03.08.2018, begutachtet im 'expedited Review' am 10.08.2018** | |
| ✓ Zwischenbericht | 01.08.2018 |
| **Dokumente eingegangen am 30.08.2019 (in der nächsten Begutachtung mitbegutachtet)** | |
| ✓ Zwischenbericht | 30.08.2019 |
| **Dokumente eingegangen am 04.09.2019, begutachtet im 'expedited Review' am 12.09.2019** | |
| ✓ Informed Consent Form 07 | 04.09.2019 |
| **Dokumente eingegangen am 14.09.2020 (in der nächsten Begutachtung mitbegutachtet)** | |
| ✓ Zwischenbericht | 10.09.2020 |
| **Dokumente eingegangen am 21.10.2020 (in der nächsten Begutachtung mitbegutachtet)** | |
| ✓ CV Prüfer Gattringer Oktober 2020 | |
| ✓ Sonstiges: Bestätigung - Übernahme der Studie Prof. Gattringer | 21.10.2020 |
| **Dokumente eingegangen am 11.11.2020, begutachtet im 'expedited Review' am 13.11.2020** | |
| ✓ Informed Consent Form 08 | 11.11.2020 |
| **Dokumente eingegangen am 20.09.2021, begutachtet im 'expedited Review' am 06.10.2021** | |
| ✓ Zwischenbericht | 20.09.2021 |
| **Dokumente eingegangen am 09.03.2022, begutachtet im 'expedited Review' am 23.03.2022** | |
| ✓ Originalprotokoll 06 | 08.03.2022 |
| ✓ Informed Consent Form 09 | 08.03.2022 |
| ✓ Sonstiges: EK-Meldeformular - Amendment | 08.03.2022 |
| **Dokumente eingegangen am 28.09.2022, begutachtet im 'expedited Review' am 05.10.2022** | |
| ✓ Zwischenbericht | 28.09.2022 |

**Datum Erstvotum: 30.09.2013**

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

**zusätzliche Auflagen:** Die behördlich vorgeschriebenen Maßnahmen hinsichtlich der COVID-19 Pandemie müssen beachtet werden. Der Prüfer und der Sponsor müssen in ihrem jeweiligen Wirkungskreis unter allfälliger Beachtung von Leitlinien gewährleisten, dass keine zur Bekämpfung der Pandemie benötigten Ressourcen gebunden werden bzw. ausreichend Personal vorhanden ist und die TeilnehmerInnen durch ihre Studienteilnahme keiner zusätzlichen Infektionsgefahr ausgesetzt werden.

Graz, 05. Oktober 2022

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

Univ.Prof.Dr.Hans Peter Dimai
Stv. Vorsitzender

**Achtung:** Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!