UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE EFFECTS OF ACUTE MELATONIN SUPPLEMENTATION ON CENTRAL AND PERIPHERAL RESPONSES IN HEALTHY HUMANS DURING SYMPATHETIC STIMULI

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

By

SARAH SKILLETT

Norman, Oklahoma

2023

THE EFFECTS OF ACUTE MELATONIN SUPPLEMENTATION ON CENTRAL AND PERIPHERAL RESPONSES IN HEALTHY HUMANS DURING SYMPATHETIC STIMULI

A THESIS APPROVED FOR THE

DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY THE COMMITTEE CONSISTING OF

Dr. J. Mikhail Kellawan, Chair

Dr. Debra A. Bemben

Dr. Daniel J. Larson

TABLE OF CONTENTS

List of Tables

List of Figures

Abstract

There is evidence that melatonin has more functions other than regulating sleeping tendencies such as anti-hypertensive and antioxidant functions, however how melatonin exerts these functions is unknown. **PURPOSE:** To determine if acute melatonin supplementation alters central and peripheral responses to mild hypovolemia at rest and during rhythmic exercise in healthy humans. **METHODS:** 8 healthy young adults (23±3, 3 females) participated in a randomized, single blind, crossover protocol. Subjects ingested 5 mg of melatonin (MEL) or placebo (mint water) as a sublingual spray 30 minutes before data collection. Cardiovascular variables (SV, TPR, and HR) measured by ECG and finger photoplethysmography, and vascular hemodynamics (FBF and FVC) measured by doppler ultrasound during –20mmHg stimulus from LBNP and rhythmic handgrip exercise. Paired t-tests were performed to compare data between conditions, significance was set as $p \leq 0.05$. **RESULTS:** Data are presented as mean±SD. No significant differences were found for SV (P:102.80±31.08 vs M:102.08±21.68, p=0.78,r=0.07), TPR (P:102.00±31.57 vs M:102.16±18.53, p=0.89,r=0.04), FBF (P:217.70±112.41 vs. M: 213.99±148.39, p=0.89,r=0.04), or FVC (P:180.47±93.19 vs. M:179.40±119.93, p=0.67,r=0.11) during exercise, except a significant decrease in HR in the MEL condition (P:74.58±10.09 vs M: 68.69±7.79, p=0.03,r=0.56). There were no significant differences found for SV (P:87.28±24.61, vs. M:91.02±23.24, p=0.58,r=0.14), TPR (P:112.70±35.43 vs. M:112.11±23.33, p=1.00,r=0.00), FBF (P:224.48±119.69 vs. M:229.46±147.33, p=0.48,r=0.18), or FVC (P:180.95±88.93 vs M:188.67±119.73, p=0.58,r=0.14) during exercise with LBNP except a significant decrease in HR in the MEL condition (P:78.08±11.76 vs. M:71.06±9.34, p=0.05,r=0.49). **CONCLUSION:** Our data indicates that melatonin does not alter central or peripheral responses in healthy humans during sympathetic stimuli.

Acknowledgements

I would like to first acknowledge my advisor Dr. Kellawan and express how grateful I am for everything that he has taught me. I have grown so much in the past 4 years with being in your lab and I want to thank you for pushing me to be the best scientist and student that I can be even when things got really hard. Next, I would like to thank my committee, Dr. Larson and Dr. Bemben, for being patient with me and my thesis journey and for serving on my committee despite their busy schedules.

Next, I want to thank my lab mates for being there for me and helping me complete my thesis, I would not have been able to accomplish anything without you guys. Finally, I would like to thank my family and friends for everything you have done for me. You all had checked in and made sure that I ate something, or that I slept (ironically considering the main subject of this study). My online community of friends who would play games with me outside of lab to make sure I took breaks and relaxed. Without your support, I would not have been able to finish and for that I am extremely grateful and love all of you with all my heart.

These past four years of being in the Human Cardiovascular Research Laboratory have been amazing, and I am so glad for all the people that I have gotten to meet and know in the program. Despite the rough parts of life and graduate school, I am so happy to have persevered through it all and achieved everything that I have during my time here at the University.

I am so excited to see what the future holds and for the next adventure that awaits me.

Chapter I

Introduction

Cardiovascular disease is the leading cause of death worldwide, this is typically associated with sedentary behaviors that lead to the development of chronic diseases, such as atherosclerosis, causing issues that either increase the risk for developing cardiovascular disease or worsening other chronic conditions (Vaduganathan et al., 2022). Studies in patients with cardiovascular disease have reported low nocturnal levels of melatonin in a variety of cardiometabolic diseases such as coronary heart disease, congestive heart failure and myocardial infarcts when compared to healthy subjects (Yaprak et al., 2003; Arangino et al., 1999; Brugger et al., 1995). Suggesting that low melatonin production is associated with higher risk of developing cardiovascular disease.

The hormone melatonin is secreted from the pineal gland and is part of regulating the sleep cycle in the body. This hormone is negatively regulated through sympathetic innervation of beta-adrenergic receptors with secretion highest at night when sympathetic activity is lower and lowest during the day when sympathetic activity is higher (Moore, 1996). Further, light plays a role in the suppression of melatonin secretion through sympatho-excitation and vagal suppression, with low light conditions decreasing inhibition signals for melatonin secretion (Baker & Kimpinski, 2018). Melatonin synchronizes endogenous rhythms and environmental cycles and thereby is important for regulating functions in the body such as endocrine function and propensity to sleep (Lewy and Sack, 1997; Cagnacci et al., 2001). There are two receptor subtypes for melatonin designated as MT1 and MT2, where MT2 is primarily found in the brain and retina and MT1 is primarily found throughout the brain and peripheral organs (Ekmekcioglu et al., 2001; Ekmekcioglu et al., 2003). These receptors are also found in coronary arteries and in

the vasculature of both rats and humans leading to the implication that melatonin has more functions than just circadian rhythm regulation. Although the chronobiological effects of melatonin have been studied and accepted, the other physiological effects of melatonin have not been widely accepted (Ekmekcioglu et al., 2003). This is due to a lack of conclusive research on understanding the role of melatonin in other systems and functions of the body.

Oxidative stress is part of many pathophysiological conditions and has been associated with cardiovascular diseases. Interestingly, melatonin has been found to limit the formation of free radicals and increasing other antioxidative reserves in both animal and human models (Girouard et al., 2004; Anwar et al., 2001; Koziróg et al., 2011; Reiter et al., 2003). Improvements in oxidative stress from melatonin have caused lower blood pressure and inhibiting contractile responses in smooth muscle (Anwar et al., 2001). As stated earlier, melatonin receptors are found throughout vasculature in animal models and humans which supports a role of melatonin in blood pressure regulation (Baker & Kimpinski, 2018). Evidence for a contribution of melatonin to blood pressure regulation is found in animal studies which observed that pineal gland removal resulted in vasoconstriction and subsequent hypertension which was reversed with exogenous melatonin supplementation (Cunnane et al., 1980; Zanoboni et al., 1967; Holmes & Sugden, 1976). There is also evidence in human studies that low doses of melatonin in healthy men and women have caused significant decreases in systolic, diastolic, and mean blood pressures (Arangino et al., 1999; Cagnacci et al., 1997; Cagnacci et al., 1998; Cagnacci et al., 2001; Nishiyama et al., 2001). This finding has implications that melatonin could be used as an antihypertensive treatment, but the mechanisms behind how melatonin regulates blood pressure have yet to be clear.

Exercise is important for prevention and maintenance of cardiovascular disease and is a big part of treatment processes (Fiuza-Luces et al., 2018). This causes beneficial physiological changes that work in conjunction with medication depending on the treatment plan. Research in hypertensive rats has shown that melatonin has an anti-hypertensive effect during exercise along with increasing vasorelaxation and improvement in oxidative stress (Qui et al., 2018). Research in healthy humans indicates that sympathetic responses to lower body negative pressure (LBNP) are decreased and there are changes in forearm vascular control during handgrip exercise with melatonin supplementation (Ray, 2003). Although research is promising, there is not a conclusive answer as to how melatonin exerts these effects in humans, especially during exercise. In a study with hypertensive rats, melatonin was found to inhibit alpha receptors in both the experimental and control groups (Girouard, 2004). In addition to antioxidant properties, this finding suggests that a possible mechanism behind melatonin function in the body is through inhibiting sympathetic activity by blunting alpha receptors. Improvement in vascular parameters in humans is evident through melatonin supplementation, but the suggested mechanism of melatonin blunting vascular responsiveness to sympathetic activity during rest or exercise is unknown (Baker & Kimpinski, 2018).

Purpose

This leads to the purpose of this experiment which is to determine if acute melatonin supplementation alters central and peripheral responses to mild hypovolemia at rest and during rhythmic exercise, therefore providing evidence that melatonin inhibits alpha receptors in healthy humans.

Research Questions

- Does acute supplementation of melatonin inhibit alpha receptors as evidenced by affecting forearm blood flow and conductance during rest and handgrip exercise in healthy individuals?
- Does melatonin affect central cardiovascular variables and blood pressure at rest, during mild hypovolemia, and during handgrip exercise in healthy individuals?

Research Hypotheses

- Acute supplementation of melatonin does inhibit alpha receptors in healthy humans as evidenced by increases in forearm blood flow and vascular conductance and preserved muscle oxygenation during mild hypovolemia and exercise.
- Melatonin decreases heart rate, blood pressure, maintains stroke volume and increases muscle oxygenation in the forearm during handgrip exercise in healthy individuals.

Delimitations

- All subjects were recruited from the Norman and Oklahoma City area.
- All participants were considered healthy with no cardiovascular conditions.
- All participants are between the ages of 18-35 years.
- All participants have no history of autonomic dysfunction.
- All participants do not use any nicotine products.
- All participants do not use melatonin products and do not regularly take melatonin.
- Female subjects were tested between days 1 and 7 of the follicular phase in their menstrual cycle.
- All participants were below the waist circumference requirements.

Limitations

- Melatonin is administered sublingually and not intra-arterially or venously.
- Data may not be representative of individuals in areas other than Norman.
- Data might not be applicable to other populations other than young and healthy individuals.

Assumptions

- All subjects adhered to pre-protocol requirements.
- Sublingual melatonin is absorbed into the bloodstream as previous pharmacokinetic data has described (Bartoli et al., 2013).
- All subjects were not able to distinguish between placebo and melatonin.

Operational Definitions

- **FinaPres NOVA:** System that measures continuous arterial blood pressure through infrared light and pressure signaling on the fleshy part of the middle phalanx (Favilla et al., 2023).
- **OxiplexTS NIRS:** Instrument that uses near-infrared spectroscopy on the flexor digitorum muscle of the non-dominant arm to determine oxygenated and deoxygenated hemoglobin levels (Abozguia et al., 2008).
- **Ultrasonography**: Device that uses soundwaves for imaging arteries and veins and determining the blood velocity (Limberg et al., 2020).
- **Hand grip dynamometer**: Instrument used to measure maximal voluntary contraction (MVC) with the nondominant arm.
- **HbO_2 :** Oxygenated hemoglobin.

- **HHb:** Deoxygenated hemoglobin.
- **tHb:** Total hemoglobin.
- **Sympathetic Nervous System (SNS)**: Part of the autonomic nervous system that prepares the body for activity by altering autonomic functions.
- **Functional sympatholysis:** The ability to blunt sympathetic vasoconstrictors in working muscle during exercise (Saltin & Mortenson, 2012).
- **Heart Rate (HR):** The amount of times the heart beats per minute (BPM).
- **Mean Arterial Pressure (MAP):** Average blood pressure in the arterioles during one cardiac cycle.
- **Cardiac Output (CO);** The volume of blood ejected by the heart per minute (L/min).
- **Stroke Volume (SV):** The amount of blood ejected by the left ventricle per heartbeat (ml/beat).
- **Total Peripheral Resistance (TPR):** Total resistance to blood flow within the systemic circulation.
- **Forearm Muscle Blood Flow (FBF):** The volume of blood moving through the brachial artery to the forearm per minute. Calculated via (mean blood velocity x 60 x π x (brachial diameter/2)^2) (ml/min).
- **Forearm Vascular Conductance (FVC):** The amount of FBF relative to 100 mmHg of arterial pressure. Use as an assessment of dilation vs constriction in the forearm circulation. Calculated via (FBF/MAP) ´ 100mmHg (ml/min/100mmHg).
- **Placebo (PLA):** Will consist of a mixture of one microliter of McCormicks Pure Mint extract and 29 mL of filtered water that will be given as a sublingual spray.

- **Melatonin (MEL):** 5 mg of sublingual melatonin ingested as 10 sprays underneath the tongue (Onnit, Austin, TX).
- **Lower Body Negative Pressure (LBNP):** Device that redistributes blood from the upper body to the lower body by inducing negative pressure to the lower half of the body sealed into the chamber, thus causing a sympathetic response to occur through changes in SV (Hinojosa-Laborde et al., 2014).

Chapter II

Literature Review

Introduction

Melatonin is a hormone secreted by the pineal gland and has been accepted for regulating circadian rhythm and sleep patterns in the body (Cagnacci et al., 2001). Recent evidence has suggested that melatonin has more functions in the body although the exact mechanism of how these functions work is unknown (Baker & Kimpinski, 2018). These functions include anti-hypertensive, antioxidative and anti-inflammatory properties that improve autonomic function in both human and animal models. One suggestion as to how melatonin exerts its effects is through the inhibition of α-adrenergic vasoconstriction (Baker & Kimpinski, 2018). The purpose of this literature review is to examine the effects that melatonin supplementation can have on peripheral vasculature during acute exercise and evaluate the evidence that melatonin can inhibit α-adrenergic vasoconstriction

In this chapter we will go over, regulation of vascular control, muscle blood flow during moderate intensity exercise, natural production and secretion of melatonin in the body, the doses and pharmaceutics of melatonin supplementation, proposed interaction with tissues involved with cardiovascular control, endothelial dependent vasodilation, free radical scavengers, suppression of inflammation, parasympathetic and sympathetic inhibition, and possible clinical implications.

Regulation of vascular control/exercise muscle blood flow during moderate intensity exercise

Neural Control of Exercise Pressor Response

The exercise pressor response is activated at the start of exercise and anticipates the changes that the body will undergo to maintain exercise. At the start of exercise, the body shifts from mainly parasympathetic activation to sympathetic activation through several mechanisms of both feedforward and feedback responses. Central command is a feedforward response that involves higher brain centers that activate cardiovascular, muscular, and ventilatory functions in anticipation of change that occurs with exercise (Fu & Levine, 2013). This is activated through increases in sympathetic nervous activity and decreases in parasympathetic nervous activity. The other two feedforward responses would be the mechanoreflex and the muscle pump. During the mechanoreflex, mechanoreceptors sense changes in stretch of muscle during the beginning of muscular contractions and initiate withdrawal of parasympathetic activation by sending signals to regulatory centers in the brain through Group III and IV afferent neurons (Smith et al., 2015). The muscle pump works in conjunction with the mechanoreflex, by emptying the blood vessels as the muscles contract, thus creating a bigger pressure gradient that will increase blood flow to maintain pressure (Laughlin et al., 2012).

When exercise begins, the body's feedback responses will meet the necessary functions to sustain exercise depending on the intensity. These feedback responses consist of the metaboreflex and the baroreflex. The metaboreflex works with metaboreceptors that sense the concentration of metabolites in the blood (Crisafulli, 2017). As intensity of exercise increases there will be an increased concentration of metabolites signaling that there is a mismatch between oxygen delivery to oxygen demand. This leads to an increase in sympathetic activity

which in turn increases cardiac output that will increase blood flow to decrease the concentration of metabolites in the blood and increase oxygen delivery (Crisafulli, 2017). The baroreflex functions through baroreceptors which sense the pressure from mean arterial pressure (MAP) in the heart and aortic arch from transmural pressure and send afferent signals from pressure changes (Wehrwein & Joyner, 2013). This reflex alters parasympathetic and sympathetic output through efferent pathways to the heart and blood vessels depending on the amount of pressure on the arterial walls thus regulating MAP (Wehrwein & Joyner, 2013). These mechanisms work together to meet the demands of exercise and are crucial in the activation of the exercise pressor response. Without the exercise pressor response, the body would not be able to properly adjust to increases in sympathetic activity and blood pressure and therefore would not be able to maintain exercise. As changes occur from the exercise pressor response, the cardiovascular and ventilatory system adjust to meet these changes and maintain homeostasis through vasodilation.

Exercise Vasodilation

Blood vessels change their radius during exercise as a response to the increase in oxygen demand from the working muscles by relative changes in vascular smooth muscle contraction or relaxion. Vasodilation can occur either directly or indirectly, causing changes in vascular conduction. Direct vasodilation occurs from vascular smooth muscle relaxation causing the vessels to dilate due to changes in intravascular pressure (Laughlin, 2012). Indirect vasodilation occurs through signaling molecules that interact with endothelium to release other vasodilators that diffuse into vascular smooth muscle causing it to relax leading to vasodilation (Laughlin, 2012). These vasodilators are ATP, NO, K^+ ions, PGs, and EDHF.

Adenosine triphosphate (ATP) is a molecule that has many different functions throughout the body, primarily used as energy in metabolic pathways, but is also known as a local

vasodilator during exercise (Ellsworth et al., 2010). At the start of exercise, the concentration of oxygen is decreased in working muscles to meet immediate oxygen demands, red blood cells in the capillaries feeding the working muscle with saturated hemoglobin now enters a low O2 environment initiating a greater offload of ATP and O2 causing the hemoglobin to change shape (Ellsworth & Sprague, 2012). The deformed red blood cells generate a signal that there is an imbalance in oxygen delivery leading to a rise in sympathetic activity to increase heart rate and flow, while ATP causes local vasodilation (Ellsworth & Sprague, 2012). When released into the vasculature, ATP attaches to P2X and P2Y purinergic receptors on the endothelium stimulating the release of several vasodilators that creates local vasodilation and increases flow to that area to maintain O2 pressure (Ellsworth et al., 2010). ATP is a potent vasodilator that increases vasodilation through release of several vasodilators to maintain oxygen delivery to working muscle and O2 pressure in vessels during exercise.

Nitric Oxide (NO) is another important vasodilator that regulates vascular tone and blood flow in the body (Tejero et al., 2019). There are enzymatic and non-enzymatic sources that produce NO within and near vessels. For enzymatic sources, the production of NO is through nitric oxide synthase (NOS) enzymes that create NO from oxygen and L-arginine (Mortensen and Saltin, 2014). There are several isoforms of NOS that provide different functions for the body, the main three are eNOS, nNOS, and iNOS (Tejero et al., 2019). The eNOS isoform is the most abundant and provides the most impact on vascular function, which is due to this isoform being located in the vascular endothelium (Mortensen and Saltin, 2014). The non-enzymatic sources are through nitrate and nitrite concentrations, and through blood proteins that can preserve and release NO. Production of NO through nitrate and nitrite is usually through nitrate being broken down to nitrite, which is absorbed into the circulation that is reduced to NO

through several proteins (Tejero et al., 2019). Another source that contributes to NO production is through the offloading of ATP from erythrocytes in the blood that attach to receptors on the endothelium that release NO (Chen et.al., 2008). Typically, this pathway is through local vasodilation but is still a big contributor to the overall increase in vasodilation during exercise.

Potassium ions (K^+) contribute to the regulation of blood flow and blood pressure through potassium channels that induce hyperpolarization of vascular smooth muscle cells (Haddy et al., 2006). There are three different types of potassium channels that contribute to vascular function, which are KATP, KCa, and Kv channels. The hyperpolarization of the membrane causing an action potential opens KATP channels which closes voltage dependent calcium channels creating a decrease in calcium leading to vasodilation (Laughlin et al., 2012). KCa channels consist of different sizes with large conductance channels (BKCa) being the most prominent in the vasculature. The opening of KCa channels increases vasodilation and is activated through vasodilator substances that stimulate cGMP-dependent protein kinase (PKG) and cAMP-dependent protein kinase (PKA) that stimulate the opening of these channels (Laughlin et al., 2012). Lastly Kv channels open when the membrane depolarizes and thereby decreases vasoconstriction, and these channels are sensitive to changes in oxidative stress and the activity of beta-adrenoceptors (Laughlin et al., 2012). The opening of these potassium channels in vascular smooth muscle cells leads to vasodilation through the relaxation of smooth muscle in the vasculature thereby inducing vasodilation. During exercise there is increased activity of potassium channels inducing vasodilation to maintain blood flow and pressure in the vasculature of working muscle to meet oxygen demands (Haddy et al., 2006).

Prostaglandins are another potent vasodilator that contributes to local vasodilation in systemic circulation (Laughlin et al., 2012). The formation of prostaglandins is through the

conversion of arachidonic acid by the enzyme cyclooxygenase (COX) and has many isoforms that perform different functions throughout the body (Zarghi & Arfaei, 2011). The two main isoforms of prostaglandins that mediate vasodilation in the endothelium are prostaglandin E2 (PGE2) and prostacyclin (PGI2) which are both increased during the venous efflux of exercising muscle, while PGI2 increases during exercise (Mortensen & Saltin, 2014). There are two COX enzymes, COX-1 and COX-2, that both appear in the pulmonary system while COX-1 enzymes are mainly expressed in endothelial cells in systemic vasculature (Laughlin et al., 2012). Along with functions in the cardiovascular system, prostaglandins also have anti-inflammatory effects in the body when tissue damage or inflammation occurs (Zarghi & Arfaei, 2011). When COX pathways are inhibited, exercise blood flow is not inhibited but when simultaneously inhibited with NOS pathways blood flow is reduced by 30% showing that there is a compensatory function when one pathway is compromised (Mortensen & Saltin, 2014). Prostaglandins are important in vascular dilation during exercise and contribute significantly to local vasodilation in exercising muscle.

Endothelial derived hyperpolarizing factor (EDHF) is a proposed pathway that plays a role in local vasodilation. This hypothesis is due to studies that found local vasodilation was not completely blocked despite COX and NOS inhibitors that prevented the synthesis of the vasodilators NO and prostaglandins (Laughlin et al., 2012). The suggested candidate behind EDHF is endothelium-derived cytochrome P450 from arachidonic acid which causes hyperpolarization of membranes through the activation of KCa channels thus increasing vasodilation (Ozkor et al., 2011). Whether EDHF acts independently or is a NOS backup has yet to be made clear, when cytochrome P450 and NOS are inhibited, there is a decrease in exercise blood flow, but when EDHF is blocked via potassium channel blocker TEA along with inhibition

of NOS and COX, exercise blood flow was not as reduced as when cytochrome P450 and NOS were inhibited (Mortensen & Saltin, 2014). While the pathway for EDHF and all its functions have yet to be fully elucidated, there is evidence that EDHF plays a role in local vasodilation in the muscle during exercise.

Functional Sympatholysis

During exercise, sympathetic activity is increased causing blood vessels to become vasoconstricted, however vessels in working muscle are vasodilated due to the blunting effect of sympathetic vasoconstrictors, this mechanism is termed functional sympatholysis (Saltin & Mortenson, 2012). With increases in sympathetic outflow during exercise, cardiac output (CO) is also increased thus causing an increase in blood flow. The increase in vascular conductance in exercising muscle is to meet elevated demands of metabolism during exercise to maintain oxygen delivery (Tschakovsky et al., 2002). The mechanism of functional sympatholysis vasoconstricts vessels in resting muscle but blunts vasoconstrictor activity in working muscle causing blood flow to be redistributed to exercising muscle to maintain blood pressure (Mortensen et al., 2014). In combination with increased blood flow and improved vascular conductance, blood is redistributed from resting muscles to working muscles to maintain perfusion pressure. This contributes to another function of functional sympatholysis which is regulating blood pressure.

Studies have found that hypertensive and older individuals have impaired functional sympatholysis during exercise (Mortensen et al., 2014). Both populations have endothelial dysfunction typically due to inactivity. In a study comparing normotensive and hypertensive individuals, alpha adrenergic responsiveness is not altered in hypertensive individuals meaning that there is no blunting effect (Mortensen et al., 2014). Although with exercise, the mechanism

of functional sympatholysis is improved with observations in reduced alpha-adrenergic responsiveness and decreases in blood pressure in hypertensive individuals. These results display that functional sympatholysis plays a role in blood pressure regulation due to improvements in hypertensive individuals with exercise training (Mortensen et al., 2014). While exercise improves functional sympatholysis by reducing alpha-adrenergic responsiveness, there is still not a clear answer as to why exercise allows us to dilate regardless of an increased sympathetic vasoconstriction signal. The answer to this question was thought to be one of the potent vasodilators such as NO or ATP, but even with the inhibition of these molecules there is not a reduction in exercise hyperemia or blunting of sympathetic vasoconstriction (Saltin & Mortensen, 2012; Rosenmeier et al., 2003). It is thought that maybe these substances work together to mediate an effect in functional sympatholysis but again has yet to be made clear.

Although the mechanism behind how functional sympatholysis works is unclear, what is clear is that exercise training improves this function in older and diseased individuals. These populations have increased sympathetic vasoconstrictor activity and decreased blood flow during exercise (Vongpatanasin et al., 2011; Proctor et al., 1998). Although, in chronically active older individuals functional sympatholysis remains intact which provides sufficient blood flow to maintain exercise (Saltin & Mortensen, 2012). This suggests that exercise improves functional sympatholysis and therefore maintains blood flow and autonomic function during exercise. In another study with hypertensive individuals, blood flow was normalized in response to exercise after 8 weeks of high intensity exercise (Nyberg et al., 2012). While the exact function behind functional sympatholysis is unknown, we know that improvements of this mechanism has something to do with exercise training. Overall, functional sympatholysis plays a role in autonomic and blood pressure regulation and blood flow redistribution during exercise. This is

through the blunting effects of alpha-adrenergic receptors during exercise, maintaining blood pressure in the vasculature, and redistributing blood flow from resting to exercising muscle.

Natural Production and Secretion of Melatonin

Production From the Pineal Gland

Melatonin is released from the pineal gland under low light conditions (Baker & Kimpinski, 2018). This process is completed through a cascade of activation that mediates the production and secretion of melatonin from the pineal gland. When light passes through the retina, this activates the suprachiasmatic nuclei (SCN) and inhibits the paraventricular nucleus (PVN)(Baker & Kimpinski, 2018). This projects from the PVN to the sympathetic intermediolateral (IML) nucleus which then projects to the superior cervical ganglia that goes to the pineal gland to increase melatonin production and secretion (Baker & Kimpinski, 2018). The secretion and production of melatonin is dependent on the amount of light that comes to the retina, with more light there is an increase in inhibition of the PVN thus blocking the activation of the pineal gland (Baker & Kimpinski, 2018). With less light, there is less inhibition of the PVN which will continue the rest of the activation pathway to stimulate production and secretion of melatonin from the pineal gland. Therefore, melatonin production and secretion reach its peak between 2 and 4 AM at night, and slowly decreases with time as the sun starts to rise (Tordjman et al., 2017). The production and secretion of melatonin functions in a circadian rhythm that is regulated through light with high peaks at night and lowest peaks during the day (Cagnacci, 1996).

Production From Extra-Pineal Glands

The production of melatonin is found in the pineal gland, but production has also been found outside of the pineal gland such as bone marrow cells, skin, lymphocytes, immune system and gastrointestinal tracts (Tordjman et al., 2017). Since these extra-pineal tissues do not have day and night to regulate synthesis, this suggests that there are other pathways that regulate the production of melatonin from these tissues (Acuna-Castroviejo et al., 2014). This suggests that synthesis acts more locally as a protective mechanism against oxidative and inflammatory damage (Acuna-Castroviejo et al., 2014). Although many of these extra-pineal tissues also produce higher concentrations of melatonin, they are not released into the systemic circulation and is why it is suggested that it acts more locally (Acuna-Castroviejo et al., 2014). It is suggested that there is a mechanism, such as binding to a protein, that works to maintain melatonin concentrations in the cell to ensure none is released into the circulation, but the answer as to why melatonin created through extra-pineal sources does not have access to the circulatory system has yet to be discovered (Acuna-Castroviejo et al., 2014). Melatonin is very lipophilic allowing easy passage through cell membranes and is how its functions are able to be spread throughout the body (Zhao et al., 2017).

Function of Melatonin

While the main function of melatonin is to regulate circadian rhythm and sleep patterns, studies have found that melatonin has other potential functions that improve blood pressure, oxidative stress and inflammation (Tordjman et al., 2017). With these findings, melatonin has become increasingly recognized in the pathophysiology of cardiovascular diseases, especially with the finding of melatonin receptors throughout the cardiovascular system (Imenshahidi et al., 2020). More evidence supports this with hypertension and peripheral vasoconstriction occurring

in pinealectomized rats (Imenshahidi et al., 2020; Baker & Kimpinski, 2018). This could have clinical implications for treatment and prevention of cardiovascular diseases and other chronic conditions where inflammation and oxidative stress are the main contributors.

The Effect of Exercise on Cardiovascular Disease

Exercise has been proven to have health benefits in cardiometabolic functions, decreasing risk for developing cardiovascular diseases, and overall reducing all-cause mortality in adults (Fiuza-Luces et al., 2018). Most prognoses of cardiovascular diseases involve a combination of medication and exercise due to exercise training improving vascular endothelial function and helps to decrease the risk of mortality (Fiuza-Luces et al., 2018). Individuals that participate in repetitive bouts of aerobic exercise are found to have lower measures of arterial stiffness, increased protection against oxidative stress and inflammation, and increased production of NO causing improvements in endothelial-dependent vasodilation (Lavie et al., 2015). Along with improvements in endothelial function, exercise training also improves vagal tone through regulating autonomic activity in the body (Fiuza-Luces et al., 2018). This is through restoring balance to sympathetic and parasympathetic activity in the body that becomes dysregulated in cardiovascular conditions, this can be measured through heart rate variability (HRV). Exercise also improves efficiency in metabolic function by decreasing adiposity and improving lipid profiles thereby increasing sensitivity to insulin and reducing inflammation (Lavie et al., 2015). Overall, these physiological adaptations decrease the risk for developing cardiovascular disease and reducing mortality risk (Lavie et al., 2015). The body becomes dysregulated with the development of chronic conditions and exercise reverses some of these effects and improves the body's homeostasis.

The Effect of Melatonin on Cardiovascular Disease

With evidence of melatonin receptors in the cardiovascular system, recent studies suggest that melatonin plays a major role in various cardiovascular diseases such as myocardial ischemia-reperfusion injury, atherosclerosis, hypertension and heart failure (Sun et al., 2016). Melatonin plays a role in both antioxidant and anti-inflammatory effects of the body however the mechanism in which melatonin exerts these effects is still unknown (Chitimus et al., 2020). Typically, disturbances in sleep are an indicator of disease and can be an increased risk for developing cardiovascular disease (Buxton & Marcelli, 2010). This can be observed in studies that compared melatonin synthesis at night between patients with coronary artery disease (CAD) and healthy subjects, and CAD patients had a markedly decreased amount of melatonin secretion than the healthy control subjects (Yaprak et al., 2003; Sakotnik et al., 1999; Brugger et al., 1995). Melatonin has also been found to protect against ischemia/reperfusion injury, hypertension, and myocardial infarction with its antioxidant mechanism (Chitimus et al., 2020). Another study by Kozirog et al., 2011 found that supplementation of melatonin to patients with metabolic syndrome improved blood pressure, serum lipid profile, and antioxidative status. There are also studies that have found that melatonin has an antihypertensive effect in reducing blood pressure in patients with hypertension (Sun et al., 2016). Although the mechanism is unknown, melatonin is clearly involved with cardiovascular regulation and therefore plays a role in prevention and development of cardiovascular disease.

The Effect of Combined Exercise with Melatonin on Cardiovascular Disease

The mechanisms behind the function of melatonin during exercise are conflicting in studies but show beneficial effects in different cardiovascular diseases (Escames et al., 2012). Research done in rats has shown that the combination of exercise with melatonin exerts a

hypotensive effect in hypertensive rats as well as increasing vasorelaxation and improving oxidative stress (Rahman et al., 2017; Qiu et al., 2018). Although the dose of the effect is dependent on when exercise is performed, in the morning melatonin concentration is higher than in the afternoon and has greater effect on cardiovascular variables (Escames et al., 2012). This suggests that depending on the time of day, the relationship between exercise mediated sympathetic activity and melatonin secretion is altered (Escames et al., 2012). Overall, exercise influences melatonin release and typically prolongs the period of peak secretion therefore increasing levels in the body (Qiu et al., 2018). Therefore, with higher concentrations of melatonin it can exert its anti-hypertensive effects on the body for longer periods of time. There are not conclusive results on how melatonin exerts these effects, but data suggests that the combination of melatonin and exercise could be beneficial for individuals with cardiovascular disease.

Doses and Pharmaceutics of Melatonin Supplementation

Research completed on the safety of exogenous melatonin has shown very low toxicity of high doses of melatonin. The reduction of endogenous melatonin shows greater damage than studies who give high doses of melatonin (Acuna-Castroviejo et al., 2014). Any adverse effects that were reported from clinical trials were small and did not last very long, these were fatigue, mood changes and poor neurocognitive performance (Imenshahidi et al., 2020). No adverse effects were seen in studies that used higher doses ranging from 20 to 100 mg, and most studies have an average dose range between 1 and 5 mg (Imenshahidi et al., 2020). Typically, administration of melatonin during the daytime results in lower body temperature due to vasodilation in distal parts of the body but is still safe to take during the day or at night (Emet et al., 2016). From the clinical studies performed, this shows that melatonin supplementation is safe

even at high doses with easily manageable adverse effects if they do occur. The three most common pathways of administration for melatonin studies are oral tablets, venous infusions and sublingual sprays. After oral or intravenous supplementation, melatonin is quickly metabolized in the liver and kidneys and is excreted through urine (Tordjman et al., 2017). For venous infusions, melatonin is quickly distributed and eliminated while oral supplementation typically reaches its peak within 60 minutes and allows melatonin levels to reach 10 to 100 times higher than usual nocturnal levels (Tordjman et al., 2017). With sublingual supplementation, the first pass effect of metabolism in the liver is avoided which increases the amount of melatonin that reaches the systemic circulation compared to oral tablets (Bartoli et al., 2013). This pathway is preferred compared to the other two types since sublingual spray improves absorption creating a higher concentration in the systemic circulation after ingestion. Overall, melatonin is considered non-toxic at low doses which can be used safely for both clinical and recreational situations.

Melatonin Proposed Interaction with Tissues Involved in Cardiovascular Control

Vasculature

Arteries and veins are the vessels that carry oxygenated and deoxygenated blood throughout the body along with nutrients and removal of waste. Both arteries and veins have similar anatomy, the blood vessel comes in layers starting with the innermost layer called the endothelium which is the layer that comes into direct contact with blood. The next layer of the vessel is the basement membrane, which covers the outside layer of the endothelium and is important for vessel function (Khalilgharibi & Mao, 2021). The next layer of the vessel is smooth muscle which is thicker in arteries for vasoconstriction and vasodilation compared to

veins. The last layer is adventia which is the outermost layer that protects vessels from the rest of the body. Although the anatomy of vessels are similar, there are some differences that apply to different functions for each type. For arteries, there are internal and external elastic layers around the vessel to help with compliance of blood flow and they carry oxygenated blood. For veins, they have a smaller layer of smooth muscle with valves to prevent the backflow of blood and they carry deoxygenated blood. The vascular system is a huge network of arteries, veins, and capillaries that all work together throughout the body. Oxygenated blood is ejected from the left ventricle of the heart that then goes through arteries to supply oxygen to the body. The transition of arteries to veins is a connection through capillaries. Capillaries consist of a single layer of endothelial cells and is where the exchange of nutrients, solutes, and water between the blood and surrounding tissues occurs (Pugsley & Tabrizchi, 2000). Which then transitions into the venous system with deoxygenated blood going back to the right atrium of the heart. Blood flow moves through the body by changes in pressure, with blood flowing from high to low pressures.

The autonomic system consists of sympathetic and parasympathetic activity in the body. Sympathetic activation increases during exercise which increases the release of neurotransmitters epinephrine and norepinephrine, that bind to alpha-receptors leading to increases in vasoconstriction or bind to beta-receptors leading to vasodilation in the systemic circulation. Parasympathetic activation is at its highest during times of rest and increases vasorelaxation of the smooth muscle in arteries (Wehrwein et al., 2016). The regulation of mean arterial pressure (MAP) is important and is maintained through the vasoconstriction and vasodilation of arteries which is the reason behind a thicker layer of smooth muscle. During exercise, the vessels in exercising muscle are vasodilated and the vessels in other areas of the body that are not being used during exercise are vasoconstricted. This will meet the changes in oxygen demand to

oxygen delivery in the body during exercise while also maintaining MAP and blood distribution. Blood vessels are important to maintain autonomic function of the body and keep tissues properly oxygenated during exercise.

Melatonin Receptors

There are three types of melatonin receptors that have been identified in the human body (Baker & Kimpinski, 2018). MT1 and MT2 receptors are G protein coupled receptors and have been associated with vascular reactivity, and the MT3 receptor is part of quinone reductases which plays a role in preventing oxidative stress (Imenshahidi et al., 2020). After receptor binding for both MT1 and MT2 types, intracellular signaling is affected through the regulatory proteins adenylate cyclase, guanylate cyclase, phospholipase C, and through calcium channels (Imenshahidi et al., 2020). These two receptors also inhibit cAMP production through Gi-coupled inhibition of the protein adenylate cyclase (Baker & Kimpinski, 2018). They also activate phospholipase C which results in an increase in intracellular calcium concentrations through Gq coupled activation (Baker & Kimpinski, 2018). Additionally, MT2 receptor also inhibits cGMP production through affecting the protein guanylyl cyclase (Ekmekcioglu, 2006). Typically, when MT1 receptors are activated vasoconstriction occurs and when MT2 receptors are activated vasodilation occurs (Ekmekcioglu, 2006; Emet et al., 2016). Besides protection against oxidative stress, there is not a lot of information behind the functions of the MT3 receptor in current literature (Ekmekcioglu, 2006). Due to the hydrophilic and lipophilic nature of melatonin, it can interact with molecules inside of the cell without binding to a melatonin receptor and still cause an effect to occur. This is called non-receptor-mediated actions (Zhao et al., 2017).

Melatonin receptors have been located throughout the body including the cardiovascular system, kidneys, liver, gastrointestinal system, adipocytes, and in both male and female reproductive systems (Tordjman et al., 2017). MT1 and MT2 receptors have been found more heavily in the brain specifically in the anterior pituitary, suprachiasmatic nucleus (SCN) of the hypothalamus, hippocampus, cortex, thalamus, cerebellum, paraventricular nucleus, cornea and retina (Tordjman et al., 2017). MT3 receptors have been found in the cytoplasm of the liver, lungs, kidneys, eyes, heart, brown adipose tissue, intestines and muscle cells. In summary, melatonin can exert its effects through binding to receptors in the body, or through non-receptor mediated actions in the cell. These receptors are found throughout several systems in the body and even have secretion from tissues other than the pineal gland. Although melatonin receptors are found throughout the body, there are clear effects seen in the pathophysiology of cardiovascular diseases.

Melatonin Receptor Function in Cardiovascular System

As discussed earlier, melatonin receptors have been found in the peripheral and central tissues in the cardiovascular system, mostly MT1 and MT2 receptors that cause vasoconstriction and vasodilation respectively. How these receptors mediate the effects on the cardiovascular system remains to be fully elucidated, although there are several functions that are clear even though the mechanism is not fully understood. The responses of vasoconstriction and vasodilation from MT1 and MT2 receptors may be from receptor stimulation with a higher potentiation from MT1 receptors for vessels to constrict (Doolen et al., 1998). Although at higher concentrations of melatonin there is an increase in vasodilation which could be from a higher affinity for MT2 receptors instead of MT1 receptors (Doolen et al., 1998). This could be an explanation as to why lower concentrations of melatonin are seen in hypertensive and coronary

artery disease (CAD) patients. Vascular tone is important in the body to maintain pressure which is regulated through different dilatory and constrictor agents that depend on the influx of calcium (Pogan et al., 2002). Another effect melatonin has on the vasculature is through the regulation of vascular tone, and the suggested mechanism for this is improved calcium signaling through enhancement of calcium mobilization and through capacitative calcium entry (Pogan et al., 2002). Melatonin also has antioxidant properties that reduce free radicals which decreases the suppression of calcium influx in the endothelium, and therefore is another way that melatonin improves calcium signaling (Pogan et al., 2002). With both vasodilatory and vasoconstrictor properties, melatonin plays an important role in the cardiovascular system by regulating vascular tone and reducing oxidative stress. This suggests that a dysfunction in melatonin secretion leading to decreased melatonin levels could increase the risk for developing cardiovascular disease.

Melatonin Effects on Endothelial-dependent Vasodilation

The result of melatonin having anti-hypertensive properties is through increased vasodilation by improving NO availability, NO synthase (NOS), cGMP production, and vascular conductance. In a study on spontaneous hypertensive rats, melatonin supplementation improved NO pathways through enhanced NOS, and reduced blood pressure (Pechanova et al., 2007). From MT2 activation on the endothelium, the increase in NOS activity led to increases in NO and cGMP production (Girouard et al., 2001). It is suggested that the increase in cGMP levels could possibly be from the inhibition of cGMP phosphodiesterase from melatonin (Satake et al., 1991). This is supported through evidence of NOS inhibition by L-NAME-induced hypertension in which melatonin was not able to improve vascular function due to the NOS pathway being blocked (Paulis et al., 2010). With melatonin supplementation, endothelin and angiotensin II

both had decreased, diminishing their hypertensive effects while also increasing NO and eNOS levels (Shao et al., 2017). The suggestion of the mechanism behind the improvement in NO availability is through enhancing NOS activity by melatonin acting partially through melatonin receptors on the endothelium (Zhao et al., 2017).

In addition, melatonin has been found to increase Ca^{2+} signaling in vascular endothelial cells through improving the mobilization of Ca^{2+} and capacitative Ca^{2+} entry in the endothelium (Pogan et al., 2002). There is further evidence that the anti-hypertensive effect of melatonin depends partially on the endothelium and is affected when there is an inhibition of Ca^{2+} channels with calcium channel blockers (Satake et al., 1991). Again, the exact mechanism behind melatonin function is unknown, but a suggested mechanism is that melatonin both directly and indirectly increases Ca^{2+} signaling (Zhao et al., 2017). Direct activation could be due to melatonin's lipophilic nature where it can pass through cell membranes, indirect activation would be through MT1 and MT2 receptors on the smooth muscle of vascular endothelium (Zhao et al., 2017). Altogether, the signaling cascade for vasorelaxation is improved through melatonin either directly or indirectly by stimulating MT2 receptors leading to increases in Ca^{2+}, that increase eNOS activity, thereby increasing NO, also increasing cGMP levels, ultimately leading to vasodilation (Paulis et al., 2010; Zhao et al., 2017; Satake et al., 1991). These observations are supported with melatonin supplementation in humans through observing improvement in cardiovascular variables such as reduced blood pressure, reduced pulse wave velocity (PWV), reduced pulsatility markers, and reduced vascular resistance (Cagnacci et al., 1998; Cagnacci et al., 1997; Yildiz et al., 2006). The vasodilatory function of melatonin reduces blood pressure thus providing protection from the detriments of hypertension, leading to reductions in oxidative stress and inflammation.

Melatonin as an Antioxidant and Effects on Free Radical Scavengers

Along with anti-hypertensive effects, melatonin also has antioxidant effects in the body through decreasing reactive oxygen species (ROS) and scavenging free radicals (Pogan et al., 2002). This is seen through healthy rat endothelial cells where melatonin reversed the effects of superoxide on Ca^{2+} signaling by preventing the ROS generating system (Pogan et al., 2002). Melatonin has been shown to scavenge hydroxyl radicals, hydrogen peroxide, superoxide, and hypochlorous acid, and with non-radical oxidants such as singlet oxygen and peroxynitrite (Chitimus et al., 2020; Baker &Kimpinski, 2018).

Oxidative damage in the body is a result of oxygen not being properly utilized by cells which is then converted to a free radical, due to its highly reactive state, it either steals or donates an electron causing damage to the surrounding molecules (Reiter et al., 2004). With excessive generation of free radicals, oxidative damage occurs which increases the risk for many chronic diseases. Fortunately, the body has developed an antioxidative defense system against ROS which consists of both enzymatic and non-enzymatic mechanisms (Tomas-Zapico and Coto-Montes, 2005). The enzymes involved are superoxide dismutases (SOD), Glutathione perioxidase (GPx), and catalase (CAT), while some non-enzymatic antioxidants or free radical scavengers are vitamins E and C, glutathione (GSH), beta-carotene, and now melatonin (Tomas-Zapico and Coto-Montes, 2005). Melatonin regulates oxidative damage in both direct and indirect ways. It is known as a free radical scavenger because it directly removes ROS as well as non-radical oxidants and is more efficient than other antioxidants due to its widespread distribution in tissues as well as its lipophilicity to cross membranes (Tomas-Zapico and Coto-Montes, 2005). The indirect way of regulation is through increasing the activation of oxidant enzymes SOD, GPx, and CAT (Baker & Kimpinski, 2018; Kozirog et al., 2011). After two

months of melatonin supplementation to patients with metabolic syndrome, the results showed improvements in catalase activity, lipid profile and reduction in blood pressure thereby decreasing the patients' hypertension and oxidative stress (Kozirog et al., 2011). With the reduction of oxidant processes, melatonin can restore NO levels thereby preventing hypertension and improving endothelial function (Shao et al., 2017; Pechanova et al., 2007). The antioxidative effects of melatonin play a role in not only regulating oxidative stress but working together in anti-hypertensive effects.

Melatonin Suppress of Inflammation

Along with antioxidant and anti-hypertensive functions, melatonin has been found to also have anti-inflammatory properties that assist in preventing oxidative stress (Prado et al., 2018). Inflammation plays a role in the development of hypertension and other cardiovascular diseases, due to the increased immune response from the immune system that creates dysfunction in the body leading to damage from increased blood pressure and oxidative stress (Liu et al., 2017). There are several functions that melatonin participates as an anti-inflammatory namely through reducing cyclooxygenase synthase, prostanoid production, and pro-inflammatory cytokines (Prado et al., 2018). Hypertrophy of adipose tissue causes dysfunction that leads to an increase in inflammation by activating inflammasome pathways that leads to the pathogenesis of metabolic and vascular diseases (Liu et al., 2017). Another anti-inflammatory function of melatonin that has been found was to ameliorate inflammatory processes by inhibiting inflammasome pathways and pyroptosis, an inflammatory form of regulated cell death (Liu et al., 2017). The overproduction of ROS contributes to increases in inflammation, but melatonin's free radical scavenging properties is another function that prevents inflammatory processes (Favero et al., 2017). Overall, the anti-inflammatory effects of melatonin work together with anti-hypertensive

and antioxidative functions to prevent the pathogenesis of cardiovascular disease through endothelial dysfunction.

Melatonin Effects on the Autonomic Nervous System

The beneficial effects of melatonin in the vascular system are through the inhibition of alpha-receptors during sympathetic activation and stimulate beta-receptors causing increases in parasympathetic activity. There are MT1 and MT2 receptors found in the paraventricular nucleus (PVN) and suprachiasmatic nuclei (SCN) in the hypothalamus, these structures connect to premotor sympathetic neurons in the rostral ventrolateral medulla (RVLM), which has extensive projections to sympathetic preganglionic cell bodies in the intermediolateral cell column (Baker & Kimpinski). Gamma aminobutyric acid (GABA) signaling is how central sympathetic structures are inhibited, this is shown through animal model studies where injections of melatonin decreased the release of sympatho-excitatory glutamate and increased the release of sympathoinhibitory GABA which led to decreases in MAP (Baker & Kimpinski; Xia et al., 2008). In the study by Girouard et al., 2003, they investigated melatonin as an antioxidant treatment that resulted in decreased norepinephrine (NE) concentrations to healthy levels, normalized NE release, and normalized beta-adrenoreceptor sensitivity in spontaneously hypertensive rats (SHR). They suggested that the altered NE concentrations and beta-adrenoreceptor function is due to enhanced oxidative stress in the SHR model. Melatonin has also been found to decrease sympathetic nervous activity in response to orthostatic stress from a lower body negative pressure machine in healthy humans (Ray, 2003). The effects of sympathetic inhibition can be observed in human models where melatonin supplementation ranging from 1 mg to 3 mg results in decreased MAP, NE, dopamine, blood pressure and

pulsatility index (Cagnacci et al., 1997; Cagnacci et al., 1998; Cagnacci et al., 2001; Nishiyama et al., 2001; Arangino et al., 1999).

Not only is sympathetic activity inhibited, but parasympathetic activity is stimulated and increases with melatonin supplementation. In rats, melatonin decreases serotonin release which leads to an increase in parasympathetic activation, causing decreased heart rate and arterial pressure (Chuang et al., 1993). Acetylcholine (Ach) production, which is an important neurotransmitter in the parasympathetic nervous system, was restored in older rats with melatonin, further providing evidence that melatonin increases PNS activity (Brusco et al., 1998). In a study with healthy humans, melatonin decreased blood pressure, NE, and dopamine while increasing PNS parameters of heart rate variability (HRV) indicating that melatonin increases cardiac vagal tone (Nishiyama et al., 2001). Melatonin increases parasympathetic activity through regulating the release of neurotransmitters that attach to beta-receptors in the vasculature inducing vasodilation. Overall, melatonin inhibits sympathetic activity and stimulates parasympathetic activity through regulation of neurotransmitters and sensitivity of alpha- and beta- receptors.

Melatonin and Clinical Implications

Sleep

The most well known function of melatonin is regulation of the sleep cycle through chronobiological functions, the highest secretion is at night and the lowest secretion is during the day (Sun et al., 2016). Sleep is important for maintaining hormonal circadian rhythm and for immune functions carried out in the body (Cappuccio et al., 2010). Insufficient sleep has been associated with metabolic dysfunction, obesity, diabetes, and development of cardiovascular

diseases (Buxton & Marcelli, 2010). There is a U-shaped curve of increased risk of cardiovascular morbidity and mortality where short sleep duration is less than 5 hours and long sleep duration is more than 8 hours per night (Cappuccio et al., 2010). The mechanisms behind these associations are not fully elucidated, although low grade inflammation occurs during short sleep duration which increases risk for chronic conditions, and for long sleep duration comorbidities such as depression or other chronic diseases contribute to the increased risks of cardiovascular mortality (Cappuccio et al., 2010). Disturbances in circadian rhythm of melatonin secretion reduces its antioxidant and anti-inflammatory effects in the body (Prado et al., 2018). These rhythms are synchronized by light and dark cycles and ensure correct physiological processes are carried out, disruptions that occur are usually from lifestyle changes of increased wakefulness and artificial light (Szewczyk-Golec et al., 2015). Dysregulation of melatonin can lead to increased risk for developing cardiovascular and chronic diseases.

Hypertension and Cardiovascular Disease

The improper regulation of melatonin can cause hypertension and other cardiovascular diseases due to decreased concentration. Patients with coronary artery disease (CAD) were found with significantly lower levels of melatonin than the healthy control group (Brugger et al., 1995; Arangino et al., 1999). The risk of developing comorbidities such as metabolic syndrome or other cardiovascular diseases is increased after development of hypertension (Fiuza-Luces et al., 2018). Hypertension occurs in pinealectomized rats which was reversed with exogenous melatonin supplementation (Holmes & Sugden, 1975; Cunnane et al., 1980; Simko & Paulis, 2007; Reiter et al., 2009). A study with rats that developed metabolic syndrome from high fructose diet, resulted in decreased melatonin secretion and hypertension development, but with exogenous melatonin supplementation hypertension was eliminated (Leibowitz et al., 2008).

Another study with type 2 diabetic rats showed that melatonin intake with exercise decreased blood pressure and improved hyperglycemia due to a decrease in proinflammatory cytokines and improved antioxidant function compared to diabetic rats with no melatonin supplementation (Rahman et al., 2017). When comparing melatonin with the ACE-inhibitor captopril, melatonin reduced SBP better than an ACE-inhibitor in rats with hypertension from constant light exposure (Simko et al., 2014). Melatonin treatment with hypertensive rats reduced oxidative load, improved NO availability through increased eNOS expression, and reduced blood pressure (Simko et al., 2009). Supplementation with melatonin increases antioxidant and anti-inflammatory properties and vasodilatory stimuli that help regulate vascular to prevent the development and progression of hypertension. These studies provide evidence that melatonin plays a role in the pathogenesis of hypertension and can be used as a preventative measure for the development of other cardiovascular diseases. (Simko & Paulis, 2007).

Conclusion

Melatonin plays more roles in the body than just circadian rhythm and sleep regulation, from the finding of melatonin receptors in the systemic circulation. These roles include anti-hypertensive, anti-inflammatory, and antioxidant functions throughout the body through improvements in vascular function and oxidative stress. While the exact mechanism behind these roles has yet to be fully understood, there is evidence that the dysregulation of melatonin contributes to the pathogenesis of cardiovascular diseases. Exercise is important for treatment and prevention of cardiovascular diseases, by improving vascular function and blood pressure. Melatonin could be another potent vasodilator due to its effects on decreasing sympathetic activity and increasing parasympathetic stimulation. However, there are not a lot of studies on the combined effects of exercise and melatonin in humans.

Chapter III

Methodology

The purpose of this study was to determine if acute melatonin supplementation changes central and peripheral responses to sympathetic stimuli. We hypothesized that melatonin will inhibit alpha-receptors during forearm exercise and stimulus from LBNP, therefore increasing blood flow, vascular conductance, and blood oxygenation in exercising muscle. This chapter discusses ethical approval, participants recruited, inclusion and exclusion criteria, overall study design, description of materials and equipment used, threats to validity, and data processing and analyses.

Ethical Approval

This study was approved by the Institutional Review Board at the University of Oklahoma Health Sciences Center (IRB #13084). All participants were thoroughly informed of the experimental protocol and procedures of this experiment and provided signed informed consent documents before participating in the study.

Participants

Before we started recruitment, a power analysis was performed to estimate a total sample size of 8 subjects to achieve an estimated power of 0.95 for statistical analyses. We recruited a total of 8 participants, 5 males and 3 females. The participants we recruited were young healthy subjects between the ages of 18 to 35. Recruiting methods for subjects included posting flyers around the Norman campus of the University of Oklahoma and through word of mouth. Individuals that were recruited for the study were enrolled after meeting all inclusion criteria and none of the exclusion criteria.

Inclusion Criteria

- Males and Females between the ages of 18-35 years.
- Individuals with resting systolic blood pressure <130 mmHg, and diastolic blood pressure <85 mmHg.
- No known cardiovascular or metabolic diseases.
- Individuals free of autonomic dysfunction.
- Females are premenopausal and have a regular menstrual cycle (26-30 days).
- Individuals under waist circumference cutoffs (Males <102 cm, Females <88 cm).

Exclusion Criteria

- Individuals with history of autonomic dysfunction.
- Individuals with cardiovascular or metabolic disease.
- Subjects who use any form of tobacco/nicotine.
- Individuals with blood pressure ≥ 130/85.
- Individuals taking sex hormone replacement (e.g. testosterone, estrogen, progesterone).
- Subjects with regular melatonin use (≥ 1 use/week).
- If individuals have an allergy to melatonin.

Study Design

Our research design consisted of a single blind, randomized, crossover study design. Participants came in for a total of three study visits. Visit one consisted of informed consent, collection of demographic information, eligibility determination, and familiarization of the experimental procedure. Visits two and three consisted of experimental visits where subjects

were instrumented with equipment and then given either melatonin or a placebo and completed the experimental protocol that was shown during the first visit.

Experimental Protocol

The purpose of visit one was to determine subject eligibility as well as familiarize them with the experimental protocol. Before being enrolled into the study, subjects were informed of the risks and benefits and then sign an IRB approved informed consent document as well as a Health Insurance Portability and Accountability Act (HIPPA) authorization form. After that subjects completed a health history questionnaire to help determine eligibility, the International Physical Activity Questionnaire (IPAQ) to determine the subject's physical activity level, and the Pittsburgh Sleep Quality Index (PSQI) questionnaire to determine the subject's sleeping habits. Once all paperwork was completed, a pregnancy test was completed (for females), then subjects laid down for 10 minutes before blood pressure measurements were taken. Then anthropometric data was measured to ensure subjects met the inclusion criteria and none of the exclusion criteria. Anthropometric data recorded was height, weight, waist and hip circumference, we also recorded arm dominance, and maximal handgrip strength of the non-dominant arm. Once eligibility was confirmed for the study, subjects then had a dual-energy x-ray absorptiometry (DXA) scan completed to assess body composition. Finally, subjects were then familiarized with the experimental protocol including being fit into the LBNP device, practicing handgrip exercise with the metronome, and a brief recording of the brachial artery velocity and diameter using the ultrasound machine to screen for bifurcation of the brachial artery.

For the second and third experimental visits, subjects were given either melatonin (Onnit, Austin, TX) or the placebo (one microliter of McCormicks Pure Mint extract and 29 mL of

filtered water), and then switched for the next experimental visit. Upon subject arrival, subjects gave verbal confirmation that all pre-testing guidelines were adhered to (abstaining from NSAIDs and vigorous exercise for 24 hours, caffeine for 12 hours, were fasted for 8 hours, and if there were no new medical conditions since last visit). After confirming all pre-testing guidelines were adhered to, subjects were reviewed the experimental protocol again before beginning instrumentation. Once the protocol was reviewed, subjects were then instructed to use the restroom and put on the Equivital vest (Equivital, New York, NY). Then instrumentation began by first having subjects sit in a chair with a hand warmer in their dominant hand. Subjects were then given 10 sublingual sprays of either melatonin (Onnit, Austin, TX) or placebo (one microliter of McCormicks Pure Mint extract and 29 mL of filtered water). Time of ingestion was recorded, and the lights were then turned off before fitting the VO2 mask (Hans Rudolph, Shawnee, KS) on the subject. Once the mask is secured on the subject's head, they are then carefully moved to a supine position in the LBNP machine. We then finished instrumentation by adding a Finapres finger cuff device (Finapres Medical Systems, Enschede, Netherlands) and blood pressure cuff on the dominant arm. On the non-dominant arm, we added a NIRS device, the OxiplexTS (ISS, Champaign, IL), on the belly of the flexor digitorum profundus muscle, and a Hokanson cuff (Hokanson, Bellevue, WA) on the upper arm. During the experimental protocol, we also performed an ultrasound (GE Healthcare, Madison, WI, USA) of the brachial artery on the non-dominant arm. Lastly, we added a small gas analyzer tube and gas collection tube to the mask going through a gas analyzer (Gemini, ADInstruments, Colorado Springs, CO), and SpO2 monitor attached to the subject's finger of their dominant hand.

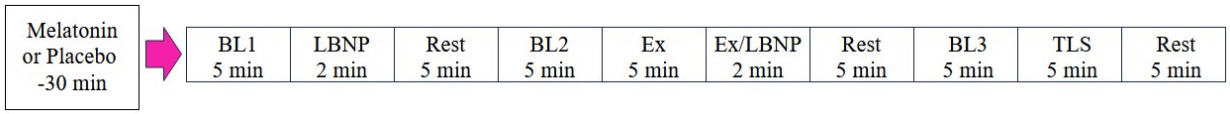


Figure 1: Experimental protocol during visits two and three, baseline (BL), lower body negative pressure (LBNP), exercise (Ex), exercise with LBNP (Ex/LBNP), total liable signal (TLS).

Once instrumentation was finished, the subject rested until it had been 30 minutes since the time of ingestion. The experiment started with baseline measurements for 5 minutes with the subject resting. Next the LBNP was turned on for 2 minutes at –20 mmHg, then rested for 5 minutes before another 5-minute baseline was taken. Then subjects began 7 minutes of continuous handgrip exercise at 20% of their maximal voluntary contraction at a rate of 20 contractions per minute. During the last two minutes of exercise the LBNP was turned back on at –20 mmHg until the end of the exercise period. After that, another 10-minute rest period followed and finally there was a 5-minute occlusion. After the occlusion, 5 minutes of rest was given to the subject to ensure levels returned to normal. Once the protocol was finished, the subject was de-instrumented and the next visit was scheduled.

Instrumentation

- Oxiplex TS (ISS, Champaign, IL): Wired Near-Infrared Spectroscopy (NIRS) measuring forearm muscle oxygenation (TSI%) on the flexor digitorum profundus of the non-dominant arm.
- Equivital Life Monitor (Equivital, New York, NY): Measures heart rate (HR), and respiratory rate (RR) with fitted vest.

- Finapres (Finapres Medical Systems, Enschede, Netherlands): Non-invasive finger photoplethysmography that measures mean arterial blood pressure (MAP), cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR).
- Handgrip dynamometer (Takei, Tokyo, Japan): measures maximal voluntary contraction (MVC) of the non-dominate hand by taking the highest trial value out of 3 maximal squeezes and calculating 20% of their MVC.
- Ultrasound (GE Healthcare, Madison, WI, USA): measures forearm blood flow of the brachial artery.
- Hans Rudolph mask (Hans Rudolph, Shawnee, KS): Mask that covers the subject's nose and mouth and collects gas that goes through Gemini gas analyzer.
- Hokanson cuff (Hokanson, Bellevue, WA): Cuff that goes on the upper arm and is used for blood flow occlusion. The pressure was set to 230 mmHg during this stage.
- Lower Body Negative Pressure (LBNP)(Techavance Inc, Austin, TX, USA): Device that induces sympathetic stimulus with subject being sealed into the chamber that is connected to a vacuum producing a negative pressure at –20 mmHg.
- Dual Energy X-Ray Absorptiometry (DXA) (GE Lunar, Madison, WI): Method for measuring total body composition by x-rays moving through tissues, and will be used for specifically analyzing forearm fat free mass of the non-dominant forearm with a custom region of interest (ROI) analysis.

Threats to Validity

Threats to internal validity included technician error and equipment malfunction. To control for these threats, we ensured that each technician was properly trained before data collection began. This included going over how to properly use all equipment and knowing how

to instrument each subject. Lastly, all technicians were reviewed the protocol and knew what events occurred at important time points. For this specific experiment, checklists were created for each piece of equipment with detailed instructions and reminders of important time-points. This reduced inconsistency errors and ensured consistent data collection with each subject. To avoid equipment malfunction, all equipment that was used was properly calibrated and ensured it was functioning properly before data collection began. Technicians came in before subject arrival to perform calibration and check all equipment functions. A threat to external validity could come from data not being applicable to other populations. This study tested healthy young adults and results might not be applied to older or special populations.

Data Processing

All data collected was recorded and stored in the software LabChart (ADInstruments, CO, USA) and was numerical and continuous. Cardiovascular variables, LBNP pressure, forearm muscle oxygenation and pulse velocity data were all collected through Power Lab (ADInstruments, CO, USA) that fed into LabChart. Blood velocity waveforms collected from the ultrasound on the upper arm were collected continuously with a qDAT audio converter in to PowerLab (Limberg et al., 2020). The cardiovascular variables and blood oxygenation data collected through LabChart were averaged beat by beat then transferred to an Excel file that processed 3 second bins for data analysis. Hemodynamic data from the forearm was averaged in the last 30 seconds of each stage except for the Rest with LBNP stage which was averaged between minute 1:30 and 2:00. Brachial artery diameter collected from ultrasound was collected through a capture card and the video was processed through cutting the video into small clips of each stage needed for analysis. Then each video was processed through a brachial analyzer software that calculated the diameters needed for data analysis. The following equations were

used to calculate forearm blood flow (FBF), forearm vascular conductance (FVC), and percent functional sympatholysis (%FS).

FBF (ml/min)= mean blood velocity x 60 x π x (brachial diameter/2)^2

FVC (ml/min/100mmHg)= (FBF/MAP) x 100mmHg

FBF or FVC- Rest-LBNP (Δ%): ([LBNP – rest]/rest) x 100
FBF or FVC- Rest-Exercise (Δ%): ([Ex – rest]/rest) x 100
FBF or FVC- Ex-Ex+LBNP (Δ%): ([Ex+LBNP – exercise]/ exercise) x 100

%FS= ((ΔHbO2 (%TLS rest) - ΔHbO2 (%TLS exercise)) / (ΔHbO2 (%TLS rest) x 100

ΔHbO2 (%TLS rest) = (ΔHbO2 (rest) - ΔHbO2(rest and LBNP)) / (ΔHbO2 (max) - ΔHbO2 (nadir)) x 100

ΔHbO2 (%TLS exercise) = (ΔHbO2 (exercise) - ΔHbO2 (exercise and LBNP)) / ((ΔHbO2 (max) - ΔHbO2 (nadir)) x 100

Data Analyses

Collected data was downloaded from LabChart and analyzed using SPSS statistics, version 28.0 (IBM SPSS, Armonk, NY). The statistical tests that were used for this study were paired samples t-tests comparing differences in raw and Δ cardiovascular variables between melatonin and placebo conditions during experimental stages baseline, LBNP, exercise, and exercise with LBNP. Paired samples t-tests were also used to compare differences in raw and Δ vascular hemodynamic variables between melatonin and placebo conditions during experimental stages baseline, LBNP, exercise and exercise with LBNP. Paired samples t-tests were used to determine the differences in Δ% FBF, Δ%FVC and %FS between melatonin and placebo conditions. Normality was tested for all variables with the Shapiro-Wilks test and the Wilcoxon non-parametric test was used as alternative for paired t-tests. Cohen's *d* was used for estimating

effect sizes for normally distributed data, and the wilcoxon effect size (r) was used for estimating effect sizes for not normally distributed data. The level of significance was set at $p \leq 0.05$.

Chapter IV

Results

Subject Characteristics

We had a total of 8 participants (5 males, 3 females) finish all three visits. There were 5 participants that did not return after visit 1 or 2, and 3 participants that failed to react to the sympathetic stimuli of the study. All subject characteristics labeled in Table 1 are of young healthy adults with no known cardiovascular or metabolic diseases, non-obese (BMI<30), non-smokers, and did not have regular melatonin use. Of the 8 participants, 6 identified as right hand-dominant and used their left hand for exercise and vice versa for the 2 left-hand dominant individuals.

Table 1: Subject Characteristics

	Total (n=8)	**Men (n=5)**	**Women (n=3)**
Age (yrs)	22.5±2.83	23.60±3.13	20.67±0.58
Height (cm)	177.50±8.59	181.60±8.53*	170.67±0.58*
Weight (kg)	78.41±11.16	84.48±7.97	68.3±7.96
BMI (m2/kg)	24.79±2.08	25.60±1.37	23.43±2.65
Waist circumference (cm)	82.26±7.88	85.40±7.03	77.03±7.27
Hip Circumference (cm)	104.50±4.05	105.70±3.55	102.50±4.77
IPAQ (MET min/week)	4997.88±3336.10	3434.00±2515.00	7588.33±3181.69
PSQI	6.75±2.71	8.00±2.74*	4.67±0.58*
MVC (kg)	41.45±13.41	50.56±6.13*	26.27±1.17*
MVC (20%)	8.29±2.69	10.12±1.22*	5.23±0.15*
SBP (mmHg)	114.13±7.88	118.40±6.77*	107.00±2.00*
DBP (mmHg)	69.13±2.64	68.60±3.29	70.00±1.00
HR (bpm)	58.13±8.41	58.60±5.55	57.33±13.58
FFFM (g)	1001.25±372.77	1236.80±209.11*	608.67±170.35*

*Values are displayed as mean ± SD. *stands for significant difference between men and women when p≤0.05. BMI= Body Mass Index, IPAQ= International Physical Activity Questionnaire, PSQI= Pittsburgh Sleep Quality Index, MVC= Max Voluntary Contraction, SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, HR= Heart Rate, FFFM= Forearm Fat Free Mass*

Central Cardiovascular Variables

Raw Values

The data for raw central cardiovascular variables shown in Table 2, displays no significant differences for cardiac output (CO) between conditions at rest (p=0.48, r=0.18), LBNP (p=0.48, r=0.18), Exercise (p=0.89, r=0.04), or Exercise with LBNP (p=1.00, r=0.00). There were also no significant differences for stroke volume (SV) between conditions at rest (p=0.48, r=0.18), LBNP (p=1.00, r=0.00), Exercise (p=0.78, r=0.07), or Exercise with LBNP (p=0.58, r=0.14). No significant differences were found for total peripheral resistance (TPR) between conditions at rest (p=0.58, r=0.14), LBNP (p=0.58 r=0.14), Exercise (p=0.89, r=0.04), or Exercise with LBNP (p=1.00, r=0.00). There was a significant decrease found in heart rate (HR) in the melatonin condition compared to placebo during Exercise (p=0.03, r=0.56) and Exercise with LBNP (p=0.05, r=0.49), but not at rest (p=0.67, r=0.11) or LBNP (p=0.33, r=0.25).

Table 2: Raw Central Cardiovascular Variables

Variable	Rest	LBNP	Exercise	Exercise+LBNP
CO (L/min)				
PLA	6.30±1.50	6.14±1.50	7.21 ±1.23	6.74±1.29
MEL	6.60±2.21	6.03±1.70	7.96±3.10	7.14±2.44
SV (mL)				
PLA	96.03±22.68	83.02±19.96	102.80±31.08	87.28±24.61
MEL	96.06±17.42	83.64±16.27	102.08±21.68	91.02±23.24
TPR				
PLA	99.43±26.71	104.16±25.52	102.00±31.57	112.70±35.43
MEL	97.77±16.17	100.84±17.05	102.16±18.53	112.11±23.33
HR (BPM)				
PLA	65.61±8.24	70.41±9.44	74.58±10.09*	78.08±11.76*
MEL	62.55±7.78	66.09±9.14	68.69±7.79*	71.06±9.34*

*Values are displayed as mean±SD. *stands for significant difference between conditions when p≤0.05. MEL= Melatonin, PLA= Placebo, LBNP= Lower Body Negative Pressure stimulus at –20 mmHg,*

Exercise= handgrip exercise at 20% of MVC, Exercise+LBNP= handgrip exercise with LBNP stimulus at –20 mmHg stimulus.

Delta Values

The Δ values for central cardiovascular variables shown in Table 3 display no significant differences for ΔRest-LBNP between conditions for CO (p=0.24, *d*=0.46), SV (p=0.83, *d*=0.08), TPR (p=0.65, *d*=0.17), or HR (p=0.72, *d*=0.13). There were also no significant differences found for ΔEx-ExLBNP between conditions for CO (p=0.31, *d*=0.39), SV (p=0.27, *d*=0.42), TPR (p=0.82, *d*=0.09), or HR (p=0.73, *d*=0.13).

Table 3: Central Cardiovascular Δ Values

Variable	ΔRest-LBNP	ΔEx-ExLBNP
CO (L/min)		
PLA	-0.57±0.71	-0.81±0.84
MEL	-0.16±0.34	-0.47±0.30
SV (mL)		
PLA	-13.01±8.72	-15.52±12.24
MEL	-12.42±7.37	-11.05±5.24
TPR		
PLA	4.73±9.22	10.70±12.66
MEL	3.07±5.41	9.94±7.20
HR (BPM)		
PLA	4.79±5.00	3.50±6.82
MEL	3.54±7.49	2.37±3.80

*Values are displayed as mean±SD. *stands for significant difference between conditions when p≤0.05. MEL= Melatonin, PLA= Placebo, LBNP= Lower Body Negative Pressure stimulus at –20 mmHg, Ex= handgrip exercise at 20% of MVC, ExLBNP= handgrip exercise with LBNP stimulus at –20 mmHg stimulus.*

Vascular Hemodynamic Variables

Raw Vascular Variables

The data for raw vascular hemodynamic variables are shown in Table 4, displays a significant increase of brachial artery diameter in the melatonin condition at rest ($p=0.05$, $r=0.50$) but not during LBNP ($p=0.36$, $r=0.23$), Exercise ($p=0.40$, $r=0.21$), or Exercise with LBNP ($p=0.40$, $r=0.21$). There were no significant differences for brachial artery velocity between conditions at rest ($p=0.21$, $r=0.32$), LBNP ($p=0.16$, $r=0.35$), Exercise ($p=0.40$, $r=0.21$), or Exercise with LBNP ($p=0.40$, $r=0.21$). There were also no significant differences in forearm blood flow (FBF) between conditions during rest ($p=0.33$, $r=0.39$), LBNP ($p=0.21$, $r=0.32$), Exercise ($p=0.89$, $r=0.04$), or Exercise with LBNP ($p=0.48$, $r=0.18$). For mean arterial pressure (MAP) between conditions there were no significant differences found during rest ($p=0.78$, $r=0.07$), LBNP ($p=1.00$, $r=0.00$), Exercise ($p=0.78$, $r=0.07$), or Exercise with LBNP ($p=0.89$, $r=0.04$). Lastly, there were no significant differences found for forearm vascular conductance (FVC) between conditions at rest ($p=0.33$, $r=0.25$), LBNP ($p=0.26$, $r=0.28$), Exercise ($p=0.67$, $r=0.11$), or Exercise with LBNP ($p=0.58$, $r=0.14$).

Table 4: Raw Vascular Hemodynamic Variables

Variables	Rest	LBNP	Ex	Exercise+LBNP
Diameter (mm)				
PLA	3.93±0.73*	3.85±0.72	3.96±0.73	3.96±0.63
MEL	3.99±0.74*	3.93±0.72	4.02±0.77	4.03±0.79
Velocity (cm/s)				
PLA	5.10±2.78	3.55±1.96	29.05±9.02	29.14±9.05
MEL	6.63±3.82	5.30±3.39	24.99±9.35	26.62±8.73
FBF (mL/min)				
PLA	37.75±21.62	25.06±14.47	217.70±112.41	224.48±119.69
MEL	46.77±22.41	37.01±23.16	213.99±148.39	229.46±147.33
MAP (mmHg)				
PLA	101.14±14.16	99.78±14.68	120.99±12.27	123.00±10.13
MEL	99.25±8.68	99.22±10.32	120.80±12.62	123.06±12.46
FVC (mL/min/100mm Hg)				
PLA	37.43±21.00	25.44±14.94	180.47±93.19	180.95±88.93
MEL	44.12±21.40	37.50±25.44	179.40±119.93	188.67±119.73

*Values are displayed as mean±SD. *stands for significant difference between conditions when p≤0.05. MEL= Melatonin, PLA= Placebo, MAP= Mean Arterial Pressure FBF= Forearm Blood Flow, FVC= Forearm Vascular Conductance, LBNP= Lower Body Negative Pressure stimulus at –20 mmHg, Exercise= handgrip exercise at 20% of MVC, Exercise+LBNP= handgrip exercise with LBNP stimulus at –20 mmHg stimulus.*

Delta Values

The Δ values for vascular hemodynamic variables displayed in Table 5, show no significant differences for ΔBaseline-LBNP between conditions for Diameter (p=1.00, r=0.00), Velocity (p=0.89, r=0.04), FBF (p=0.67, r=0.11), MAP (p=0.48, r=0.18), or FVC (p=0.26, r=0.28). There were also no significant differences for ΔExercise-Exercise with LBNP between conditions for Diameter (p=0.67, r=0.11), Velocity (p=0.48, r=0.18), FBF (p=0.67, r=0.11), MAP (p=0.58, r=0.14), or FVC (p=0.40, r=0.21).

Table 5: Vascular Hemodynamic Δ Variables

Variables	Δ Rest-LBNP	Δ Ex-ExLBNP
Diameter (mm)		
PLA	-0.09±0.19	0.00±0.24
MEL	-0.07±0.14	0.02±0.06
Velocity (cm/s)		
PLA	-1.54±1.38	0.09±3.24
MEL	-1.32±2.20	1.63±4.57
FBF (mL/min)		
PLA	-12.69±9.22	6.77±27.11
MEL	-9.76±14.12	15.47±43.83
MAP (mmHg)		
PLA	-1.36±2.12	2.01±6.13
MEL	-0.03±2.31	2.25±2.59
FVC (mL/min/100mmHg)		
PLA	-11.99±8.92	0.47±23.01
MEL	-6.62±13.75	9.27±34.24

*Values are displayed as mean±SD. *stands for significant difference between conditions when p≤0.05. MEL= Melatonin, PLA= Placebo, MAP= Mean Arterial Pressure, FBF= Forearm Blood Flow, FVC= Forearm Vascular Conductance, LBNP= Lower Body Negative Pressure stimulus at –20 mmHg, Ex= handgrip exercise at 20% of MVC, ExLBNP= handgrip exercise with LBNP stimulus at –20 mmHg stimulus.*

Forearm Blood Flow as Δ%changes

Rest with LBNP

The Δ%change of FBF during rest and LBNP decreased similarly with no significant differences between conditions (PLA: -31.36±10.71%, MEL: -26.36±31.44%) (p=0.58, r=0.14) with a stimulus of LBNP at –20 mmHg.

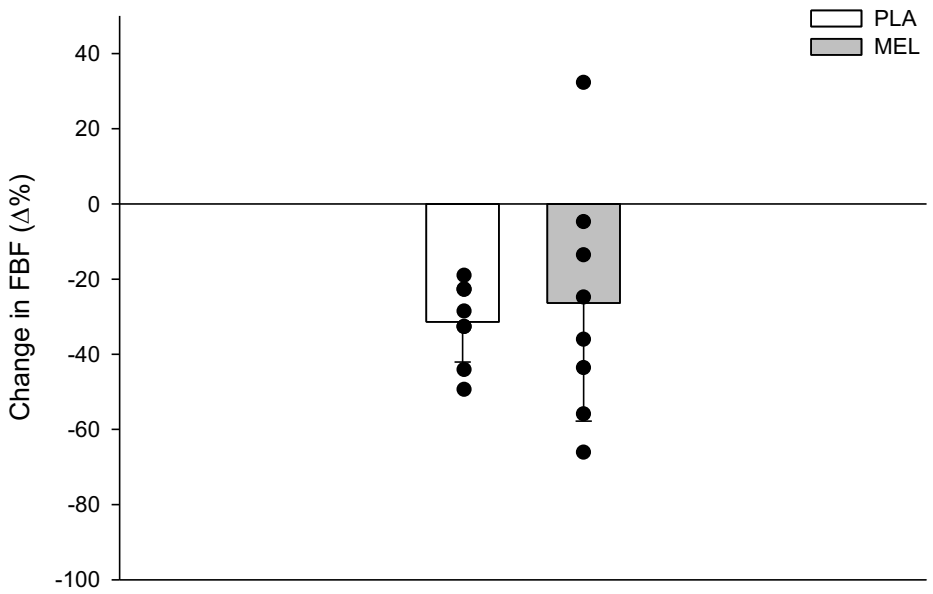


Figure 2: Group mean Δ %changes ±SD with individual data points in forearm blood flow for PLA and MEL conditions from rest to LBNP at –20 mmHg.

Exercise

The Δ%change of FBF during rest to Exercise increased similarly with no significant differences between conditions (PLA: 1104.42±1820.72%, MEL: 498.33±537.73%) (p=0.26, r=0.28) with rhythmic handgrip exercise at 20% of the participants' MVC.

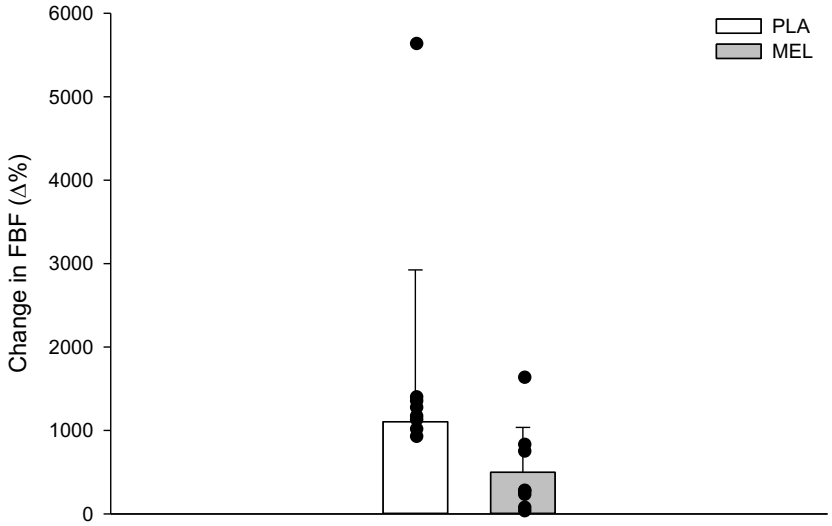


Figure 3: Group mean %changes ±SD with individual data points in forearm blood flow for PLA and MEL conditions from rest to exercise.

Exercise with LBNP

The Δ%change of FBF during exercise to exercise with LBNP increased similarly with no significant differences between conditions (PLA: 2.63±16.90%, MEL: 10.16±23.99%) (p=0.67, r=0.11) with rhythmic handgrip exercise at 20% of the subject's MVC in addition to LBNP stimulus at –20 mmHg.

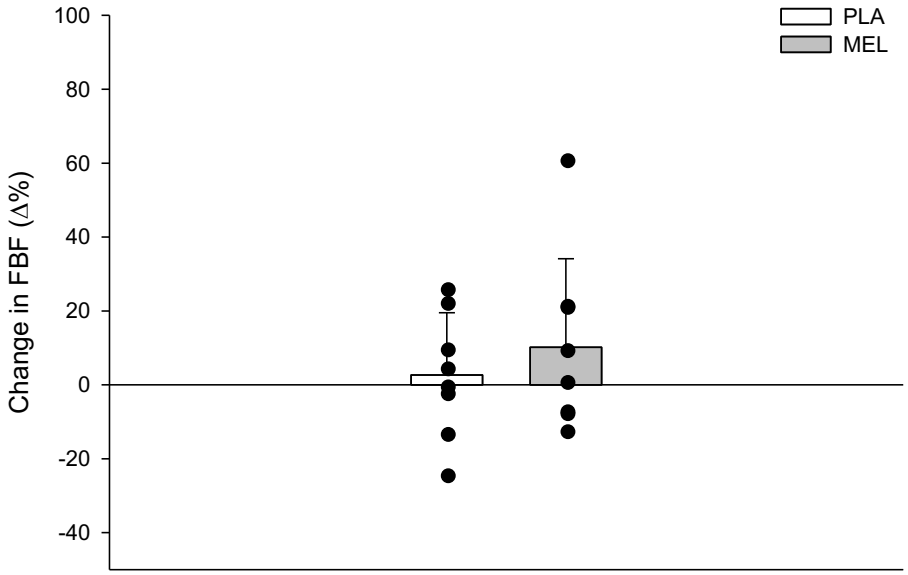


Figure 4: Group mean %changes ±SD with individual data points in forearm blood flow for PLA and MEL conditions from exercise to exercise with LBNP.

Forearm Vascular Conductance

Rest with LBNP

The Δ%change of FVC during rest and LBNP decreased similarly with no significant differences between conditions (PLA: -30.39±10.60%, MEL: -21.76±32.53%) (p=0.40, r=0.21) with a stimulus of LBNP at –20 mmHg.

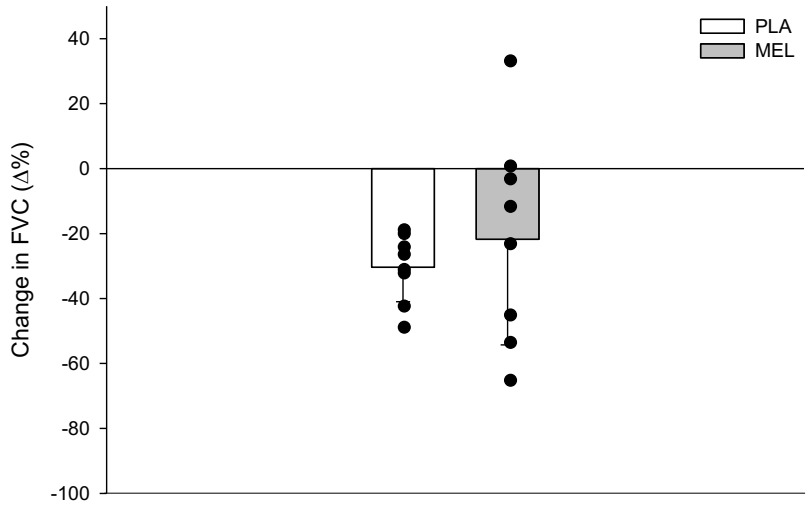


Figure 5: Group mean %changes ±SD with individual data points in forearm vascular conductance for PLA and MEL conditions from rest to LBNP at –20 mmHg.

Exercise

The Δ%change of FVC during rest to Exercise increased similarly with no significant differences between conditions (PLA: 860.79±1409.36%, MEL: 428.54±465.41%) (p=0.58, r=0.14) with rhythmic handgrip exercise at 20% of the participants' MVC.

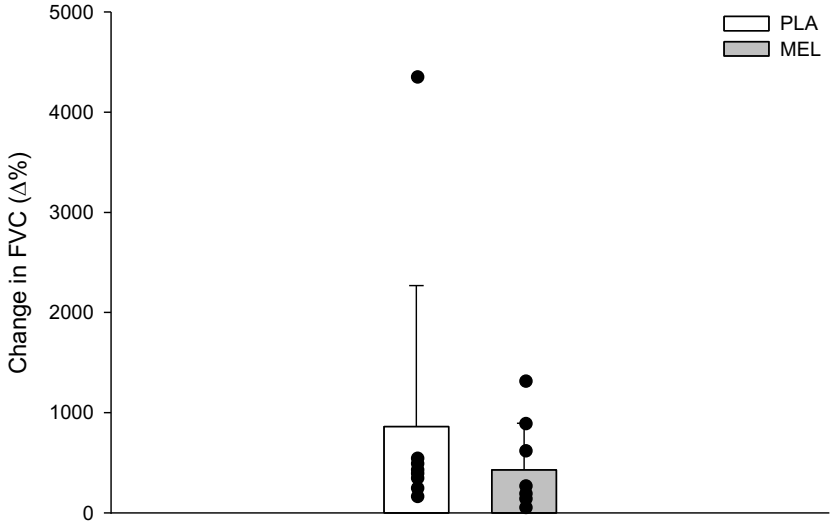


Figure 6: Group mean %changes ±SD with individual data points in forearm vascular conductance for PLA and MEL conditions from rest to exercise.

Exercise with LBNP

The Δ%change of FVC during exercise to exercise with LBNP increased similarly with no significant differences between conditions (PLA: 0.60±15.25%, MEL: 8.14±23.54%) (p=0.40, r=0.21) with rhythmic handgrip exercise at 20% of the subject's MVC in addition to LBNP stimulus at –20 mmHg.

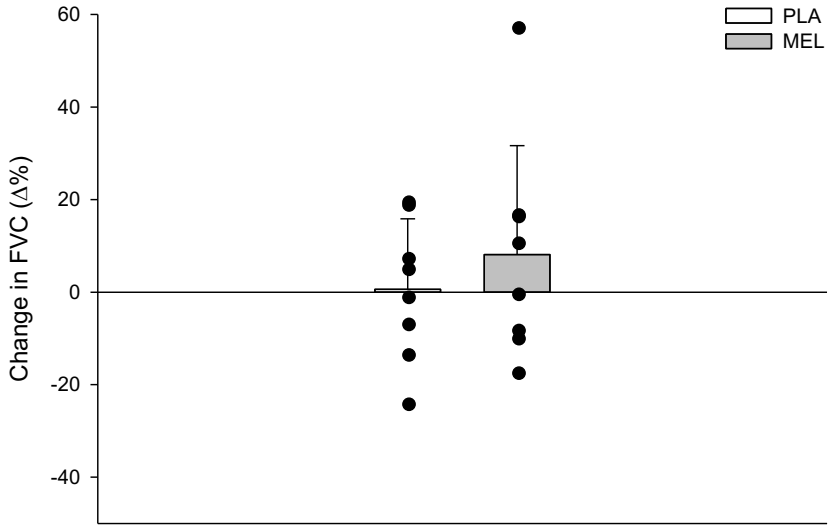


Figure 7: Group mean %changes ±SD with individual data points in forearm vascular conductance for PLA and MEL conditions from exercise to exercise with LBNP.

Functional Sympatholysis

The calculated %FS from oxyhemoglobin concentrations have no significant differences between conditions (PLA: 64.94±89.05%, MEL: 10.70±80.63%) (p=0.25, *d*=0.44). Figure 8 displays the average percent oxyhemoglobin of total liable signal (TLS) throughout the experimental protocol for each condition which is used for the %FS calculation.

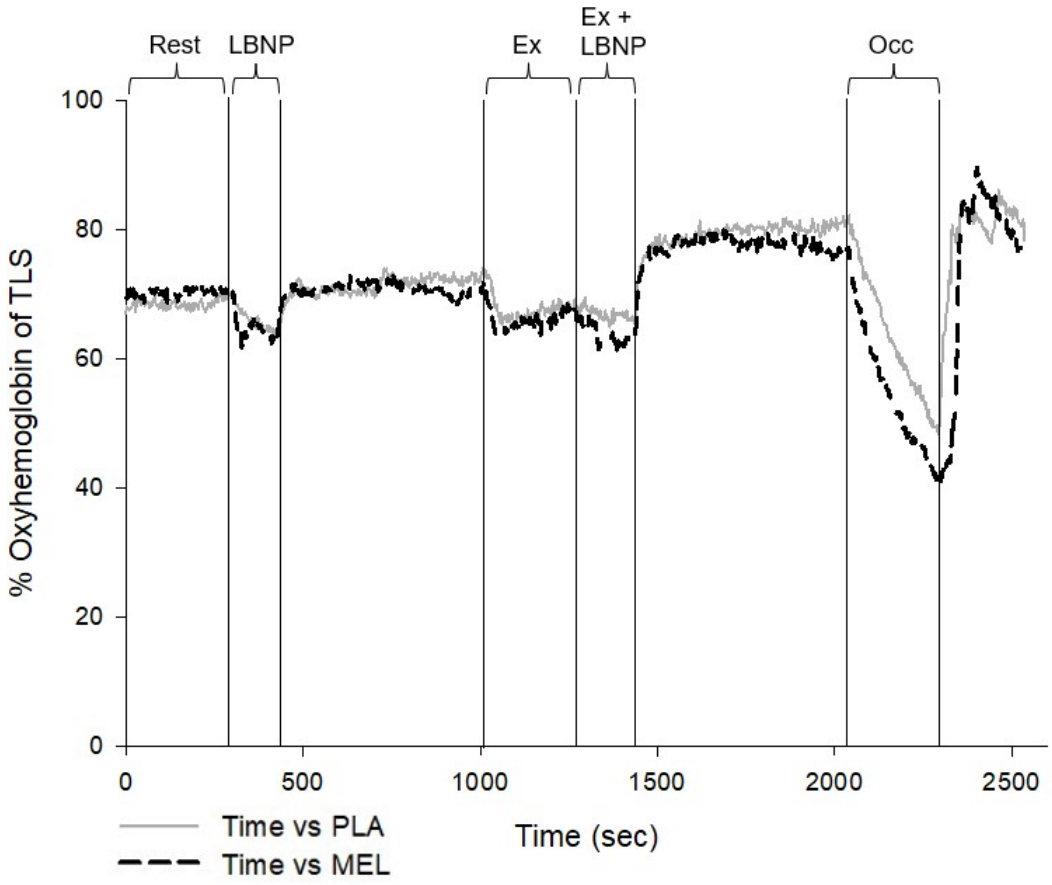


Figure 8: Tracing of average oxyhemoglobin concentration throughout experimental protocol for PLA and MEL conditions. LBNP= lower body negative pressure at -20 mmHg, Ex= rhythmic handgrip exercise, Ex+LBNP= rhythmic handgrip exercise with lower body negative pressure at -20 mmHg, Occ= occlusion at 230 mmHg.

Chapter V

Discussion

This study focused on the effects of acute melatonin supplementation in the cardiovascular system on sympathetic stimuli in healthy young adults. The main finding was that there were no significant differences found for forearm blood flow or forearm vascular conductance during mild hypovolemia, handgrip exercise or when combined simultaneously. However, it is observed that melatonin has an effect on decreasing heart rate during exercise and exercise with mild hypovolemia.

Cardiovascular Variables

For the central cardiovascular variables studied there were no significant differences found during baseline measurements between melatonin and placebo conditions (Table 2). These findings match the results where resting HR does not change during relaxation (Cagnacci et al., 1997; Nishiyama et al., 2001). There were also no significant differences found between any of the central cardiovascular variables during LBNP between conditions and when comparing baseline to LBNP (Tables 2 and 3). No differences observed during LBNP between conditions could be due to not having a big enough stimulus, this is seen in a study by Ray, 2003 where -10mmHg LBNP stimulus did not change HR but –40 mmHg showed a significant increase. A bigger sympathetic stimulus might be needed to fully investigate the effects of melatonin.

There were no significant differences found for the cardiovascular variables CO, SV, and TPR during only exercise and exercise with LBNP between either condition (Table 2). However, there were significant decreases found with HR in the melatonin condition compared to placebo during the exercise and exercise with LBNP stages (Table 2). There are also no differences found

in SV showing that this variable was maintained throughout sympathetic stimuli. Studies completed on melatonin supplementation in healthy humans have found that melatonin enhances heart rate variability (HRV) which supports the argument that melatonin plays a role in cardiac vagal tone by suppressing sympathetic activity (Nishiyama et al., 2001; Escames et al., 2012). Enhanced cardiac vagal tone would explain why a lower HR found in the melatonin condition during sympathetic stimuli in this study. The reason why there are not any significant differences seen with other variables could be due to the small sample size. Since there is a significant decrease seen in HR with melatonin, it is reasonable that with a bigger sample there could also be significant differences with other variables as well.

Vascular Hemodynamic Variables

There were no significant differences found for the vascular hemodynamic variables MAP, brachial artery velocity, FBF, or FVC during baseline between either condition. This contradicts previous data where there are decreases in MAP and velocity at rest after melatonin supplementation (Arangino et al., 1999; Yildiz et al., 2006). Although, there is a significant increase found in brachial artery diameter at rest during the melatonin condition. There are also no significant differences found for any of the vascular hemodynamic variables measured between either condition during the LBNP stage (Table 4). These results agree with a study from Ray, 2003 where they found no effects of melatonin on forearm blood flow or vascular resistance during two different stimuli at –10 mmHg and –40 mmHg. While our study used one stimulus at –20 mmHg for LBNP, we still had the same outcome, suggesting that a greater sympathetic stimulus might be needed.

For vascular hemodynamic variables measured during the exercise only and exercise with LBNP stages, there were no significant differences displayed between conditions. There are very

little studies on the effects of melatonin and exercise in healthy young individuals, since many of these studies are completed in rats with hypertension or other chronic diseases (Rahman et al., 2017; Qui et al., 2018). Within these studies, there is a hypotensive effect seen with an increase in vasorelaxation and improvements in oxidative stress with the combination of melatonin supplementation and exercise. These same effects observed in rats might not be transferrable to humans. It's also important to note that our study was conducted in healthy humans while previous studies are completed with a chronic disease model in rats, which leads to the suggestion that melatonin plays a role in endothelial dysfunction.

Implication of Work

There is a lack of research in melatonin supplementation and exercise in healthy humans, and while there were not a lot of significant differences found there are still implications that can still be taken from this study. With majority of melatonin studies completed either in patients with cardiovascular disease or in rats with a chronic disease model, the effects of melatonin can be observed through improving antioxidant, anti-inflammatory, and anti-hypertensive mechanisms in the body (Chitimus et al., 2020; Kozirog et al., 2011; Sun et al., 2016). These effects can be observed since patients with cardiovascular disease typically have lower amounts of melatonin secretion than healthy subjects and supplementing melatonin improves conditions (Yaprak et al., 2003; Sakotnik et al., 1999). This could explain why we do not observe any differences in healthy humans since there is a lack of dysfunction. Leading to the implication that melatonin does not work independently but through enhancing other functions in the body such as the NOS pathway (Girouard et al., 2001). This would explain how improvements can be observed in patients with cardiovascular disease and in rat models when supplemented with melatonin.

Limitations

There were a small amount of limitations found throughout data collection for this experimental protocol. Firstly, there were a few participants that were excluded due to high tolerance of the LBNP stimulus suggesting that the –20 mmHg was not a strong enough stimulus to generate a large sympathetic response. To avoid this in the future, a tolerance test should be given during the first visit to exclude subjects that are tolerant to the specific stimulus to ensure that all subjects respond appropriately (Fadil et al., 2023; Lightfoot & Tsintgiras, 1995; Rickards et al., 2011). There are confirmed studies that melatonin given as a sublingual spray increases melatonin concentration in the body however, due to the supplement having to go through the digestion system, the highest concentration of melatonin in the body could have been reached at a different time depending on the individual. We also did not test melatonin concentration before or after administering the supplement to ensure that there is an increased amount in the system. There was also not a direct measurement for sympathetic activity during the different sympathetic stimuli, which could have provided clearer answers instead of the indirect indicators from typical cardiovascular responses.

Lastly, our study reached the estimated sample size needed from the power analysis completed at the start of the study, however our low effect sizes prove that we need a bigger sample size to further assess results found in this study. This study also focuses on a young healthy population which our results might not be applicable to other populations to which melatonin studies typically focus on especially in patients with cardiovascular disease.

Future Directions

Our results indicate that we need a bigger sample size and should continue to recruit more subjects to fully research the main purpose of this study. With a bigger sample size, we will be able to achieve a higher power and will provide clearer results found from the data. This also opens the opportunity to study sex differences since there is a significant difference with women having better sleep quality scores than men and leaves reasonable room for an investigation. There should be continued studies on older populations and in patients with cardiovascular disease since there are more observed effects of melatonin supplementation within these populations that results remain to be fully elucidated.

Also in future considerations, using intravenous administration of supplementing melatonin will bypass digestion time for the body. As well as including pre- and post- testing of melatonin concentration after administration will ensure that an increase in concentration is achieved. Furthermore, there should be considerations for comparing melatonin testing either in the morning or later in the evening. Melatonin concentration fluctuates throughout the day and supplementation could have a bigger effect on sympathetic responses during exercise at different times of the day. Lastly, using MSNA (Muscle Sympathetic Nerve Activity) as a direct measurement of sympathetic activity would better answer the research questions that were asked in the present study.

Chapter VI

Conclusion

The present study found that acute melatonin supplementation does not inhibit alpha receptors or alter central and peripheral responses in healthy humans. Therefore, we reject the first hypothesis that melatonin supplementation inhibits alpha receptors through increases in forearm blood flow and vascular conductance and preserved muscle oxygenation during mild hypovolemia and exercise. We also reject the second hypothesis that melatonin decreases blood pressure, maintains stroke volume, and increases muscle oxygenation in the forearm during handgrip exercise in healthy individuals, however we do accept that melatonin decreases heart rate during handgrip exercise and when combined with LBNP.

In conclusion, we found that acute supplementation of melatonin does not affect cardiovascular variables during different sympathetic stimuli in young healthy humans. Melatonin plays a multitude of roles in the body with previous findings showing that low melatonin concentrations are associated with cardiovascular diseases. However, the mechanism behind how melatonin carries out these functions in the body has yet to be fully understood. In addition, there are also a lack of research surrounding the effects of melatonin and exercise in healthy humans. Further studies should be conducted to discover the function of melatonin which could be beneficial for prevention and therapeutic interventions for individuals with cardiovascular disease.

References

Abozguia, K., Phan, T. T., Shivu, G. N., Maher, A. R., Ahmed, I., Wagenmakers, A., & Frenneaux, M. P. (2008). Reduced in vivo skeletal muscle oxygen consumption in patients with chronic heart failure—A study using Near Infrared Spectrophotometry (NIRS). *European Journal of Heart Failure*, *10*(7), 652–657. https://doi.org/10.1016/j.ejheart.2008.05.009

Acuña-Castroviejo, D., Escames, G., Venegas, C., Díaz-Casado, M. E., Lima-Cabello, E., López, L. C., Rosales-Corral, S., Tan, D.-X., & Reiter, R. J. (2014). Extrapineal melatonin: Sources, regulation, and potential functions. *Cellular and Molecular Life Sciences*, *71*(16), 2997–3025. https://doi.org/10.1007/s00018-014-1579-2

Anwar, M. M., Meki, A. R., & Rahma, H. H. (2001). Inhibitory effects of melatonin on vascular reactivity: Possible role of vasoactive mediators. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, *130*(3), 357–367. https://doi.org/10.1016/s1532-0456(01)00261-7

Arangino, S., Cagnacci, A., Angiolucci, M., Vacca, A. M. B., Longu, G., Volpe, A., & Melis, G. B. (1999). Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. *The American Journal of Cardiology*, *83*(9), 1417–1419. https://doi.org/10.1016/S0002-9149(99)00112-5

Baker, J., & Kimpinski, K. (2018). Role of melatonin in blood pressure regulation: An adjunct anti-hypertensive agent. *Clinical and Experimental Pharmacology and Physiology*, *45*(8), 755–766. https://doi.org/10.1111/1440-1681.12942

Bartoli, A. N. (2013). Bioavailability of a New Oral Spray Melatonin Emulsion Compared with a Standard Oral Formulation in Healthy Volunteers. *Journal of Bioequivalence & Bioavailability*, *04*(07). https://doi.org/10.4172/jbb.1000120

Brugger, P., Marktl, W., & Herold, M. (1995). *Impaired nocturnal secretion of melatonin in coronary heart disease*. https://doi.org/10.1016/S0140-6736(95)92600-3

Brusco, L. I., García-Bonacho, M., Esquifino, A. I., & Cardinali, D. P. (1998). Diurnal rhythms in norepinephrine and acetylcholine synthesis of sympathetic ganglia, heart and adrenals of aging rats: Effect of melatonin. *Journal of the Autonomic Nervous System*, *74*(1), 49–61. https://doi.org/10.1016/S0165-1838(98)00134-9

Buxton, O., & Marcelli, E. (2010). *Short and long sleep are positively associated with obesity, diabetes, hypertension, and cardiovascular disease among adults in the United States | Elsevier Enhanced Reader*. https://doi.org/10.1016/j.socscimed.2010.05.041

Cagnacci, A. (1996). Melatonin in relation to physiology in adult humans. *Journal of Pineal Research*, *21*(4), 200–213. https://doi.org/10.1111/j.1600-079X.1996.tb00287.x

Cagnacci, A., Arangino, S., Angiolucci, M., Maschio, E., Longu, G., & Metis, G. B. (1997). Potentially beneficial cardiovascular effects of melatonin administration in women.

Journal of Pineal Research, *22*(1), 16–19. https://doi.org/10.1111/j.1600-079X.1997.tb00297.x

Cagnacci, A., Arangino, S., Angiolucci, M., Maschio, E., & Melis, G. B. (1998). Influences of melatonin administration on the circulation of women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *274*(2), R335–R338. https://doi.org/10.1152/ajpregu.1998.274.2.R335

Cagnacci, A., Arangino, S., Angiolucci, M., Melis, G. B., Facchinetti, F., Malmusi, S., & Volpe, A. (2001). Effect of exogenous melatonin on vascular reactivity and nitric oxide in postmenopausal women: Role of hormone replacement therapy. *Clinical Endocrinology*, *54*(2), 261–266. https://doi.org/10.1046/j.1365-2265.2001.01204.x

Cappuccio, F. P., D'Elia, L., Strazzullo, P., & Miller, M. A. (2010). Sleep Duration and All-Cause Mortality: A Systematic Review and Meta-Analysis of Prospective Studies. *Sleep*, *33*(5), 585–592. https://doi.org/10.1093/sleep/33.5.585

Chen, K., Pittman, R. N., & Popel, A. S. (2008). Nitric Oxide in the Vasculature: Where Does It Come From and Where Does It Go? A Quantitative Perspective. *Antioxidants & Redox Signaling*, *10*(7), 1185–1198. https://doi.org/10.1089/ars.2007.1959

Chitimus, D. M., Popescu, M. R., Voiculescu, S. E., Panaitescu, A. M., Pavel, B., Zagrean, L., & Zagrean, A.-M. (2020). Melatonin's Impact on Antioxidative and Anti-Inflammatory Reprogramming in Homeostasis and Disease. *Biomolecules*, *10*(9), 1211. https://doi.org/10.3390/biom10091211

Chuang, J. I., Chen, S. S., & Lin, M. T. (1993). Melatonin decreases brain serotonin release, arterial pressure and heart rate in rats. *Pharmacology*, *47*(2), 91–97. https://doi.org/10.1159/000139083

Crisafulli, A. (2017). The Impact of Cardiovascular Diseases on Cardiovascular Regulation During Exercise in Humans: Studies on Metaboreflex Activation Elicited by the Post-exercise Muscle Ischemia Method. *Current Cardiology Reviews*, *13*(4), 293–300. https://doi.org/10.2174/1573403X13666170804165928

Cunnane, S. C., Manku, M. S., Oka, M., & Horrobin, D. F. (1980). Enhanced vascular reactivity to various vasoconstrictor agents following pinealectomy in the rat: Role of melatonin. *Canadian Journal of Physiology and Pharmacology*, *58*(3), 287–293. https://doi.org/10.1139/y80-049

Doolen, S., Krause, D. N., Dubocovich, M. L., & Duckles, S. P. (1998). Melatonin mediates two distinct responses in vascular smooth muscle. *European Journal of Pharmacology*, *345*(1), 67–69. https://doi.org/10.1016/S0014-2999(98)00064-8

Ekmekcioglu, C. (2006). Melatonin receptors in humans: Biological role and clinical relevance. *Biomedicine & Pharmacotherapy*, *60*, 97–108. https://doi.org/10.1016/j.biopha.2006.01.002

Ekmekcioglu, C., Haslmayer, P., Philipp, C., Mehrabi, M. R., Glogar, H. D., Grimm, M., Leibetseder, V. J., Thalhammer, T., & Marktl, W. (2001). EXPRESSION OF THE MT 1 MELATONIN RECEPTOR SUBTYPE IN HUMAN CORONARY ARTERIES. *Journal of Receptors and Signal Transduction*, *21*(1), 85–91. https://doi.org/10.1081/RRS-100107144

Ekmekcioglu, C., Thalhammer, T., Humpeler, S., Mehrabi, M. R., Glogar, H. D., Holzenbein, T., Markovic, O., Leibetseder, V. J., Strauss-Blasche, G., & Marktl, W. (2003). The melatonin receptor subtype MT2 is present in the human cardiovascular system. *Journal of Pineal Research*, *35*(1), 40–44. https://doi.org/10.1034/j.1600-079X.2003.00051.x

Ellsworth, M. L., Ellis, Christopher G, Goldman, Daniel, Stephenson, Alan H, Dietrich, Hans H, & Sprague, Randy S. (2010). *Erythrocytes: Oxygen Sensors and Modulators of Vascular Tone in Regions of Low PO2*. 18.

Ellsworth, M. L., & Sprague, R. S. (2012). Regulation of blood flow distribution in skeletal muscle: Role of erythrocyte-released ATP: Erythrocytes contribute to vascular control. *The Journal of Physiology*, *590*(20), 4985–4991. https://doi.org/10.1113/jphysiol.2012.233106

Emet, M., Ozcan, H., Ozel, L., Yayla, M., Halici, Z., & Hacimuftuoglu, A. (2016). A Review of Melatonin, Its Receptors and Drugs. *The Eurasian Journal of Medicine*, *48*(2), 135–141. https://doi.org/10.5152/eurasianjmed.2015.0267

Escames, G., Ozturk, G., Baño-Otálora, B., Pozo, M. J., Madrid, J. A., Reiter, R. J., Serrano, E., Concepción, M., & Acuña-Castroviejo, D. (2012). Exercise and melatonin in humans: Reciprocal benefits. *Journal of Pineal Research*, *52*(1), 1–11. https://doi.org/10.1111/j.1600-079X.2011.00924.x

Fadil, R., Verma, A. K., Sadeghian, F., Blaber, A. P., & Tavakolian, K. (2023). Cardio-respiratory interactions in response to lower-body negative pressure. *Physiological Measurement*. https://doi.org/10.1088/1361-6579/acb7c6

Favero, G., Franceschetti, L., Bonomini, F., Rodella, L. F., & Rezzani, R. (2017). Melatonin as an Anti-Inflammatory Agent Modulating Inflammasome Activation. *International Journal of Endocrinology*, *2017*, 1–13. https://doi.org/10.1155/2017/1835195

Favilla, C. G., Carter, S. C., Atluri, P., Reehal, N., & Genuardi, M. V. (2023). Noninvasive finger plethysmography for continuous blood pressure monitoring in patients with left ventricular assist device. *Artificial Organs*. https://doi.org/10.1111/aor.14560

Fiuza-Luces, C., Santos-Lozano, A., Joyner, M., Carrera-Bastos, P., Picazo, O., Zugaza, J. L., Izquierdo, M., Ruilope, L. M., & Lucia, A. (2018). Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. *Nature Reviews Cardiology*, *15*(12), 731–743. https://doi.org/10.1038/s41569-018-0065-1

Fu, Q., & Levine, B. D. (2013). Exercise and the autonomic nervous system. In *Handbook of Clinical Neurology* (Vol. 117, pp. 147–160). Elsevier. https://doi.org/10.1016/B978-0-444-53491-0.00013-4

Girouard, H., Chulak, C., Lejossec, M., Lamontagne, D., & de Champlain, J. (2001). Vasorelaxant effects of the chronic treatment with melatonin on mesenteric artery and aorta of spontaneously hypertensive rats: *Journal of Hypertension*, *19*(8), 1369–1377. https://doi.org/10.1097/00004872-200108000-00004

Girouard, H., Chulak, C., LeJossec, M., Lamontagne, D., & de Champlain, J. (2003). Chronic antioxidant treatment improves sympathetic functions and beta-adrenergic pathway in the spontaneously hypertensive rats. *Journal of Hypertension*, *21*(1), 179–188. https://doi.org/10.1097/00004872-200301000-00028

Girouard, H., Denault, C., Chulak, C., & Dechamplain, J. (2004). Treatment by -acetylcysteine and melatonin increases cardiac baroreflex and improves antioxidant reserve. *American Journal of Hypertension*, *17*(10), 947–954. https://doi.org/10.1016/j.amjhyper.2004.06.009

Haddy, F. J., Vanhoutte, P. M., & Feletou, M. (2006). Role of potassium in regulating blood flow and blood pressure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *290*(3), R546–R552. https://doi.org/10.1152/ajpregu.00491.2005

Hinojosa-Laborde, C., Shade, R. E., Muniz, G. W., Bauer, C., Goei, K. A., Pidcoke, H. F., Chung, K. K., Cap, A. P., & Convertino, V. A. (2014). Validation of lower body negative pressure as an experimental model of hemorrhage. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *116*(4), 406–415. https://doi.org/10.115/japplphysiol.00640.2013

Holmes, S. W., & Sugden, D. (n.d.). *The effect of melatonin on pinealectomy- induced hypertension in the rat*. 2.

Imenshahidi, M., Karimi, G., & Hosseinzadeh, H. (2020). Effects of melatonin on cardiovascular risk factors and metabolic syndrome: A comprehensive review. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *393*(4), 521–536. https://doi.org/10.1007/s00210-020-01822-4

Khalilgharibi, N., & Mao, Y. (2021). To form and function: On the role of basement membrane mechanics in tissue development, homeostasis and disease. *Open Biology*, *11*(2), rsob.200360, 200360. https://doi.org/10.1098/rsob.200360

Koziróg, M., Poliwczak, A. R., Duchnowicz, P., Koter-Michalak, M., Sikora, J., & Broncel, M. (2011). Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome: Melatonin in metabolic syndrome. *Journal of Pineal Research*, *50*(3), 261–266. https://doi.org/10.1111/j.1600-079X.2010.00835.x

Laughlin, M. H., Davis, M. J., Secher, N. H., van Lieshout, J. J., Arce-Esquivel, A. A., Simmons, G. H., Bender, S. B., Padilla, J., Bache, R. J., Merkus, D., & Duncker, D. J. (2012). Peripheral Circulation. In *Comprehensive Physiology* (pp. 321–447). John Wiley & Sons, Ltd. https://doi.org/10.1002/cphy.c100048

Lavie, C. J., Arena, R., Swift, D. L., Johannsen, N. M., Sui, X., Lee, D., Earnest, C. P., Church, T. S., O'Keefe, J. H., Milani, R. V., & Blair, S. N. (2015). Exercise and the Cardiovascular System: Clinical Science and Cardiovascular Outcomes. *Circulation Research*, *117*(2), 207–219. https://doi.org/10.1161/CIRCRESAHA.117.305205

Leibowitz, A., Peleg, E., Sharabi, Y., Shabtai, Z., Shamiss, A., & Grossman, E. (2008). The Role of Melatonin in the Pathogenesis of Hypertension in Rats With Metabolic Syndrome. *American Journal of Hypertension*, *21*(3), 348–351. https://doi.org/10.1038/ajh.2007.60

Lewy, A. J., & Sack, R. L. (1997). Exogenous Melatonin's Phase-Shifting Effects on the Endogenous Melatonin Profile in Sighted Humans: A Brief Review and Critique of the Literature. *Journal of Biological Rhythms*, *12*(6), 588–594. https://doi.org/10.1177/074873049701200614

Lightfoot, J. T., & Tsintgiras, K. M. (1995). Quantification of tolerance to lower body negative pressure in a healthy population. *Medicine & Science in Sports & Exercise*, *27*(5), 697.

Limberg, J. K., Casey, D. P., Trinity, J. D., Nicholson, W. T., Wray, D. W., Tschakovsky, M. E., Green, D. J., Hellsten, Y., Fadel, P. J., Joyner, M. J., & Padilla, J. (2020). *Assessment of resistance vessel function in human skeletal muscle: Guidelines for experimental design, Doppler ultrasound, and pharmacology*. https://doi.org/10.1152/ajpheart.00649.2019

Liu, Z., Gan, L., Xu, Y., Luo, D., Ren, Q., Wu, S., & Sun, C. (2017). Melatonin alleviates inflammasome-induced pyroptosis through inhibiting NF-κB/GSDMD signal in mice adipose tissue. *Journal of Pineal Research*, *63*(1), e12414. https://doi.org/10.1111/jpi.12414

Moore, R. (1996). *Neural control of the pineal gland*. https://doi.org/10.1016/0166-4328(96)00083-6

Mortensen, S. P., Nyberg, M., Gliemann, L., Thaning, P., Saltin, B., & Hellsten, Y. (2014). Exercise training modulates functional sympatholysis and α-adrenergic vasoconstrictor responsiveness in hypertensive and normotensive individuals. *The Journal of Physiology*, *592*(Pt 14), 3063–3073. https://doi.org/10.1113/jphysiol.2014.273722

Mortensen, S. P., & Saltin, B. (2014). Regulation of the skeletal muscle blood flow in humans. *Experimental Physiology*, *99*(12), 1552–1558. https://doi.org/10.1113/expphysiol.2014.081620

Nishiyama, K., Yasue, H., Moriyama, Y., Tsunoda, R., Ogawa, H., Yoshimura, M., &Kugiyama, K. (2001). Acute effects of melatonin administration on cardiovascular autonomic regulation in healthy men. *American Heart Journal*, *141*(5), 13A-17A. https://doi.org/10.1067/mhj.2001.114368

Nyberg, M., Jensen, L. G., Thaning, P., Hellsten, Y., & Mortensen, S. P. (2012). Role of nitric oxide and prostanoids in the regulation of leg blood flow and blood pressure in humans with essential hypertension: Effect of highintensity aerobic training. *J Physiol*, 14.

Ozkor, M. A., Murrow, J. R., Rahman, A. M., Kavtaradze, N., Lin, J., Manatunga, A., & Quyyumi, A. A. (2011). Endothelium-Derived Hyperpolarizing Factor Determines Resting and Stimulated Forearm Vasodilator Tone in Health and in Disease. *Circulation*, *123*(20), 2244–2253. https://doi.org/10.1161/CIRCULATIONAHA.110.990317

Paulis, L., Pechanova, O., Zicha, J., Barta, A., Gardlik, R., Celec, P., Kunes, J., & Simko, F. (2010). Melatonin interactions with blood pressure and vascular function during l-NAME-induced hypertension. *Journal of Pineal Research*, *48*(2), 102–108. https://doi.org/10.1111/j.1600-079X.2009.00732.x

Pechánová, O., Zicha, J., Paulis, L., Zenebe, W., Dobesová, Z., Kojsová, S., Jendeková, L.,Sládková, M., Dovinová, I., Simko, F., & Kunes, J. (2007). The effect of N-acetylcysteine and melatonin in adult spontaneously hypertensive rats with established hypertension. *European Journal of Pharmacology*, *561*(1–3), 129–136. https://doi.org/0.1016/j.ejphar.2007.01.035

Pogan, L., Bissonnette, P., Parent, L., & Sauvé, R. (2002). The effects of melatonin on Ca(2+) homeostasis in endothelial cells. *Journal of Pineal Research*, *33*(1), 37–47. https://doi.org/10.1034/j.1600-079x.2002.01890.x

Prado, N. J., Ferder, L., Manucha, W., & Diez, E. R. (2018). Anti-Inflammatory Effects of Melatonin in Obesity and Hypertension. *Current Hypertension Reports*, *20*(5), 45. https://doi.org/10.1007/s11906-018-0842-6

Proctor, D. N., Shen, P. H., Dietz, N. M., Eickhoff, T. J., Lawler, L. A., Ebersold, E. J., Loeffler, D. L., & Joyner, M. J. (1998, July 1). *Reduced leg blood flow during dynamic exercise in older endurance-trained men | Journal of Applied Physiology*. https://journals-physiology-org.ezproxy.lib.ou.edu/doi/full/10.1152/jappl.1998.85.1.68?rfrdat=crpub++0pubmed&urlver=Z39.88-2003&rfrid=ori%3Arid%3Acrossref.org

Pugsley, M. K., & Tabrizchi, R. (2000). The vascular system An overview of structure and function. *Journal of Pharmacological and Toxicological Methods*, 8.

Qiu, F., Liu, X., Zhang, Y., Wu, Y., Xiao, D., & Shi, L. (2018). Aerobic exercise enhanced endothelium-dependent vasorelaxation in mesenteric arteries in spontaneously hypertensive rats: The role of melatonin. *Hypertension Research*, *41*(9), 718–729. https://doi.org/10.1038/s41440-018-0066-9

Rahman, Md. M., Kwon, H.-S., Kim, M.-J., Go, H.-K., Oak, M.-H., & Kim, D.-H. (2017). Melatonin supplementation plus exercise behavior ameliorate insulin resistance, hypertension and fatigue in a rat model of type 2 diabetes mellitus. *Biomedicine & Pharmacotherapy*, *92*, 606–614. https://doi.org/10.1016/j.biopha.2017.05.035

Ray, C. A. (2003). Melatonin attenuates the sympathetic nerve responses to orthostatic stress in humans. *The Journal of Physiology*, *551*(3), 1043–1048. https://doi.org/10.1113/jphysiol.2003.043182

Reiter, R. J., Tan, D.-X., Gitto, E., Sainz, R. M., Mayo, C., Leon, J., Manchester, L. C., & Kilic, Ü. (2004). PHARMACOLOGICAL UTILITY OF MELATONIN IN REDUCING OXIDATIVE CELLULAR AND MOLECULAR DAMAGE. *Pol. J. Pharmacol.*, 12.

Reiter, R. J., Tan, D.-X., Manchester, L. C., Lopez-Burillo, S., Sainz, R. M., & Mayo, J. C. (2003). Melatonin: Detoxification of oxygen and nitrogen-based toxic reactants. *Advances in Experimental Medicine and Biology*, *527*, 539–548. https://doi.org/10.1007/978-1-4615-0135-0_62

Reiter, R. J., Tan, D.-X., Paredes, S. D., & Fuentes-Broto, L. (2010). Beneficial effects of melatonin in cardiovascular disease. *Annals of Medicine*, *42*(4), 276–285.https://doi.org/10.3109/07853890903485748

Rickards, C. A., Ryan, K. L., Cooke, W. H., & Convertino, V. A. (2011). Tolerance to central hypovolemia: The influence of oscillations in arterial pressure and cerebral blood velocity. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *111*(4), 1048–1058. https://doi.org/10.1152/japplphysiol.00231.2011

Rosenmeier, J. B., Fritzlar, S. J., Dinenno, F. A., & Joyner, M. J. (2003). *Exogenous NO administration and α-adrenergic vasoconstriction in human limbs*. https://doi.org/10.1152/japplphysiol.00634.2003

Sakotnik, A., Liebmann, P., Stoschitzky, K., Lercher, P., Schauenstein, K., Klein, W., & Eber, B. (1999). Decreased melatonin synthesis in patients with coronary artery disease. *European Heart Journal*, *20*(18), 1314–1317. https://doi.org/10.1053/euhj.1999.1527

Saltin, B., & Mortensen, S. P. (2012). Inefficient functional sympatholysis is an overlooked cause of malperfusion in contracting skeletal muscle: Muscle blood flow in healthy ageing. *The Journal of Physiology*, *590*(24), 6269–6275. https://doi.org/10.1113/jphysiol.2012.241026

Satake, N., Oe, H., & Shibata, S. (1991). Vasorelaxing action of melatonin in rat isolated aorta; possible endothelium dependent relaxation. *General Pharmacology: The Vascular System*, *22*(6), 1127–1133. https://doi.org/10.1016/0306-3623(91)90589-X

Shao, G., Zhang, S., Nie, J., Li, J., & Tong, J. (2017). Effects of melatonin on mechanisms involved in hypertension using human umbilical vein endothelial cells. *Journal of Toxicology and Environmental Health, Part A*, *80*(23–24), 1342–1348. https://doi.org/10.1080/15287394.2017.1384171

Simko, F., & Paulis, L. (2007). Melatonin as a potential antihypertensive treatment. *Journal of Pineal Research*, *42*(4), 319–322. https://doi.org/10.1111/j.1600-079X.2007.00436.x

Simko, F., Pechanova, O., Pelouch, V., Krajcirovicova, K., Mullerova, M., Bednarova, K., Adamcova, M., & Paulis, L. (2009). Effect of melatonin, captopril, spironolactone and simvastatin on blood pressure and left ventricular remodelling in spontaneously hypertensive rats. *Journal of Hypertension. Supplement: Official Journal of the International Society of Hypertension*, *27*(6), S5-10. https://doi.org/10.1097/01.hjh.0000358830.95439.e8

Simko, F., Pechanova, O., Repova Bednarova, K., Krajcirovicova, K., Celec, P., Kamodyova, N., Zorad, S., Kucharska, J., Gvozdjakova, A., Adamcova, M., & Paulis, L. (2014). Hypertension and Cardiovascular Remodelling in Rats Exposed to Continuous Light: Protection by ACE-Inhibition and Melatonin. *Mediators of Inflammation*, *2014*, 1–10. https://doi.org/10.1155/2014/703175

Smith, S. A., Leal, A. K., Murphy, M. N., Downey, R. M., & Mizuno, M. (2015). MUSCLE MECHANOREFLEX OVERACTIVITY IN HYPERTENSION: A ROLE FOR CENTRALLY-DERIVED NITRIC OXIDE. *Autonomic Neuroscience : Basic & Clinical*, *188*, 58–63. https://doi.org/10.1016/j.autneu.2014.12.004

Sun, H., Gusdon, A. M., & Qu, S. (2016). Effects of melatonin on cardiovascular diseases: Progress in the past year. *Current Opinion in Lipidology*, *27*(4), 408–413. https://doi.org/10.1097/MOL.0000000000000314

Szewczyk-Golec, K., Woźniak, A., & Reiter, R. J. (2015). Inter-relationships of the chronobiotic, melatonin, with leptin and adiponectin: Implications for obesity. *Journal of Pineal Research*, *59*(3), 277–291. https://doi.org/10.1111/jpi.12257

Tejero, J., Shiva, S., & Gladwin, M. T. (2019). SOURCES OF VASCULAR NITRIC OXIDE AND REACTIVE OXYGEN SPECIES AND THEIR REGULATION. *Physiological Reviews*, *99*(1), 311–379. https://doi.org/10.1152/physrev.00036.2017

Tomás-Zapico, C., & Coto-Montes, A. (2005). A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *Journal of Pineal Research*, *39*(2), 99–104. https://doi.org/10.1111/j.1600-079X.2005.00248.x

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., & Fougerou, C. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, *15*(3), 434–443. https://doi.org/10.2174/1570159X14666161228122115

Tschakovsky, M. E., Sujirattanawimol, K., Ruble, S. B., Valic, Z., & Joyner, M. J. (n.d.). Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J. Physiol.*, 13.

Vaduganathan, M., Mensah, G., Varieur Turco, J., Fuster, V., & Roth, G. (2022, December). *The Global Burden of Cardiovascular Diseases and Risk*. Journal of the American College of Cardiology. https://doi.org/10.1016/j.jacc.2022.11.005

Vongpatanasin, W., Wang, Z., Arbique, D., Arbique, G., Adams-Huet, B., Mitchell, J. H., Victor, R. G., & Thomas, G. D. (2011). Functional sympatholysis is impaired in hypertensive humans: Functional sympatholysis and hypertension. *The Journal of Physiology*, *589*(5), 1209–1220. https://doi.org/10.1113/jphysiol.2010.203026

Wehrwein, E. A., & Joyner, M. J. (2013). Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. In *Handbook of Clinical Neurology* (Vol. 117, pp. 89–102). Elsevier. https://doi.org/10.1016/B978-0-444-53491-0.00008-0

Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2016). Overview of the Anatomy, Physiology, and Pharmacology of the Autonomic Nervous System. In *Comprehensive Physiology* (pp. 1239–1278). John Wiley & Sons, Ltd. https://doi.org/10.1002/cphy.c150037

Xia, C.-M., Shao, C.-H., Xin, L., Wang, Y.-R., Ding, C.-N., Wang, J., Shen, L.-L., Li, L., Cao, Y.-X., & Zhu, D.-N. (2008). EFFECTS OF MELATONIN ON BLOOD PRESSURE IN STRESS-INDUCED HYPERTENSION IN RATS. *Clinical and Experimental Pharmacology and Physiology*, *35*(10), 1258–1264. https://doi.org/10.1111/j.1440-1681.2008.05000.x

Yaprak, M., Altun, A., Vardar, A., Aktoz, M., Ciftci, S., & Ozbay, G. (2003). *Decreased nocturnal synthesis of melatonin in patients with coronary artery disease*. https://doi.org/10.1016/S0167-5273(02)00461-8

Yildiz, M., Sahin, B., & Sahin, A. (2006). *Acute effects of oral melatonin administration on arterial distensibility, as determined by carotid-femoral pulse wave velocity, in healthy young men*. *11*(4), 3.

Zanoboni, A., & Zanoboni-Muciaccia, W. (1967). Experimental hypertension in pinealectomized rats. *Life Sciences*, *6*(21), 2327–2331. https://doi.org/10.1016/0024-3205(67)90043-4

Zarghi, A., & Arfaei, S. (2011). *Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships*. 30.

Zhao, T., Zhang, H., Jin, C., Qiu, F., Wu, Y., & Shi, L. (2017). Melatonin mediates vasodilation through both direct and indirect activation of BKCa channels. *Journal of Molecular Endocrinology*, *59*(3), 219–233. https://doi.org/10.1530/JME-17-0028

Appendices

IRB Approved Study Documents

COLLEGE OF ARTS AND SCIENCES
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE
The UNIVERSITY of OKLAHOMA

The Effects of Acute Melatonin Supplementation on Cardiovascular Responses to Sympathetic Activation

Are you interested in helping us determine if

Taking Melatonin improves cardiovascular function at rest and during exercise?

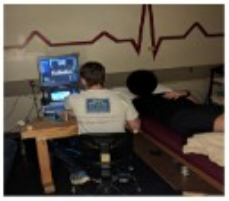
What is Melatonin? Melatonin is natural hormone that helps regulate sleep. Recent studies have also found that taking melatonin which is available as an over-the-counter supplement may have positive benefits for the cardiovascular system. This experiment is looking to understand if Melatonin improves cardiovascular responses and how it may do so.

Time commitment: 3 visits (~9 hours total)

What will be measured: Brain and Muscle Blood flow and Oxygenation, Blood Pressure, Heart Rate, Breathing Rate. ***All measures are noninvasive***.

Eligibility: Healthy women* and men between the ages of 18-35 years who do not regularly take melatonin, are not allergic to melatonin, do not use nicotine/tobacco products.

**Women need to have a regular menstrual cycle*



- Brain Oxygenation
- Muscle Oxygenation
- Handgrip Exercise

Compensation: A gift card valued at **$50** for Amazon.com will be given for completing **all** study requirements.

If interested, please contact:

MS student: Sarah Skillett; sarah.skillett@ou.edu

Principal Investigator: Dr. Mikhail Kellawan; kellawan@ou.edu; (405) 325-9028

OR Scan the QR CODE!

The University of Oklahoma is an equal opportunity institution (IRB# 13084)

IRB NUMBER: 13084
IRB APPROVAL DATE: 01/26/2023

Consent Form to Participate in a Research Study
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma- Norman Campus

Study Title: The Effects of Acute Melatonin Supplementation on Cardiovascular Responses to Sympathetic Activation

Sponsor: Department of Health and Exercise Science

Principal Investigator: J. Mikhail Kellawan
Phone Number: 405-325-9028

KEY INFORMATION ABOUT THE RESEARCH STUDY

You are being asked to participate in a research study. Research studies are voluntary and include only people who choose to take part. This consent form begins with a 'Key Information' section to provide important information to help you decide whether or not to participate in this study. More detailed information is provided after the key information. Please take your time, discuss this with family and friends, and ask the investigator and study team any questions you may have.

WHY HAVE I BEEN ASKED TO PARTICIPATE IN THIS STUDY?

You are being asked to participate in this research study because you are a healthy individual that does not use melatonin and is not allergic to melatonin.

WHY IS THIS STUDY BEING DONE AND HOW LONG WILL IT LAST?

The purpose of this study is to find out what effects (good and bad) on the cardiovascular system that melatonin has on you and other healthy people. We think that you will be in the study for as little as 1 week or as long as three months dependent on when your schedule allows you to complete the three study visits.

WHAT WILL I BE ASKED TO DO IN THIS STUDY?

If you decide to participate in this study, you will be asked to complete three study visits. The first visit is to ensure you qualify for study and to measure your cardiovascular responses to: a) placing your lower body into a device that causes suction during rest and during handgrip exercise b) rebreathing a gas mixture that has higher levels of oxygen and carbon dioxide than the outside air. Putting your lower body in this device and causing suction while measuring cardiovascular responses is a common non-invasive test called "Lower Body Negative Pressure or LBNP". The breathing of the high oxygen, high carbon dioxide mix while measuring cardiovascular responses is a common non-invasive test called vascular reactivity test or "hypercapnia" test. You will also complete a Dual-energy X-ray absorptiometry (DXA) scan to determine the amount of fat, muscle and bone is in your body. The second and third visits are to test if a dose of melatonin, which is an "over-the-counter" supplement used to help with sleep, changes cardiovascular responses to LBNP during rest and during handgrip exercise and to hypercapnia. In visits two and three you will be randomly chosen to receive either melatonin treatment or a placebo treatment. Both treatments are a liquid spray under your tongue. You will not know which treatment you are receiving on any visit. However, whatever treatment you receive on visit two, you will receive the opposite treatment on visit three.

WHY MIGHT I WANT TO PARTICIPATE IN THIS STUDY?

If you agree to take part in this study, there is no direct medical benefit to you. We hope that the information learned from this study will benefit people with diseases that affect the cardiovascular system in the future.

WHY MIGHT I NOT WANT TO PARTICIPATE IN THIS STUDY
You may not want to be exposed to the radiation of a DXA scan. DXA uses less than one-tenth of the dose of radiation than that used in a standard chest X-ray, which is considered extremely low and is generally safe for most people. This amount of radiation is similar to the amount of background radiation received by an average person over a typical day. However, any amount of radiation may harm an unborn baby. Excessive radiation exposure may increase cancer risk, but the amount of radiation used in a DXA scan remains relatively low, and the benefits often outweigh potential cancer risks. You may not want to participate because some of the procedures/measurement may cause you some discomfort. However, the researchers will do everything possible to minimize your discomfort. Further, there is a rare possibility that you will have an adverse response to melatonin). The researchers do not know all the side effects that could happen. For a complete description of known risks, refer to the Detailed Information section of the consent form.

WHAT OTHER OPTIONS ARE THERE?
You may choose to receive no therapy at this time and receive only care to help you feel more comfortable. You may choose not to participate in this study. Please talk to your regular doctor about these and other options.

HOW WILL PARTICIPATING IN THE STUDY AFFECT ME FINANCIALLY?
There is no additional cost to you if you participate in this study.
If you chose to participate and complete all three study visits you will receive a gift card for $50 for Amazon.com.

DETAILED INFORMATION ABOUT THE RESEARCH STUDY

The following pages of the consent form will provide you with more information about this study. Please take your time in reviewing this information and ask the investigator and study team any questions you may have.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
About *150* people will take part in this study.

WHAT IS THE STATUS OF THE SUPPLEMENT USED IN THIS STUDY?
Melatonin is an "Over-the-counter" supplement that is typically used as a sleep aid. It falls under the US Food and Drug Administration's Dietary health and Education Act as a **dietary supplement**. Therefore, melatonin is not an investigational drug and not approved or regulated by the US Food and Drug Administration

WHAT IS INVOLVED IN THE STUDY?
If you agree to be in this research, you will be asked to complete three study visits. The first visit is to ensure you qualify for study and to measure your cardiovascular responses to lower body negative pressure (LBNP) during rest and during handgrip exercise and during hypercapnia. Putting the lower part of your body in chamber and adding suction while measuring cardiovascular responses is a common non-invasive test to challenge your cardiovascular systems to regulate blood pressure. Breathing the hypercapnia gas responses is a common non-invasive test that causes your blood vessels to open wider. The second and third visits are to test if a dose of melatonin, which is an "over-the-counter" supplement used to help with sleep, changes cardiovascular responses to LBNP during rest and during handgrip exercise and to hypercapnia. In visits two and three you will be randomly chosen to receive either melatonin treatment or a placebo treatment. Both treatments are a liquid spray under your tongue. You will not know which treatment you are receiving on any visit. However,

whatever treatment you receive on visit two, you will receive the opposite treatment on visit three. Female participants will take a pregnancy test prior to any experimental visit.

Visit 1 (Screening): ~2 hours. The screening visit will include informed consent, measurements of height, weight, waist and hip circumference, and questionaries about your health history, sleeping, and physical activity. . Women will also have to complete a pregnancy test. These are done to ensure you are eligible for the study and that it is safe for you to participate. If you qualify, you will then undergo a complete a time control experiment. Once you have been deemed eligible to participate, you will complete a Dual-energy X-ray absorptiometry (DXA) scan to determine the amount of fat, muscle and bone is in your body. After the DXA scan, your forearm grip strength in your non-dominate arm will be measured. Additionally, the proper positioning of the handgrip dynamometer will be determined for forearm exercise. The proper position of a transcranial doppler ultrasound device will also be determined.

Visit 2 & 3 (Study day): ~3 hours. On these study days you will receive a dose of melatonin or placebo via a spray under your tongue. You will not be told on which visit you will receive melatonin and which day you will receive the placebo. Next, we will setup and attach multiple pieces of equipment which measure several cardiovascular variables. All pieces of equipment used are described in the list below under "Instrumentation". You will rest for 30 minutes while all pieces of equipment are setup to start the experiment. After the rest period, a lower body negative pressure (LBNP) machine will be activated for 2 minutes. LBNP will cause a redistribution of your blood from your upper body to the lower body as well as an increase in heart beats per minute. You will then rest for 10 more minutes after which, you will undergo 2 trials of hypercapnic rebreathing separated by 5minutes of rest. After hypercapnia, you will have another 10min of rest. Then you will start handgrip exercise at 20% of your maximal grip strength. You will squeeze a handgrip exercise device for 1 second and relax for 2 seconds in time with a metronome. You continue this exercise for 7 minutes. 5 minutes into the exercise an investigator will start the LBNP for 2 minutes. You will continue to exercise during the LBNP exposure for the remaining 2 minutes. After exercise is completed, you will rest for 10 minutes. Then a blood pressure cuff will inflate around the arm that you were exercising for 5 minutes. After 5 minutes, the cuff will deflate, and recovery measurements will be collected - after which, the visits end.

Instrumentation: These are the devices what we will place on you. All of these devices are non-invasive.

- Forearm and Brain Oxygenation: These are measured via Near-Infrared Spectroscopy (NIRS). We will place probes on your forehead and forearm secured with a tensor bandage
- Heart Rate (HR): will be measured with a heart rate monitor that is you strap on your torso directly on your skin
- Blood Pressure: Is measured non-invasively via Finger photoplethysmography. In which a larger blood pressure cuff is placed on both of your upper arm and small blood pressure cuff on your middle finger which is connected to monitor that is strapped on your wrist
- Blood Oxygenation: Is measured via infrared light with a clip on your ear
- End-Tidal Carbon Dioxide ($EtCO_2$): End-Tidal Carbon Dioxide ($EtCO_2$) will be measured non-invasively using a mask and recorded using a metabolic cart.
- Cerebral Blood Velocity (MCAv): A non-invasive Transcranial Doppler Ultrasound with bilateral 2MHz robotic probes will be used for MCA_v on the left and right portions of the brain
- Forearm Blood Flow: Non-invasive Echo and Doppler Ultrasound with a linear array probe in B-mode will be used to measure brachial artery diameter and blood velocity.
- Maximal Voluntary Contraction (MVC): using a Handgrip dynamometer. Using your non-dominate hand you will squeeze the dynamometer as hard as you can 3 sperate times.
- Handgrip Exercise: Using your non-dominant hand at 20% MVC, 1:2s contraction-relaxation cycle, for 7 minutes

- Lower Body Negative Pressure (LBNP): Your lower body up to the top of your hips will be inserted into a plexiglass chamber attached to a vacuum. When turned on the device will "suck" at a pressure of -20 mmHg for 2 minutes.
- Hypercapnia (HC): While lying down you will breathe through a mouthpiece connected to a three-way sliding valve. A meteorological ballon filled with hyperoxic (%40 O_2), hypercapnic (3% CO_2) mix of gas will be filled to a volume 1 liter above estimated vital capacity based on sex, height, and age. The valve will be switched such that you will rebreathe the mixture until you $EtCO_2$ levels are 10mmHg above baseline levels (~2min)
- Pregnancy Test: Female participants will take a commercially available pregnancy test prior to participation in any experimental visit.
- Body Composition: Total body composition will be determined by use of a Dual-energy X-ray absorptiometry (DXA) scan
- Placebo: 1 µL McComicks Pure Mint Extract mixed with 29 ml filter water, which you will spray under your tongue
- Melatonin: 5mg commercially available melatonin spray (Onnit, Mint Flavor), which you will spray under your tongue

CAN I WITHDRAW FROM THE STUDY?
You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first. To withdraw from the study, simply inform one of the researchers of your decision.

There may be circumstances under which your participation may be terminated by the investigator without your consent.

- He/She feels that it is in your medical best interest.
- New information becomes available.
- You fail to follow study requirements.

WHAT ARE THE RISKS OF THE STUDY?
In addition to the risks described in the Key Information section, you may also be at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. Other drugs may be given to make side effects less serious and uncomfortable. Many side effects go away shortly after the melatonin/fasting/exercise/LBNP are stopped. The treatment or procedure may involve risks that are currently unforeseeable.

Risks and side effects related to melatonin and the procedures we are studying include:

This experiment is non-invasive. Therefore, we do not anticipate any risk or benefit to you personally. However, nothing is without risk, there is a rare chance that you may have an adverse event. Some risks associated with the study may include:

a. Melatonin supplement: Melatonin is an "Over-the-counter" supplement that is typically used as a sleep aid. Adverse responses to melatonin are rare but include headache, short-term feelings of depression, daytime sleepiness, dizziness, stomach cramps, nausea, and irritability. *We recommend that you do not drive or use machinery for four to five hours after taking melatonin.*
b. 8-hour fast: Side effects of an 8-hour fast include feeling hungry or weak. To reduce this risk, we will encourage you to eat a substantial meal prior to the fast, drink plenty of water while fasting. If you feel sign/symptoms such as tiredness, headache, stomachache, unsteadiness, anxiety, etc. that interfere with daily living, subjects should discontinue the fast. To avoid these sensations, we recommend fasting after a night-time meal and visiting the lab in the early morning.
c. Anthropometric Measurements: There are no risks to measuring anthropometrics height, weight, heart rate, etc. Measuring blood pressure with a forearm cuff and finger cuff may cause

a temporary increase in pressure at the area of skin below the cuff, however, this discomfort is temporary and subsides when the cuff pressure is reduced.

d. Abstaining from exercise, caffeine, NSAIDs: This poses minimal risk to you. Abstaining from caffeine may result in irritability and headache. Acute abstinence from exercise has no risk. There is no risk form abstaining from NSAIDs considering regular use of NSAIDS for a clinical condition would exclude you from participation.

e. NIRS: A NIRS will be used to measure oxygenation of your brain and muscle. It is a non-invasive device that continuously monitors regional tissue oxygenation safely. It is secured on your forehead and forearm with a tensor bandage. This may feel tight on your forehead and arm.

f. Blood pressure monitoring: Photoplethysmography uses a non-invasive, automatic blood pressure cuff around the upper arm and finger. Around the finger a near-infrared light is used to measure changes in pressure on a beat-by-beat basis. When both the cuffs are inflated it may feel uncomfortable while inflated but this is temporary. These blood pressure measures are considered very safe.

g. Total Liable Signal determination: Uses a non-invasive, automatic blood pressure cuff around the upper arm. When the cuff is inflated it may feel uncomfortable while inflated but this is temporary. It will be inflated for 5 minutes then released. While inflated, there may be some feeling some numbness and tingling in your hands when the cuff is inflated. Once released those sensations will subside quickly. These procedures are considered very safe

h. Radiation Exposure: If you participate in the research, you will receive a DXA scan, a type of x ray procedure. The DXA scan will be performed for research purposes only and is not required for your medical care. The amount of additional radiation to which you will be exposed is approximately the amount that you receive in one day from natural, background sources of radiation. The risk of radiation exposure is cumulative over your lifetime.

i. Lower Body Negative Pressure: Requires placing the lower half of your body (up to the top of your hips) in a chamber that when turned on creates suction at -20 mmHg for 2-5 minutes. The risk of LBNP is you may feel uncomfortable or light-headed when the machine is running.

j. Breach of Confidentiality: Personal information such as name, gender, date of birth, and medications will be stored in a locked file cabinet in the HCRL laboratory. Study records will be coded with a number and only study personnel will have access to the link connecting your name to the collected data. After the study is complete, we will remove all identifying information so that study data is coded during analysis and publication. Your information will be coded to remove any personal identifiers during data analysis or research publications.

We do not anticipate that there will be any direct benefits to you for participating.

For more information about risks and side effects, ask the researcher.

TO WHAT EXTENT WILL MY INFORMATION BE KEPT CONFIDENTIAL?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations outside the OUHSC that may inspect and/or copy your research records for quality assurance and data analysis. These organizations may include the US Food & Drug Administration and other regulatory agencies. The OUHSC Human Research Participant Program office, the OUHSC Institutional Review Board, OUHSC Office of Compliance, and other University administrative offices may also inspect and/or copy your research records for these purposes.

Posting Study on ClinicalTrials.gov:
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. However, this website will not include information that can identify you. At most, the website will include a summary of the study and results. You can search this website at any time.

Identifiable Private Information:
Your information may be used for future studies without your additional consent. We will remove direct identifiers from your information and assign a code. The key to this code will be kept separately and only the researcher for this study will have access to the code. If your information is shared with another investigator for research purposes, they will not have access to the key code and will not be able to re-identify you.

WHAT ARE THE COSTS?
There are no direct costs to participating in the study.

WHAT IF I AM INJURED OR BECOME ILL WHILE PARTICIPATING IN THIS STUDY?
In the case of injury or illness results from this study, emergency medical treatment is available.

Emergency medical treatment should be sought at the nearest medical center and the study P.I J. Mikhail Kellawan should be contacted immediately (405-325-9028, kellawan@ou.edu).

You or your insurance may be charged for this treatment.

No other funds have been set aside by the University of Oklahoma Health Sciences Center, University of Oklahoma – Norman Campus to compensate you in the event of injury, illness, or for other damages related to your event of injury or illness.

WHAT ARE MY RIGHTS AS A PARTICIPANT?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, please be sure to discuss leaving the study with the principal investigator or your regular doctor. You may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished. You consent to this temporary restriction.

DO I HAVE ANY OTHER RIGHTS OVER MY DATA?
Depending on where the sponsor for your study is located and other factors, you may have additional rights over your personal data collected in this study. For example, the European Union General Data Protection Regulation (GDPR) and some state privacy laws might apply. If the GDPR applies, generally you may have the following rights:

1. The right to request the information collected to be corrected.
2. The right to withdraw your consent for the use of your personal information at any time.
3. The right, in some circumstances, to receive your personal information in a structured, commonly used and machine-readable format and the right to provide your information to a third party.
4. The right to strict confidentiality of your personal data when it is used/shared.
5. The right to limit the use/sharing of your personal information in certain circumstances.
6. The right under some circumstances to request the erasure of your personal data.

7. The right to file a complaint with a privacy protection regulator if you believe any of the rights above have been violated.

You can receive more information regarding these rights in the Privacy Notice for Research Participants, located on the OUHSC Office of Human Research Participant Protection (HRPP) website at *https://compliance.ouhsc.edu/HRPP/Participant/Privacy-Notice*.

If you have any questions and requests, please contact the HRPP Office at 405-271-2045.

WHOM DO I CALL IF I HAVE QUESTIONS, SUGGESTIONS, OR CONCERNS?
If you have questions, concerns, or complaints about the study or have a research-related injury, contact *J. Mikhail Kellawan* at *405-325-9028*.

If you cannot reach the Investigator or wish to speak to someone other than the investigator and for questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection, at 405-271-2045.

SIGNATURE:
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18)	**Printed Name**	**Date**
SIGNATURE OF PERSON OBTAINING CONSENT	**Printed Name**	**Date**

AUTHORIZATION TO USE or SHARE HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH

An Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **[The Effects of Acute Melatonin Supplementation on Cardiovascular Responses to Sympathetic Activation**

Leader of Research Team: **J. Mikhail Kellawan**

Address: **1401 Asp AVE. Norman, OK, USA, Rm 112, 73019**

Phone Number: **405-325-9028**

If you decide to sign this document, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and [Heart rate, Forearm & Brain Oxygenation, Blood Pressure, Blood Oxygentation, End-Todal Carbon Dioxide, Brain and Forarm Blood Flow, Pregnacy test, and Body Composition during rest, exercise, and lower body negative pressure testing there is also survey data on your sleeping and physical activity habits.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to To determine if acute melatonin supplementation alters central and peripheral responses to lower body negative pressure at rest and during rhythmic exercise and to hypercapnia

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with no one else

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OUHSC.

Canceling Permission. If you give the OUHSC researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OUHSC researchers to use or share your PHI for their research will never end.]

Contacting OUHSC: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190	or	Privacy Board University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190
If you have questions, call: (405) 271-2511	or	(405) 271-2045.

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

University of Oklahoma Health Sciences CenterResearch Privacy Form 1
PHI Research Authorization

Patient/Participant Name (Print): _____

_____ _____
Signature of Patient-Participant Date
or Parent if Participant is a minor

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Participant, provide a description of the relationship to the Patient-Participant and the authority to act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB NUMBER: 13084
IRB APPROVAL DATE: 01/26/2023

PI: J. Mikhail Kellawan IRB Number: 13084

Subject ID: _____ Sex: M / W Date: / /

Medical History (General)

Body System	Have you ever had any conditions affecting these body systems?	*Only complete if 'Yes' for Diagnosed Condition*			
		Diagnosis/Condition/Surgery	Onset Date	Is it a current problem?	Are you currently taking a prescribed medication?*
Cardiovascular					
☐ Heart Attack	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Stroke	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Hypertension	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Coronary Artery Disease	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Other Cardiovascular	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Your 1st Degree Relatives (e.g. mother, brother, daughter)	☐ Yes ☐ No	List family members, their diagnosis, and approximately when they were diagnosed:			
Lungs					
☐ Asthma	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Exercise-Induced Bronchospasm	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Obstructive Lung Disease	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Other	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Musculoskeletal					
☐ Knee	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Hips	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
☐ Back					
☐ Other					
Head/Eyes/Ears/ Nose/Throat/Neck	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Endocrine/Metabolic					
Diabetes	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No

High blood sugar	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Liver	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Thyroid	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Kidney	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No
Pituitary Gland	☐ Yes ☐ No			☐ Yes ☐ No	☐ Yes ☐ No

ADDITIONAL NOTES: _____
_____
_____

Additional Questions (all subjects)

Have you recently experienced any of the following?	Yes	No	When?
Pain in the neck, jaw, or arms?			
Dizziness or fainting?			
Swelling in the ankles?			
Rapid heart rate while at rest?			
Leg pain or cramping while walking, relieved with rest?			
Has a doctor ever told you that you have a heart murmur?			
Unusual fatigue with usual activities?			
Are you allergic to Melatonin?			

Additional COVID-19 Questions (all subjects)

Have you recently experienced any of the following in the 14 days?	Yes	No	When?
Fever or Chills?			
Cough?			
Shortness of Breath or Difficulty Breathing?			
Unusual fatigue with usual activities?			
Muscle of body aches?			
Headache?			
Sore Throat?			
Congestion or runny nose?			
Nausea or vomiting?			
Diarrhea?			
If you have answered YES to any of the above, **have you been Tested for COVID-19?**			

Have you experienced any of the following?	Yes	No	When?
Reason to believe you have had been infected with COVID-19?			
Received a positive test or confirmed diagnosis of COVID-19?			
Been hospitalized for COVID-19?			
Been Infected with COVID-19 and experienced symptoms?			
Been Infected with COVID-19 and experienced **NO** symptoms (Asymptomatic)?			
If you have had a confirmed case of COVID-19, did you experience any of the following symptoms:			
Fever or Chills?			
Cough?			
Shortness of Breath or Difficulty Breathing?			
Unusual fatigue with usual activities?			
Muscle of body aches?			
Sore Throat?			
Congestion or runny nose?			
Nausea or vomiting?			
Diarrhea?			
Have you recovered from your COVID-19 infection? (if applicable)			
Has a medical professional told you that you have you recovered from your COVID-19 infection? (if applicable)			

Please list all Medications or Supplements You Take

Medications/Supplements
Prescribed medications:
Are you taking hormone replacement (e.g., estrogen or testosterone) therapy?
Do you take supplements (aspirin, vitamins, anti-oxidants, probiotics etc.)?
Do you take a melatonin supplement (it can be a spray or tablet)? ☐ Yes ☐ No If yes, do you take it once or more a week? ☐ Yes ☐ No

Do you have any reason you believe you should not participate in this research study? ☐ Yes ☐ No

Explain:

Are you currently enrolled in any other research studies or have you participated in any other research studies in the past 30 days? ☐ Yes ☐ No

If yes, when was your last study visit (MM/DD/YYYY)?

If yes, what is the date of your next visit (MM/DD/YYYY)?

Female Subjects Only ☐ N/A - subject is male

Subject currently pregnant? ☐ Yes ☐ No
Subject plans to become pregnant? ☐ Yes ☐ No

Currently using birth control? ☐ Yes ☐ No

If yes, method of birth control *[Select All That Apply]:*

☐[1] Oral Contraceptives	☐[5] NuvaRing	☐[10] Post-menopausal for ≥ 1 year
☐[2] Hormonal Injections	☐[6] Intrauterine device	☐[11] Tubal ligation, bilateral oophorectomy, or hysterectomy
☐[3] Hormonal Implants (i.e. Implanon)	☐[7] hormonal Intrauterine device	☐[12] Abstinence
☐[4] Contraceptive Patches	☐[8] non-hormonal Barrier method	☐[13] Other (specify in Reproductive field)
	☐[9] Spermicide	
Start Date of Birth Control (MM/DD/YY):	Brand Name: _____	

What is the date do you expect you next period? _____

Do you have a regular menstrual cycle (last 3 cycles consecutive)? ☐ Yes ☐ No

Past Menstrual History

Start Date of LAST menstrual cycle (MM/DD/YY):_____

End Date of LAST menstrual cycle (MM/DD/YY):_____

Have you ever consulted a doctor about menstrual problems (specifically, about irregular or missing periods)?

_____

Have you ever consulted a doctor about any problems relating to your hormonal system? If so, please explain.

_____

For HCRL Staff Only

Form Verified by:_____ **Date:** _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Name:_____ Date: _____

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. **Please answer all questions.**

1. During the past month, what time have you usually gone to bed at night? _____
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? _____
3. During the past month, what time have you usually gotten up in the morning? _____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.) _____

5. During the past month, how often have you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				
6. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				