THE ACUTE EFFECTS OF MITOQ SUPPLEMENTATION ON PULSE WAVE VELOCITY AND WAVE REFLECTION PROPERTIES IN MIDDLE AGED AND OLDER ADULTS

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ABSTRACT

THESIS: The Acute Effects of MitoQ Supplementation on Pulse Wave Velocity and Wave Reflection Properties in Middle Aged and Older Adults

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Arteriosclerosis can be defined as the thickening and stiffening of the arteries, a process that occurs naturally with age. In part, these arterial changes can be attributed to reactive oxygen species that arise from within the mitochondria of vascular smooth muscle cells. Research exploring the effects of antioxidant supplementation, such as MitoQ, on vascular health has been limited in human subjects. **PURPOSE:** To examine arterial stiffness measured via cfPWV and wave reflection properties with an acute dose of MitoQ in a population of middle aged and older adults. **METHODS:** 16 adults (9 females, 7 males) >50 years of age participated in this double-blind, placebo-controlled crossover study. Participants completed a comprehensive health screening and maximal cardiopulmonary exercise test to determine baseline health and fitness. Participants then completed two trials in a randomized order in which cfPWV and pulse wave analysis were measured before and 1 hour after consumption of either 80mg of MitoQ or a placebo. 2-way ANOVA was used to examine differences between trials. **RESULTS:** cfPWV was not different ($p>0.05$) between MitoQ and placebo trials and this lack of difference persisted when the cohort was split into groups based on the healthy vascular aging criteria of cfPWV <7.6 m/s and after normalization for mean arterial pressure. Central diastolic blood pressure (cDBP),

diastolic pressure-time index (DPTI), and subendocardial viability ratio (SEVR) were higher ($p<0.05$) in MitoQ. **CONCLUSIONS:** Acute consumption of 80mg of MitoQ did not alter cfPWV in a cohort of apparently healthy middle aged and older adults. However, MitoQ supplementation was associated with changes in pressure that appear to drive improved myocardial perfusion as noted with significantly increased cDBP, DPTI, and SEVR. Considering these pressure changes, MitoQ may acutely enhance cardiac health through improved myocardial perfusion.

CHAPTER I

INTRODUCTION

In 2020, cardiovascular disease (CVD) attributed to 19 million deaths globally, accounting for nearly a 20% increase from 2010 (1). The most common type of CVD is coronary artery disease (CAD), a diagnosis shared by over 18 million adults in the US (1, 2). CAD is caused by atherosclerosis, a specific type of arteriosclerosis that is characterized by the buildup of plaque within the arteries of the heart and other vasculature (3). Arteriosclerosis, on a broader level, is characterized by the stiffening and thickening of the elastic arteries and appears to be a typical response to aging, even in apparently healthy individuals (3). This arterial stiffening reduces the ability of the vasculature to dilate appropriately in response to changes in tissue demand, which has been correlated with higher incidence of CVD (4).

Evidence suggests that arterial stiffening is caused, in part, by endothelial dysfunction (ED) which can be defined as a decline in endothelium-dependent vasodilatory capacity (5, 6). Endothelial dysfunction has become a hallmark of CVD, and nearly every traditional CVD risk factor has been correlated with increased atherosclerosis and ED (7). Although the development of ED is multifaceted, one of the proposed mechanisms by which this dysfunction occurs is through the overproduction of reactive oxygen species (ROS) at the level of the mitochondria (2, 8).

ROS arise most commonly from within the mitochondria, particularly the electron transport chain (ETC) during cellular respiration (9). The ETC is composed of protein complexes that harness energy from electrons to produce ATP (9). Although most oxygen present along the ETC is eventually reduced to water, a small percentage is reduced to superoxide anions (O2.-) through the leakage of electrons within the ETC (9). These O2.- molecules are one example of a

variety of ROS that serve critical roles in cell signaling pathways and aide in the process of adaptation to external stimuli. In apparently healthy individuals at rest, ROS are managed by antioxidants, yet proper signaling by ROS during periods of heightened stress such as with exercise is still possible (10). These signaling cascades occur due to the increased production of ROS during exercise beyond what can be quickly neutralized by available antioxidants (10). By contrast, in metabolically unhealthy individuals, ROS production outstrips endogenous antioxidant function even at rest, resulting in chronic oxidative stress and the accumulation of ROS (9, 11, 12). Oxidative stress is defined as the redox balance between pro-oxidants and antioxidants shifting in favor of the former at rest (2).

Chronic oxidative stress manifests itself in several disease states, but also promotes vascular cell inflammation, apoptosis, and proliferation, as well as causing alterations within the extracellular matrix of vascular endothelial cells (13). These unfavorable outcomes are the result of ROS interactions with a multitude of cellular structures and enzyme cascades, yielding damage and dysfunction through oxidation (14). Superoxide anions, for example, may directly interact with nitric oxide, resulting in the formation of peroxynitrite and causing a subsequent cascade of enzyme alterations that promote the acceleration of atherosclerosis (13).

While many exogenous antioxidants have been researched in an attempt to reduce oxidative stress at rest, not all supplemental antioxidants are equally effective (5). Mitoquinone (MitoQ) is a mitochondrial-targeted antioxidant composed of the antioxidant ubiquinol attached to triphenylphosphonium (8). The triphenylphosphonium cation allows MitoQ to cross the cellular and mitochondrial membranes and accumulate within the mitochondria where it is ideally positioned to scavenge for ROS as they are produced (8).

Six weeks of MitoQ supplementation has been shown to improve endothelial function as measured by pulse wave velocity and flow-mediated dilation in apparently healthy older adults compared to a placebo (2). MitoQ has also been found to improve endothelial function and exercise tolerance acutely in peripheral arterial disease (PAD) patients (8). These changes in endothelial function do not appear to occur under acute MitoQ supplementation in younger adults, likely due to the lack of arteriosclerotic progression and endothelial dysfunction early in life (3, 15).

Pulse wave velocity (PWV) is a measure of arterial stiffness and has been established as a viable, though uncommon, method of measuring acute changes in endothelial function (16). PWV considers the response of the vascular system to the projection of blood from the heart (4). As blood passes from the large elastic arteries to the muscular arteries of the periphery, an accompanying pressure wave is produced (4). The stiffness of the elastic arteries interacts with the pressure wave, determining the speed at which blood travels through the vasculature and what resistance it meets (4). As the pressure wave moves through the arterial tree, it is partially reflected back towards the heart (17). This reflected wave returns to the heart and aides in coronary artery perfusion during late systole or early diastole (17, 18). A faster pressure wave results from stiffer arteries and yields an unfavorably high PWV measurement (4). Further, the stiffening of the arteries causes the reflected wave to return to the heart during systole, resulting in decreased coronary perfusion (17).

The relationship between acute decrease in ROS and vascular function measured by PWV in humans is not well-researched. Therefore, the primary aim of this study is to examine arterial stiffness measured via PWV and wave reflection properties with an acute dose of MitoQ

in a population of middle aged and older adults (MA/O adults). It is hypothesized that acute MitoQ supplementation will decrease resting PWV in MA/O adults compared to a placebo.

CHAPTER II

LITERATURE REVIEW

Introduction

Due to high number of deaths each year resulting from CVD, managing cardiovascular health and risk factors to minimize mortality is an important topic of discussion. Recently, reactive oxygen species, produced either through cellular respiration or exogenous sources, have gained interest due to their critical role in many cellular functions including involvement in the aging process. Chronic oxidative stress caused by an imbalance between reactive oxygen species and antioxidants has been linked to a wide variety of chronic diseases, including cardiovascular disease, cancer, and diabetes. Research to determine the impact of oxidative stress on the vascular system is ongoing, but studies have shown markers of oxidative stress to be linked with increased incidence of arterial stiffness. These discoveries motivate exploration of certain antioxidant supplements, such as MitoQ, and whether they are able to reduce oxidative stress and improve disease outcomes. To assess the effectiveness of these supplements on the vasculature, measures such as pulse wave velocity and central blood pressure may prove useful. These variables can be measured non-invasively and may provide insight into the interaction between MitoQ and an individual's preexisting cardiovascular risk factors and vascular health.

Oxidative Stress

Oxidative stress occurs when there is an imbalance between the accumulation and neutralization of reactive oxygen species (ROS). ROS occur naturally as a byproduct of oxygen metabolism and play several physiological roles in healthy cell function including cell signaling, repair, and the facilitation of apoptosis (19-21). ROS accomplish these functions through pro-inflammatory pathways, which promote clearance of pathogens and damaged tissue, but can also

result in damage to the cells and tissue if unchecked, as in the case of chronic oxidative stress (22). ROS can arise from multiple endogenous sources but are produced most prevalently via mitochondrial respiration (21). ROS can also arise from exogenous sources such as pollution, alcohol, and tobacco smoke, which can contribute to ROS production beyond antioxidant neutralization capabilities (23).

The combination of endogenous and exogenous sources causes a variety of ROS to be formed. ROS can be defined as any oxygen-containing atoms or molecules that are also reactive species (24). This definition includes superoxide (O_2.-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.), among others (5, 24). Many, but not all, ROS are volatile in nature due to the presence of at least one unpaired electron in their valence shell (5). These types of ROS are called free radicals (5). Due to their unpaired electrons, free radicals are highly reactive and seek stability from surrounding molecules (25). To become neutral, free radicals can give or receive valence electrons by interacting with surrounding stable molecules (25). This causes the surrounding molecules to become free radicals themselves (25). The result is a cascade of free radical production that can damage cellular structure and integrity (23).

In humans, the most prevalent type of ROS is superoxide, which is produced in the electron transport chain (ETC) within the mitochondrial matrix during oxidative phosphorylation (26, 27). The electron transport chain passes donated electrons from NADH and $FADH_2$ along a series of complexes in order to drive ATP synthesis (27). Complexes I, III, and IV pump protons into the intermembrane space and the resultant proton gradient allows ATP synthesis to occur (27). During this process, O_2 is reduced to H2O, except in cases where electrons leak out of the ETC and interact with O_2 to create O_2.- (27). The major site of O_2.- generation is complex III due to ubisemiquinone, a Q cycle intermediate that undergoes autooxidation, therefore donating an

electron to O_2 (27). O_2.- produced in complex III can be released into both the mitochondrial matrix and the intermembrane space, where other reactions may occur, resulting in cascades of damage and the production of other ROS from O_2.- (27). O_2.- and other ROS that are released through these processes can cause damage to the mitochondria itself and initiate degradation throughout the body (28).

Under normal physiological conditions the body also produces antioxidant defenses to neutralize ROS before they can cause damage to cells and organ systems (25). An antioxidant is a molecule that can donate an electron to a free radical in order to neutralize it, thereby preventing damage to other cell structures (25). Antioxidant molecules are stable enough to donate electrons without becoming unstable themselves (25). These molecules are categorized by type as well as by the action that they perform in the body. There are two main antioxidant defense systems. One system helps to prevent initial ROS formation and scavenges any ROS that may form (29). The second system neutralizes and repairs molecules damaged by ROS interaction before they are able to cause downstream harm (29). For example, superoxide dismutase (SOD) can scavenge for O_2.- and convert it into hydrogen peroxide, which can be further degraded so that it is not harmful to the cell (30).

When antioxidants are not able to match the rate of ROS appearance over a long period of time, chronic oxidative stress is said to be present in the body (29). The result of chronic oxidative stress is the promotion of cardiovascular diseases such as hypertension, hypercholesterolemia, heart failure, and vascular disease, as well as other chronic disease states including chronic kidney disease, cancer, diabetes, and neurodegeneration (5, 27, 29, 31, 32). These diseases may further contribute to accelerating ROS production once developed (5, 29). Furthermore, the development of these chronic diseases correlates strongly with increasing age

(33). Indeed, it has been suggested that oxidative stress is a key component of aging (33). Damage caused by ROS can facilitate loss of function across tissues and organ systems and these losses are a hallmark of the aging body (33). The mitochondrial theory of aging links ROS with damage to mitochondrial DNA. This theory postulates that the accumulation of oxidative damage to the mitochondrial DNA can cause mutations that further accelerate the degeneration of mitochondria and produce even greater numbers of ROS (34). This cycle of damage and mutation can lead to cell death and energy depletion, which has been thought to explain the origin of the disease states discussed above (34).

MitoQ

Antioxidant supplementation is a topic of interest in both scientific and lay communities due to the potential health benefits these substances boast. Many antioxidants such as vitamins C and E have been studied in an attempt to manage the excessive production of mitochondrial ROS common in chronic disease states and oxidative stress (26). Overall, these antioxidants have failed to significantly benefit individuals in chronic disease states, likely for several reasons (26). First, antioxidants small enough to be of use in the mitochondria typically become distributed throughout the body, with a limited amount being taken up by the mitochondria (26). Further, the total amount of antioxidant that can be taken up by the body may limit the therapeutic effects possible (26).

MitoQ is a supplement composed of the antioxidant ubiquinol conjugated to a triphenylphosphonium (TPP) cation (2). TPP is a lipophilic cation which can pass through the lipid bilayer of the cell membrane and into the mitochondria due to its large, hydrophobic radius compared to other cations (26, 35). TPP does not require a specific mechanism to cross the lipid bilayer and has a comparatively low activation energy (26). Due to the membrane potential, TPP

will accumulate within the cytosol of the cell where it will then be driven into the mitochondria and accumulate at about 200 times greater a rate in the mitochondrial matrix than in the cytosol (26).

Once MitoQ has entered the mitochondrial matrix, this compound is reduced by complex II of the ETC, producing active ubiquinol (26). Due to the number of carbons in the compound side chain and subsequent ability to travel deep within the inner mitochondrial membrane, MitoQ is thought to be beneficial primarily due to the prevention of lipid peroxidation (26). When ubiquinol is oxidized, it can be rapidly recycled through complex II in the form of ubiquinone, producing ubiquinol again (26). This property of MitoQ is important because the ability to be recycled in the mitochondria determines the efficacy of an antioxidant (26). While this antioxidant pathway has been heavily studied in vitro, it is still unclear whether MitoQ functions along the same pathway in vivo, and research is especially limited in human subjects (26).

Even with limited understanding of how MitoQ assists in the regulation of oxidative stress in humans, this compound has been associated with several positive outcomes in a variety of populations. When comparing MitoQ to another antioxidant, CoQ10, in healthy middle-aged men, there was evidence that both supplements suppressed hydrogen peroxide produced through respiration, although MitoQ appeared to be somewhat more effective that CoQ10 (36). In one study, healthy older adults (ages 60-79) underwent either 6 weeks of MitoQ supplementation or received a placebo (2). In this study, those who received MitoQ observed increased flow-mediated dilation of the brachial artery (42% increase), and those who had a baseline cfPWV of greater than 7.60 m/s had lower aortic stiffness after 6 weeks of supplementation compared to baseline (2). Another study focusing on endothelial function in PAD patients demonstrated that an acute dose of 80mg of MitoQ could improve vascular endothelial dysfunction, walking

capacity, and delayed claudication onset in this patient population (8). By contrast, a study done in healthy young adults using an acute MitoQ dosage of 100-160mg depending on body mass revealed no significant changes in arterial stiffness or central hemodynamics, though it should be noted that this data has not been peer reviewed. (15). Further research is needed to determine what populations, conditions, and disease states may benefit from MitoQ supplementation, as well as to elucidate the mechanisms by which MitoQ interacts with ROS in human subjects.

Arterial Stiffness

Stiffening of the arterial walls, also called arteriosclerosis, is generally considered to be a hallmark of typical aging and is strongly correlated with incidence of cardiovascular disease and other chronic diseases independent of traditional CVD risk factors (4, 37). Stiffening of the central arteries has been correlated with kidney disease, diabetes, and the acceleration of atherosclerosis (4). While increasing arterial stiffness is correlated with age, even young adults with greater arterial stiffness have been shown to have an increased incidence of metabolic syndrome, increased left ventricular mass, and insulin resistance (38, 39). It has been suggested that arteriosclerosis is an early marker of disease presence, rather than a risk factor for disease, even in adults who appear to be generally healthy (40). This is due to the multitude of diseases that have been linked to the acceleration of arteriosclerosis such as diabetes and hypertension (40).

Arteriosclerosis describes a condition wherein the arterial walls become harder and thicker (37). This process has been thought to occur primarily due to alterations in the composition of load bearing elements in the vascular extracellular matrix including the ratio of collagen to elastin (41). While collagen is constantly repaired and remodeled in the medial layer of the arteries, elastin appears to be deposited during fetal growth and infancy alone (41). It is

thought that elastin has a half-life of approximately 40-50 years, meaning that arteries will eventually stiffen naturally as collagen, the stiffer component of the cellular matrix, becomes the more prominent structure (41). While this process alone produces significant changes in the large elastic arteries, a multitude of other components are thought to contribute to the stiffening of these arteries with increasing age (41). Focus has recently broadened to the role of vascular smooth muscle cell changes (37, 40). The exploration of vascular smooth muscle cell changes includes research on increased mitochondrial oxidative stress as well as changes in gene expression and mutation within these cells (40).

While oxidative stress has been implicated in aortic stiffening, the link between aging, chronic oxidative stress, and the effect on arterial smooth muscle cell function is not yet fully understood (30). Much of the research linking mitochondrial oxidative stress to arteriosclerosis thus far has been conducted in mice. In one study, deletion of the SOD2 gene in mice resulted in lower aortic compliance and decreased vasorelaxation over their lifespan (30). SOD2 deficiency also resulted in higher collagen levels and lower elastin levels in the aortic smooth muscle cells (30). The SOD2 deficient mice also had aortic smooth muscle cells that were more susceptible to apoptosis and less able to preserve their mitochondrial integrity (30).

Another study found that mice that had been genetically altered to express NADPH oxidase 4 (NOX4) 5-fold above typical levels had similar results to the SOD deficient mice (42). NADPH oxidases are another major source of ROS in vascular smooth muscle cells, along with mitochondrial sources (42). The mice that expressed NOX4 at the highest rates saw increased vascular smooth muscle cell stiffness, remodeling of the extracellular matrix, and lower compliance, as well as increased incidence of DNA damage, apoptosis, and inflammatory protein expression (42).

While research is more limited in humans, studies attempting to isolate markers of oxidative stress have been conducted (43, 44). In one study done on patients with sickle cell disease or sepsis, two conditions known to cause oxidative stress, cysteine was isolated as a sensitive biomarker for oxidative stress in the plasma (43). In this study, cysteine was sensitive to the oxidative stress and to the antioxidant treatment effectiveness (43). In another study done on 169 people free of traditional cardiovascular disease risk factors, the presence of cystine was associated with increased arterial stiffness (44). These results suggest that oxidative stress may result in vascular dysfunction even in individuals who are considered to be otherwise healthy (44).

In addition to general markers of oxidative stress in the blood, arterial stiffness has also been linked to endothelial dysfunction via imbalances in the production of nitric oxide. Nitric oxide (NO) produced in the vascular endothelial cells has many important functions for appropriately maintaining the vasculature (45). These critical functions include vascular dilation, inhibition of platelet aggregation, and alteration of the ability of leukocytes to adhere to the vessel walls (45). When less NO is available, these important functions may be impaired. Reduced availability of NO can occur through several pathways, including interactions with ROS produced within the cells (45). NO is highly reactive due to its unpaired electron and in the presence of ROS, such as O_2.-, will readily react to form reactive nitrogen species such as peroxynitrate (45). The creation of peroxynitrate can inhibit the generation of additional NO, thereby resulting in even greater oxidative stress (45). This cycle can cause damage to the endothelial cells and prevent them from functioning optimally, especially with respect to their vasodilatory and antithrombotic properties (45).

Although more research is needed to fully elucidate the impact of oxidative stress on arterial stiffness, the evidence discussed here suggests several mechanisms by which oxidative stress plays a role in detrimental changes in the endothelium.

Pulse Wave Velocity

Pulse wave velocity (PWV) is the most widely used measure of arterial stiffness and has been used preferentially due to its non-invasive, cost-effective, and reproducible nature (3). While the degree to which PWV can stand as an independent risk factor for CVD is heavily debated, the association between PWV and arterial stiffness, arterial compliance, and distensibility has been clearly established (3, 18, 46).

To assess PWV non-invasively, an automated device can be used. These devices collect and calculate the desired variables through several different means. One such device, the SphygmoCor XCEL, can take automated blood pressure as well as measuring volumetric displacement of peripheral pressure waveforms to estimate central waveforms (47, 48). When using this device to assess carotid femoral PWV (cfPWV), a cuff is placed around the upper thigh to measure volumetric displacement of the peripheral waveform (47). Applanation tonometry is simultaneously used to acquire the peripheral waveform at the carotid artery (47). These waveforms, along with distance measures along the surface of the body allow the SphygmoCor XCEL to calculate cfPWV (47). This measure has been extensively tested and validated using the SphygmoCor XCEL (47). Further, cfPWV has been commonly used in research and has been named the gold standard for estimating changes to the central vasculature (47, 49). This measurement roughly follows the path of the aorta rather than estimating from the extremities, making it favorable over other methods of assessing PWV (47, 49).

PWV refers to the velocity with which blood travels through the vessels after systole (4). The contraction of the heart causes blood to move through the large elastic arteries and into the muscular arteries of the periphery following a pressure wave, or pulse wave (4, 18). With each heartbeat, a new forward-propagating wave is generated, traveling through the arterial tree to deliver blood under high pressure (4). Under optimal circumstances, the interaction of the propagated wave with the aorta results in a pulse wave with a relatively low velocity. As the pulse wave moves distally, it encounters areas of impedance including changes in vessel wall properties and diameter (17). These areas of impedance mismatch amplify the pulse wave and partially reflect the wave back towards the heart (17). This reflected wave returns to the heart during early diastole or late systole, aiding in coronary perfusion and augmenting diastolic blood pressure (17, 18). The reflected wave is also advantageous because it lessens the pulsatile energy that will reach distally to the microcirculation, a critical function for mitigating end-organ damage (17).

PWV has been shown to increase in the presence of vascular aging, which includes changes to the arterial walls caused by atherosclerosis, endothelial dysfunction, and changes in the elastin to collagen ratio in the vessel walls (49). These PWV changes are predominantly found within the central arteries which are composed of a higher percentage of elastin (50). The change in PWV may be present, but is less prominent, in the muscular arteries of the periphery (50). While lifestyle does have an impact on an individual's ability to maintain healthy vascular aging, the deterioration of vascular health can only be delayed by healthy choices, not halted entirely (51). These vascular changes seem to accelerate after the age of 50 and are especially true of those over 70 (17, 51).

With the stiffening of the central arteries, the reflected wave returns to the heart sooner, arriving during systole and causing diastolic blood pressure to drop while systolic blood pressure rises (49). These changes in blood pressure result in a greater workload for the heart (49). In addition, increased stiffness in the central arteries translates to greater pulsatile flow to the peripheral tissue, resulting in end-organ damage (17). This damage is thought to be furthered by changes to the microcirculation (17).

While much research is still being conducted on the factors impacting cfPWV, this measure has been used to set the normative values for PWV across a multitude of studies. A study published in the European Heart Journal used a population of over 11,000 European men and women with no diagnosed cardiovascular disease, diabetes, or treated hypertension or hyperlipidemia to establish normative values for PWV (52). These values increase substantially with age, especially after age 60, where the mean PWV was 10.3 m/s compared to an average of 8.3 m/s in the fifth decade of life (52). While this research supports the statement that PWV increases with age regardless of other risk factors for CVD, other studies have attempted to define healthy vascular aging and whether those vascular changes can be prevented (51, 52). Using the Framingham Cohort, one study defined healthy vascular aging as the absence of hypertension and a cfPWV of less than 7.6 m/s in adults 50 years and older (51). In research on vascular aging, the cfPWV of 7.6 m/s has become an accepted cutoff for determining a healthy PWV measurement.

Central Blood Pressure and Pulse Pressure

Blood pressure, measured at the brachial artery, has been long established as a risk factor for cardiovascular events and can also be viewed as a marker of heightened arterial stiffness that occurs with increasing age (53). Central blood pressure (cBP) has been found to be even more

valuable than brachial blood pressure when attempting to predict mortality from CVD due to the direct interaction between the heart and stiffening aorta (54). Central blood pressure is the product of a multitude of factors, including arterial stiffness, age, sex, heart rate, and body height, among others (54). One study used a validated oscillometric device to provide 24-hour ambulatory assessment of central blood pressure in over 2,500 subjects, producing evidence that males have a higher central blood pressure than females (55). Further, central systolic blood pressure (cSBP) and heart rate were highest with middle age, whereas central pulse pressure (cPP) was highest with older age (55). This study also suggests that central blood pressure varies throughout the day, with the average values across the population being 128/79 mmHg during the day and 125/72 mmHg at night (55).

While the factors that influence central blood pressure are still being explored, there is evidence that damage to other organs, such as the brain and kidneys, is also linked to central hemodynamics, particularly heightened cPP (56, 57). cBP has also been found to correlate strongly with left ventricular mass and hypertrophy (54). In particular, the kidneys, heart, and brain seem to be most greatly affected by elevated central pulse pressure because they are directly exposed to it through the arterial tree, rather than being exposed to peripheral pressures (56). Further, it is the central blood pressure at the aorta that interacts directly with the heart to determine both afterload and coronary perfusion (58).

Pulse pressure (PP) is defined as the difference between systolic and diastolic blood pressure, where systolic blood pressure (SBP) is the highest pressure measured during the contraction of the left ventricle, and diastolic blood pressure (DBP) is the lowest pressure experienced which occurs while the heart is relaxed (59). With age, especially after midlife, PP tends to increase, indicating a larger range between SBP and DBP, mostly due to increased SBP

(53, 54). Age-related arterial stiffness results in the heart contracting against less compliant arteries and this change primarily produces higher SBP values (53, 59). Increasing SBP drives PP upward, which may eventually translate to end organ damage (59). Furthermore, PP is impacted by the reflected wave, the diameter of the aorta, and the aortic flow, which can increase the difference between SBP and DBP even more dramatically (53). PP varies throughout the arterial tree, and the difference between SBP and DBP tends to widen as blood travels towards the periphery (60).

When considering these changes in PP, it is important to understand that SBP is amplified as the pressure wave travels away from the heart (57). In fact, SBP at the brachial artery will be as much as 40 mmHg greater than at the aorta, while DBP remains mostly unchanged (57). This is due to increasing vascular stiffness and interaction with the reflected wave when moving distally (57). The interaction between the forward propagating wave and reflected wave has been termed "augmentation pressure" and helps to describe the degree that the reflected wave raises blood pressure above and beyond what would occur in the presence of the forward propagating wave alone (61). With age, the difference in central and peripheral pressures begins to narrow, mainly due to an increase in central blood pressure (60).

The most direct and accurate measurement of cBP is produced invasively using a catheter, but this method is not practical for regular assessment of cBP (57, 58). Several methods to estimated cBP reliably and non-invasively have been developed, each finding slightly different ways to account for the factors that influence central and peripheral pressures, and the differences between them (57, 58). One technique uses applanation tonometry at the radial or carotid artery and calibrates this measurement to brachial blood pressure (58). The calibrated

measurements are applied to a generalized transfer function that produces an estimate of cBP (58).

Another technique for assessing cBP uses an oscillometric cuff that automatically inflates to produce the needed measures. The cuff first assesses brachial blood pressure by sensing oscillations in cuff pressure (62). Inflation techniques and algorithms to produce a final measurement may vary depending on the model (62, 63). After brachial pressure has been assessed, the cuff reinflates, usually near the level of the diastolic measurement (63). Here, the cuff uses pulse volume plethysmography to sense subtle changes in arm volume as blood flows through the arterial tree (62, 63). After these measurements have been collected, the values are calibrated to the initial brachial blood pressure and a generalized transfer function can be applied to produce a cBP estimation (63, 64).

Although these techniques are more convenient than invasive means of cBP measurement, they are estimations that depend on several factors that may introduce sources of error. First, generalized transfer functions are not specific to the individual and may not accurately reflect the difference between central and peripheral pressures (63). Additionally, using brachial blood pressure cuff measurements to calibrate introduces population wide assumptions and algorithms in the case of an oscillometric device (62, 63). Furthermore, all methods of assessing cBP require training and quality control (58). Even considering these sources of error, non-invasively measured cBP has been correlated to incidence of future cardiovascular events (57, 65).

Furthermore, non-invasive measures of cBP have been used to determine the impact of certain anti-hypertensive medications on cBP and pulse pressure amplification (PPA) with respect to peripheral pressure measurements (65). One study found that 12 weeks of treatment

with amlodipine, candesartan, or indapamide SR were able to reduce cBP and cPP, which appeared to be largely due to changes in wave reflections (65). While all three drugs produced changes in cBP in subjects with essential hypertension, only indapamide SR was able to significantly decrease PPA (65).

Another study used over 2000 participants with hypertension (untreated or treated but not well controlled), who also had at least 3 other CVD risk factors to examine the differences in central and peripheral blood pressure responses to treatment with amlodipine or atenolol (66). In the amlodipine group, perindopril was added if blood pressure was not responsive to the initial dosage (66). In the atenolol group, bendroflumethiazide K was added as needed (66). The participants were assessed via tonometry and pulse wave analysis at baseline and at least twice more prior to the end of the follow up period (~3 years later) (66). The results demonstrated that brachial blood pressure response was similar between treatment groups, but amlodipine significantly reduced cBP and cPP compared to atenolol (66). By comparison, atenolol was found to increase cBP, likely due the differences in the mechanism of action between the drug types (66). These results demonstrate that brachial and central blood pressures do not always change in unison, and even suggest that using certain medication groups such as beta blockers for the treatment of blood pressure may need to be considered more carefully (66).

Conclusion

Measures of vascular stiffness and vascular health such as central blood pressure and pulse wave velocity have been linked to health outcomes including increased risk of CVD and end organ damage, especially with increasing age. The mechanisms by which these measures increase with age are not yet fully understood and include a multitude of components. Because these variables have been found to be responsive to certain medications and supplements in

specific disease states, but their responsiveness to antioxidant supplements such as MitoQ has not been explored in the general adult population, further study is warranted. The study of MitoQ in apparently healthy adults may improve the understanding of specific mechanisms of arterial stiffness arising from oxidative stress through observed changes to central hemodynamics.

CHAPTER III

METHODOLOGY

Participants

This study included a sample of 16 participants (9 females, 7 males) at least 50 years of age with no known clinical disease. Participants were recruited via face-to-face interaction, flyers posted around Ball State University's campus, and through mass email. Individuals with a body mass index (BMI) of greater than 35 kg/m2, resting blood pressure greater than 160/100 mmHg, or who were smokers, as well as individuals prescribed any metabolic medication, such as metformin or another type of medication used for glucose control, were excluded from the study. Individuals with signs and symptoms suggestive of cardiovascular or metabolic disease were also excluded. Women were post-menopausal defined as cessation of menses for at least 1 year. Each participant provided written informed consent as approved by the Ball State University Institutional Review Board.

Study Design

This study used a double-blind, placebo-controlled crossover design to examine the effects of acute MitoQ supplementation on resting arterial stiffness and wave reflection properties. Participants visited the Clinical Exercise Physiology Laboratory within the Ball State University Human Performance Laboratory four times. The participant was instructed to fast from food and drink (other than water) for a minimum of 12 hours prior to each visit. Participants were asked to track their food intake prior to each trial and to avoid high fat meals (>50g) for 12 hours prior to each visit. Further, they were asked to abstain from NSAIDs for 24 hours prior and aspirin for 72 hours prior.

Before the first visit, each participant completed a health history questionnaire to confirm eligibility. The first visit involved the collection of baseline resting data including anthropometrics, DXA scan, resting ECG, a blood glucose and lipid panel, and assessment of vascular hemodynamics. Visit two involved a maximal cardiopulmonary exercise test (CPET) using a cycle ergometer and a ramp protocol where subjects were encouraged to exercise to volitional fatigue. Upon arriving in the CEP lab for visits three and four, height and weight were measured, followed by a supine rest of at least 10 minutes. Baseline pulse wave analysis (PWA), and PWV measures were collected after the rest period. Following the collection of baseline measures, the subjects were given either 80mg of MitoQ supplement (16 capsules) or 16 placebo capsules to ingest. The placebo consisted of empty pill capsules. Participants were given 15 minutes to consume either the MitoQ or placebo capsules. Following ingestion, participants rested supine for at least 60 minutes before PWA and PWV measures were repeated.

Clinical Measures

Anthropometrics and Body Composition:

Height in centimeters was measured using a calibrated stadiometer to the nearest 0.1 cm. Weight in kilograms was collected from a calibrated electronic scale to the nearest 0.1 kg. These measures were used to calculate body mass index (BMI, kg/m^2). Waist circumference was measured with an inelastic tape measure above the iliac crest and hip circumference was measured at the widest part of the hip. From these measures, waist-to-hip ratio was calculated. Dual X-ray absorptiometry was used to assess regional body fat percentage, fat mass, and fat-free mass.

Resting Heart Rate, Blood Pressure, and Electrocardiogram:

A resting heart rate was obtained from a finger pulse oximeter after 5 minutes of quiet rest. Following the collection of resting heart rate, no less than 2 measurements of resting brachial blood pressure were collected, at least 1 minute apart. Measurements differing by >5mmHg resulted in additional measurements as needed. All measurements were collected on the left arm. A supine 12-lead ECG was completed to screen for abnormal heart rhythms.

Pulse Wave Analysis:

Resting central and brachial supine blood pressure were assessed using SphygmoCor Xcel (Atcor Medical). After 10 minutes of supine rest and collection of FMD data that is documented elsewhere, the participant was fitted with a blood pressure cuff on the right upper arm. The researcher ensured proper fit of the cuff against the bare arm of the participant (47). Automated blood pressure and PWA were then conducted, yielding brachial and central blood pressures and pulse pressures, as well as many additional hemodynamic values that were automatically calculated by the Xcel (47, 67, 68). These measures were collected at least twice to obtain two measures within an acceptable range. The criteria to determine acceptable variance between measures was brachial systolic blood pressure within 5 mmHg, an AIx75 within 5%, and system quality control approval. In order for the measures to be accepted, all three criteria must be met. The two acceptable measures were averaged.

Pulse Wave Velocity:

Upon completion of the PWA test, the cfPWV measure was collected using the Sphygmocor Xcel. A femoral blood pressure cuff was fitted to the participant's right upper thigh and the participant was asked to locate their own femoral pulse, with assistance from the researcher as needed. The carotid pulse was palpated by the researcher and marked at its

strongest point. An inelastic tape measure was used to measure the distance (in mm) of the carotid pulse to the suprasternal notch, the suprasternal notch to the top edge of the femoral cuff, and the top edge of the femoral cuff to the femoral pulse (47, 67, 68). The three measurements, as well as the last recorded brachial blood pressure from PWA were recorded in the system. A tonometer was placed on the marked area of the carotid pulse and was held in place by a researcher for the duration of the test, while the femoral cuff automatically inflated (47, 67, 68). This procedure was repeated until two acceptable measures of PWV within 0.5 m/s were recorded. The two measures were then averaged.

Statistical Analysis

Statistical analysis was conducted using Prism (version 8). Descriptive statistics were generated to provide a summary of baseline data in the entire cohort. Two-way ANOVA tests with repeated measure on time were generated to examine the presence of time by group interactions in the following variables between the placebo and supplement groups across the cohort: PWV, bSBP, bDBP, bPP, cSBP, cDBP, cPP, mean arterial pressure (MAP), PP amplification, AP, augmentation index (AIx), augmentation index at 75% of maximal heart rate (AIx@HR75), systolic pressure time integral (SPTI), diastolic pressure time integral (DPTI), subendocardial viability ratio expressed as a percentage (SEVR), and ejection duration expressed as a percentage (ED). Absolute change in PWV between trials was examined using a mixed effects model to determine if a baseline PWV above or below 7.6 m/s influenced change in PWV between supplement groups. An unpaired T-test was used to further examine the absolute change in PWV in the MitoQ group alone. A matched-pairs T-test was conducted to examine any difference between supplement groups with respect to PWV adjusted for MAP. The threshold for significance was $p<0.05$.

CHAPTER IV

RESULTS

Descriptive statistics for the cohort (n = 16) can be found in **Table 1**. This cohort was comprised of 7 males and 9 females, with an average age of 63.7 ± 2.7 years. At baseline, all participants had resting brachial blood pressure measurements within normal limits. Four subjects reported taking a blood pressure medication (n=1 calcium channel blocker; n=1 angiotensin II blocker; n=2 ace inhibitor). No subject reported more than one medication for blood pressure control. Lipid values varied between subjects with 4 subjects reporting taking a statin drug for lipid management.

Table 1. Subject Characteristics

	Mean	SE
M/F	7/9	-
Age, yrs.	63.7	2.7
HR_{rest}, bpm	60.8	2.6
bSBP, mmHg	117.3	3.0
bDBP, mmHg	71.9	2.7
BMI, kg/m^2	26.4	0.9
BF, %	33.4	2.3
WTHR	0.89	0.0
TC, mg/dL	204.8	11.9
LDL, mg/dL	128.4	8.7
HDL, mg/dL	60.4	4.5
Triglycerides, mg/dL	88.1	6.0
Glucose, mg/dL	94.8	1.7
HbA1c, %	5.6	0.1
CRF*, $mL \bullet kg \bullet min^{-1}$	27.0	2.4
Estimated VO2max	25.8	2.4

HR_{rest}, resting heart rate; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; BMI, body mass index; BF, body fat percentage; WTHR, waist to hip ratio; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; CRF, cardiorespiratory fitness; estimated VO2max using the Whaley equation. *n=14 for these values.

Table 2 presents data on central and brachial hemodynamic and wave reflection properties before and after supplementation with either 80mg of MitoQ or placebo. When examining group by time interactions, MitoQ supplementation significantly increased cDBP ($p = 0.05$), DPTI ($p = 0.03$), and SEVR ($p = 0.04$) compared to a placebo. Increased bDBP and MAP values trended towards significance but did not meet the criteria of $p < 0.05$ ($p = 0.07$ and 0.08, respectively).

There was no significant difference in cfPWV between trials when considering the entire cohort ($p = 0.20$). **Figure 1** and **Figure 2** demonstrate change in cfPWV when the cohort was divided into groups based on the healthy vascular aging criteria of cfPWV <7.6 m/s at baseline for each trial. When split into these groups, no significant difference in cfPWV was found between trials ($p = 0.28$). As can be seen in **Figure 3**, cfPWV for the whole cohort was also normalized to MAP (PWV/MAP), and no significant difference was found between or among groups ($p = 0.83$).

Table 2. Hemodynamic and wave reflection properties before and 1 hour after consumption of either MitoQ or placebo

		Pre	1 Hour Post
cfPWV, m/s	MitoQ	7.4 ± 0.3	7.9 ± 0.3
	Placebo	7.6 ± 0.3	7.8 ± 0.3
bSBP, mmHg	MitoQ	124.2 ± 3.5	132.0 ± 3.7
	Placebo	124.7 ± 3.2	130.2 ± 3.3
bDBP, mmHg†	MitoQ	74.3 ± 1.5	79.0 ± 1.4
	Placebo	75.2 ± 1.8	76.8 ± 1.4
bPP, mmHg	MitoQ	49.8 ± 2.7	53.0 ± 3.1
	Placebo	49.4 ± 2.3	53.3 ± 2.6
cSBP, mmHg	MitoQ	115.0 ± 3.1	121.3 ± 3.2
	Placebo	116.2 ± 3.0	120.2 ± 3.0
cDBP, mmHg*	MitoQ	75.1 ± 1.5	79.9 ± 1.4
	Placebo	76.1 ± 1.9	77.4 ± 1.4
cPP, mmHg	MitoQ	39.9 ± 2.2	41.4 ± 2.6
	Placebo	40.0 ± 2.0	42.8 ± 2.4
MAP, mmHg†	MitoQ	89.5 ± 2.0	94.2 ± 2.0
	Placebo	90.4 ± 2.2	92.2 ± 1.9
PP AMP	MitoQ	1.3 ± 0.02	1.3 ± 0.02
	Placebo	1.2 ± 0.02	1.3 ± 0.02
AP, mmHg	MitoQ	12.9 ± 1.4	11.4 ± 1.2
	Placebo	13.6 ± 1.6	13.3 ± 1.6
AIx, %	MitoQ	31.9 ± 3.0	28.0 ± 2.6
	Placebo	33.2 ± 3.3	30.7 ± 3.6
AIx@HR75, %	MitoQ	23.8 ± 3.6	18.4 ± 3.2
	Placebo	24.6 ± 4.1	21.2 ± 4.4
SPTI, mL	MitoQ	2181 ± 101	2230 ± 104
	Placebo	2154 ± 101	2213 ± 88
DPTI, mL*	MitoQ	3181 ± 76	3423 ± 63
	Placebo	3263 ± 84	3339 ± 73
SEVR,%*	MitoQ	150.8 ± 8.4	158.6 ± 7.9
	Placebo	156.6 ± 8.3	154.9 ± 6.9

Data presented as mean ± SE. * denotes main effect for interaction where $p < .05$. † denotes main effect for interaction where $p < .10$.

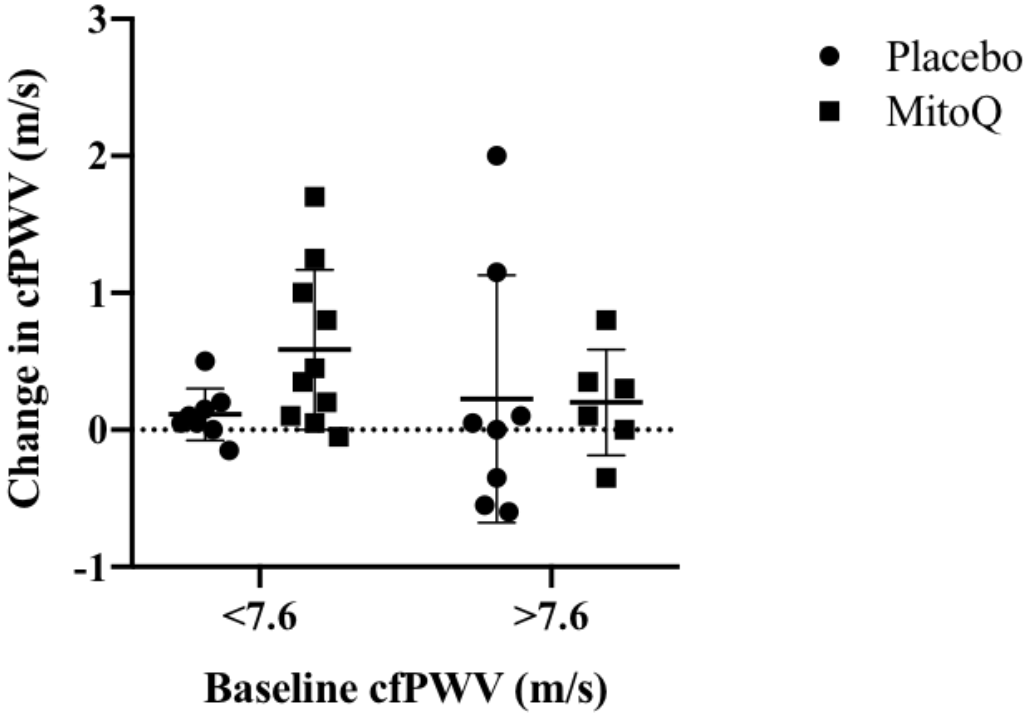

Figure 1. Change in cfPWV with respect to baseline cfPWV. The cohort was divided using baseline cfPWV for each trial and the healthy vascular aging value of 7.6 m/s.

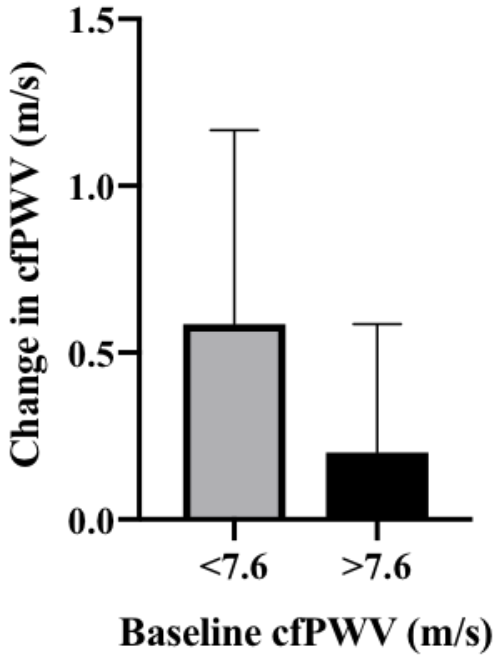

Figure 2. Change in cfPWV with MitoQ supplementation. Data are presented as mean ± SE.

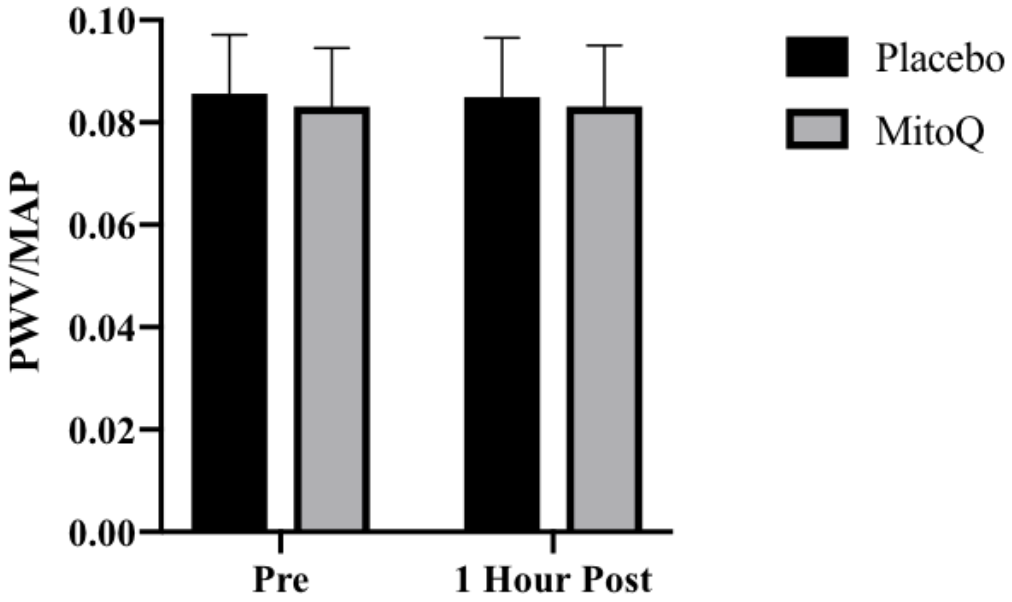

Figure 3. PWV normalized to MAP before and after supplementation. Data are presented as mean ± SE.

CHAPTER V

DISCUSSION

The primary aim of this study was to examine the effect of acute MitoQ supplementation on vascular function and cfPWV. It was hypothesized that a dose of 80mg of MitoQ would acutely decrease resting cfPWV in middle aged and older adults compared to a placebo. To our knowledge this was the first study to examine the impact of an acute dose of MitoQ on cfPWV in MA/O adults absent of diagnosed cardiovascular or metabolic disease. These data suggest that there is no effect of MitoQ supplementation, regardless of baseline PWV, even when subjects were divided into groups using the 7.6 m/s cutoff for healthy vascular aging (51). Furthermore, cfPWV remained insignificant when normalized to MAP. Pressure changes did contribute to a significant increase in SEVR with MitoQ supplementation, suggesting improved myocardial perfusion.

The results of this study add to the limited body of literature regarding acute MitoQ supplementation and cfPWV changes in human subjects. Our results are consistent with finding that no significant change in cfPWV was present in apparently healthy young adults receiving an acute MitoQ dosage of 100-160 mg (15). Considering the overall cardiorespiratory fitness of the cohort and minimal cardiovascular disease risk factors present on average, it is possible that 80 mg of MitoQ was not a sufficient stimulus to elicit a significant response in our cohort, similar to the cohort of healthy young adults. 80 mg of MitoQ was chosen because previous human research reports minimal side effects with this acute dose in older adults (8).

The average cfPWV measured at baseline during the MitoQ trial was 7.4 ± 0.3 m/s, while the baseline cfPWV during the placebo trial was 7.6 ± 0.3 m/s. When considering the average age of the cohort, 63.7 ± 2.7 years, the normative values for healthy vascular aging suggest that our

cohort would be expected to experience higher PWV than our data shows on average (52). According to the European Heart Journal, the mean PWV in the normal values population for healthy adults ages 60-69 years is 10.3 m/s. This lower-than-expected cfPWV may be partially explained by a higher-than-average CRF in this cohort (27.0 ± 2.4 mL•kg•min^{-1}), which is consistent with the findings of a study conducted using the BALLST cohort that demonstrated an association between higher CRF and healthy vascular aging (69).

In one study, healthy older adults with a baseline cfPWV of >7.6 saw a significant decrease in aortic stiffness after 6 weeks of MitoQ supplementation (2). While this study is inconsistent with our findings, it should be noted that an acute dose may not elicit the same response as 6 weeks of daily supplementation. Taken together, these findings suggest that MitoQ may be beneficial for certain individuals when taken chronically, but appears insignificant when taken acutely, even in a larger dose. The official MitoQ website states that 10mg of MitoQ taken daily is an effective dose for improving health and managing oxidative stress, but more research is needed to determine the timeline of these benefits and who may benefit most.

While no significant difference was found between groups with regard to arterial stiffness, cDBP, DPTI, and SEVR significantly increased from baseline to one hour post in the MitoQ trial compared to a placebo. Antioxidant therapy has been considered in research as a possible treatment for hypertension, so the acute increase in cDBP is counterintuitive, especially since significant changes are typically seen in SBP, while DBP is altered non-significantly (70). Although the increase in cDBP is significant, it should also be noted that the average cDBP across the cohort remained within the normal range of less than 80 mmHg. DPTI is defined as the area between the aortic curve and the left ventricular end diastolic pressure with respect to diastolic time (71). DPTI accounts for certain factors that affect coronary blood flow including

coronary artery blood pressure, the gradient between diastolic coronary artery and left ventricular pressure, and diastolic time (71). SEVR expresses balance between coronary perfusion and arterial load as the ratio between systolic and diastolic time integrals (71). SEVR is usually expressed as a decimal value but can also be expressed as a percentage. Higher values are considered better, as low SEVR is an indication of poor cardiovascular outcomes due to imbalanced oxygen supply and demand (71, 72). It has been suggested that 0.5 (50%) is the critical value for SEVR as ischemia is typically experienced between 0.4-0.5 (40-50%) (72). By contrast, our cohort had SEVR values well above 100%, further indicating the overall health of the cohort with respect to myocardial perfusion.

Significant changes in all three of these variables appear to be positive, as increased cDBP, DPTI, and SEVR indicate improved myocardial perfusion. The mechanism by which acute MitoQ supplementation has increased these variables is unclear as research in humans is limited and even the pathways by which MitoQ manages mitochondrial ROS are not fully elucidated. It is possible that MitoQ acts directly on the heart to produce these changes, as this supplement has been shown to be absorbed into the mitochondria of mouse donor hearts in order to decrease ischemia-reperfusion injury during and after transplant (73). Furthermore, MitoQ was able to act directly on the cardiac tissue of severely septic rodents to prevent cardiac dysfunction (74). MitoQ can also cross the blood brain barrier and change certain behaviors and brain functions in rats (75-77). It is possible that these changes in cDBP, DPTI, and SEVR between supplement groups could be partially explained by MitoQ influencing brainstem function acutely. More research in human subjects is needed to determine the pathways by which MitoQ has produced these acute changes in our cohort.

Limitations

This study has several limitations, including that the majority of our cohort was non-Hispanic white. Further, this study allowed individuals on certain blood pressure medications to take part in the study, which may have influenced the measured outcomes due to possible interactions between MitoQ and other medications. This study was also undertaken as part of a larger research project, resulting in PWA/PWV measurements being collected directly after collection of flow mediated dilation data. It is possible that the sheer stress exposure during the flow mediated dilation protocol influenced vascular behavior during PWA/PWV measurement.

Conclusions

An acute dose of 80mg of MitoQ did not significantly alter cfPWV compared to a placebo in a cohort of apparently healthy middle aged and older adults. cDBP, DPTI, and SEVR, which are associated with improved myocardial perfusion, significantly increased with an acute dose of MitoQ. These findings suggest that MitoQ may acutely improve cardiac health by facilitating advantageous pressure changes that result in increased myocardial perfusion.

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