ACUTE DOSE RESPONSE EFFECTS OF AEROBIC EXERCISE ON CEREBROVASCULAR HEMODYNAMICS AND ARTERIAL STIFFNESS

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ACUTE DOSE RESPONSE EFFECTS OF AEROBIC EXERCISE ON CEREBROVASCULAR HEMODYNAMICS AND ARTERIAL STIFFNESS

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Accumulating evidence suggests that aerobic exercise benefits cerebrovascular hemodynamics and arterial stiffness. Few studies have evaluated acute effects of aerobic exercise on cerebral blood flow and even fewer on cerebral pulsatile flow. Furthermore, it has been proposed that cerebral blood flow is more pulsatile with higher arterial stiffness, though little is known about this relationship, particularly in response to exercise. PURPOSE: To evaluate acute effects of aerobic exercise on cerebrovascular hemodynamics and arterial stiffness. METHODS: Fifteen middle-aged adults were recruited for this randomized crossover study comprised of three experimental visits: 30 minutes of sitting (SIT), 20 minutes of sitting followed by a 10-minute exercise bout (EX10), and a 30-minute exercise bout (EX30). Cerebrovascular hemodynamics and arterial stiffness were measured before the experimental session and at 30- and 60-minute post-session. Cerebrovascular hemodynamics were measured using Transcranial Doppler ultrasonography at the middle cerebral artery (MCA). Arterial stiffness was measured by pulse wave velocity (cfPWV and crPWV) and pulse pressure. RESULTS: Pulsatility index was marginally higher in EX30 versus SIT (4.7%, P=0.08) at the 30-minute post-session assessment. Cerebrovascular blood flow velocity was not different across conditions (P>0.10). Pulse pressure was lower in EX10 (β =-2.79 mmHg, P=0.01) and in EX30 (β=-3.85 mmHg, P=0.001) versus

SIT at the 60-minute post-session assessment. Neither cfPWV nor crPWV were different from SIT following EX30 (P>0.05). However, compared to SIT, cfPWV was higher in EX10 at the 30-minute post-session assessment (β =0.30 m/s, P=0.02) and crPWV was lower in EX10 at the 60-minute post-session assessment (β =-0.88 m/s, P=0.004). Neither changes in cfPWV nor crPWV were associated with changes in cerebral pulsatile flow (P>0.05). Higher pulse pressure was associated with higher cerebral pulsatile flow (β =0.004, P=0.02) at the 30- but not the 60-minute post-session assessment. CONCLUSIONS: Based on our results, aerobic exercise does not improve cerebrovascular hemodynamics in the MCA within an hour of aerobic exercise. Future research should include more frequent and longer post-exercise observation periods, different cerebral regions and vessels, and relationships with arterial stiffness. Additionally, our results suggest 10- and 30-minute exercise bouts have differential effects on PWV which also warrants further research.

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PREFACE

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1.0 INTRODUCTION

1.1 BACKGROUND

Cerebrovascular aging is associated with adverse structural and functional changes in the brain such as impairment of vascular function and reduced cerebral blood flow (CBF) [1, 2]. Reduced CBF can lead to serious consequences owing to the brain's critical need for constant blood flow. Since the brain lacks energy stores, neurons are dependent on adequate blood flow to provide nutrients and oxygen as well as remove waste for proper brain function. When blood flow is unable to meet the increased neuronal metabolic needs, neural dysfunction and subsequent disease can occur. These adverse alterations in cerebrovascular hemodynamics and reduced cerebrovascular function are associated with cognitive decline, increased risk of stroke and neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), as well as premature mortality [1]. Systemic increases in arterial stiffness are thought to contribute to impaired cerebrovascular hemodynamics. Specifically, these systemic vascular changes can lead to similar adverse changes in cerebral arteries including increases in stiffness, reduced CBF and pulsatile blood flow (i.e., blood flow with large fluctuations between systole and diastole that can be harmful to arteries) [3, 4] . Both cross-sectional and longitudinal studies have established these associations between central, peripheral, and cerebral blood vessels [4-6].

Healthy elastic central arteries (e.g., the aorta and carotid artery) expand and recoil to cushion the pulsatile blood flow generated by the left ventricle of the heart and allow for continuous, relatively non-pulsatile blood flow to reach the peripheral organs (e.g., the brain) [3, 6] . With increasing age or the presence of cardiovascular risk factors, central arteries stiffen and are less capable of cushioning pulsatile flow. This results in increased pulsations and pressure gradients in central arteries, which in turn exposes the delicate, more distal microvasculature of the brain to the same potentially harmful flow patterns [7]. Since the brain demands a high blood flow to function [1, 8], and has low vascular resistance, cerebral arteries are particularly vulnerable to such flow patterns and can be damaged when exposed to high pulsatile flow that has not been cushioned by the supplying arteries [9]. Increased cerebral pulsatile blood flow is linked to ruptures of small vessels, leading to lesions in the white matter [5], thus increasing the risk of stroke [7, 10], cognitive decline, dementia [11] and Alzheimer's disease [12]. Considering the deleterious consequences of pulsatile blood flow in the cerebral vessels, and the established associations between arterial stiffness and cerebral pulsatile blood flow, interventions targeting decreased arterial stiffness could be a potential method for improving cerebrovascular function.

Physical activity is known to reduce arterial stiffness resulting in an improved ability of the arteries to expand and recoil [13]. Consequently, the arteries are thought to be better able to buffer pulsatile flow, preventing such flow from reaching the brain. Further, physical activity is known to improve vascular function (e.g., endothelial function) and this leads to improved CBF and decreased cerebrovascular disease [14]. Therefore, physical activity may improve cerebrovascular hemodynamics, in part through systemic decreases in arterial stiffness [3]. However, cerebrovascular hemodynamics and the impact of changes in arterial stiffness following acute aerobic exercise have not been well established.

The American College of Sports Medicine recommends participating in moderate-intensity physical activity for 30 minutes per day for a total of 150 minutes per week in bouts of at least 10 continuous minutes [15, 16]. A single 30-minute bout of aerobic exercise has been shown to acutely reduce arterial stiffness [17]. Though recommended as the minimum duration for health benefits, the acute effects of a 10-minute exercise bout on arterial stiffness have not been evaluated. Furthermore, though cross-sectional studies have associated arterial stiffness with cerebrovascular hemodynamics [5, 18], it is unclear whether a single bout of exercise improves cerebral pulsatile blood flow or whether such improvements track with the effects of exercise on arterial stiffness.

1.2 SPECIFIC AIMS

Specific Aim I: Compare 1-hour trajectories of cerebrovascular pulsatile blood flow after 3 conditions: rest, 10-minute aerobic exercise and 30-minute aerobic exercise.

Specific Aim II: Compare 1-hour trajectories of peak systolic, mean, and end diastolic blood flow after 3 conditions: rest, 10-minute aerobic exercise and 30-minute aerobic exercise.

Specific Aim III: Compare 1-hour trajectories of arterial stiffness (measured by pulse wave velocity and pulse pressure) after 3 conditions: rest, 10-minute aerobic exercise and 30-minute aerobic exercise.

Specific Aim IV: Evaluate whether changes in arterial stiffness are related to changes in cerebral pulsatile blood flow over 1-hour trajectories after the 3 conditions.

1.3 HYPOTHESES

We hypothesized that cerebrovascular hemodynamics would improve in a dose-response manner following a single bout of exercise. Specifically, we expected a 10-minute exercise bout to result in improvements over rest and for a 30-minute exercise bout to result in improvements over rest and a 10-minute bout. We hypothesized that arterial stiffness (as measured by pulse wave velocity and pulse pressure) would improve in a similar manner. Further, we hypothesized that improvements in cerebrovascular hemodynamics would occur concurrently with changes in arterial stiffness that result from a single bout of exercise. In other words, changes in arterial stiffness would be related to changes in cerebrovascular hemodynamics, and particularly cerebral pulsatile blood flow.

1.4 THEORETICAL FRAMEWORK

Figure 1 illustrates the aims of the study which evaluated whether single bouts of exercise of different durations (10-minute and 30-minute bout) could elicit improvements in cerebrovascular hemodynamics (Aims I and II) and arterial stiffness (Aim III). Additionally, this study examined whether reductions in arterial stiffness explain improvements in cerebrovascular hemodynamics (Aim IV). As noted in the figure, this study investigated several novel effects and relationships (noted by single arrows) and built upon known acute effects of 30 minutes of exercise on arterial stiffness (double arrow).

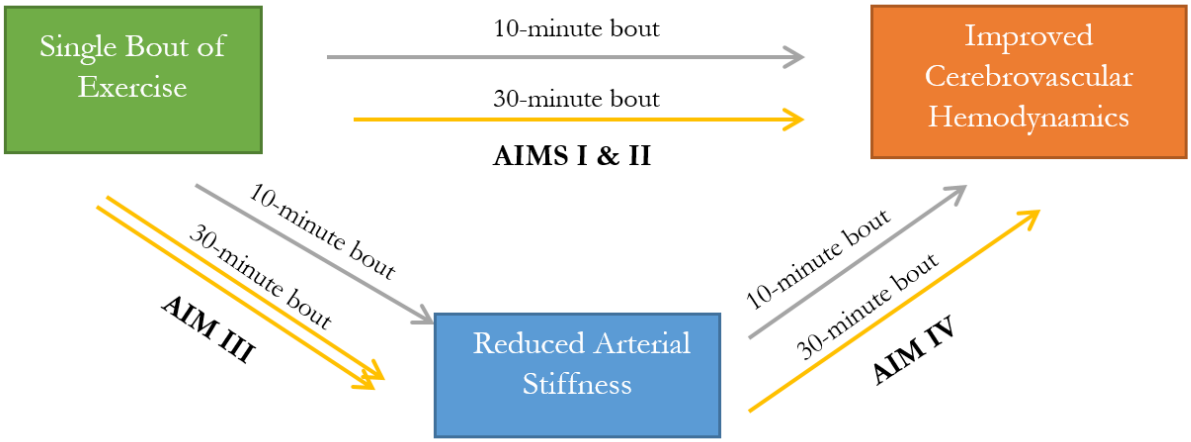

Figure 1 Theoretical Framework of the Acute Effects of a Single Bout of Exercise
Single arrows denote unknown relationships while double arrows denote relationships that have already been studied.

1.5 SIGNIFICANCE

The present study aimed to address whether aerobic exercise can acutely improve cerebrovascular hemodynamics and provide better insight into the relationship between cerebrovascular health and systemic vascular health. Specifically, we examined cerebrovascular health as measured by cerebrovascular blood flow and pulsatile flow in the middle cerebral artery as it relates to arterial stiffness as measured by pulse pressure and pulse wave velocity.

These results shed light on whether cerebrovascular hemodynamics respond as acutely to exercise as arterial stiffness. Further, we evaluated whether a shorter 10-minute bout is sufficient to elicit these beneficial changes in cerebral and systemic vascular function. These research questions address a gap in the literature regarding the acute time course and dose-response relationship of exercise-induced improvements in arterial stiffness and cerebrovascular hemodynamics. Further, this research provides insight into the mechanisms through which acute

benefits accumulated via habitual exercise could reduce the risk of cerebrovascular disease and improve cerebrovascular function.

Additionally, if bouts of exercise lasting 10 or 30 minutes have beneficial effects on cerebrovascular hemodynamics, these results would support the American College of Sports Medicine recommendation of participating in physical activity for 30 minutes on most days of the week in bouts lasting at least 10 continuous minutes to meet a goal of 150 minutes per week (rather than reaching this goal over a couple of days). If beneficial effects are only observed after longer, 30-minute bouts, this would inform exercise interventions with the aim of improving cerebrovascular health to not recommend 10-minute exercise bouts, but rather longer bouts for acute benefits.

Lastly, this study serves as a foundation for future research. Future studies could investigate the effects of longer bouts of exercise, different modes and intensities. Additionally, future studies could explore acute changes in cerebral microvasculature. Further research could evaluate if improvements in cerebrovascular hemodynamics are related to acute improvements in cognition known to occur following an exercise bout. Also, future studies could examine whether longer term exercise training improves cerebrovascular hemodynamics and evaluate associations between chronic adaptations in cerebrovascular hemodynamics and arterial stiffness that result from an exercise intervention.

2.0 REVIEW OF LITERATURE

2.1 CEREBROVASCULAR HEALTH

2.1.1 Cerebrovascular circulation

The brain is one of the most highly perfused organs in the body [19] due to its high metabolism and lack of energy stores. Though the brain only makes up 2% of total body mass, it receives 20% of cardiac output, consumes 20% of the body's oxygen supply and 25% of the body's glucose [20]. Neurons are dependent on blood vessels to provide oxygen and nutrients, as well as to remove carbon dioxide and waste for normal function [1]. Neural activation elicits local increases in blood flow to support increased neural metabolism (functional hyperemia) and thus continuous CBF is critical [8]. In fact, the brain has several protective mechanisms to ensure that the brain has sufficient blood flow providing needed nutrients and energy metabolites. These mechanisms include neurovascular coupling, the blood brain barrier, autoregulation, and collateral blood flow.

Neurovascular coupling is the primary mechanism to ensure adequate nutrient supply to the brain. It occurs via an intricate network of vascular cells (endothelium, pericytes, vascular smooth muscle cells), glial cells (astrocytes and microglia) and neurons that form the neurovascular unit [1, 8]. Proper cell to cell signaling and interaction is necessary for

neurovascular coupling to occur. For example, neurotransmitters and mediators released from neurons indirectly alter blood vessel tone through activation of vascular smooth muscle cells and astrocytes [21]. Specifically, the release of glutamate (the main neurotransmitter in the brain) results in upregulation of nitric oxide (NO) synthase. Greater NO synthase leads to the release of neuronal NO and astrocyte arachidonic acid, which results in vasodilation and an increase in cerebral blood flow, thereby meeting increased neuronal metabolic needs [22].

Another mechanism to ensure sufficient nutrient delivery is the blood brain barrier. The neurovascular unit endothelial cells together form a "highly specialized" membrane around blood vessels which underlines the blood brain barrier. This is a selective barrier that regulates the delivery of energy metabolites and nutrients and prevents potentially neurotoxic and vasculotoxic molecules from entering into the central nervous system [1]. Thus, the integrity of this barrier is crucial for normal neuronal and synaptic function.

Autoregulation is another important protective mechanism to ensure constant cerebral blood flow. Autoregulation refers to the intrinsic property of the cerebral blood vessels to maintain flow at approximately 50 mL per 100 g of brain tissue per minute despite changes in mean arterial pressure within 60 mmHg and 150 mmHg. This occurs through cerebrovascular reserve (measured by vasoreactivity), which is the ability of cerebral blood vessels to respond to increased metabolic demand and other chemical or mechanical stimuli [8]. Thus, cerebral autoregulation is dependent upon the vasculature's ability to vasoconstrict and vasodilate to maintain constant cerebral blood flow.

Yet another mechanism to ensure constant blood flow is a circulatory anastomosis (cross connection) of cerebral blood vessels called the Circle of Willis. The Circle of Willis typically contains vessels which allow for collateral blood flow between the cerebral hemispheres. The

arterial blood supply to the brain typically consists of two internal carotid and two vertebral arteries. The vertebral arteries join to form the basilar artery, which then anastomoses with the internal carotid arteries via the posterior communicating arteries, forming this anastomotic ring at the base of the brain [19, 20]. The Circle of Willis allows for blood to flow to compensate for a lack of blood flow in one brain region in the event of a blockage. Each of the internal carotid arteries provide approximately 40% of blood flow to the brain and give rise to the middle cerebral artery (MCA) and the anterior cerebral artery, which mainly supply the cerebrum. The basilar artery provides the remaining 20% of blood flow and gives rise to the posterior cerebral arteries, which mainly supply the cerebellum and brain stem [19]. The middle, anterior and posterior cerebral arteries divide into increasingly smaller arteries and arterioles until they reach the brain tissue to supply blood to neurons in their respective regions.

These mechanisms work together so that the brain receives sufficient blood flow to support neural metabolism and prevent neuronal damage that could occur from toxic molecules and fluctuations in blood flow or mean arterial pressure. They also highlight the importance of cerebrovascular function in normal brain function. The following sections describe how aging and disease can impair these protective mechanisms and lead to maladaptive cerebrovascular hemodynamics and reduced brain function.

2.1.2 Cerebrovascular aging

Aging is associated with changes in cerebral hemodynamics. Specifically, changes include decreases in resting CBF and increases in pulsatile flow [23]. After the third decade, CBF declines by approximately 4 mL min^{-1} yr^{-1} [24]. These age-related decreases in blood flow occur in both the major cerebral arteries and in regional cerebral perfusion [23, 25]. Though the

underlying mechanisms have yet to be elucidated, structural changes in the cerebrovasculature and increases in reactive oxygen species may contribute to the decline in cerebral blood flow.

Aging brings about several alterations in the cerebrovasculature. Vessels in both cerebral gray and white matter undergo structural changes with increased tortuosity of vessels, significant rarefaction of arterioles, pericyte and capillary degeneration, loss of endothelium and thickening of the basement membrane [2, 26]. These vascular changes could have detrimental effects on the affected regions [2]. For example, with increasing vascular tortuosity, CBF becomes perfusion-dependent in the deep white matter regions, leaving this area susceptible to chronic hypoperfusion (inadequate blood flow) [27].

Additionally, reactive oxygen species (ROS) are known to increase with age. ROS are thought to decrease NO bioavailability (resulting in endothelial dysfunction), and this reduction is associated with a decline in basal cerebral perfusion [28]. Moreover, ROS promotes vascular inflammation of the cerebral blood vessels [29, 30]. This could further increase ROS, leading to increased blood-brain barrier permeability to proinflammatory cytokines, vascular damage, and further reduced blood flow [8].

Another alteration in cerebrovascular hemodynamics that occurs with age is an increase in cerebral pulsatile blood flow [23]. Pulsatile flow is characterized by blood flow with large fluctuations between systole and diastole that can be harmful to arteries. The primary mechanism thought to result in such flow in the brain is arterial stiffness and these relationships are a focus of the proposed research and are discussed in detail below (**Section 2.3**).

These age-related declines in CBF emphasize the need for interventions to attenuate such cerebrovascular alterations as these can lead to cerebrovascular disease.

2.1.3 Cerebrovascular disease

Cerebrovascular disease refers to any disorder that affects the supply of blood flow to the brain and can occur in both large and small arteries (microcirculation). Common cerebrovascular diseases include stroke, transient ischemic attack, small vessel disease, and dementia. Cerebrovascular disease risk factors (e.g. hypertension, diabetes, hypocholesteremia) exacerbate aging effects and can lead to serious cerebral damage and disease. Specifically, cerebrovascular disease risk factors alter vascular structure by promoting stiff, narrow, thick and tortuous vessels [29]. These changes are related to pathological alterations in resting cerebral blood flow, perturbations in CBF regulation and increases in cerebral pulsatile blood flow [29]. Major pathways through which cerebrovascular disease risk factors exert their harmful effects are oxidative stress and vascular inflammation leading to reductions in NO bioavailability [31, 32]. This loss of vasoregulatory capability of endothelial NO results in vasoconstriction and diminished NO-dependent vascular responses, leading to an impaired regulation of microvascular flow [32]. In addition, ROS increases the permeability of the blood-brain barrier also contributing to microvascular disease [33].

Stroke is a major cerebrovascular disease where blood flow and oxygen supply are interrupted from neurons (ischemia). This occurs most commonly due to an occluded vessel (embolic) but can also result from a ruptured vessel (hemorrhage) [29]. If ischemia occurs for more than a few minutes, irreversible damage with subsequent cell death and permanent symptoms occur (infarction). Symptoms vary depending on the size, severity and location of the affected area(s). For example, a single stroke can result in dementia if it occurs in key cognition regions while multiple, less severe strokes in the same regions can result in more gradual cognitive decline [29]. Transient ischemic attacks (TIA), sometimes called brief “mini” or

“warning” strokes, last only a few minutes and resolve before the ischemic tissue damage becomes irreversible [34]. Though TIAs have a short duration, they increase the likelihood of more severe future strokes.

Small vessel disease is a group of pathological processes that affect the cerebral small arteries, arterioles, venules and capillaries and it is also referred to as microvascular disease [11]. One such disorder results in small (lacunar) strokes in the small vessels deep within the brain. In addition to lacunar strokes, microvascular diseases also include white matter lesions (seen as white matter hyperintensities on MRI) and microbleeds [11]. Microvascular disease is thought to be the most common neuropathology and plays a major role in stroke, vascular dementia and Alzheimer’s disease [33]. Furthermore, it is the leading cause of cognitive decline and functional loss in the elderly [11]. Of note, cerebral pulsatile blood flow is heavily implicated in microvascular disease. Since microvascular disease is thought to commonly underlie other cerebrovascular disorders, targeting cerebral pulsatile blood flow could be an important means by which to prevent cerebrovascular disease.

Dementia is a clinical syndrome characterized by gradual cognitive decline and loss of function and independence [35] . Alzheimer’s disease, the most prevalent type of dementia, is a devastating neurodegenerative disease in which a progressive deterioration of brain structure and function occurs particularly in cognition [36]. Two main neuropathological features of Alzheimer’s disease are deposition of amyloid-β plaques and tau tangles. Amyloid-β accumulates in the presence of impaired waste clearance capabilities of the blood brain barrier, emphasizing the importance of the integrity of the blood brain barrier [1]. Furthermore, endothelial dysfunction, arterial stiffness, hypoxia and a decline in CBF have been implicated in the pathophysiology leading to Alzheimer’s disease [37].

Another form of dementia is vascular dementia, which is a progressive decline in cognitive function thought to be caused by chronic cerebral hypoperfusion (inadequate blood flow) and ischemia. Inadequate blood flow results in impairments of protein synthesis, synaptic activity, and glutamate excitocity, thereby affecting neuronal function and potentially leading to neuronal apoptosis [38, 39]. Though the etiology of vascular dementia and Alzheimer's disease are thought to be different, vascular risk factors and dysfunction are heavily implicated in both [37].

These disorders highlight the importance of the health of the cerebral vasculature and of the cardiovascular system supplying the blood for normal cerebral function. Specifically, maintaining continuous CBF and protecting the brain from potential damage that could result from physiological insults such as toxic molecules and pulsatile blood flow.

2.1.4 Cerebrovascular imaging methods

2.1.4.1 Transcranial Doppler ultrasonography

Transcranial Doppler (TCD) is a non-invasive ultrasound that measures CBF velocity (CBFv), a validated measure of CBF[40]. CBFv is assessed by placing a small, low frequency transducer (2 MHz) on the scalp over a specific acoustic window where bone is the thinnest [41]. Several locations can be insonated depending on which artery is being assessed. The most commonly insonated artery is the MCA [42]. The MCA velocity is accessed utilizing the transtemporal window by the tragus above the zygomatic arch [41, 43]. The Doppler probe emits high-frequency sound waves that are reflected off moving red blood cells. These reflected waves are then detected by the transducer resulting in a frequency shift, with this shift being proportional to blood velocity [44, 45]. This frequency shift refers to the difference between emitted and

reflected waves: if the red blood cells are moving toward the transducer, the sound waves are compressed and the frequency increases while the opposite happens when red blood cells are moving away from the transducer [45].

From the spectral waveform display, peak systolic and end diastolic blood flow velocity are directly measured. Mean blood flow velocity is estimated by:

$$Mean\ Velocity = \frac{Peak\ Systolic\ Velocity\ + End\ Diastolic\ Velocity\ x\ 2}{3}$$

These values are obtained over a cardiac cycle and are automatically derived from the spectral analyzers in TCD [45]. In addition to these values, the pulsatility index is also calculated as a function of the CBFv parameters as follows:

$$Pulsatility\ Index = \frac{Peak\ Systolic\ Velocity - End\ Diastolic\ Velocity}{Mean\ Velocity}$$

Pulsatility index is a common measure to describe the shape of the waveform [45]. For example, fast upstrokes of flow velocity and short peak velocity latencies generally result in a higher pulsatility index. Moreover, a higher pulsatility index reflects an increased cerebrovascular stiffness [2].

TCD allows for non-invasive dynamic monitoring of CBFv and vessel pulsatility with a high temporal resolution and over extended periods of time. It is also relatively inexpensive, repeatable, and portable [45, 46]. TCD is not without limitations, however, with the main limitation being that it is highly operator dependent [45] and there is a steep learning curve. Further, 10-20% of individuals have inadequate acoustic windows and spatial resolution is low due to variations in thickness and porosity of the bone [45, 46]. In addition, CBFv is only proportional to CBF when the vessel size remains constant. In this way, TCD values are an

indirect measure of flow [47]. One way to address this limitation, however, is by capturing a more comprehensive picture of cerebrovascular hemodynamics by also measuring the supplying artery, internal carotid artery. This allows for comparisons between flow velocities in MCA and the internal carotid artery.

Despite limitations, TCD has several clinical applications and is widely utilized for monitoring CBF during or following critical conditions such as sickle cell disease vasospasms, stenosis, intracranial occlusions, thrombosis, traumatic brain injury, stroke, and brain death [42, 44, 46]. It has also been used extensively in physiological research studies [5, 48]

2.1.4.2 Other cerebrovascular imaging methods

Like TCD, several other methods measure some proxy of cerebral blood flow. Some of the most widely used methods in physiological research are near infrared spectroscopy (NIRS), arterial spin labeling (ASL), and functional magnetic resonance imaging (fMRI).

NIRS is a non-invasive method used to measure regional cerebral tissue oxygen saturation and thus oxygen extraction and consumption. NIRS utilizes the specific absorption of near-infrared light by oxygenated, deoxygenated, and total hemoglobin [49]. This method has been used to evaluate brain activation during cognitive tasks and to compare neural metabolism in different populations (inactive vs aerobically trained). NIRS has excellent temporal resolution, but a primary limitation that skull, adipose/muscle tissue and scalp blood flow may create noise in the signal [49]. NIRS, unlike TCD, is an assay of the more distal microvasculature.

ASL is an MRI technique to measure tissue perfusion by using the magnetization of water in the blood [50]. A 180-degree radiofrequency inversion pulse is applied to modify the magnetization of blood water, thereby tagging it and measuring the delivery of blood. ASL has also been used to evaluate differences in cerebral perfusion, both in quantity and location, in

different populations. This method provides an absolute quantification of perfusion, however it is expensive and the temporal resolution is poor due to the long latency (4 seconds) required to acquire a tagged and control image [50].

Another non-invasive MRI method which uses blood-oxygen dependent contrast imaging (BOLD) is fMRI. This method utilizes the contrast in the magnetic properties of oxygenated versus deoxygenated hemoglobin as a measurement of metabolism (neural activity) denoted by a change in BOLD from baseline [51]. fMRI is often used in cognitive research to evaluate differential neural activation in different populations (e.g., aging) or after an intervention (e.g., exercise). fMRI provides a detailed view of the brain in different dimensions; however, it is expensive and the results can vary widely based on parameters chosen.

2.2 ARTERIAL STIFFNESS

2.2.1 Pathophysiology

Arterial stiffness is a pathological process which results from changes in the structural and cellular elements of the vessel wall. The main alterations are a loss of the elastic properties and remodeling of the vessel wall via enlargement and wall thickening [52]. Such changes result in a 'hardened' artery with a reduced capability to expand and recoil in response to changes in pressure and flow [53]. Traditional cardiovascular risk factors such as aging, hypertension, diabetes mellitus, and hyperlipidemia are thought to promote arterial stiffness through several mechanisms [54].

Aging and hypertension, for instance, are thought to increase arterial stiffness through their effects on collagen and elastin. Collagen and elastin are two scaffolding proteins that are crucial to the stability, resilience and compliance of the vascular wall. However, an imbalance of these proteins can have adverse vascular effects [54]. Aging results in an overproduction of collagen and reduction of elastin [55], while hypertension can result in replacement of elastin with collagen as a response to excess pressure being exerted on the vessel wall by blood. Both processes lead to increased arterial stiffness. Furthermore, accumulation of advanced glycation end products (AGE) secondary to hyperglycemia can alter the physical properties of proteins and form cross-links between proteins such as collagen [56, 57]. This AGE-linked collagen is stiffer and can further contributes to the loss of elasticity in vessels. Aging also results in an increased calcium content in the arterial wall, which may contribute to arterial stiffness by reducing distensibility. In addition, metabolic dysfunction such as hyperinsulinemia and hyperlipidemia can cause endothelial dysfunction, exacerbating arterial stiffness due to the pivotal role the endothelium plays in vascular tone [52]. Also, hormones such as angiotensin II and aldosterone may lead to increased smooth muscle cell tone, hypertrophy of smooth muscle cells, and fibrosis, resulting in a loss of elasticity [52, 58]. Lastly, increases in sympathetic nerve activity can increase vascular smooth muscle cell tone and, subsequently, contribute to arterial stiffness [58] .

Aortic stiffness causes an increased load on the heart, resulting in higher systolic arterial pressure, while the loss of elastic recoil reduces diastolic blood pressure. Together these effects combine to increase pulse pressure, which is considered a surrogate measure of arterial stiffness for this reason [58]. This increased load requires more energy for a given ejection flow [52]. Chronic ejection into stiffer vessels leads to a loss of efficiency in cardiac ejection and reduced perfusion of the heart itself [52].

Arterial stiffness has been established by numerous longitudinal epidemiological studies as an independent predictor of cardiovascular morbidity and mortality [59]. This has been demonstrated after adjusting for classical cardiovascular risk factors, for example those in the Framingham Risk score, which suggests that arterial stiffness has an added predictive value for detecting early cardiovascular disease progression [59, 60]. Moreover, arterial stiffness can be measured non-invasively, thus making it an ideal subclinical measure of cardiovascular disease in clinical research and practice. Methods for measuring arterial stiffness are discussed below.

2.2.2 Methods for assessing arterial stiffness

2.2.2.1 Pulse wave velocity

During systole, the left ventricle contracts to eject blood into the ascending aorta, acutely dilating the aortic wall and generating a pressure wave that travels along the arterial tree [61]. The velocity of this wave provides a measurement of arterial compliance since stiff arteries inherently have a reduced capacity to dilate [53]. The pulse wave is reflected back from the peripheral vessels mainly from branching points. In elastic arteries, the pressure wave travels slowly and is reflected back during diastole. In more stiff arteries, the pulse travels faster and is reflected back prematurely. This reflection of the wave during systole results in an increased cardiac load [62].

Carotid-femoral pulse wave velocity (cfPWV) is the gold-standard non-invasive measure of central (aortic) arterial stiffness. The reliability and validity of cfPWV against direct invasive measures on central arterial stiffness have been well established [59, 63]. cfPWV is assessed by dividing the estimated aortic distances measured externally using a measuring tape by the difference in transit time of the pressure waves [62, 64, 65]. Piezoelectric sensors are placed

superficially over the carotid and femoral artery sites to capture the pressure waves. The foot of each wave is used as a reference point for the arrival of the pressure wave to each site and the difference in time between sites serves as the denominator for calculation of cfPWV [59].

Mounting evidence has established cfPWV as an independent predictor of cardiovascular, stroke [66] and all-cause mortality [67, 68]. A recent meta-analysis of 17,635 participants, revealed that each 1 standard deviation increase in cfPWV, was associated with a 23% increase in the risk for CHD events, 30% for CVD events, 28% for stroke, 28% for CVD mortality and 17% for all-cause mortality even after adjusted for traditional CVD risk factors [69] .

PWV is measured peripherally at other superficial arterial sites such as the radial, brachial, posterior tibialis or dorsalis pedis arteries [59]. Though less studied, some evidence suggests that peripheral PWV is a comparable predictor of coronary heart disease and stroke compared to cfPWV [70]. Further, these smaller vessels may be more responsive to interventions and are thus often utilized in clinical research.

2.2.2.2 Pulse pressure

As described earlier, pulse pressure is another measure of arterial stiffness and is calculated as the difference in systolic and diastolic blood pressure. Pulse pressure depends on left ventricular ejection fraction and the properties of the arterial wall, and therefore increases with higher stroke volume or increases in arterial stiffness [71]. In healthy compliant arteries, each cardiac contraction by the left ventricle ejects blood into the central arteries which is “accommodated” by stretching of the vessel walls in systole. An elastic recoil follows in late systole and diastole [72]. Pulse pressure is widened when arterial stiffness increases, particularly that of the aorta, due to the inherent reduced capacity of the arterial wall to expand and recoil to in response to increases in volume or pressure [71].

Like PWV, pulse pressure is an independent predictor of cardiovascular outcomes [73] and total mortality [74]. Some studies even suggest that pulse pressure better predicts outcomes as compared to other blood pressure parameters such as systolic, diastolic or mean arterial pressure [71, 75, 76]. Of note, in a study of over 19,000 men with low cardiovascular risk, a wide pulse pressure was found to be a significant predictor of all-cause and cardiovascular mortality over a mean follow-up of 19.5 years [77]. Similarly, in 1,243 chronic hemodialysis patients, every 10 mmHg increase in pulse pressure was associated with a 12% increase in the hazard of mortality over a nine year follow-up [78].

2.2.2.3 Other methods for assessing arterial stiffness

Common alternative methods to measuring arterial stiffness are ultrasound and pulse wave analysis. Ultrasound is used to measure distensibility and compliance of large and accessible arteries. Thus, it is mainly used on the brachial, carotid, femoral, and abdominal aorta arteries. Ultrasound captures several images of the vessel wall per cardiac cycle. Vessel wall tracking edge-detection software calculates the minimum and maximum wall vessel areas [79]. Ultrasound of the common carotid artery is the most widely used. First a B-mode (2D) ultrasonography is captured parallel to the carotid, after which m-mode (moving) is placed perpendicular to the vessel, and a number cardiac cycles are recorded [80]. The primary limitation for the use of ultrasound to measure arterial stiffness is its limited resolution, which can lead to difficulty in detecting small changes in vessel diameter [79]. Additionally, it is technician dependent and only moderately repeatable [79].

Another method is pulse wave analysis which can be performed utilizing applanation tonometry (the same method used to measure PWV) or echo tracking. Pulse wave analysis provides indices of wave reflection such as augmentation pressure (the difference between the

first and second systolic peaks of a pressure waveform) and augmentation index (the quotient of augmentation pressure on pulse pressure expressed as a percentage) [54]. Of note, pulse wave analysis is complementary to PWV rather than interchangeable [59].

2.3 RELATIONSHIP BETWEEN ARTERIAL STIFFNESS AND CEREBROVASCULAR HEALTH

2.3.1 Associations between arterial stiffness and cerebrovascular health

Accumulating evidence suggests that central and peripheral arterial stiffness is associated with deleterious consequences in the brain. Increased arterial stiffness has been shown to be related to decreased CBF[81] and an increased risk of transient ischemic attacks [82], cerebral small vessel disease [7, 83, 84], stroke [66], deterioration of white matter neuronal fiber integrity [85], impaired cerebrovascular reactivity [86, 87], cognitive decline [88], vascular dementia, cortical brain atrophy, and Alzheimer's disease [89]. For example, in 692 older adults (mean age 63) with no history of stroke, transient ischemic attack or signs of neurological disease, those in the highest quartile of pulse pressure (> 54mmHg) had 2.55 (95% confidence interval of 1.03-6.30) times the odds of advanced white matter lesions [90]. Higher odds of white matter lesions have also been reported with increased PWV [91]. Thus, arterial stiffness is related to reduced cerebral function and cerebrovascular health.

2.3.2 Arterial stiffness and cerebral pulsatile blood flow

MCA blood flow is known to be more pulsatile in the presence of arterial stiffness. One study measured arterial stiffness by brachial and central pulse pressure, cfPWV, and brachial-ankle (peripheral) PWV, and MCA pulsatility using TCD in 334 adults. MCA pulsatility index significantly increased with all indices of arterial stiffness even after adjusting for age, sex, smoking, alcohol intake, and 24-hour heart rate and mean arterial pressure (**Table 1**) [5]. Similarly, central and peripheral pulse pressure were found to be significantly associated with higher MCA pulsatile flow in 160 healthy adults ($P<0.05$) [4].

Table 1. Cross-sectional Correlations between Arterial Stiffness and Middle Cerebral Artery Pulsatile Flow [5]

Arterial Stiffness Measure	n	*r*	P-value
Carotid-femoral PWV	334	0.12	0.036
Brachial-ankle PWV	334	0.23	<0.001
Brachial Pulse Pressure	334	0.49	<0.001
Central Pulse Pressure	334	0.34	<0.001
24-Hour Pulse Pressure	334	0.44	<0.001

The mechanism through which arterial stiffness is thought to increase pulsatile blood flow is through impaired Windkessel function [92]. Healthy elastic arteries expand and recoil to cushion the pulsatile blood flow generated by the left ventricle of the heart and allow for relatively non-pulsatile blood flow to reach the peripheral organs (e.g., the brain). With increasing age or the presence of cardiovascular risk factors, central arteries stiffen and are less capable of cushioning pulsatile flow [61]. This results in increased pulsations and pressure

gradients in central arteries, which in turn exposes the delicate, more distal microvasculature of the brain to the same harmful flow patterns. Due to the brain's high demand for blood flow and its low vascular resistance, cerebral arteries can be damaged when exposed to high pulsatile flow that has not been cushioned by the supplying arteries [3].

Pulsatile flow accelerates the alterations in cerebral hemodynamics known to occur with age, such as reduced cerebral blood flow. In fact, high pulsatile pressure and flow can tear smooth muscle cells and endothelial cells off the vessel wall, resulting in hemorrhage or thrombosis in small arteries, arterioles and capillaries [61]. Age-related weakening of the arterial walls in concert with this pulsatile stress can further induce thrombosis, lacunar infarction, endothelial damage, fluid "exudation", microbleeds and microaneurysms [61, 93]. These events can lead to more serious cerebral disease, for example, microbleeds can cause a buildup of amyloid plaques [94], which are a hallmark of Alzheimer's disease [95].

In summary, cerebral pulsatile flow can lead to cerebrovascular pathology that may result in deleterious consequences such as white matter lesions [96, 97] and Alzheimer's disease [12]. Due to these serious consequences of cerebral pulsatile blood flow, it is crucial to better understand the mechanisms and effective prevention or treatment methods. We hypothesized that reducing arterial stiffness through physical activity could result in reduced pulsatile blood flow. However, as will be discussed in the following section, limited research has investigated relationships between physical activity, arterial stiffness and cerebrovascular hemodynamics.

2.4 BENEFITS OF PHYSICAL ACTIVITY

2.4.1 General health benefits

It is well established that physical activity is effective in the primary and secondary prevention of a variety of chronic diseases such as cardiovascular disease, diabetes mellitus, cancer, hypertension, obesity, mental illness (anxiety, depression, schizophrenia), sleep apnea (and other sleep disturbances), osteoporosis and premature mortality [16, 98-101]. The American College of Sports Medicine recommends participating in moderate-intensity aerobic physical activity for 30 minutes per day on most days of the week, in bouts of at least 10 continuous minutes, for a total of 150 minutes per week [15]. Due to the fact that individuals may be more likely to adhere to intermittent bouts of 10 minutes rather than a 30-minute continuous bout [102], studies have been conducted evaluating whether these intermittent bouts have the same health benefits as continuous 30-minute bouts of equal overall volume. Exercise intervention studies have found that exercise prescriptions of three 10-minute bouts accumulated throughout the day are as effective as one 30-minute bout per day for improving cardiovascular risk factors such as cardiorespiratory fitness, blood pressure, lipid metabolism and cholesterol levels [102-106]. It is unknown, however, whether 10-minute bouts have acute or long-term benefits to arterial stiffness, pulse pressure or cerebrovascular hemodynamics.

2.4.2 Cerebrovascular health benefits

Exercise leads to both structural and functional changes in the brain such as increases in grey (neurogenesis) and white matter (myelinated axons) volume [107], as well as improvements in

white matter integrity. A specific cerebrovascular benefit is increased basal CBF (perfusion), which is thought to occur through both acute and chronic effects of exercise. Acute effects include NO bioavailability and antioxidant activity, while chronic adaptations include angiogenesis and attenuation of tortuous vessels.

One mechanism through which exercise may acutely increases CBF is increased NO bioavailability. NO is known to enhance endothelial function by regulating vessel tone at rest and during activation [3], which is key to the regulation of cerebral blood flow. Increases in vascular sheer stress may upregulate endothelial NO synthase expression, leading to increased NO bioavailability and NO-dependent vasodilation [108]. In addition to sheer stress, NO bioavailability is preserved through exercise-related decreases in ROS, which are known to have deleterious effects on NO bioavailability. Exercise is associated with increased antioxidant activity and decreased oxidative stress, and thus exercise can attenuate the negative effects ROS has on NO bioavailability [8]. This NO-mediated enhancement of endothelial function may facilitate improved neurovascular coupling and, consequently, cerebrovascular health [37].

Chronic adaptations to increased CBF due to exercise are thought to result from collateral blood vessel recruitment and angiogenesis [8, 109]. Per the vascular niche hypothesis, neurogenesis cannot occur without corresponding angiogenesis to support its metabolism [109-111]. Both neurogenesis and angiogenesis result from insulin-like growth factor 1, brain-derived neurotropic factor, vascular endothelial growth factor and bone marrow-derived endothelial progenitor and CD34+ cells , all which are known to increase with exercise [8, 109].

Another chronic adaptation through which exercise may improve cerebrovascular health is through an attenuation of tortuous vessels [112]. Tortuosity increases the length of the vessel, and there is a loss of kinetic energy with each ‘turn and loop.’ Consequently, more tortuous

vessels require an increase in blood pressure to maintain adequate blood flow [27]. Cerebrovascular tortuosity is known to increase with age, hypertension and disease [113, 114]. Yet, vessel tortuosity has been found to be attenuated in individuals who engage in physical activity [112].

Though much less studied, and thus the focus of the proposed study, exercise may also have a beneficial effect on cerebral pulsatile flow. One small intervention study (n=16) found that decreases in cerebral pulsatile flow were associated with increases arterial compliance (the inverse of arterial stiffness) post-intervention, [6]. Though cerebral pulsatile flow did not change significantly, potentially secondary to small sample and limited power, this preliminary association between arterial stiffness and cerebral pulsatile flow supports our hypotheses and warrants further research.

These beneficial effects on cerebrovascular hemodynamics are thought to result in an improved regulation of CBF through endothelial function via NO, increased collateral flow, and healthier less tortuous vessels, all of which improve the ability of the cerebral vessels to meet the metabolic needs to neurons (neurovascular coupling). Improved neurovascular coupling and overall cerebrovascular health can attenuate the effects of aging and progression of the cerebrovascular disorders discussed in the previous sections, highlighting the importance of exercise. Evidence that exercise improves CBF includes observational and intervention studies discussed below. The effects of a single bout of exercise on cerebrovascular hemodynamics are less clear, and these were evaluated in this study.

2.4.2.1 Observational studies

Cross-sectional studies find that physical activity and cardiorespiratory fitness are positively associated with greater cerebral blood flow. In healthy men ages 18-79 (n=153 inactive, n=154

endurance trained), blood flow velocity in the middle cerebral artery (MCA CBFv) (a surrogate measure of cerebral blood flow) was consistently elevated by 9.1 ± 3.3 cm s^{-1} (17% higher) in those who participated in frequent vigorous aerobic training (>4 times per week over the previous two years), compared to their inactive counterparts [115]. Of note, this resulted in a 10-year reduction of estimated cerebrovascular hemodynamic 'age'. In the same study, age-related reductions in CBF were attenuated in the endurance trained men. Another study in 81 healthy males reported a positive linear relationship between VO_2max and MCA CBFv (r=.58-.59, P<0.05) and similar attenuations in age-related declines in MCA CBFv with higher cardiorespiratory fitness (P<0.001). Like in the previous study, exercise-trained participants had an 11-year younger cerebrovascular hemodynamic 'age' versus the inactive participants. These participants were stratified into 1 of 4 groups based on their age (young ≤30 years vs old ≥ 60 years) and lifetime physical activity (achieving ≥ 150 minutes per week of aerobic physical activity sustained during adult lifespan vs. not). MCA CBFv was highest in young active (64±13 cm/s), followed by young inactive (52±11 cm/s), old active (46±11 cm/s) and old inactive (37±8 cm/s) [116]. Yet another study found that more frequent physical activity and greater cardiorespiratory fitness was associated with better regulation of CBF in young healthy adults (n=55) [117].

Similar results have been observed using other neuroimaging methods such as NIRS and ASL. Higher fit women, regardless of age, were found to have improved cerebrovascular health as measured by greater regional cerebral oxygenation (NIRS) [118]. Similarly, endurance-trained middle aged adults were found to have greater regional cerebral perfusion (via ASL) compared to their inactive counterparts [119].

These studies demonstrate improved cerebrovascular health in individuals who have higher cardiorespiratory fitness and greater physical activity levels. These benefits to subclinical cerebrovascular health measures are thought to explain observed relationships between physical activity and reduced risks of cerebrovascular disorders such as stroke and Alzheimer's disease.

2.4.2.2 Intervention studies

Intervention studies have confirmed the relationships between physical activity and MCA CBFv found in cross-sectional studies. Twenty inactive, healthy, postmenopausal women (mean age 60 years) were assigned to either an 8-week aerobic exercise intervention or a control group. The exercise group participated in mostly supervised aerobic exercise on 3-6 days per week, beginning with 30 minutes per day at 60% age-predicted maximum heart rate and progressing to 40-60 minutes per day at 70-75% age-predicted maximum heart rate. The average frequency and duration were approximately 4 days per week with each exercise bout lasting approximately 47 minutes. The control group was instructed to continue with their usual level of physical activity. The women in the intervention group showed significant increases in peak systolic, mean systolic and diastolic MCA CBFv as well as a significant decrease in cerebrovascular resistance (calculated as mean blood pressure divided by mean MCA CBFv). Further, though pulsatility index did not change significantly, changes in the pulsatility index were associated with changes in carotid artery compliance (a measure of arterial stiffness) [48].

Similarly, in inactive young and older healthy adults, a mostly supervised 12-week aerobic exercise training intervention began with 20-30 minute sessions, 3 times per week, at 65% heart rate range, and progressed to 40-50 minute sessions, 4 times per week, at 65-80% heart rate range. This intervention resulted in an increased resting MCA CBFv and improved cerebrovascular autoregulation [120].

Improvements in cerebrovascular hemodynamics and cerebrovascular function with exercise training are not limited to healthy individuals. For example, a 6-month aerobic exercise training intervention in hemiparetic stroke survivors (mean age of 60) was found to significantly improve ipsilesional and contralesional cerebrovascular autoregulation [121]. Due to the applicability of exercise in different populations for improvements in cerebrovascular health, it is important to understand the chronic and acute effects to develop population and disease appropriate interventions. Single bouts of exercise are an important starting point for understanding acute mechanisms and how, over time, these effects could lead to chronic adaptions. The limited research regarding the acute effects of a single bout of exercise on cerebrovascular hemodynamics is discussed below.

2.4.2.3 Single bout of exercise

Few studies have evaluated the acute effects of exercise on cerebral blood flow. Using ASL, global CBF[122] has been reported to increase acutely following exercise. However, regional decreases and increases have also been reported using other methods such as single-photon emission computed tomography and ASL [123-126]. Furthermore, studies using TCD have found inconsistent changes in MCA CBFv following a single bout of exercise. In one study, a 40-minute bout at 60% of VO_2max did not change MCA CBFv at 10, 30, or 60 minutes post-exercise. It should be noted, however, that participants in this study were injected with sodium nitroprusside (a vasodilator) to induce hypotension and this could have affected the results [127]. In another study, 11 participants performed two identical high intensity cycling sessions of 30 minutes on separate occasions. Following one of the exercise sessions (n=11), there was a significant 7% decrease in MCA CBFv at 6 minutes post-exercise. Following the other session (n=7), a nonsignificant 3% decrease in MCA CBFv was observed [128]. Yet another study

measured supine MCA CBFv pre- and post-marathon (within 4 hours of completion) and found no significant differences [129]. These inconsistencies may be due to limited sample sizes, differences in measurement methodology and timing, as well as variable exercise intensities. Also, these experimental studies have focused on mostly young, healthy individuals who are likely to have healthy CBF and these results may reflect a ceiling effect.

In addition to the limited research on CBF acutely post-exercise, the acute effects of a single bout of exercise on cerebrovascular pulsatility have not previously been examined. Thus, we aimed to evaluate the effects of a single bout of exercise on cerebral hemodynamics, specifically CBF and pulsatile flow. Additionally, we focused on healthy, middle-aged adults as they are at a higher risk for subclinical cerebrovascular alterations and may be more likely to improve from a single bout of aerobic exercise.

2.4.3 Effects on arterial stiffness

It has been well established that aerobic exercise elicits beneficial cardiac and vascular effects [130]. Exercise-induced benefits on arterial stiffness occur acutely as well as chronically. Similar to acute mechanisms of cerebrovascular benefit described earlier, increased vascular sheer stress results in increased NO bioavailability as well as other vasodilators. NO is a potent vasodilator and reduces the vascular smooth muscle cell tone, thereby decreasing arterial stiffness[61]. Vasodilation of vascular muscle cells transfers stress from collagen fibers to elastin proteins [131]. In addition, the increased arterial pressure and heart rate from exercise may result in physical forces deforming the large blood vessels, thereby reversing and inducing chronic improvements in arterial stiffness such as connective tissue linking [132]. Furthermore, arterial baroreflexes may play a role in augmenting the vagal tone of the heart [132]. Lastly, afferent

nerves in the aortic arch and carotid artery send signals to the brain stem when distension occurs in these arteries during systole, resulting in inhibition of sympathetic outflow [132]. Repeated bouts of exercise induce larger vessel diameter and better endothelial function [133, 134]. Moreover, habitual exercise results in increased antioxidant activity and enhanced resistance to oxidative stress, all which can increase NO bioavailability [135]. These acute and chronic benefits on arterial stiffness due to exercise are supported by both cross-sectional and intervention studies. Less research has been conducted on the acute effects of a single bout of exercise on arterial stiffness. This evidence is discussed below.

2.4.3.1 Observational studies

Cross-sectional studies have established inverse relationships between arterial stiffness and physical activity or cardiorespiratory fitness. For example, central and peripheral arterial stiffness were greater in 405 young adults with poorer cardiorespiratory fitness [136]. After adjusting for age, gender, mean arterial pressure, anthropometrics, physical activity and other health behaviors, higher VO_2max remained significantly associated with lower central (β= -0.18, P=0.008) and peripheral arterial stiffness (β= -0.20, P=0.002). Similar inverse relationships between cardiorespiratory fitness or physical activity and arterial stiffness have been reported in children [137], adolescents [138], middle-aged adults and older adults [139, 140].

2.4.3.2 Longitudinal studies

Aerobic exercise is known to reduce arterial stiffness as measured by PWV with interventions ranging in duration from 4 weeks to 6 months. These interventions include diverse populations and exercise prescriptions ranging from 30 to 60 minutes per session at 40-80% maximum aerobic capacity. They include healthy adults [13, 141] and individuals with diabetes [142, 143],

pre-and stage-1 hypertension [143, 144], hypercholesterolemia [143] and metabolic syndrome [141]. Shorter studies have found that exercise can decrease arterial stiffness in as little as 24 hours [145] to 6 days [146].

Extensive research has been conducted on the effects of exercise on blood pressure reductions (on average by 2-5 mmHg systolic and 1-4 mmHg diastolic blood pressure) [147], though fewer studies have investigated effects on pulse pressure. Reductions in blood pressure are not necessarily mirrored in pulse pressure, however systolic blood pressure has been shown to explains most of the pulse pressure variance [148]. Preliminary evidence suggests that pulse pressure is reduced with exercise training. For example, one study using pulse pressure as a primary target of exercise intervention found that an 8 weeks of 45-60 minutes at 60-79% of age-predicted maximum heart rate significantly reduced pulse pressure [148] .

2.4.3.3 Acute effects of a single bout of exercise

A single bout of exercise has been shown to have acute effects on both central and peripheral PWV and pulse pressure. Twenty-three young, healthy men underwent a 60-minute bout of aerobic exercise at 65-75% of heart rate reserve. cfPWV as well as brachial and aortic pulse pressure were significantly reduced at 20- and 50-minute post-exercise (**Table 2**) ($P<0.05$) [149]. Similarly, central and peripheral PWV significantly decreased 20 minutes ($P<0.05$) [18] and 30 minutes ($P= 0.01-0.04$) [17] following a 30-minute bout of aerobic exercise at 65% peak VO_2max in moderately active as well as inactive young adults. In contrast, several studies have not found PWV to change following aerobic exercise [150-154]. There is evidence to suggest that three accumulated bouts of 10 minutes throughout the day can reduce blood pressure with chronic training, however, whether a single 10-minute bout has acute effects on blood pressure has yet to be evaluated. Furthermore, the acute effects of a 10- vs. 30-minute bout of exercise are

unknown on arterial stiffness, and thus we aimed to study these and examine whether these elicit benefits in dose-response manner.

Table 2. Reductions in Arterial Stiffness 20 and 50 Minutes Following an Acute Exercise Bout [149]

Arterial Stiffness Measure	**Baseline**	**20 minutes**	**50 minutes**
Carotid-femoral Pulse Wave Velocity (m/s)	7.1±2.7	6.9±1.5	6.8±1.2
Brachial Pulse Pressure (mmHg)	51.0±6.0	48.0±5.0	49.0±7.0
Central Pulse Pressure (mmHg)	31.0±4.0	28.0±3.0	28.0±4.0

2.4.4 Relationship between exercise-induced improvements in systemic and cerebrovascular hemodynamics

The cardiovascular benefits of aerobic exercise, particularly those on arterial stiffness, are thought to enhance Windkessel function (the ability of vessels to recoil and expand and buffer pulsatile flow). This may improve cerebrovascular hemodynamics by attenuating the pulsatile flow reaching the brain [3, 131] and, consequently, reduce the risk for the cerebrovascular disorders thought to originate from pulsatile flow. Furthermore, exercise may attenuate the reductions in CBF believed to occur as a result of systemic pulsatile flow [81] and could potentially lower the risk for the cerebrovascular disorders (e.g. stroke and vascular dementia). Preliminary research supports this mechanism, though the limited understanding motivated this project.

2.4.4.1 Cross-sectional studies

Though few studies have been conducted, it appears arterial stiffness is associated with cerebral hemodynamics. Associations between CBF and arterial stiffness were evaluated in master athletes and inactive middle-aged adults. Master athletes were individuals participating in moderate-to-vigorous aerobic exercise for 7.5 hours per week while inactive individuals reported participating in exercise less than once a week for the past year. Master athletes had lower arterial stiffness and greater regional blood blow (measured by ASL) compared to their inactive counterparts [119]. However, no differences in brachial or central pulse pressure were found across groups and the relationship between pulse pressure and cerebrovascular hemodynamics were not evaluated.

2.4.4.2 Intervention

Our hypotheses are also supported by an exercise training study. A 16-week endurance training intervention was implemented in young tennis players with all participants engaging in the intervention. All participants attended 60-90 minute sessions three times per week at 65-75% and 90% heart rate reserve before their mandatory 2-hour tennis practice. The intervention progressed over three phases including long distance running and short interval training. Interestingly, cerebrovascular hemodynamics (blood flow and pulsatile flow) were unchanged. However, carotid compliance was found to increase post-training and larger increases in compliance were associated with improved cerebrovascular hemodynamics. Specifically, higher CBF and greater attenuations of pulsatile flow in the MCA. Brachial pulse pressure was unchanged and its relationship to cerebrovascular hemodynamics was not evaluated [6].

2.4.4.3 Single bout of exercise

To be best of our knowledge, there are no studies evaluating whether a single bout of exercise effects cerebrovascular hemodynamics through reductions in arterial stiffness and pulse pressure. Thus, evaluating this relationship was an aim of this study.

2.5 GAPS IN THE LITERATURE

There is limited research on the acute effects of a single bout of exercise on arterial stiffness and cerebrovascular hemodynamics. The effect of a 30-minute bout of exercise on cerebral hemodynamics is unclear and the effects of a 10-minute bout are unknown. Additionally, more research is needed regarding the benefits of exercise on cerebral pulsatile flow.

Preliminary evidence suggests that arterial stiffness is reduced acutely following a 30-minute exercise bout; however, the effects of a 10-minute bout have not been studied. Furthermore, whether the acute effects of a single bout of exercise on arterial stiffness and cerebrovascular hemodynamics are related is unknown. The aims of the proposed study were to evaluate whether single bouts of exercise of different durations (10-minute and 30-minute bout) can elicit improvements in cerebrovascular hemodynamics and arterial stiffness. Additionally, this study examined whether reductions in arterial stiffness explain the improvements in cerebrovascular hemodynamics.

3.0 METHODS

3.1 RECRUITMENT AND SCREENING PROCEDURES

Subjects were recruited using flyers (**Appendix A)** which instructed interested individuals to contact the Physical Activity and Weight Management Research Center. To determine initial eligibility, trained staff provided a description of the study and, after verbal consent, proceeded to the phone screening (**Appendix B**). Questions determined initial eligibility based on demographic information, medical history, general physical health and ability to complete study procedures. In addition, interested participants completed a Physical Activity Readiness Questionnaire (PAR-Q) [155] as part of the phone screening to confirm that they were able to exercise safely. If "yes" was answered to one or more of the PAR-Q questions, participants were ineligible to participate. Those found to be initially eligible after the phone screen were asked to attend an orientation session led by the principal investigator.

3.2 SUBJECTS

Fifteen middle-aged adults (ages 35-59 years old) were recruited to participate in this study. Middle-aged adults were of interest due to their increased risk for arterial stiffness, which predicts subclinical cerebrovascular disease, cognitive decline, and increased risk of dementia

later in life [88, 156]. Exclusion and inclusion criteria (**Table 3**) were assessed by self-report during the phone screening (**Appendix B**) and the baseline visit. Blood pressure was measured at the baseline visit. There were no exclusion criteria regarding level of physical activity due to the lack of evidence to suggest that those that are more physically active will have differential cerebral hemodynamics in response to an exercise stimulus compared to those who are inactive.

Table 3. Inclusion/Exclusion Eligibility Criteria

Inclusion Criteria	**Exclusion Criteria**
• Middle-aged adults (ages 35-59 years old) • Ability to provide informed consent • Willingness to watch National Geographic content • Ability to attend all four lab visits • Able to complete exercise sessions safely • Resting systolic blood pressures < 140 mmHg and diastolic < 90 mmHg • Resting systolic blood pressures 140-159 mmHg and diastolic 90-99 mmHg with PCP medical clearance	• Inability to complete exercise sessions for any reason • Presence or history of cardiovascular or cerebrovascular disorders • Currently taking medication to control heart rate or blood pressure • For women, those currently pregnant, trying to get pregnant, or planning on becoming pregnant in the next 2 months

3.3 ASSESSMENT PROCEDURES

Measurements at the baseline visit included blood pressure, height, weight, body mass index, PWV distances, arm circumference and waist circumference. Outcome measurements included blood pressure and heart rate, PWV, and cerebrovascular hemodynamics (via TCD). These measurements were assessed at the beginning of each experimental visit and at 30 and 60 minutes following the experimental condition (**Table 4**). Vascular assessment time points were chosen to be comparable to several previous studies [150-154, 157].

Table 4. Assessment Schedule

Measurement	**Baseline Assessment**	**30-Minute Post-Session Assessment**	**60-Minute Post-Session Assessment**
Resting Blood Pressure	X	X	X
TCD	X	X	X
PWV (carotid-femoral, carotid-radial)	X	X	X

3.3.1 Body height, weight and body mass index

Height and weight were measured at the baseline visit while the participant wore lightweight clothing and no shoes. Bodyweight was measured to the nearest 0.1 kg on a Tanita WB 110A digital scale, and height was measured using a wall-mounted stadiometer. Two measurements of each were made and, if measurements differed by ≥ 0.5 cm (height) or ≥0.2 kg (weight),

additional measurements were made until two were within range. The average of measurements within range was used for data analyses. Body mass index was computed as kg/m^2.

3.3.2 Arm circumference

To determine proper blood pressure cuff size (**Table 5**), a Gulick measuring tape was used. The arm circumference was measured at the midpoint between the acromion process and the olecranon process on the right arm while the subject was standing upright, shoulders relaxed and right arm hanging loosely. Two measurements were made to the nearest millimeter. A third measurement was made if the first two measurements differed by more than 1.0 cm. The average of the measurements within range was used to select the appropriate cuff.

Table 5. Blood Pressure Cuff Sizes

Arm Circumference	**Cuff Size**
17.0 to < 24.0 cm	Adult small
24.0 to <33.0 cm	Adult
33.0 to <41.0 cm	Large Adult

3.3.3 Waist circumference

Waist circumference was measured while the subject was standing using a Gulick measuring tape. The hip area was located at the right ilium [158] and a horizontal line was drawn just above the uppermost lateral border of the right ilium. Then, a vertical line was drawn to cross the

horizontal line at the midaxillary line. The measuring tape was placed around the trunk in a horizontal plane at the level of the drawn line. The tape was parallel to the floor and was snug but did not compress the skin. Two measurements were made at the end of a normal expiration and were recorded to the nearest millimeter. A third measurement was made if the first two measurements differed by more than 1.0 cm. The average of the measurements within range were used for data analyses.

3.3.4 Level of physical activity

Physical activity levels were measured using the Paffenbarger Exercise Habits Questionnaire [159] (**Appendix C**) which included questions regarding number of stairs climbed and participation in recreational sports. Total weekly exercise minutes were calculated from the Paffenbarger Exercise Habits Questionnaire by adding minutes spent walking briskly and minutes spent in sport, fitness or recreational activities per week.

3.3.5 Fitness

Fitness was estimated using the fitness calculator created by the K. G. Jebsen Center of Exercise in Medicine at the Norwegian University of Science and Technology [160]. This calculator estimates VO_2max using gender, age, height, weight, resting heart rate, waist circumference, age-predicted maximum heart rate and level of physical activity (**Appendix D**). This equation was found to explain 61% and 56% of variance in VO_2peak with standard error of the estimate of 5.70 and 5.14 ml/min/kg for men (n=2067) and women (n=2193), respectively [161]. It is recommended as an estimate of fitness by the American Heart Association [162].

3.3.6 Resting blood pressure and heart rate

Brachial artery blood pressure was measured in duplicate by an automated blood pressure monitor (Omron HEM-705) after a 10-minute supine rest. Pulse pressure was calculated by subtracting diastolic blood pressure from systolic blood pressure. Pre-condition and post-exercise time course measures of blood pressure included two blood pressure measurements with a 1-minute rest between measures. These were averaged.

3.3.7 Heart rate

Participants wore a heart rate monitor (Polar A1) continuously throughout each experimental visit. Heart rate is an important covariate of cerebrovascular hemodynamics when measured by TCD. Thus, heart rate was recorded every minute during TCD measurements. Heart rate was also monitored and recorded every minute during the workload estimation, the 10-minute and the 30-minute exercise sessions. Additionally, heart rate was monitored every 5 minutes while watching National Geographic during the seated session (**Section 3.4.2.1**) and the seated portion of the 10-minute exercise (**Section 3.4.2.2**) session.

3.3.8 Carotid-femoral and carotid-radial distances

Using a tape measure, aortic distance was estimated by subtracting the distance from the carotid artery site to the sternal notch from the distance of the sternal notch to the femoral artery site [65]. Carotid radial distance was estimated by measuring from the carotid artery site to the radial artery site with the arm in anatomical position [65].

3.3.9 Carotid-femoral and carotid-radial pulse wave velocity

Central and peripheral arterial stiffness were measured with the subject in a supine position using tonometry and the Complior Analyse (Alam Medical). Sensors were placed on the right side of the body by the principal investigator (inter- and intra-technician ICCs for PWV were 90% and 94-98%, respectively). Piezoelectric sensors were placed on the skin surface over the carotid and radial artery sites, and over thin fabric (e.g., cotton pants, spandex) on the femoral artery where the pulse was most strongly felt. Sensors were held in place until 10 valid waveforms were captured for each scan with low error, ideally $\leq$ 5% but up to 10% was accepted. Average carotid-radial and carotid-femoral PWV (m/s) were calculated as the distance divided by the average time differential between the foot of the waveform at the carotid and radial or femoral sites [59, 62, 64, 65]. Three scans were measured at each site and were averaged to reduce measurement error.

3.3.10 Transcranial Doppler ultrasonography

Cerebrovascular blood flow velocity of the MCA and extracranial internal carotid artery (ICA) was measured bilaterally for one minute using a 2 MHz noninvasive ultrasound probe (Terumo; Spencer Technologies) by the principal investigator (intra-rater reliability was 85-97% across TCD measures in 2016). Changes in cerebrovascular blood flow can be inferred from changes in blood flow velocity. To insonate the MCA, the probe was placed on the temporal bone window and readings at depths of 40-65 mm were made [41, 43]. The extracranial ICA was insonated at shallower depths of 35-50 mm using the submandibular approach (below jaw) [41]. The depth, location of probe, and bony landmarks were recorded so that the TCD recording position

remained the same in each subject and the same approach was used for each condition as well as between conditions. Quality of the measurements was rated from poor to excellent based on how well the envelope captured the waveforms, the strength of the signal, the direction and depth that is consistent with MCA flow. Any scan with a rating below adequate was not used. Cerebrovascular pulsatile flow was calculated as the difference between peak systolic and diastolic flow velocities divided by mean flow velocity.

3.4 PARTICIPANT VISITS

All enrolled participants attended four separate visits: one baseline visit and three 2.5-hour experimental visits. Participants attended these visits between March 2017 and May 2017 at the Physical Activity and Weight Management Research Center.

3.4.1 Orientation and baseline visit

The principal investigator reviewed the study protocol with participants and answered any questions before participants provided informed consent. Height, weight, arm and waist circumference and distances from the carotid artery site to the radial and femoral artery sites were measured. Participants completed a Paffenbarger Exercise Habits Questionnaire to report their level of physical activity (**Appendix C**) [159].

Participants were also familiarized with the treadmill and underwent a workload estimation (**Appendix E**) to establish their starting speed to reach their heart rate target zone (70-75% of age-predicted maximum heart rate) during the exercise sessions. Age-predicted

maximum heart rate was calculated as 220 – age in years [163]. During the workload estimation, the treadmill speed and grade were adjusted based on heart rate response for the individual until the target heart rate of 70-75% of age-predicted maximum was attained. For this session, the speed started at 2.4 mph and 0% grade (elevation). Speed increased by 0.2 mph each minute until the target heart rate was attained or a walking speed of 3.4 mph was attained. If the target heart rate was not achieved at a walking speed of 3.4 mph, the grade of the treadmill was increased by 0.5% each minute until the target heart rate was achieved. This target heart rate zone represents a moderate-to-vigorous intensity and was chosen for comparability to previous studies [145, 149].

3.4.2 Overview of experimental sessions

All participants completed each experimental session (**Figure 2**) at separate visits in a randomized order (**Section 3.4.3**). Visits to the lab were at least 48 hours apart and no more than seven days apart. Additionally, each participant had the same appointment start time for each of their three experimental visits (**Section 3.4.4**). Upon arrival to each visit, adherence to instructions to abstain from moderate-to-vigorous physical activity for 24 hours, caffeine, and alcohol, nicotine for 12 hours, and a 4-hour fast was verbally confirmed and recorded. Participants were then instructed to put on a heart rate monitor which they wore for the duration of each experimental visit. Participants were prompted to use the restroom prior to and following each experimental visit. If a restroom break was necessary, it was recorded. At the end of each session, a bottle of water was provided to the participant for consumption ad libitum.

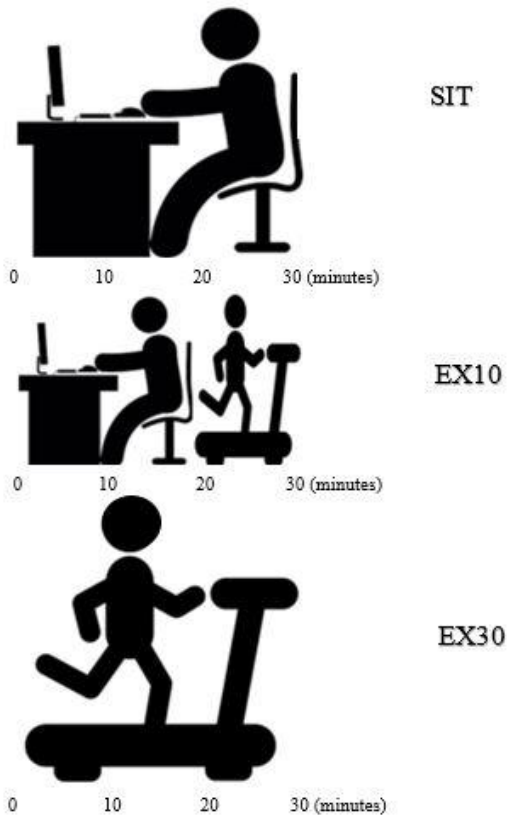

Figure 2. Overview of Experimental Sessions

3.4.2.1 Seated session (SIT)

Immediately following baseline assessments, participants were asked to sit and watch a preselected documentary series from the National Geographic Channel website for 30 minutes. National Geographic Channel was selected due to its options for neutral content which would be less likely to affect outcomes such as blood pressure and heart rate. Willingness to watch National Geographic content was determined during the phone screening (**Appendix B**).

3.4.2.2 10-minute exercise bout (EX10)

Immediately following baseline assessments, participants watched the same documentary series for 20 minutes, after which they began their 10-minute moderate-to-vigorous exercise bout. The

10-minute exercise bout started at the speed and grade established at the baseline visit (**Section 3.4.1**). Thereafter, heart rate was monitored each minute, with speed or grade adjusted to maintain the target of 70-75% of age-predicted maximal heart rate. Speed and grade were adjusted based on each individual's heart rate response using an algorithm developed by Dr. John Jakicic at the Physical Activity and Weight Management Research Center (**Appendix F)**. Minutes 4 and 5 of the first 5-minute interval were averaged and the appropriate adjustment was made if this average heart rate was outside of the 70-75% heart rate zone. For example, if the average heart rate for minutes 4-5 was below the 70-75% heart rate zone, speed was increased by 0.2 mph. The participant then walked at this speed for minutes 6-10. If speed was already at 3.4 mph and heart rate was below the targeted range, then grade was adjusted by 0.5%. If heart rate was within the prescribed zone, no adjustments were made. All adjustments were recorded.

3.4.2.3 30-minute exercise bout (EX30)

Immediately following baseline assessments, participants walked on the treadmill for 30 minutes. The exercise session started at the speed and grade established at the baseline visit (**Section 3.4.1**) Thereafter, heart rate was monitored each minute, with speed or grade adjusted according to the procedures noted above for the 10-min exercise condition. These procedures were repeated at minutes 9-10, 14-15, 19-20, and 24-25 (**Appendix G**). All adjustments were recorded.

3.4.3 Randomization and allocation procedures

There were six possible orders. Using 1 as SIT, 2 as EX10 and 3 as EX30, potential orders of conditions are as follows: 123, 132, 213, 231, 312, 321. To achieve counterbalance of all orders,

two participants were randomly assigned to each of the six experimental orders (n=12) and the remaining three were randomly assigned an order.

3.4.4 Standardization and timing

The timing of the experimental visits was standardized such that they all took place between 11:40am and 5:00pm and standardized within participant such that each participant had the exact same appointment start time for each of their three experimental conditions. This was based on previous research in our laboratory that suggests diurnal variation in cerebrovascular hemodynamics across the day [164]. In addition, protocol timing was tightly controlled and monitored (**Appendix H**). Timeframes were allotted for measuring each outcome (**Table 6**) and these times were adhered to. After each experimental condition, participants laid in a supine position until it was time to begin the post-condition measurements. To limit potential hemodynamic influences of postural changes, participants remained supine until the end of the last 60-minute post-session measurement.

Table 6. Standardized Timing

Baseline Measurement	**Transitions**	**30- and 60-minute Post-Session**
Supine Rest (10 min) Duplicate Blood Pressures (3 min) TCD (10 min) Each MCA (3 min) Each ICA (2 min) Supine PWV (5 min)	From PWV to computer (3 min) From room to treadmill (2-5 min) From treadmill to room (2-5 min)	Duplicate Blood Pressures (3 min) TCD (10 min) Each MCA (3 min) Each ICA (2 min) Supine PWV (5 min)

3.4.5 Participant compensation

Participants were compensated $100 for completing the 3 experimental sessions (SIT, EX10 and EX30), each on a separate visit. If a participant did not complete all sessions, they were paid $25 for each session completed.

3.5 STATISTICAL ANALYSES

Paired t-tests or Wilcoxon signed-rank tests were performed, as appropriate, to evaluate differences in heart rates and parameters of experimental sessions. To evaluate specific aims I-III, linear mixed models estimated effects for cerebrovascular hemodynamics (systolic, diastolic, mean and pulsatile blood flow), and arterial stiffness (pulse pressure and PWV) over the 1-hour trajectories and across conditions. Analyses investigated whether outcome measures differed by group, time point, or differ as a function of group and time point (i.e., interaction) and adjusted for order of condition, age, and gender. MCA and ICA CBFv were additionally adjusted for heart rate. Overall linear effects of time were not evaluated due to variable non-linear effects over time. Rather, pairwise differences at each time points were evaluated if no group by time point interactions were observed. Pairwise differences at each time point were also analyzed (at 30-and 60-minute post-session assessments) in linear mixed models restricted to the single post-session time point and adjusted for baseline value, order of condition, age, and gender. Linear mixed models were also used to assess differences in baseline values across conditions adjusted for order of condition, age and gender. To evaluate Specific Aim IV, mixed linear regression

models evaluated whether changes in arterial stiffness were related to changes in cerebral pulsatile blood flow.

3.6 POWER ANALYSIS

Sample size was calculated using G*Power (Universität Düsseldorf). Because this is the first study to evaluate whether an acute bout of exercise yields decreases in cerebrovascular pulsatile blood flow (measured by pulsatility index), we estimated sample size assuming 80% power, two-sided α=0.05, a within-subjects correlation of 0.80 based on data from another randomized crossover study conducted in our laboratory[164] (r=0.80-0.95), and assuming a small between-condition effect size (SIT, EX10, or EX30) of 0.25 (corresponding to $\eta 2$ = 6%). Given these assumptions, 12 subjects were required. Based on a 20% estimate of missing data (e.g., rare inability to insonate vessels), 15 subjects were recruited.

4.0 RESULTS

The purpose of this study was to evaluate acute effects of aerobic exercise on 1-hour trajectories of cerebrovascular hemodynamics and arterial stiffness across three conditions: sitting (SIT), 10-minute exercise bout (EX10) and 30-minute exercise bout (EX30). This study used a randomized crossover design. The results are presented below, starting with participants and experimental sessions, and following with results organized by specific aim.

4.1 PARTICIPANTS

4.1.1 Recruitment and enrollment

Telephone screenings were conducted on a total of 27 interested individuals. Of these potential participants, five were ineligible due to an inability to participate in all study visits (n=3), being outside of age range (n=1) or reporting chest pain when not doing physical activity in the month prior (n=1). Of those eligible based on the phone screening, four were unable to be scheduled for the baseline visit due to scheduling conflicts (n=2) or nonresponsiveness (n=2). One participant became ineligible at the baseline visit due to resting blood pressure in the Stage II hypertensive range. One became ineligible due to resting blood pressure in the Stage I hypertensive range and unwillingness to obtain primary care physician (PCP) clearance to participate. Finally, one

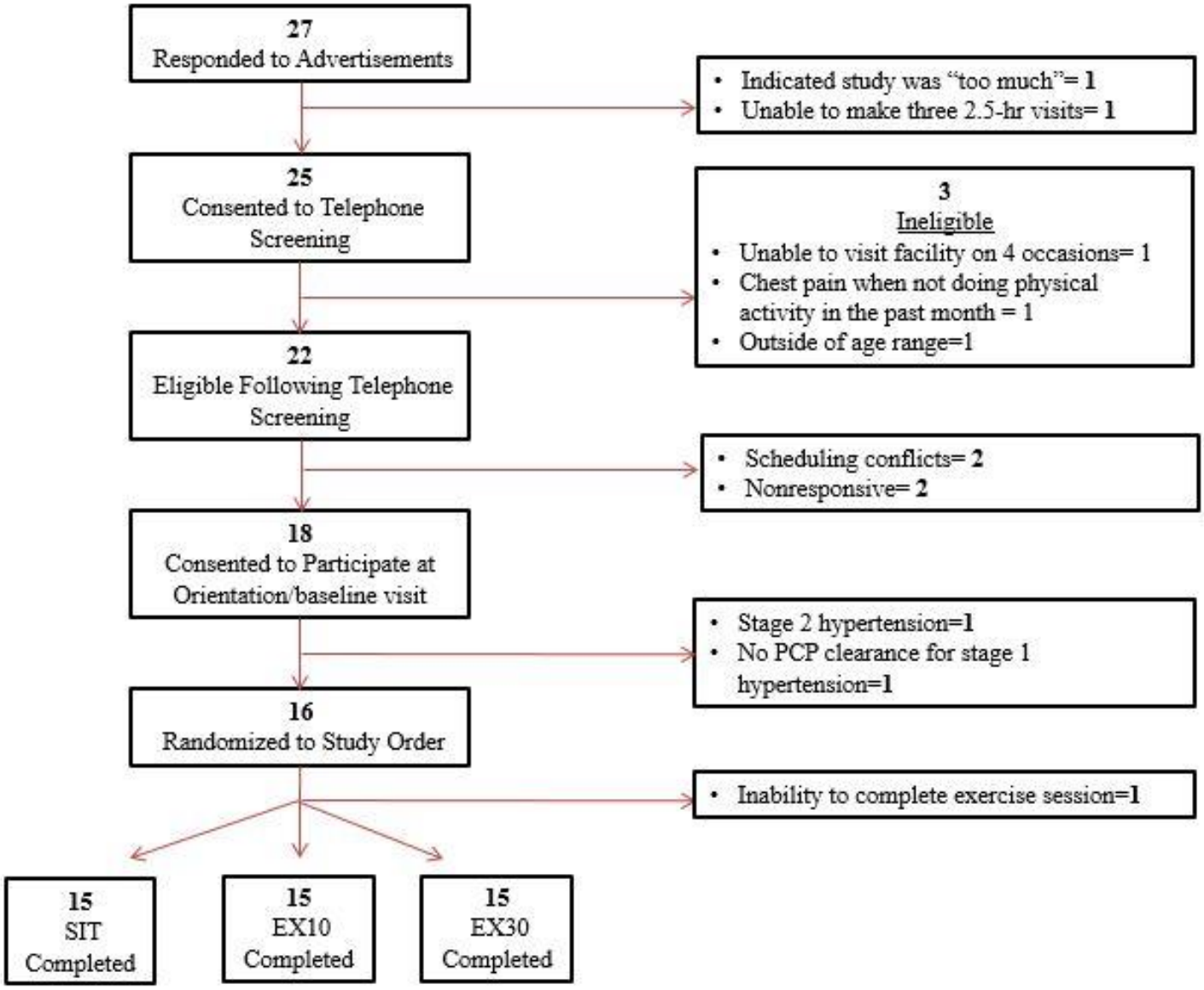

Figure 3. CONSORT Diagram

participant became ineligible after being randomized due to an inability to complete the exercise session during their first experimental visit (EX30). Figure 2 displays the CONSORT diagram.

4.1.2 Participant characteristics

Fifteen adults between the ages of 36-59 years completed all research procedures in this crossover study. Participants had a mean age of 45.4 (8.9) years, a mean BMI of 26.4 (4.2) kg/m^2 and were mostly non-Hispanic (73.3%), white (66.7%) women (87.0%). Additionally, most

Table 7. Participant Characteristics

	Mean (SD) or n, %	Range
Age, years	45.4 (8.9)	36-59
Height (cm)	165.5 (10.2)	149.1-184.9
Weight (kg)	73.0 (18.4)	53.3-116.8
Body Mass Index, kg/m^2	26.4 (4.2)	21.5-34.5
Waist Circumference (cm)	91.1 (12.6)	75.7-112.1
Gender		
Female	13, 87.0%	
Male	2, 13%	
Ethnicity		
Hispanic	4, 26.7%	
Non-Hispanic	11, 73.3%	
Race		
White or Caucasian	10, 66.7%	
Black or African American	1, 6.7%	
Asian	3, 20.0%	
Other	1, 6.7%	
Education		
High school	1, 7.0%	
College	8, 53.0%	
Post graduate	6, 40.0%	
Occupational Status		
Full-time	9, 60.0%	
Part-time	1, 7.0%	
Student	3, 20.0%	
Homemaker	2, 13.0%	
Cardiorespiratory Fitness and Physical Activity		
Estimated VO_2max (ml/min·kg)	37.0 (5.3)	30.0-45.0
Duration of Workload Estimation (minutes)	14.5 (8.6)	2.0-30.0
Weekly Exercise (minutes)	201.0 (224.8)	0.0-765.0

participants worked full time (60.0%) and were educated with a college degree (53.0%) or a post-graduate degree (40.0%). Demographic characteristics are reported in **Table 7**. Participants had a wide range of fitness and physical activity levels. The mean estimated VO_2max was 37.0 (5.3) ml/min·kg while the mean workload estimation duration was 14.5 (8.6) minutes. Participants reported climbing 13.2 (11.4) flights per day (range 2-45), walking briskly for at least 10 minutes in duration for the purpose of transportation or exercise for 113.3 (108.6) minutes per week (range 0-360) and participating in sports, fitness or recreational activities not including household or occupational activities for 87.6 (134.2) minutes per week (range 0-450). Combining minutes spent walking briskly and minutes spent in sport, fitness or recreational activities per week (**Table 7**), 46.7% of participants met American College of Sports Medicine physical activity guidelines of 150 minutes per week.

4.2 WORKLOAD ESTIMATION

During the baseline visit, participants completed a workload estimation (**Appendix E**) to establish their exercise sessions' starting speed corresponding to their heart rate target zone (70-75% of age-predicted maximum heart rate) (**Section 3.4.1**.). Across the workload estimation, the average speed was 3.3 (0.2) mph and the average grade was 4.4 (4.1) %. Average 70-75% of age-predicted maximum heart rate, speed, grade at termination and time to termination are reported in **Table 8**.

Table 8. Exercise Parameters for the Workload Estimation

	Mean (SD)	Range
70% of Age-Predicted Max Heart Rate (bpm)	122.2 (6.0)	113.0-129.0
75% of Age-Predicted Max Heart Rate (bpm)	131.2 (6.4)	121.0-138.0
Average Termination Heart Rate (bpm)	124.3 (6.6)	114.0-134.0
Percent APMHR of Average Termination HR (%)	71.2 (1.5)	69.8-75.1
Average Termination Speed (mph)	3.3 (0.2)	2.6-3.4
Average Termination Grade (%)	4.4 (4.1)	0.0-12.0
Average Termination Time (minutes)	14.5 (8.6)	2.0-30.0

4.3 EXPERIMENTAL SESSIONS

4.3.1 Comparison of sitting heart rates (SIT vs EX10)

Immediately following baseline testing, participants watched a pre-selected neutral National Geographic series for 30 minutes in SIT and 20 minutes in EX10. Bathroom breaks were allowed if necessary; however, no bathroom breaks were taken while watching the series.

Though participants watched the same National Geographic series in both SIT and EX10, average heart rate was compared to demonstrate comparability. A paired t-test revealed average heart rate during SIT was not different from average heart rate during the 20-minute sitting portion of EX10 (P= 0.54). Average heart rate during each session is reported in **Table 9**.

Table 9. Comparing Sitting Heart Rates (SIT vs EX10)

	Mean (SD)	Range
Average Heart Rate for SIT (bpm)	66.2 (11.3)	44.3-89.6
Average Heart Rate for the Sitting Portion of EX10 (bpm)	67.2 (10.2)	53.4-90.6

Values are reported as Mean (SD). *P<0.05 compared to EX10.**P<0.01 compared to EX10.

4.3.2 Comparison of exercise session parameters (EX10 vs EX30)

Immediately following baseline testing, participants completed a 30-minute exercise bout in EX30. In EX10, participants completed a 10-minute exercise bout immediately following the 20-minute sitting portion. These exercise sessions began at the predetermined speed from the workload estimation. Speed or grade were adjusted based on the average heart rate of the last two minutes of the each 5-minute interval (**Appendices F and G**). **Table 10** displays average heart rate for each 5-minute interval and its corresponding percentage of age-predicted maximal heart rate for EX10 and EX30.

Average heart rate during EX10 was 124 (7) beats per minute while the average percent of age-predicted maximum heart rate was 71%. Additionally, an average of 6 out of 10 minutes were within the target range (61% of the exercise bout) and 1 minute was above the target range (14% of the exercise bout). Average speed was 3.4 (0.2) mph and the average grade was 4.8 (4.1) %. Average heart rate during EX30 was 128 (7) beats per minute while the average percent of age-predicted maximum heart rate was 73%. Additionally, an average of 13 out of 30 minutes

were within the target range (45% of the exercise bout) and 11 minutes above the target range (37% of the exercise bout). Average speed was 3.4 (0.2) mph and the average grade was 4.7 (4.2) %. These values are reported in **Table 11**.

Table 10. Average Heart Rate and Age-Predicted Maximal Heart Rate Percentage Across 5-Minute Intervals in EX10 and EX30

	EX10		**EX30**	
	Mean (SD)	HR%	Mean (SD)	HR%
Minutes 1-5 Average Heart Rate	120.9 (7.7)	69.3 (2.9)	118.9 (7.9)	68.1 (3.8)
Minutes 6-10 Average Heart Rate	127.5 (8.3)	73.0 (2.4)	125.9 (8.7)	72.1 (3.6)
Minutes 11-15 Average Heart Rate	____	____	128.9 (9.2)	73.8 (3.4)
Minutes 16-20 Average Heart Rate	____	____	131.4 (8.7)	75.2 (2.7)
Minutes 21-25 Average Heart Rate	____	____	132.5 (6.6)	75.9 (1.9)
Minutes 26-30 Average Heart Rate	____	____	131.9 (6.4)	75.5 (1.5)

Values are reported as Mean (SD).

Though both the 10- and 30-minute exercise bouts in EX10 and EX30 had the same starting speed and grade, average heart rate, average corresponding percent of age-predicted maximum heart rate, speed, grade and minutes below, within and above range as well as their corresponding percentage were compared to determine if EX10 and the first 10 minutes of EX30 were similar. Additionally, these parameters were compared between EX30 (overall) and EX10. **Table 11** displays these values.

Table 11. Parameters of EX10 vs EX30 (First 10 Minutes and Overall)

	EX10	EX30 (first 10 min)	EX30 (overall)
Average Heart Rate (bpm)	124.2 (7.4)	122.4 (7.7)	128.3 (7.2)**
Average Corresponding Percent of APMHR	71.1 (2.1)	70.1 (3.3)	73.5 (2.2) **
Average Minutes Below Range	2.5 (2.2)	4.5 (3.3)*	5.3 (4.6)*
Average Minutes Within Range	6.1 (2.3)	4.2 (2.9)	13.4 (5.0)**
Average Minutes Above Range	1.4 (1.7)	1.2 (2.5)	11.2 (7.2)**
Average Percent of Minutes Below Range	25.3 (21.7)	44.7 (32.9)*	17.8 (15.4)
Average Percent of Minutes Within Range	60.7 (22.5)	42.0 (28.8)	44.7 (16.7)
Average Percent of Minutes Above Range	14.0 (17.2)	12.0 (25.1)	37.3 (24.0)*
Average Speed (mph)	3.4 (0.2)	3.3 (0.2)	3.4 (0.1)
Average Grade (%)	4.8 (4.1)	4.9 (4.2)	4.7 (4.2)

Values are reported as Mean (SD). *P<0.05 compared to EX10.**P<0.01 compared to EX10.

Comparing the first 10 minutes of EX30 to EX10, average heart rate was not significantly different in EX10 compared to the first 10 minutes of EX30 (P=0.18). Similarly, neither average speed (P=0.32) nor grade (P=0.09) were significantly different during the first 10 minutes of EX30 and EX10. Average corresponding percent of age-predicted maximum heart rate was not significantly different (P=0.20). Average minutes below range was significantly higher in the first 10 minutes of EX30 compared to EX10 (P=0.03), while minutes within range approached significance (P=0.07) and above range were not different (P=0.77). Average percent of minutes below the target range was significantly higher in the first 10 minutes of EX30 compared to EX10 (P=0.03) while average percent of minutes within the target range approached significance (P=0.07) and average percent of minutes above the target range were not different (P=0.39).

Comparing the full 30 minutes of EX30 to EX10, average heart rate was significantly lower in EX10 compared to EX30 (P=0.001). Neither average speed (P=0.38) nor grade (P=0.79) were different. Average corresponding percent of age-predicted maximum heart rate was significantly higher in EX30 (P=0.001) compared to EX10. However, it should be noted that the average percent of age-predicted heart rate maximum was within the targeted 70-75% range for both exercise sessions and only differed by 2% between conditions. Average minutes below (P=0.02) within (P=0.0003) and above (P=0.001) the target range were significantly higher in EX30 compared to EX10; however, of note, there were more minutes in the 30-minute bout than there are in the 10-minute bout of exercise. Average percent of minutes below (P=0.10) the target range were not different, while average percent of minutes within the target range was approaching significance (P=0.06) and average percent of minutes above range were higher in EX30 compared to EX10 (P=0.001).

4.4 ANALYSIS OF DATA BY SPECIFIC AIM

Baseline values across conditions are presented first. Then, blood pressure and heart rate results are presented as they are important covariates. Following these, results are reported by specific aim.

4.4.1 Baseline values across condition

Most baseline values were similar across condition (P>0.05) except for MCA peak systolic and mean CBFv as well as heart rate. Baseline MCA peak systolic CBFv was significantly higher in

EX10 compared to SIT (β =4.22 cm/s, P=0.02) and EX30 (β =4.41 cm/s, P=0.02). Similarly baseline MCA mean CBFv was significantly higher in EX10 compared to SIT (β =3.04 cm/s, P=0.04) and EX30 (β =3.05 cm/s, P=0.04). Baseline heart rate was higher in EX10 compared to SIT (β =2.61 bpm, P=0.02). **Table 12** reports individual baseline values in SIT, EX10 and EX30. To reduce the potential influence of baseline differences, all analyses evaluating pairwise differences at the 30- and 60-minute post session assessment in the following sections were adjusted for baseline values.

Table 12. Comparison of Baseline Values Across Conditions

	SIT	EX10	EX30
Systolic Blood Pressure (mmHg)	111.3 (11.2)	110.7 (8.0)	108.5 (7.7)
Diastolic Blood Pressure (mmHg)	70.0 (6.9)	69.2 (7.2)	68.37 (7.9)
Pulse Pressure (mmHg)	41.3 (6.7)	41.5 (4.8)	40.2 (4.8)
Heart Rate (bpm)	59.6 (10.7)	62.2 (11.1)*	61.7 (10.4)
MCA Cerebral Pulsatile Flow	0.679 (0.088)	0.678 (0.074)	0.671 (0.079)
MCA Peak Systolic CBFv (cm/s)	86.7 (13.6)	90.6 (13.7)*†	86.4 (15.2)
MCA Mean CBFv (cm/s)	62.4 (10.4)	65.3 (10.8) *†	62.4 (11.0)
MCA Diastolic CBFv (cm/s)	44.5 (7.8)	46.8 (7.3)	44.7 (7.7)
ICA Pulsatile Flow (cm/s)	0.683 (0.102)	0.678 (0.100)	0.665 (0.078)
ICA Peak Systolic CBFv (cm/s)	56.4 (7.3)	55.1 (8.8)	56.9 (7.0)
ICA Mean CBFv (cm/s)	39.8 (4.9)	39.1 (5.4)	40.7 (4.6)
ICA Diastolic CBFv (cm/s)	29.3 (3.8)	28.6 (3.6)	29.9 (3.4)
cfPWV (m/s)	6.98 (0.71)	6.80 (0.66)	6.93 (0.74)
crPWV (m/s)	8.98 (1.02)	8.53 (1.42)	8.68 (1.37)

Table 12 continued

Values are reported as Mean (SD). *Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01. † Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01

4.4.2 Trajectories of blood pressure and heart rate after SIT, EX10 and EX30

Systolic Blood Pressure

A linear mixed model adjusting for condition order, gender and age revealed no significant condition by time interaction (P=0.23); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments, with adjustment for baseline systolic blood pressure, condition order, gender and age. **Figure 4** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline systolic blood pressure, condition order, gender and age.

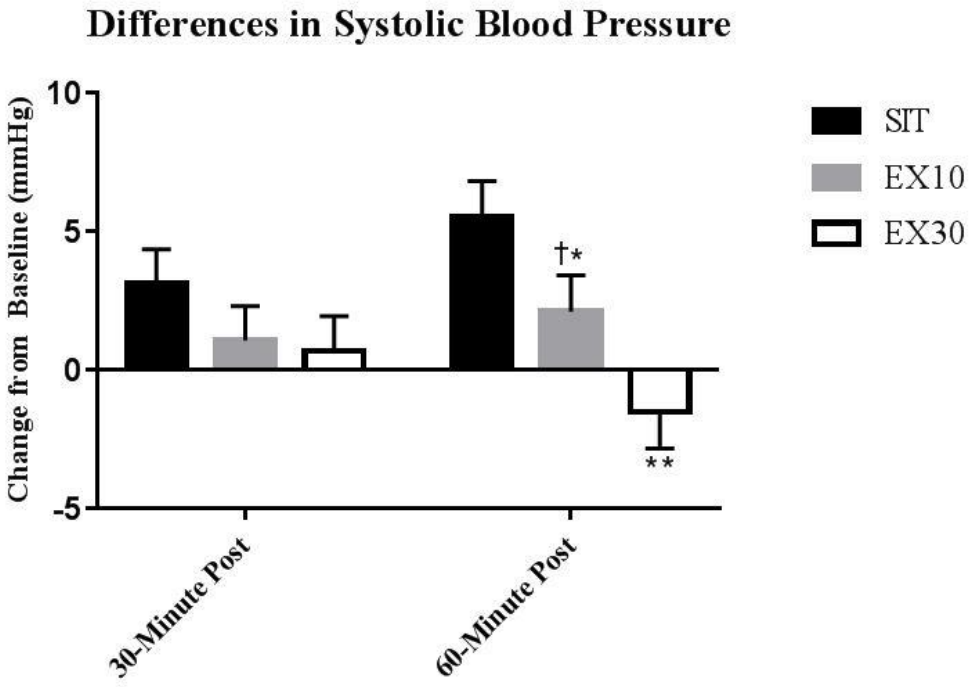

Figure 4. Differences in Systolic Blood Pressure
Differences are least square means adjusted for baseline systolic blood pressure condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

Systolic blood pressure did not differ by condition at the 30-minute post-session assessment. Compared to SIT, systolic blood pressure was nonsignificantly lower in EX10 (β =-2.05 mmHg, P=0.11) and EX30 (β =-2.42 mmHg, P=0.07). EX10 and EX30 were not different (β =-0.37 mmHg, P=0.78). Of note, differences from SIT to EX30 were approaching significance with P<0.10.

Systolic blood pressure differed by condition at the 60-minute post-session assessment. Systolic blood pressure was lower in EX10 (β =-3.40 mmHg, P=0.04) and in EX30 (β=-7.03 mmHg, P<0.001) when compared to SIT and lower in EX30 compared to EX10 (β =-3.64 mmHg, P=0.03).

Diastolic Blood Pressure

A linear mixed model adjusting for condition order, gender and age revealed no significant condition by time interaction (P=0.60); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline diastolic BP, condition order, gender and age. **Figure 5** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline diastolic BP.

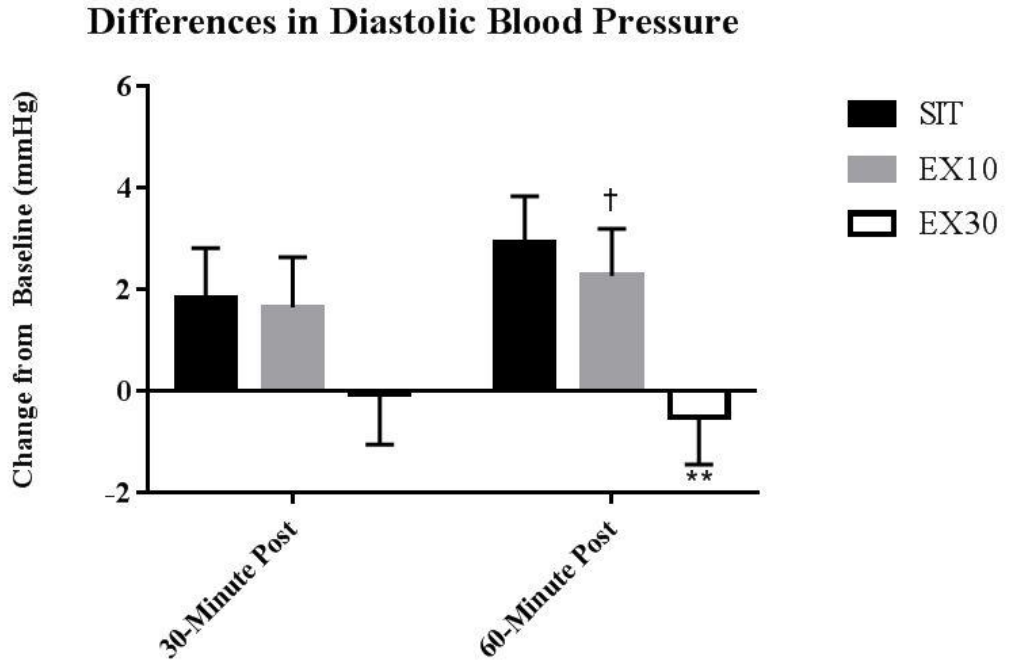

Figure 5. Differences in Diastolic Blood Pressure
Differences are least square means adjusted for baseline diastolic blood pressure, condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

Diastolic blood pressure did not differ by condition at the 30-minute post-session assessment. Compared to SIT, EX10 (β =-0.18 mmHg, P=0.89) and EX30 (β =-1.89 mmHg, P=0.17) were not different. EX30 was not different from EX10 (β =-1.71 mmHg, P=0.21).

Diastolic blood pressure differed by condition at the 60-minute post-session assessment. Compared to SIT, diastolic blood pressure was not different in EX10 (β =-0.64 mmHg, P=0.54) but was lower in EX30 (β=-3.43 mmHg, P=0.001). Diastolic blood pressure was also lower in EX30 compared to EX10 (β =-2.79 mmHg, P=0.01).

Mean Arterial Pressure

A linear mixed model adjusting for baseline condition order, gender and age revealed no significant condition by time interaction (P=0.37); therefore, condition effects were evaluated

separately at the 30- and 60-minute post-session assessments for baseline mean arterial pressure, condition order, gender and age. **Figure 6** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline mean arterial pressure condition order, gender and age.

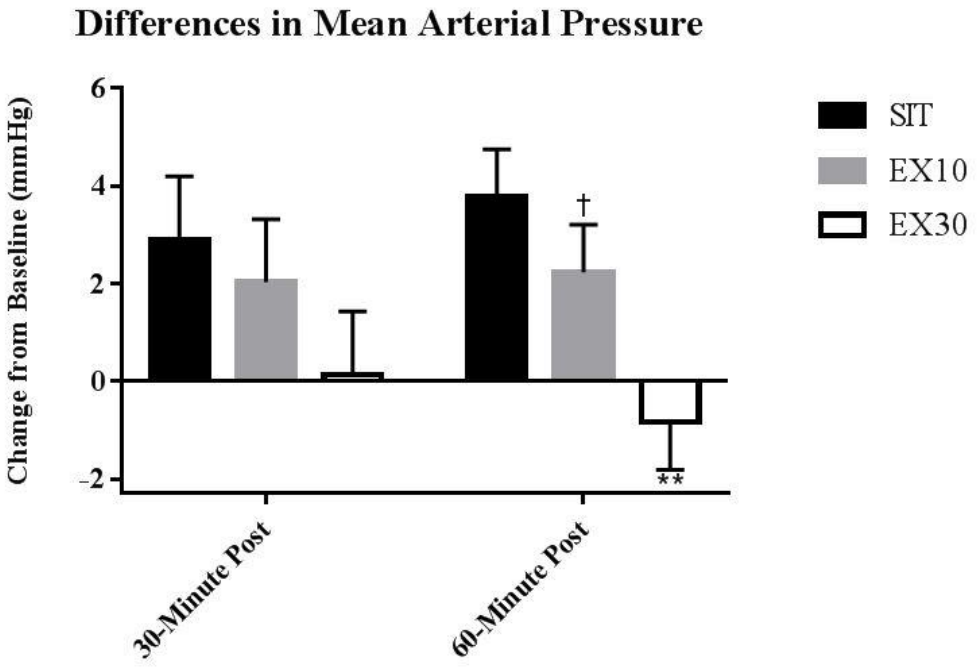

Figure 6. Differences in Mean Arterial Pressure
Differences are least square means adjusted for baseline mean arterial pressure for baseline MAP, condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

assessment. Compared to SIT, mean arterial pressure was not different in EX10 (β =-0.87 mmHg, P=0.60) but was nonsignificantly lower in EX30 (β =-2.77 mmHg, P=0.09). EX30 was not different from EX10 (β =-1.90 mmHg, P=0.25). Of note, differences from SIT to EX30 were approaching significance with P<0.10.

Mean arterial pressure differed by condition at the 60-minute post-session assessment. Compared to SIT, mean arterial pressure was not different in EX10 (β =-1.54 mmHg, P=0.19),

but was lower in EX30 (β=-4.60 mmHg, P<0.001). Mean arterial pressure was lower in EX30 compared to EX10 (β =-3.07 mmHg, P=0.01).

Heart Rate

A linear mixed model adjusting for condition order, gender and age revealed a significant condition by time interaction (P<0.0001). *Post-hoc* testing revealed heart rate in EX10 (P=0.001) and EX30 (P<0.0001) was significantly higher compared to SIT. Additionally, heart rate in EX30 was significantly higher compared to EX10 (P=0.0002). Heart rate responded in a dose-response manner such that heart rate was highest in EX30 and lowest in SIT. **Figure 7** displays the trajectories of heart rate across SIT, EX10 and EX30.

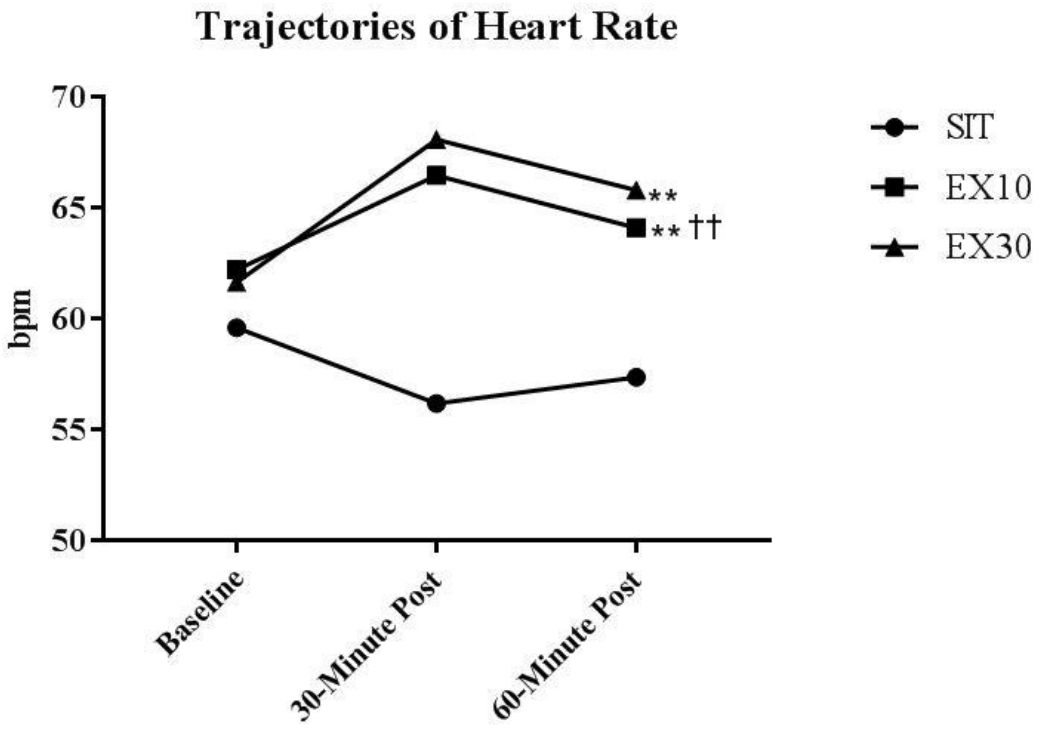

Figure 7. Trajectories of Heart Rate.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

4.4.3 Specific aim I: comparing trajectories of cerebrovascular pulsatile flow after SIT, EX10 and EX30

The primary aim of this study was to compare trajectories of cerebrovascular pulsatile flow after SIT, EX10 and EX30. Pulsatility index of the MCA was automatically calculated (as difference between MCA peak systolic and diastolic flow velocities divided by mean flow velocity) by TCD and was used as a measure of cerebrovascular pulsatile flow. Similarly, pulsatility index of the ICA (as a measure of ICA pulsatile flow) was measured to better understand cerebral pulsatile flow, as ICA supplies the MCA.

4.4.3.1 Cerebral pulsatile flow

A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.50); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline cerebrovascular pulsatile flow, heart rate, condition order, gender and age. **Figure 8** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline MCA pulsatility index values, heart rate, condition order, gender and age. Of note, higher pulsatility index indicates less healthy cerebrovascular hemodynamics.

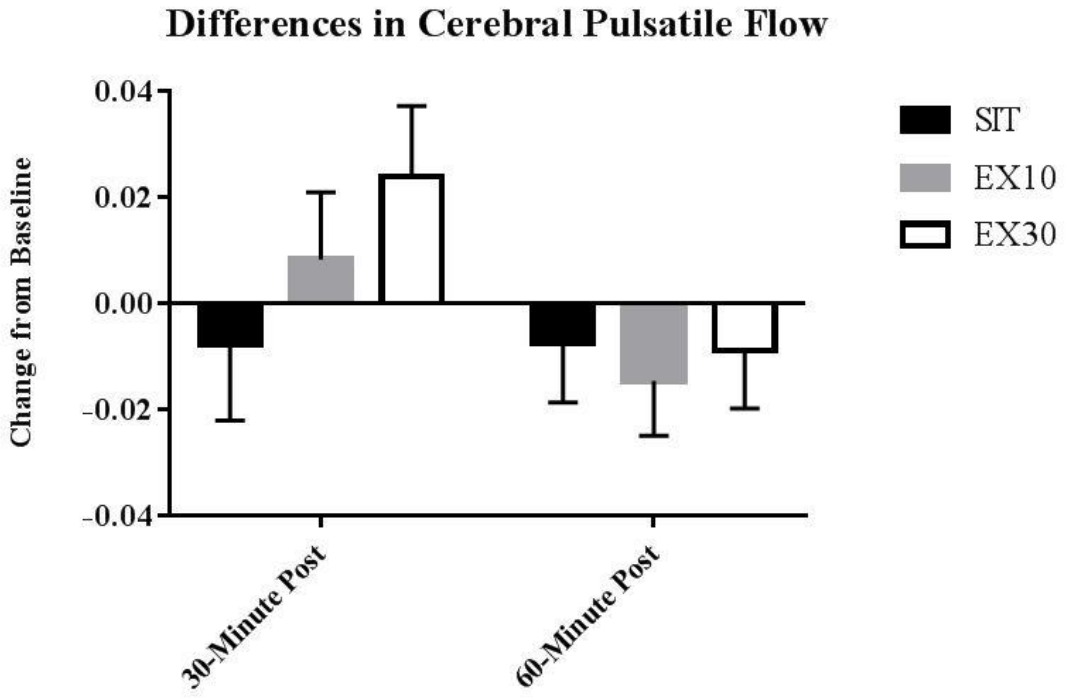

Figure 8. Differences in Cerebral Pulsatile Blood Flow.
Differences are least square means adjusted for baseline cerebral pulsatile blood flow condition order, heart rate, gender and age. MCA pulsatility index was used as a measure of cerebral pulsatile blood flow. No significant differences found.

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MCA pulsatility index did not differ by condition at the 30-minute post-session assessment. Compared to SIT, MCA pulsatility index was not different in EX10 (β=0.016, P=0.33) but was nonsignificantly higher in EX30 (β =0.032, P=0.08). MCA pulsatility index was not different in EX30 compared to EX10 (β =0.016, P=0.27). Of note, higher MCA pulsatility in EX30 compared to SIT approached significance with P<0.10.

MCA pulsatility index did not differ by condition at the 60-minute post-session assessment. Compared to SIT, MCA pulsatility index was not different in EX10 (β =-0.007, P=0.59) or EX30 (β=-0.001, P=0.92). MCA pulsatility index was not different in EX10 compared to EX30 (β=-0.006, P=0.64).

4.4.3.2 Pulsatile flow in the ICA

Interestingly, pulsatile flow in the ICA had similar responses to those in the MCA. A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.25); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline ICA pulsatile flow, heart rate, condition order, gender and age. **Figure 9** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline ICA pulsatile flow, heart rate, condition order, gender and age.

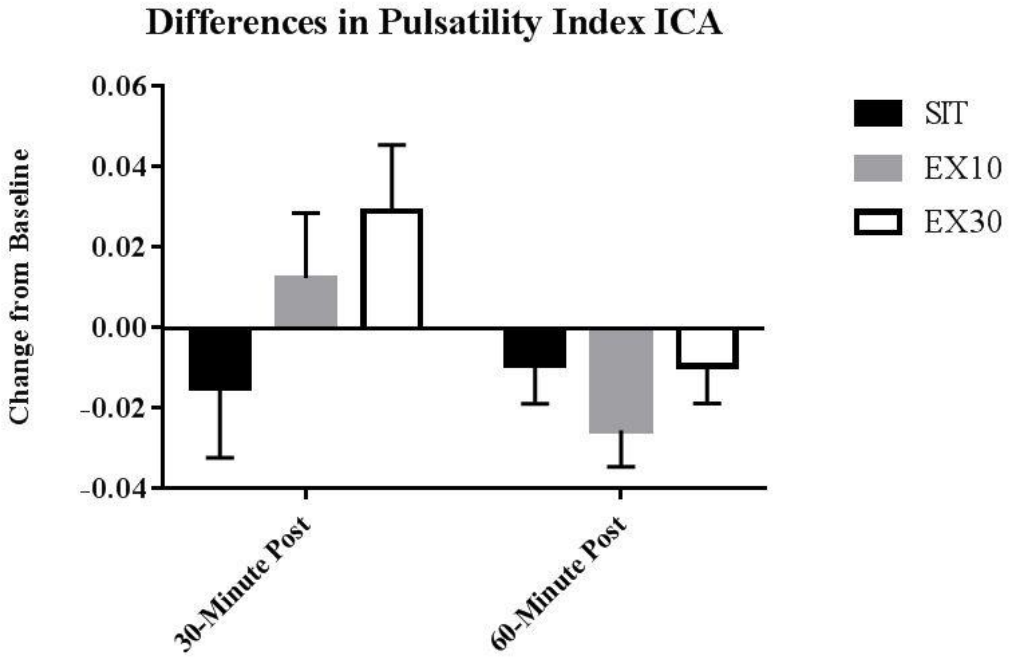

Figure 9. Differences in ICA Pulsatility Index.

Differences are least square means adjusted for baseline ICA pulsatile flow, heart rate, condition order, gender and age. ICA pulsatility index was used as a measure of pulsatile blood flow. No significant differences found.

ICA pulsatility index did not differ by condition at the 30-minute post-session assessment. Compared to SIT, ICA pulsatility index was not different in EX10 (β=0.027, P=0.26) and was nonsignificantly higher in EX30 (β =0.044, P=0.08). ICA pulsatility index was

not different in EX30 compared to EX10 (β =0.017, P=0.47). Of note, higher ICA pulsatility in EX30 compared to SIT approached significance with P<0.10.

ICA pulsatility index did not differ by condition at the 60-minute post-session assessment. Compared to SIT, ICA pulsatility index was not different in EX10 (β =-0.016, P=0.18) or EX30 (β=-0.0003, P=0.98). Compared to EX30, ICA pulsatility index was not different in EX10 (β =0.016, P=0.15).

4.4.4 Specific aim II: comparing trajectories of peak systolic, mean, and diastolic CBFv after SIT, EX10 and EX30

The second aim of this study was to compare trajectories of peak systolic, mean and diastolic CBFv after SIT, EX10 and EX30. Additionally, peak systolic, mean and diastolic CBFv of the ICA, the supplying artery, were also measured and its trajectories after SIT, EX10 and EX30 are also reported in this section.

4.4.4.1 MCA peak systolic, mean and diastolic CBFv

Peak Systolic MCA CBFv

A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.69); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline peak systolic MCA CBFv, heart rate, condition order, gender and age. **Figure 10** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline peak systolic MCA CBFv, heart rate, condition order, gender and age.

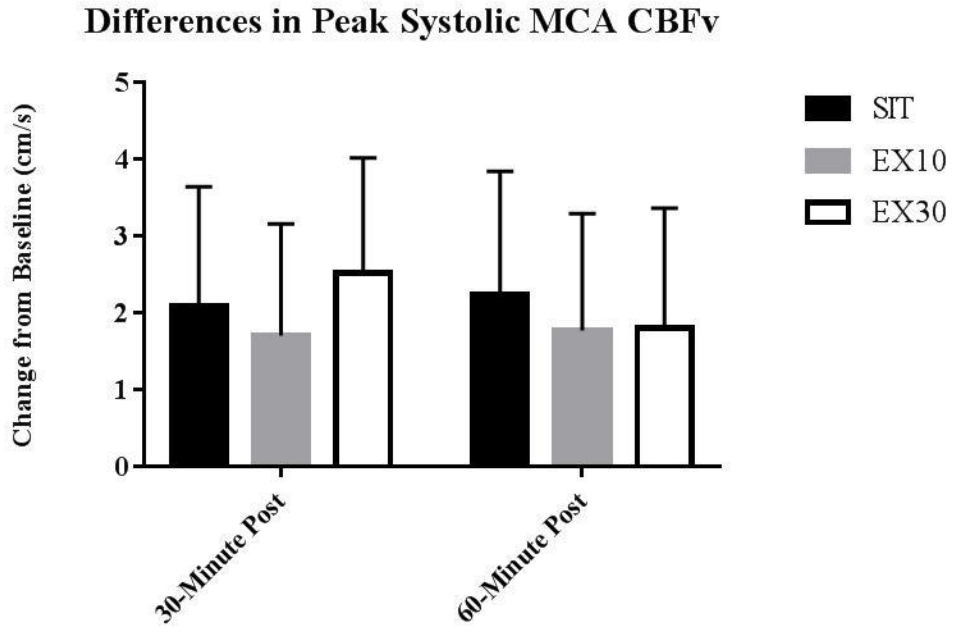

Figure 10. Differences in Peak Systolic MCA CBFv
Differences are least square means adjusted for baseline peak systolic MCA CBFv, heart rate, condition order, gender and age. No significant differences found.

Peak systolic MCA CBFv did not differ by condition at the 30-minute post-session assessment. Peak systolic MCA CBFv was not different from SIT in EX10 (β =-0.38 cm/s, P=0.86) or EX30 (β=0.44 cm/s, P=0.85) or when EX10 was compared to EX30 (β= 0.82 cm/s, P=0.69).

Similarly, peak systolic MCA CBFv did not differ by condition at the 60-minute post-session assessment. Peak systolic MCA CBFv was not different from SIT in EX10 (β=-0.48 cm/s, P=0.83) or EX30 (β=-0.44 cm/s, P=0.85) or when EX10 was compared to EX30 (β= 0.03 cm/s, P=0.99).

Mean MCA CBFv

A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.98); therefore, condition effects were evaluated

separately at the 30- and 60-minute post-session assessments adjusted for baseline mean MCA CBFv, heart rate, condition order, gender and age. **Figure 11** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline mean MCA CBFv, heart rate, condition order, gender and age.

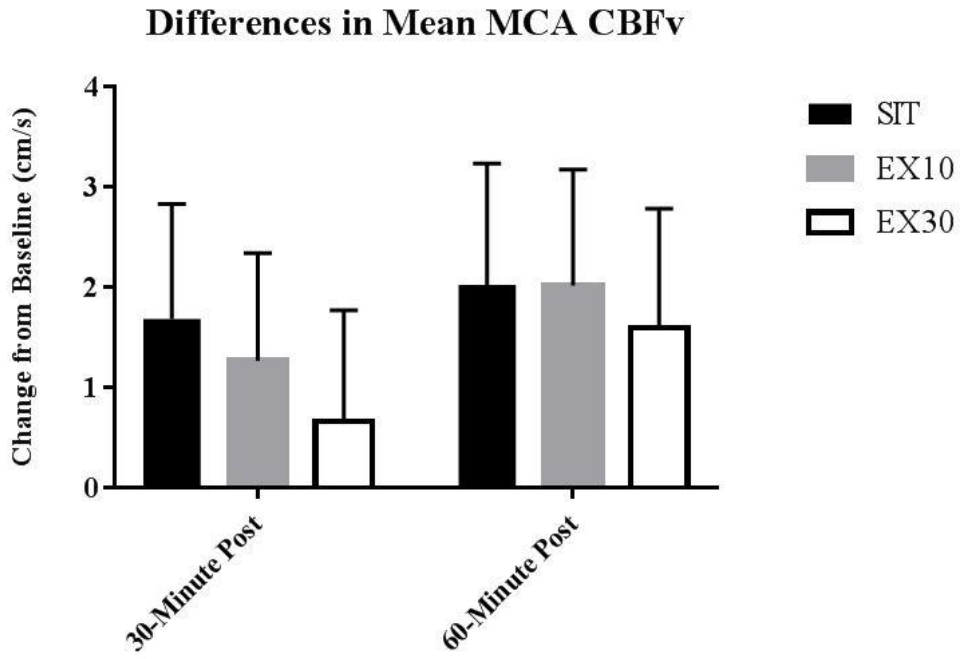

Figure 11. Differences in Mean MCA CBFv
Differences are least square means adjusted for baseline mean MCA CBFv, heart rate, condition order, gender and age. No significant differences found.

Mean MCA CBFv did not differ by condition at the 30-minute post-session assessment. Mean MCA CBFv was not different from SIT in EX10 (β =-0.41 cm/s, P=0.80) or EX30 (β=-1.02 cm/s, P=0.55) or when EX10 was compared to EX30 (β=0.60 cm/s, P=0.69).

Similarly, mean MCA CBFv did not differ by condition at the 60-minute post-session assessment. Mean MCA CBFv was not different from SIT in EX10 (β=-0.01 cm/s, P=1.00) or EX30 (β=-0.43 cm/s, P=0.81) or when EX10 was compared to EX30 (β=0.42 cm/s, P=0.80).

Diastolic MCA CBFv

A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=1.00); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline diastolic MCA CBFv, heart rate, condition order, gender and age. **Figure 12** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline diastolic MCA CBFv, heart rate, condition order, gender and age.

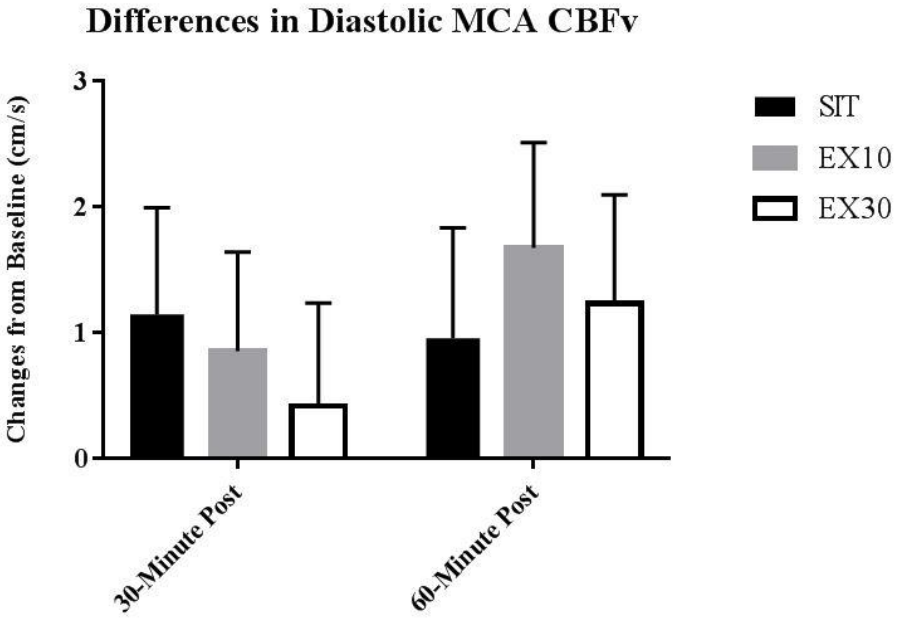

Figure 12 Differences in Diastolic MCA CBFv
Differences are least square means adjusted for baseline diastolic MCA CBFv, heart rate, condition order, gender and age. No significant differences found.

Diastolic MCA CBFv did not differ by condition at the 30-minute post-session assessment. Diastolic MCA CBFv was not different from SIT in EX10 (β =-0.29 cm/s, P=0.80) or EX30 (β=-0.73 cm/s, P=0.56) or when EX10 was compared to EX30 (β=0.43 cm/s, P=0.70).

Similarly, diastolic MCA CBFv did not differ by condition at the 60-minute post-session assessment. Diastolic MCA CBFv was not different from SIT in EX10 (β=0.72 cm/s, P=0.56) or EX30 (β=0.28 cm/s, P=0.83) or when EX10 was compared to EX30 (β=0.44 cm/s, P=0.71).

4.4.4.2 ICA CBFv

Peak Systolic ICA CBFv

A linear mixed model adjusting for heart rate, condition order, gender and age revealed a condition by time interaction (P=0.07) approaching significance (however, not statistically significant); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments baseline peak systolic ICA CBFv, heart rate, condition order, gender and age. **Figure 13** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline peak systolic ICA CBFv, heart rate, condition order, gender and age.

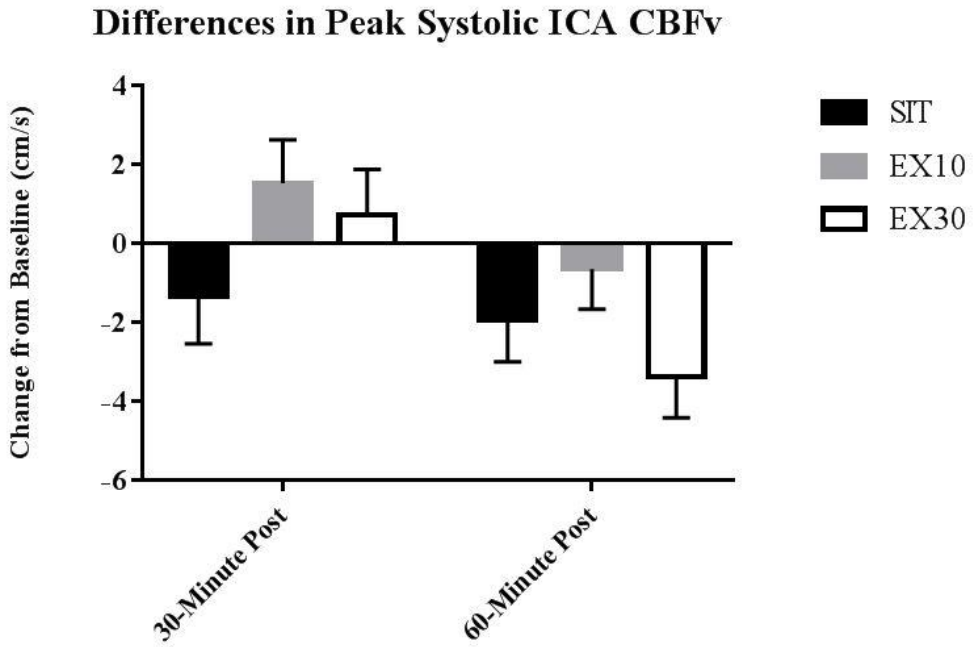

Figure 13. Differences in peak systolic ICA CBFv
Differences are least square means adjusted for baseline peak systolic ICA CBFv, heart rate, condition order, gender and age. No significant differences found.

Peak systolic ICA CBFv did not differ by condition at the 30-minute post-session assessment. Compared to SIT, peak systolic ICA CBFv was nonsignificantly higher in EX10 (β =2.85 cm/s, P=0.06) but not different from EX30 (β=2.05 cm/s, P=0.21). Peak systolic ICA CBFv was not different in EX10 compared to EX30 (β=0.81 cm/s, P=0.56). Of note, the higher peak systolic ICA CBFv in EX10 compared to SIT was approaching significance with P<0.10.

Similarly, peak systolic ICA CBFv did not differ by condition at the 60-minute post-session assessment. Compared to SIT, peak systolic ICA CBFv was not different in EX10 (β=0.48 cm/s, P=0.83) or EX30 (β=-1.28 cm/s, P=0.39). Peak systolic ICA CBFv was nonsignificantly lower in EX30 compared to EX10 (β=-2.72 cm/s, P=0.06). Of note, lower peak systolic ICA CBFv in EX30 compared to EX10 was approaching significance with P<0.10.

Mean ICA CBFv

A linear mixed model adjusting for heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.36); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline mean ICA CBFv, heart rate, condition order, gender and age. **Figure 14** displays differences from baseline to 30- and 60-minute post-session assessments adjusted for baseline mean ICA CBFv, heart rate, condition order, gender and age.

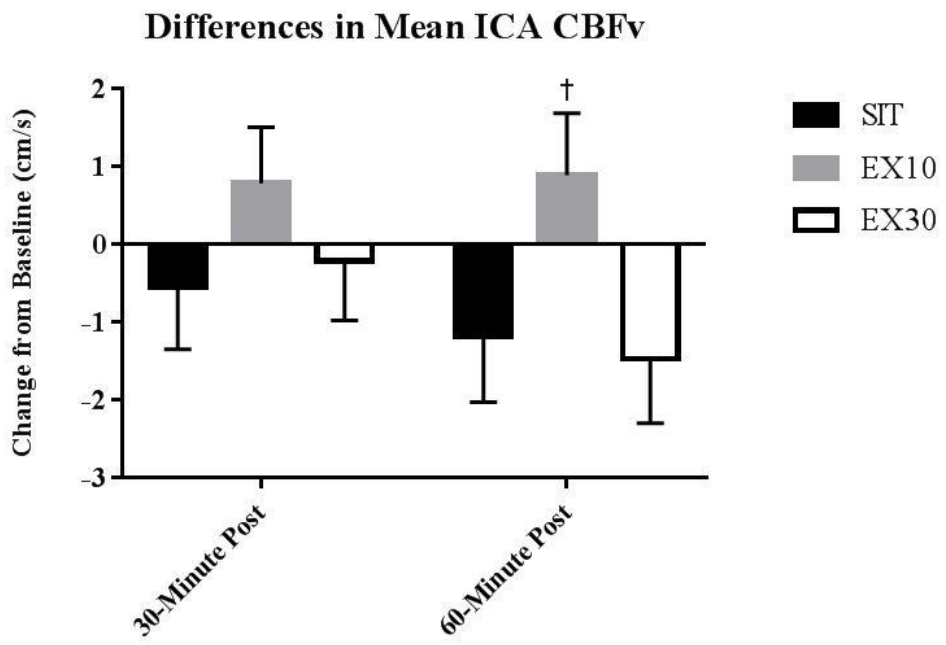

Figure 14. Differences in Mean ICA CBFv
Differences are least square means adjusted for baseline mean ICA CBFv, heart rate, condition order, gender and age *Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

Mean ICA CBFv did not differ by condition at the 30-minute post-session assessment. Compared to SIT, mean ICA CBFv was not different from EX10 (β =1.34 cm/s, P=0.16) or EX30 (β=0.34 cm/s, P=0.75). Mean ICA CBFv not different in EX30 compared to EX10 (β=-1.00 cm/s, P=0.25).

Mean ICA CBFv differed by condition at the 60-minute post-session assessment. Compared to SIT, mean ICA CBFv was nonsignificantly higher in EX10 (β=2.07 cm/s, P=0.08) but not different from EX30 (β=-0.30 cm/s, P=0.82). Of note, the higher mean ICA CBFv in EX10 compared to SIT was approaching significance with P<0.10. Mean ICA CBFv was lower in EX30 compared to EX10 (β=-2.36 cm/s, P=0.04).

Diastolic ICA CBFv

A linear mixed model adjusting for baseline diastolic ICA CBFv, heart rate, condition order, gender and age revealed no significant condition by time interaction (P=0.16); therefore,

condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted baseline diastolic ICA CBFv, heart rate, condition order, gender and age. **Figure 15** displays differences from baseline to 30- and 60-minute post-session assessments adjusted for baseline diastolic ICA CBFv, heart rate, condition order, gender and age.

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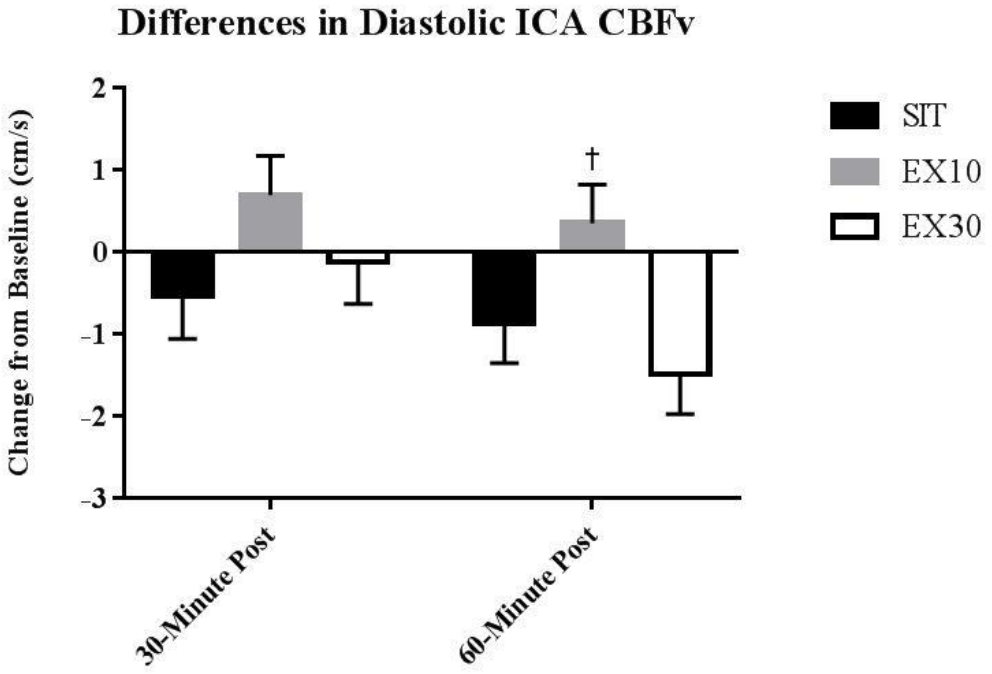


Figure 15. Differences in diastolic ICA CBFv.
Differences are least square means adjusted for baseline diastolic ICA CBFv, heart rate, condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

Diastolic ICA CBFv did not differ by condition at the 30-minute post-session assessment Compared to SIT, diastolic ICA CBFv was nonsignificantly higher in EX10 (β =1.23 cm/s, P=0.07) but not different in EX30 (β=0.41 cm/s, P=0.56). Diastolic ICA CBFv was not different in EX30 compared to EX10 (β=-0.82 cm/s, P=0.20). Of note higher diastolic ICA CBFv in EX10 compared to SIT was approaching significance with P<0.10.

Diastolic ICA CBFv differed by condition at the 60-minute post-session assessment. Compared to SIT, diastolic ICA CBFv was nonsignificantly higher in EX10 (β=1.21 cm/s, P=0.08) but not different in EX30 (β=-0.63 cm/s, P=0.39). Of note, higher diastolic ICA CBFv in EX10 compared to SIT was approaching significance with P<0.10. Diastolic ICA CBFv was lower in EX30 compared to EX10 (β=-1.84 cm/s, P=0.01).

4.4.5 Specific aim III: comparing trajectories of arterial stiffness after SIT, EX10 and EX30

The third aim of this study was to compare trajectories of arterial stiffness after SIT, EX10 and EX30. Measures of arterial stiffness included central (cfPWV) and peripheral (crPWV) PWV as well as pulse pressure calculated as systolic blood pressure minus diastolic blood pressure.

cfPWV

A linear mixed model adjusting for condition order, gender and age revealed no significant condition by time interaction (P=0.46); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted baseline cfPWV, condition order, gender and age. **Figure 16** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline cfPWV, condition order, gender and age.

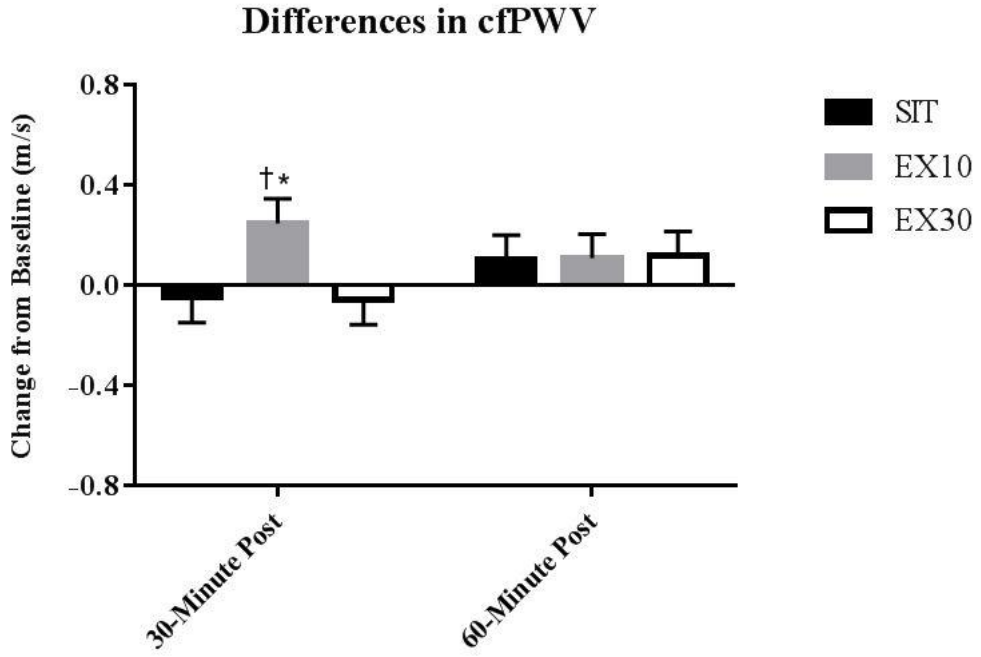


Figure 16. Differences in cfPWV
Differences are least square means adjusted for baseline cfPWV, condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

cfPWV differed by condition at the 30-minute post-session assessment. Compared to SIT, cfPWV was higher in EX10 (β =0.30 m/s, P=0.02) but not different in EX30 (β =-0.01 m/s, P=0.95). cfPWV was lower in EX30 compared to EX10 (β =-0.30 m/s, P=0.02).

cfPWV did not differ by condition at the 60-minute post-session assessment. Compared to SIT, cfPWV was not different in EX10 (β =0.004 m/s, P=0.97) or EX30 (β=0.02 m/s, P=0.89) or when EX10 was compared to EX30 (β =0.01 m/s, P=0.92).

crPWV

A linear mixed model adjusting for condition order, gender and age revealed no significant condition by time interaction (P=0.18); therefore, condition effects were evaluated separately at

the 30- and 60-minute post-session assessments baseline crPWV, condition order, gender and age.

Figure 17 displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline crPWV, condition order, gender and age.

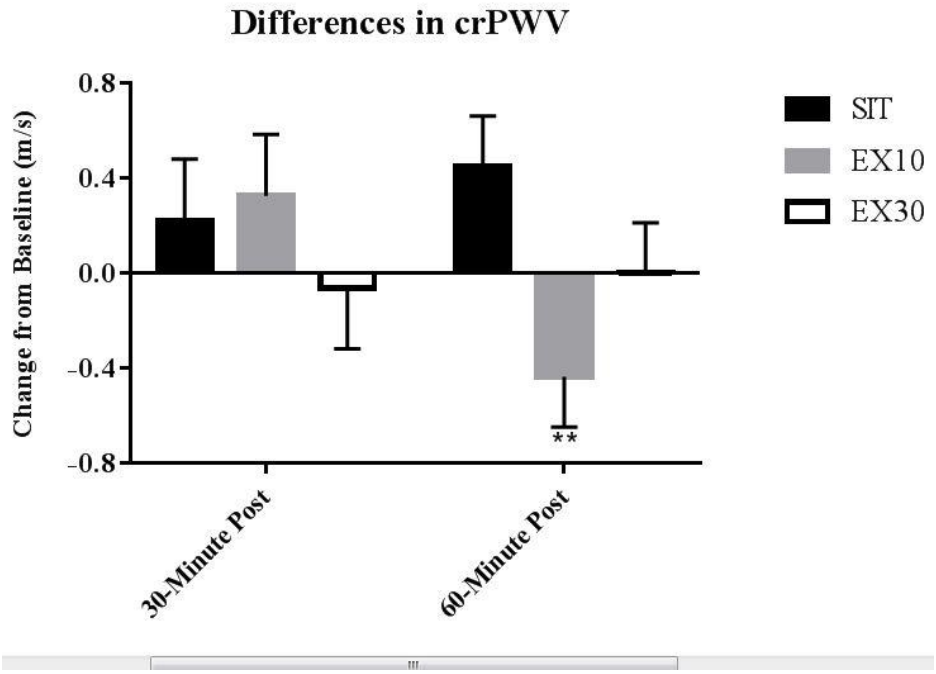


Figure 17. Differences in crPWV
Differences are least square means adjusted for baseline crPWV, condition order, gender and age.
*Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01

crPWV did not differ by condition at the 30-minute post-session assessment. Compared to SIT, crPWV did not differ from EX10 (β =0.10 m/s, P=0.77) or EX30 (β =-0.28 m/s, P=0.41). crPWV was not different in EX30 compared to EX10 (β =-0.39 m/s, P=0.26).

crPWV differed by condition at the 60-minute post-session assessment. Compared to SIT, crPWV was lower in EX10 (β =-0.88 m/s, P=0.004) but not different in EX30 (β=-0.45 m/s, P=0.14). crPWV was not different in EX30 compared to EX10 (β =0.44 m/s, P=0.15).

Pulse Pressure

A linear mixed model adjusting for baseline condition order, gender and age revealed no significant condition by time interaction (P=0.45); therefore, condition effects were evaluated separately at the 30- and 60-minute post-session assessments adjusted for baseline pulse pressure, condition order, gender and age. **Figure 18** displays differences from baseline to the 30- and 60-minute post-session assessments adjusted for baseline pulse pressure, condition order, gender and age.

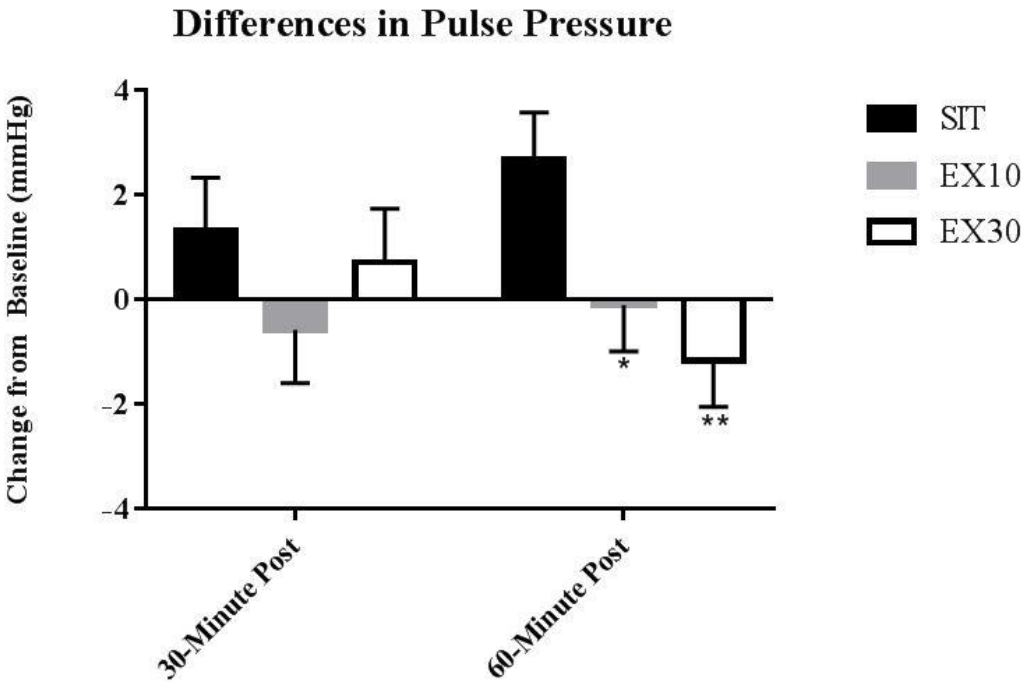


Figure 18. Differences in Pulse Pressure
Differences are least square means adjusted for baseline pulse pressure, condition order, gender and age
. *Significantly different from SIT P<0.05. **Significantly different from SIT P<0.01.
† Significantly different from EX30 P<0.05. †† Significantly different from EX30 P<0.01.

Pulse pressure did not differ by condition at the 30-minute post-session assessment. Compared to SIT, pulse pressure was not different in EX10 (β =-1.88 mmHg, P=0.13) or EX30 (β =-0.60 mmHg, P=0.63). Pulse pressure was not different in EX30 compared to EX10 (β =1.28 mmHg, P=0.31).

Pulse pressure differed by condition at the 60-minute post-session assessment. Compared to SIT, pulse pressure was lower in EX10 (β =-2.79 mmHg, P=0.01) and in EX30 (β=-3.85 mmHg, P=0.001). Pulse pressure was not different in EX30 compared to EX10 (β =-1.06 mmHg, P=0.35).

4.4.6 Specific aim IV: evaluating relationships between changes in cerebral pulsatile flow and changes in arterial stiffness

The fourth aim of the study was to evaluate relationships between changes in cerebral pulsatile flow and changes in arterial stiffness. Linear mixed models adjusting for baseline cerebral pulsatile flow, heart rate, condition order, age and gender were performed. Changes in cfPWV were not related to changes in cerebral pulsatile flow at the 30-minute post-session assessment (B=-0.02, P=0.30) or at the 60-minute post-session assessment (B= -0.006, P=0.73). Similarly, changes in crPWV were not related to changes in cerebral pulsatile flow at the 30-minute post-session assessment (B=-0.005, P=0.37) or at the 60-minute post-session assessment (B= -0.004, P=0.48). Changes in pulse pressure, however, were directly related to increases in cerebral pulsatile flow at the 30-minute post-session assessment (B=0.004, P=0.02) but not at the 60-minute post-session assessment (B= 0.0008, P=0.59). **Figure 19** displays the relationship between cerebral pulsatile flow and pulse pressure at the 30-minute post-session assessment.

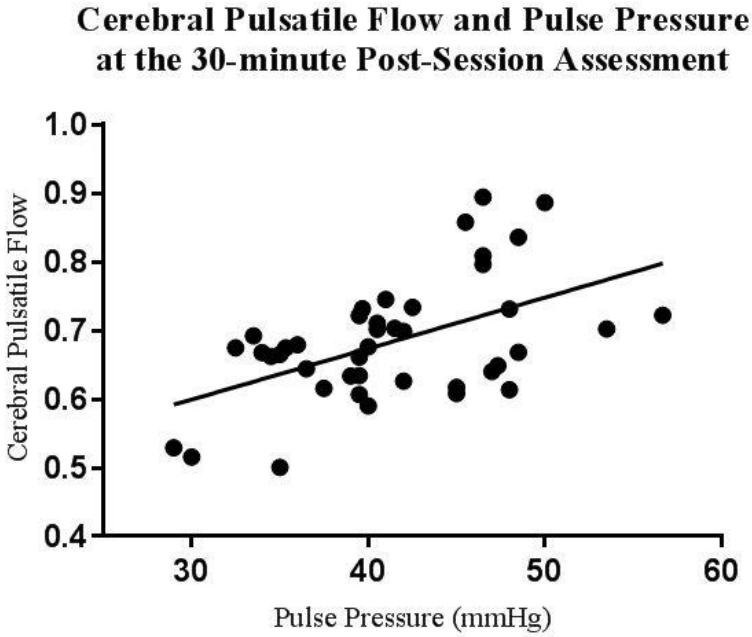


Figure 19. Relationship Between Cerebral Pulsatile Flow and Pulse Pressure at the 30-minute Post-Session Assessment

5.0 DISCUSSION

5.1 SUMMARY OF FINDINGS

The purpose of this study was to evaluate acute effects of aerobic exercise on cerebrovascular hemodynamics and arterial stiffness. This study used a randomized crossover design comprised of three separate experimental visits, each with a different experimental session: 30 minutes of sitting (SIT), 20 minutes of sitting followed by a 10-minute exercise bout (EX10) and a 30-minute exercise bout (EX30). Cerebrovascular hemodynamics and arterial stiffness were measured before the experimental session and at 30- and 60-minute post-session assessments. We hypothesized that cerebrovascular hemodynamics and arterial stiffness would respond such that these outcomes would be most improved in EX30 and least in SIT. Our goal was to keep the intensity of the aerobic exercise bouts in the moderate-to-vigorous range to isolate a duration effect of exercise. Exercise parameters (speed, grade) were comparable between EX10 and EX30, and heart rates averaged between 70-75% of age-predicted maximum heart rate (our target) which strengthens our findings.

Systolic, diastolic and mean arterial blood pressure responded in an expected dose-response manner at both the 30- and 60-minute post-session assessments. Specifically, blood pressures were highest in SIT and lowest in EX30, with these differences approaching or reaching statistical significance, particularly when comparing EX30 to SIT. There was a

significant condition by time interaction such that heart rate changed differently across conditions. Heart rate decreased in SIT and increased in EX10 and EX30, with greater increases in EX30 at both the 30-and 60-minute post-session assessments. These results are consistent with other studies reporting an acute hypotensive effect of aerobic exercise as well as acute increases in heart rate to compensate for excess post-exercise oxygen consumption [165-167]. Furthermore, these results suggest our stimuli were appropriate as they resulted in dose-response cardiovascular effects.

The primary aim of this study was to compare trajectories of cerebral pulsatile flow after SIT, EX10 and EX30. There were no significant condition effects at the 30- or 60-minute post-session assessments. However, cerebral pulsatile flow was higher in EX30 compared to SIT at the 30-minute post-session assessment and this difference was approaching statistical significance (4.7% higher, P=0.08). Similar changes were observed in ICA pulsatile flow such that it was higher at 30-minute post-session assessment in EX30 compared to SIT and this increase was also approaching statistical significance (6.5% higher, P=0.08).

The second aim of the study was to compare trajectories of additional MCA cerebrovascular hemodynamic measures including peak systolic, mean and diastolic CBFv. There were no significant condition effects at the 30- or 60-minute post-session assessments. In contrast, ICA peak systolic, mean and diastolic CBFv had borderline and significant differences such that post-session assessment blood flow velocity values were higher in EX10 but not in EX30.

The third aim of the study was to compare trajectories of arterial stiffness measures after SIT, EX10 and EX30. cfPWV (central) was significantly higher in EX10 compared to SIT (0.30 m/s higher, P=0.02) and EX30 (0.30 m/s higher, P=0.02), while there was no difference between

EX30 versus SIT at the 30-minute post-session assessment. Furthermore, cfPWV did not differ across condition at the 60-minute post-session assessment. crPWV (peripheral) was not different across conditions at the 30-minute post-session assessment. In contrast, crPWV was significantly lower in EX10 compared to SIT at the 60-minute post-session assessment (0.88 m/s lower, P=0.004). Pulse pressure was significantly lower in EX10 (2.8 mmHg lower, P=0.01) and EX30 (3.9 mmHg lower, P=0.001) compared to SIT at the 60-minute post-session assessment but did not differ by condition at the 30-minute post-session assessment.

The final and fourth aim of the study was to evaluate relationships between changes in cerebral pulsatile flow and arterial stiffness. Increases in pulse pressure were significantly related to increases in cerebral pulsatile flow at the 30- but not 60-minute post-session assessments. Neither changes in cfPWV nor crPWV were related to changes in cerebral pulsatile flow.

5.2 ACUTE EFFECTS OF EXERCISE ON PULSATILE FLOW

We hypothesized that cerebral pulsatile flow would decrease due to exercise-induced reductions in arterial stiffness, allowing for better cushioning of the pulsatile flow created by the heart, thus reducing pulsatile flow reaching the brain. Our results, however, did not support our hypothesis. We found cerebral pulsatile flow to have nonsignificant, dose response effects at the 30-minute post-session assessment with differences between SIT and EX30 approaching significance. Cerebral pulsatile flow was 2.4% higher beyond SIT following EX10 and 4.7% higher beyond SIT following EX30. We also found cerebral pulsatile flow was not different across conditions at the 60-minute post-session assessment with differences $\leq 1.1\%$. Additionally, we found pulsatile

flow in the ICA to have very similar responses such that ICA pulsatile flow was 4.1% higher beyond SIT following EX10 and 6.5% higher beyond SIT following EX30 at the 30-minute post-session assessment. Moreover, ICA pulsatile flow was a 2.4% lower in EX10 and 0.3% in EX30 compared to SIT at the 60-minute post-session assessment.

To the best of our knowledge, this is the first study to evaluate the acute effects of aerobic exercise on cerebral pulsatile flow. There are, however, studies evaluating cerebral pulsatile flow during exercise that may aid in the interpretation of our results. During 15 minutes of moderate intensity cycling in healthy young adults (n=14), pulsatility index was found to increase by 49% and 43% from baseline in the right and left MCA, respectively [168]. Similarly, during 3 minutes of pedaling an ergometer, young healthy adults (n=18) had a 24%, 18% and 16% increase in pulsatility index during minutes 1, 2 and 3, respectively. Furthermore, pulsatility index remained 20%, 8%, and 8% above baseline at recovery minutes 1, 2 and 3, respectively [169]. Our results are consistent with these studies and suggest pulsatility index increases during aerobic exercise and may remain elevated for up to 30 minutes following the exercise bout, returning to baseline by 60 minutes following exercise. Thus, while the clinical significance of the post-exercise increase in cerebral pulsatile flow is unclear, the magnitude of the increase appears to be transient and lower post-exercise as compared to during exercise.

The acute increases in cerebral pulsatile flow are likely to occur due to exercise-induced increases in cardiac output and pulse pressure, inherently increasing pulsatile flow [170-172]. It has also been proposed that pulsatility increases due to vasoconstriction of the MCA [169]. Though that may be the case during exercise, it is unlikely to be the case in the present study of post-exercise effects because we did not observe an increase in MCA CBFv at either assessment (as would be the expected with vasoconstriction).

Another potential mechanism which could explain why no significant reductions in cerebral pulsatile flow were observed is that we did not see the hypothesized reductions in PWV (specific aim III). Additionally, we found changes in cfPWV and crPWV were not related to changes in cerebral pulsatile flow. Not supporting this mechanism, however, is that we did observe reductions in pulse pressure and these changes were significantly related to cerebral pulsatile flow at 30- but not 60-minute post-session assessments. Though cerebral pulsatile flow was not lower during the hour following exercise, our results do provide novel evidence that acute changes in pulse pressure following aerobic exercise are directly related to cerebral pulsatile flow.

Another possibility is that acute changes in cerebral pulsatile flow following aerobic exercise do not occur until after our 60-minute observation period. For example, vascular benefits of a single bout of exercise may last up to 24 hours [145, 166]. Though this study is the first to evaluate post-exercise effects on cerebral pulsatile flow up to 60 minutes, it is possible that reductions in pulsatile flow could occur beyond 60 minutes. Yet another reason could be a floor effect. Our participants were healthy middle-aged adults, were active on average and had a baseline pulsatility index value of 0.67 (averaged across conditions), which is well below the clinical value for pulsatile flow of 1.2 [45]. Thus, it may be the case that their cerebral pulsatile flow could not get much lower.

Though there are no comparable studies evaluating acute relationships of cerebral pulsatile flow and arterial stiffness following exercise (specific aim IV), our findings are in line with other research. Cerebral pulsatile flow of the MCA has been shown to be more pulsatile in the presence of arterial stiffness. This relationship has been reported with both PWV and pulse pressure (central and peripheral) [4, 5]. Furthermore, decreases in arterial stiffness (increases in

carotid arterial compliance) were significantly associated with decreases in cerebral pulsatile flow following a 16-week endurance training intervention [6]. Thus, it may be that PWV is not related to cerebral pulsatile flow acutely but it is overtime.

5.2.1 Significance and future directions

In summary, and contrary to our hypothesis, we found cerebral pulsatile flow to be marginally higher in EX30 compared to SIT at the 30-minute post-session assessment and back to baseline at the 60-minute post-session assessment. Additionally, changes in cerebral pulsatile flow at the 30-minute post-session assessment were related to changes in pulse pressure.

Based on our results, aerobic exercise does not have acute benefits on cerebral pulsatile flow within an hour of an exercise bout. However, investigation of methods to reduce cerebral pulsatile blood flow, thereby reducing cerebrovascular disease risk, remains important. Exercise-induced reductions in arterial stiffness have been proposed as a method to reduce cerebral pulsatile flow and these outcomes [61, 92], at least with pulse pressure, were positively related in our study. Thus, further research with a longer duration of exercise, a longer observational period and characterization of the relationship of cerebral pulsatile flow and arterial stiffness following exercise is warranted.

5.3 ACUTE EFFECT OF EXERCISE ON PEAK SYSTOLIC, MEAN, AND DIASTOLIC CBFV

We hypothesized that MCA CBFv would increase acutely following exercise. Our results, however, did not support our hypothesis. Peak systolic, mean and diastolic MCA CBFv were not different from SIT in EX10 or EX30 at 30- or 60-minute post-session assessments. In fact, changes in peak systolic, mean and diastolic CBFv only differed by $\leq$1.6% in EX10 and EX30 as compared to SIT.

Our hypotheses were based on cross sectional studies that have established higher CBF in those with higher physical activity and cardiorespiratory fitness [115-119], intervention studies reporting that resting CBF increases following exercise interventions [48, 120, 121], accumulating evidence demonstrating CBF increases during exercise [173] and preliminary evidence suggesting increased CBF following exercise [122]. Specifically, global CBF was found to increase by 20% using arterial spin labeling (ASL) following 30 minutes of moderate intensity cycling in young healthy adults (n=5) [122].

However, the current literature describing the acute effects of aerobic exercise on CBF is inconsistent with studies reporting acute increases, decreases and no change in CBF following exercise. [123-126, 128]. Inconsistent results across studies may be due to small sample sizes or differences in neuroimaging methodology, measurement of different cerebral vessels or regions, variable duration and intensity of the exercise stimulus and post-exercise observation period. Our results are consistent with studies evaluating cerebrovascular hemodynamics following a 30- and a 40-minute bout of exercise as well as a marathon. Following a 40-minute cycling bout, MCA CBFv did not change from baseline to 10-, 30- or 60-post-exercise in twelve young healthy adults [127]. Similarly, following a 30-minute cycling bout with stepwise intensity increases

(starting at ~20% and ending at 80-90% of max capacity), MCA CBFv did not change significantly from baseline to six minutes post-exercise in seven healthy adults [128]. MCA CBFv was also unchanged within four hours of a mountain marathon in nine healthy adults [129].

It is unlikely the results in the present study are due to insufficient exercise exposure as we found dose-response effects on blood pressure and heart rate consistent with the literature [165-167], but several mechanisms could explain our null results. Most of the aforementioned studies have focused on young, healthy individuals who are likely to have healthy cerebral blood flow; studies with null results may reflect a ceiling effect. The lack of an acute effect of exercise on CBFv in our study that included middle-aged, healthy adults could also be due to a ceiling effect. Our nonsignificant changes in cerebrovascular hemodynamics could perhaps be due to a time course where MCA CBFv returns to baseline before 30 minutes post-exercise. Lastly, our results could be explained by cerebral autoregulation. Autoregulation refers to the ability of the brain to maintain a relatively constant supply of CBF despite changes in blood pressure and a wide range of metabolic demands from neuronal tissue. According to autoregulation, CBF should not change unless blood pressure falls outside of 60mmHg to 150mmHg [174], which was not the case in our participants at 30 or 60 minutes post-exercise.

Our null results do not eliminate the possibility that there are acute changes in cerebrovascular hemodynamics due to exercise. There could be changes in the more distal microvasculature which would not be captured by the TCD as we measured parent MCA, at the ICA bifurcation. Other neuroimaging methods (such as ASL and NIRS) may be able to capture changes in the microvasculature as these methodologies provide information on brain regions, not specific vessels. It may be that there are microvascular changes first, and, over time these

changes extend to the larger conduit vessels. Or it could be that the larger parent vessels can better tolerate changes in how blood is being introduced to the brain. This could explain why there seem to be acute global increases following exercise using ASL but not with TCD. Furthermore, TCD indirectly measures CBF by measuring blood flow velocity, and thus could fail to capture changes in blood flow if there were concomitant changes to vessel diameter [46].

5.3.1 Significance and future directions

In summary, and contrary to our hypotheses, we did not find differences in MCA peak systolic, mean or diastolic CBFv across conditions at the 30- or 60-minute post-session assessments. Based on our results, aerobic exercise does not have acute benefits on MCA cerebrovascular hemodynamics within an hour of an exercise bout. One implication is that our findings do not support increased MCA CBFv as a potential mechanism of cognitive improvement following exercise. Future research should evaluate less healthy and older populations, measure CBF more proximally to the exercise bout, and simultaneously measure different cerebral regions and vessels to improve our understanding of acute exercise effects on CBF.

5.4 ACUTE EFFECT OF EXERCISE ON ARTERIAL STIFFNESS

We hypothesized that arterial stiffness would decrease following EX10 and EX30 as both central and peripheral arterial stiffness have been shown to decrease following aerobic exercise [17, 18, 149]. Aerobic exercise increases the availability of NO, leading to a reduction in vascular smooth muscle cell tone, improved arterial function and vasodilation [17, 175]. Additionally, it has been

proposed that acute changes in arterial stiffness following aerobic exercise are a result of changes in the intrinsic properties of the arterial wall [17, 131]. We further hypothesized that, like blood pressure, there would be dose-response effects on arterial stiffness such that EX30 would have the largest improvement. Because effects on PWV differed substantially between EX10 and EX30, the results are discussed separately below.

5.4.1 Arterial stiffness following EX30

Our results did not support our hypotheses. We found no significant differences in cfPWV at 30- or 60-minute post-exercise following EX30 compared to SIT. These results are similar to studies reporting no change in cfPWV following aerobic exercise at 30 [150-153] and 60 minutes [151-154] post-exercise. Furthermore, a systematic review reported additional studies evaluating other measures of arterial stiffness have found similar nonsignificant results measured 10-60 minutes following exercise [157]. In contrast, a few studies have found cfPWV to decrease 20 to 50-minutes following exercise [17, 18, 149]. These inconsistent results may be due to differences in duration, intensity and modality of exercise or population characteristics.

There are fewer studies evaluating the acute effect of exercise on peripheral PWV. Though not significant, we found crPWV to be marginally lower 30 and 60 minutes following EX30 compared to SIT. One study also found nonsignificant decreases in crPWV from 6-28 minutes following a moderate-to-vigorous intensity, 30-minute cycling bout [176]. Similarly, brachial-radial PWV was found to be significantly lower every minute post-exercise for 60-minute following a maximal treadmill test [177].

Even fewer studies have evaluated acute effects of exercise on pulse pressure. We found differences in pulse pressure from baseline to be significantly lower 60 minutes following EX30

compared to SIT. One study reported a significant decrease from baseline in pulse pressure at 20-minutes and 50-minutes following a 60-minute exercise bout [149].

Our nonsignificant results of cfPWV and crPWV could partly be explained by changes in blood pressure following EX30. Blood pressure is known to influence arterial stiffness [61], and we did not observe large differences from SIT in blood pressure 30 minutes following EX30. Systolic blood pressure was 2.4 mmHg lower and diastolic blood pressure was 1.9 mmHg lower than SIT at the 30-minute post-session assessment. However, larger reductions in blood pressure were observed at 60 minutes following EX30. Systolic blood pressure was 7.0 mmHg lower and diastolic blood pressure was 3.4 mmHg lower than SIT at the 60-minute post-session assessment. These blood pressures are consistent with average post-exercise decreases which have been reported to be 8mmHg in systolic blood pressure and 9mmHg in diastolic blood pressure in normotensive individuals [167]. Others report reductions of 5-10mmHg [166]. Thus, it is unlikely that our 60-minute results reflect an inadequate hypotensive response.

It also might be the case that exercise-induced acute changes in arterial stiffness do not occur within an hour following exercise, but perhaps occur between an hour and 24 hours after the exercise bout. Previous studies have reported significant reductions in arterial stiffness 24 hours following aerobic exercise[145, 178]; however our study only measured an hour following exercise.

5.4.2 Arterial stiffness following EX10

To the best of our knowledge, the present study is the first to report the acute effects of a 10-minute bout of aerobic exercise on arterial stiffness. We found cfPWV was significantly higher 30 minutes following EX10 when compared to SIT and EX30. Additionally, we found crPWV to

be significantly lower 60 minutes following EX10 when compared to SIT. Of note, neither cfPWV nor crPWV differed from SIT at either post-session assessment. The most similar study evaluated waveforms of the carotid and radial arteries following 15 minutes of moderate intensity cycling. The study found augmentation index increased significantly 10-minutes post-exercise [179], which represents higher arterial stiffness. This is consistent with our findings at the 30-minute post-session assessment but was measured more proximally to the exercise bout.

The mechanism behind these EX10 induced results are unclear. Previous studies have demonstrated that intermittent 10-minute bouts of exercise are not as effective as a 30-minute continuous bout at improving cardiorespiratory fitness, suggesting the physiology is different during longer durations of continuous aerobic exercise [102, 180, 181]. Thirty minutes of exercise may allow for true steady state and homeostasis, while a 10-minute bout may allow for only 5-7 minutes of steady state. This shorter duration of steady state may not be enough to alter the physiological properties that influence PWV [181]. Thus, a 10-minute exercise bout may affect the factors involved in arterial stiffness such as NO bioavailability, other vasodilators, vasoconstrictors, inflammatory molecules, reactive oxygen species and antioxidants differently than a 30-minute bout [175].

A specific potential explanation for these results could be that bouts of exercise shorter than 30 minutes elicit inconsistent changes in blood pressure and do not result in hypotension following exercise in normotensive individuals [166], which could lead to inconsistent responses in PWV. We found blood pressure was not different in EX10 as compared to SIT or EX30, and thus unlikely to explain the higher cfPWV in EX10, at the 30-minute post-session assessment. At the 60-minute post-session assessment, crPWV was significantly lower in EX10 compared to

SIT while EX30 was not. Again, these results do not mimic the dose-response patterns we observed with blood pressure, arguing against this potential mechanism.

A final possible explanation may be differences in timing from initiation of exercise. At the 30-minute post-session assessment, a participant would have initiated exercise 40 minutes prior in EX10 and 60 minutes prior in EX30. We are not able to evaluate this possibility as we chose to anchor post-session assessments from the time of exercise completion.

5.4.3 Significance and future directions

In summary, and contrary to our hypotheses, neither cfPWV nor crPWV were lower than SIT following EX30. Following EX10, cfPWV was higher than SIT at the 30-minute post-session assessment. Furthermore, crPWV was lower than SIT at the 60-minute post-session assessment. Pulse pressure was lower following exercise (EX10 and EX30) at the 60 but not the 30-minute post-session assessment. Based on our results, exercise bouts of 10 and 30 minutes in duration have differential effects on cfPWV and crPWV. The mechanisms responsible for these differential effects, and especially physiological responses to 10-minute bouts of exercise, warrant further research. Given that the minimum recommended bout duration is 10 minutes [15, 16], such research could have important public health implications.

5.5 STRENGTHS AND LIMITATIONS

Strengths of this study include the randomized crossover design and standardization of the protocol as well as timing to account for known diurnal variations in CBFv. We used a 30-

minute exercise bout as this is the daily recommended duration of aerobic exercise by the American College of Sports Medicine. Moreover, we used a 10-minute exercise bout as that is the minimum duration of aerobic exercise recommended by the American College of Sports Medicine to accumulate 30 minutes of exercise [15, 16]. We used PWV to measure arterial stiffness, which is the gold standard. Additionally, we used TCD to measure cerebrovascular hemodynamics, which is widely used and has an excellent temporal resolution. We measured cerebrovascular hemodynamics of the MCA, the most measured cerebral artery, as well as at the ICA to capture a more comprehensive picture of cerebrovascular hemodynamic responses. Furthermore, a sole sonographer performed all TCD and PWV measurements, thus eliminating inter-rater variability, to which TCD is particularly sensitive. Another strength in our study is our sitting control condition (SIT) to which we compared the exercise sessions. This control condition allowed us to account for diurnal variations that are not normally accounted for in quasi-experimental studies relying on single-group pre- to post-intervention assessments.

Limitations of the study include the small sample size and a lack of measurement of other factors that could affect cerebrovascular hemodynamics such as $PaCO_2$ [173]. Additionally, this was an acute laboratory study, and thus generalizability in a real-world setting is limited. Furthermore, caution should be taken when interpreting the results as clinically meaningful, as this was an acute laboratory study. The observed effects may simply be transient returns to homeostasis following the removal of the physiological load imposed by acute exercise rather than clinically meaningful effects that could result from habitual bouts of exercise. Moreover, TCD provides CBFv which is an indirect measure of CBF [46]. Moreover, the TCD sonographer held the TCD probe in place for each minute-long recording, and thus sonographer hand movement and/or participant head movement could have also affected results. Additionally,

vascular outcomes were assessed at limited time points (30- and 60-minute post-sessions), thus potentially missing changes that could be captured with more frequent assessments and using a longer observation period. Our results could also be spurious due to multiple comparisons without Bonferroni adjustments. Given our small sample size and many outcomes at various time points and multiple conditions, we did not use such adjustments.

The experimental arrangement also had its limitations. Participants were asked to abstain from caffeine for 24 hours and from food for 4 hours to comply with vascular and exercise testing recommendations. This, combined with the extended amount of time they spent in a supine position (60-minute post experimental session), caused most participants to become sleepy, with some participants falling asleep during the rest intervals between assessments. Additionally, the time of day of the experiment (between 11:40am and 5:00pm) could have contributed to sleepiness as there is a reduction in alertness and increased sleepiness during this time frame [182]. Moreover, acute exercise is known to induce sleepiness [183]. Thus, exercising during this sensitive period could have a synergistic effect and could induce sleep even more. Participants becoming sleepy and/or falling asleep during supine rest prior to and/or during measurements of vascular outcomes could have affected the results. For example, MCA CBFv is known to decrease during stages 2-4 of sleep [184]. It is difficult to assess the level of sleepiness of participants as it is common for individuals to doze off during rest and close their eyes during vascular procedures. However, it is recommended that vascular testing be performed while participants are awake [59, 63]. Some participants appeared to be sleepy during rest but opened their eyes when testing procedures began. Other participants did not appear to be sleepy but verbally expressed they felt sleepy or could fall asleep. Measures of sleepiness during testing were not obtained; thus, a limitation is that the percentage of time during which participants were

sleepy or asleep cannot conclusively be determined. It was documented that one participant fell asleep during TCD measurements and one participant fell asleep prior to vascular testing. However, removing these participants in a sensitivity analysis did not change the results. Future research should also refine experimental protocols to minimize the effect of potential sleepiness on vascular measures created by the experimental arrangement as this could affect results. This might include time of day and a standardized stimulus during rest protocols. Furthermore, sleepiness should be evaluated during the protocol.

5.6 CONCLUSIONS

Although there is accumulating evidence suggesting that aerobic exercise improves cerebrovascular hemodynamics, few studies have evaluated the acute effects of exercise on CBF and even fewer on cerebral pulsatile flow. Based on our results, aerobic exercise does not improve cerebrovascular hemodynamics in the MCA within an hour of aerobic exercise. Additionally, our results suggest a 10- and 30-minute bout have differential effects on PWV and do not support studies reporting acute decreases in PWV following a 30-minute exercise bout. Important areas for future research include assessment of cerebrovascular hemodynamics at more frequent time points and beyond 60 minutes post-exercise, in different cerebral regions and vessels, and its relationship to concomitant changes in arterial stiffness. In addition, elucidating mechanisms and effects of shorter versus longer aerobic exercise bouts on arterial stiffness warrants further research.

APPENDIX A

RECRUITMENT FLYER

Are you between the ages of 35 and 59?

Research Study Participants Needed

The University of Pittsburgh Physical Activity and Weight Management Research Center is looking for middle-aged adults who meet the following criteria:

- 35-59 years old
- Able to exercise safely
- Able to attend 4 visits on separate occasions

The Exercise for Healthy Arteries Study is investigating whether exercise can improve the blood flow in the brain. The study involves screening and three 2.5-hour experimental visits at our research facility located in Oak Hill, near the Oakland campus. During these visits, you will either sit, exercise for 10-minutes or exercise for 30-minutes. You will be compensated up to **$100** for completing the study.

For more information and to see if you qualify, call 412-383-4035 and ask about the Exercise for Healthy Arteries Study.

APPENDIX B

PHONE SCREENING

SCREENING FORM:

1. Thank you for your interest in our program. My name is _____ and I would briefly like to tell you about this research program.

2. **Procedure for Describing the Study and Obtaining Verbal Consent to Conduct the Phone Screen:** A description of the study will be read to participants, and this description includes important components of the informed consent process (see attached script). Individuals who express an interest in participating in this study will be told the following to obtain verbal consent:
   - ***Investigators Component of Informed Consent:*** *The Exercise for Healthy Arteries Study is being conducted by Sophy Perdomo under the supervision of Dr. Bethany Barone Gibbs at the University of Pittsburgh.*
   - ***Description Component of Informed Consent****: The purpose of this randomized, cross-over study is to evaluate whether exercise has acute benefits on blood flow in the brain and on the stiffness of your blood vessels. Participants will attend one screening visit and 3 experimental sessions: one where you will be asked to sit and watch a preselected National Geographic Channel series for 30 minutes, another where you will watch the same documentary series for 20 minutes and then exercise for 10 minutes and one where you will exercise for 30 minutes. After each condition, we will measure the blood flow in your brain and how stiff your blood vessels are 2 times over a 1-hour period. The order of these experimental visits will be assigned by a process similar to flipping a coin We are interested in recruiting 15 middle aged adults, who can exercise safely and are interested in participating. If you are found to be initially eligible for the study after this phone screening, we will invite you to the laboratory near the University of Pittsburgh Oakland Campus for an orientation visit where the full details of the study will be described to you, you will have a chance to ask questions, and, if you are interested in participating, you will be asked to sign a consent document. Next, we will measure your height, weight, blood pressure, and arm and waist circumference and ask you to complete a detailed medical history and questionnaires about your level of physical activity. We will also ask you to walk briskly on the treadmill to determine at what speed you will start the exercise sessions. In preparation for the screening visit, we will ask you to abstain from physical activity, caffeine, nicotine and alcohol prior to the screening visit. It is possible that after this screening session, you may not be eligible to participate in the study. If your blood pressure readings are 140/90 or higher, you will need to obtain permission from your PCP to participate. If you are determined to be eligible after this screening visit, we will schedule a time for you attend your first experimental session. These sessions will take place in the afternoon and last approximately 2.5 hours. There should be 48 hours between each session. We will ask that for these visits you wear shorts or pants as we will be testing a blood vessel near the groin. We will also ask that you wear comfortable clothing for the exercise sessions. You may want to bring food with you as we will ask you to fast 4 hours prior to and during each 2.5 hour experimental visit, a total of 6.5 hours between meals. The link between your study ID number and personal information will be kept in a secure location by the Principal Investigator. Upon the full completion of all study procedures, you can earn $100 for completing all 3 experimental sessions. Participation in this study is voluntary and you are free to discontinue at any time.*

     If you are interested in participating in this study, I will need to ask you a few questions about your demographic background and questions about your physical health and medical history to determine if you appear to be eligible to participate in this study. It will

take approximately 5 minutes to ask you all of the questions. If we complete the interview, I will ask you for some specific information (your complete name, date of birth, and mailing address) so that we can contact you regarding your participation in this study. I will then schedule you to attend a session that will explain all of the procedures of this study in greater detail.'

"Your responses to these questions are confidential, and all information related to your health history or current behaviors that you are about to give me will be destroyed after this interview if you are found to be ineligible."

Do you have any questions related to any of the information that I have provided to you? Staff member will answer any questions or will defer these questions to the Principal Investigator or Co-Investigator when appropriate prior to proceeding. If the individual would like to think about their participation prior to proceeding with the Phone Screen, they will be provided with the telephone number that they can call if they decide to participate in the future.

- ***Voluntary Consent Component of Informed Consent:*** "Do I have your permission to ask you some questions about yourself?"

  If "YES" indicate the participant's agreement with this statement on the top of the next page, sign your name and date the form, and then complete the Phone Screen. If "NO", thank the individual for calling and do not complete the Phone Screen.

Phone Screen Interview

The caller gives verbal permission to conduct the Phone Screen:
_____ YES _____ NO

Verbal Assent was given to:

_____
Staff Member Signature

_____
Date Verbal Assent was given:

Eligible based on telephone screening: ☐ Yes ☐ No
If "No", list reason for ineligibility: _____

Discontinue Phone Screen if Participant Does NOT Meet Eligibility Criteria for a Question.
(bolded choices indicate eligible)

1. How old are you? ☐☐ **(35-59 years old)**
2. Are you able to come to our research facilities on 4 separate occasions? ☐ **Yes** ☐ No
3. Are you willing to watch National Geographic content? ☐ **Yes** ☐ No
4. Are you currently being treated for any medical condition that could limit your ability to walk on a treadmill such as cancer, end stage renal disease, or severe arthritis? ☐ Yes ☐ **No**
   If yes, describe: _____
5. Do you or have you ever had heart disease or cerebrovascular disease (such as stroke) ? ☐ Yes ☐ **No**
6. Have you ever been told by a doctor or medical professional that you should not engage in physical activity for any reason? ☐ Yes ☐ **No**
7. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? ☐ Yes ☐ **No**
8. Do you feel pain in your chest when you do physical activity? ☐ Yes ☐ **No**
9. In the past month, have you had chest pain when you were not doing physical activity? ☐ Yes ☐ **No**
10. Do you lose your balance because of dizziness or do you ever lose consciousness? ☐ Yes ☐ **No**
11. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity? ☐ Yes ☐ **No**
12. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition? ☐ Yes ☐ **No**
13. Do you know of any other reason why you should not do physical activity? ☐ Yes ☐ **No**
14. Do you use any prescription or over-the-counter medications regularly?

Medication:	Indication:

15. Would you be able to abstain from moderate-to-vigorous physical activity, caffeine, nicotine, and alcohol for 24 hours prior to each experimental session at out laboratory? ☐ **Yes** ☐ No
16. Would you be able to fast for 4 hours prior to and then during each 2.5-hour experimental condition at our laboratory? ☐ **Yes** ☐ No
17. (for females) Are you currently pregnant, trying to get pregnant, or plan to get pregnant in the next 2 months? ☐ Yes ☐ **No**

Contact Tracking Form

** THIS PAGE IS COMPLETED ONLY IF THE RESPONDANT APPEARS TO QUALIFY FOR PARTICIPATION IN THIS STUDY. **

Date: ____/____/____Staff Member Completing Form: _____

Name: _____

Street Address: _____

City: _____ State: ___ Zip Code:_____

Home Phone: _____ Work Phone: _____

Email_____

OFFICE USE ONLY:

Eligible: ☐ Yes ☐ No
Invited to Orientation: ☐ Yes ☐ No
Date of Orientation:____/____/____

APPENDIX C

PAFFENBARGER EXERCISE HABITS QUESTIONNAIRE

PAFFENBARGER EXERCISE HABITS QUESTIONNAIRE

1. Was there anything about the past week that made exercising especially different for you in terms of extended illness, injury, or vacation?

☐ 1Yes — If "YES", please complete this questionnaire about the previous "typical" week that occurred within the past 30 days.

☐ 2No — If "NO", please complete this questionnaire about this past week.

2. First, we are interested in the number of flights of stairs you climbed on average EACH DAY in this week. We only want to know the number of flights you climb going <u>UP</u> - not down.
**When answering this question, One Flight of Stairs = 10 steps if you know the number of steps.*

_____FLIGHTS PER DAY

3. We want to know how much time you spent this past week brisk walking for exercise or transportation. We are interested in bouts of walking that were at least 10 continuous minutes in duration. *This would include walking outside, at an indoor facility, or on a treadmill.*

3a. How many days this week did you walk briskly for the purpose of exercise or transportation for at least 10 continuous minutes outside, at an indoor facility, or on a treadmill?

_____ DAYS IN THE PAST WEEK

3b. On these days in which you walked briskly at least 10 continuous minutes, on average, how many minutes per day did you walk briskly?

_____ MINUTES PER DAY

4. Were there any other sport, fitness, or recreational activities in which you participated during the past week? We are interested only in time that you were physically active while performing the activity.
***Note:** Do not include "occupational" or "job related" activity as these are not considered to be sport, fitness, or recreational activity. Household activities such as cleaning, laundry, yard work and gardening are **NOT** to be included here as they are not considered to be a sport, fitness, or recreational activity.

Sport, Fitness, or Recreation	Days per Week	Average Time per Day
a.	____	_____ Minutes per Day
b.	____	_____ Minutes per Day
c.	____	_____ Minutes per Day

5. Would you say that during the past week (the week used for questions 2-4) you were:

☐ less active than usual
☐ more active than usual
☐ about as active as usual

6. In general, at least once per week, do you engage in regular activity similar to brisk walking, jogging, bicycling, etc. long enough to work up a sweat, get your heart thumping, or get out of breath?

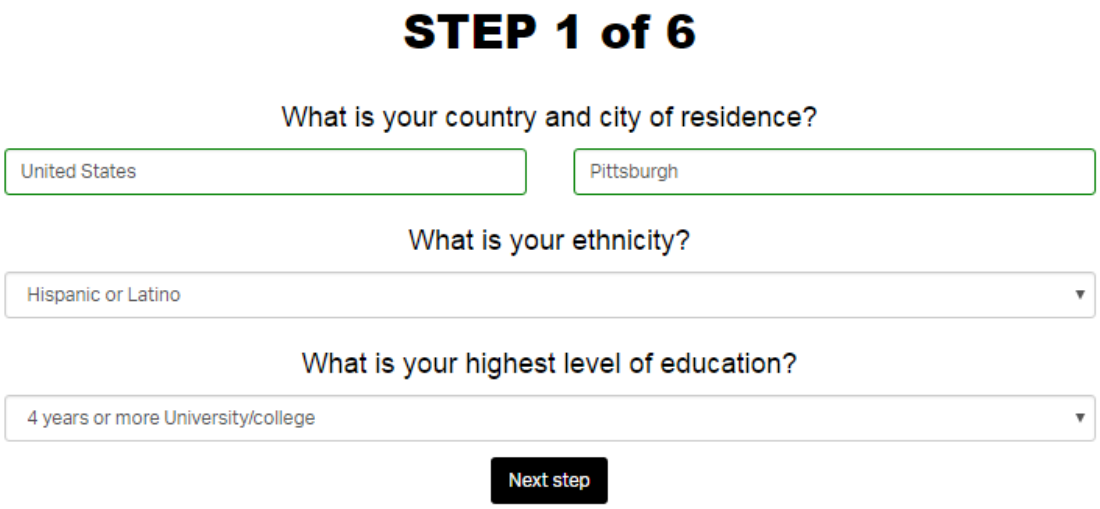
☐ Yes If "Yes", please indicate the number of days per week: ___
☐ No

7. In general, how hard do you work while you are physically active?

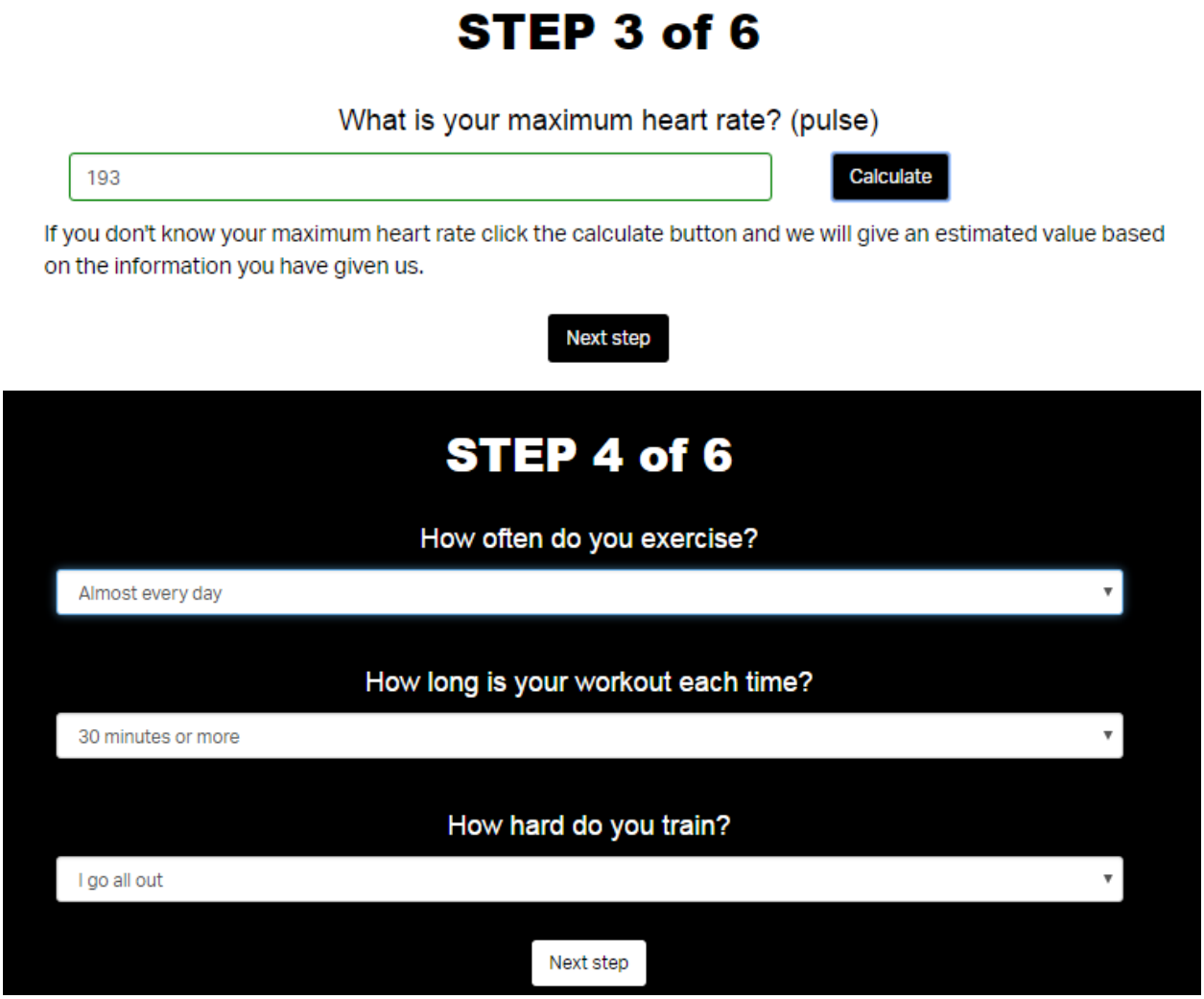
☐ take it easy without breathing hard or sweating
☐ little hard breath and sweating
☐ go all out

APPENDIX D

WORLD FITNESS LEVEL ESTIMATION OF FITNESS


STEP 1 of 6
What is your country and city of residence?
United States
Pittsburgh
What is your ethnicity?
Hispanic or Latino
What is your highest level of education?
4 years or more University/college
Next step


STEP 2 of 6
What is your gender?
Female
How old are you?
28
How tall are you?
5
2.5
feet/inches
How much do you weigh?
148
pounds
Next step


STEP 3 of 6
What is your maximum heart rate? (pulse)
193
Calculate
If you don't know your maximum heart rate click the calculate button and we will give an estimated value based on the information you have given us.
Next step
STEP 4 of 6
How often do you exercise?
Almost every day
How long is your workout each time?
30 minutes or more
How hard do you train?
I go all out
Next step

STEP 5 of 6

What does your waistline measure?

31.5 | inches

What is your resting pulse? (number of beats per minute)

58

Next step

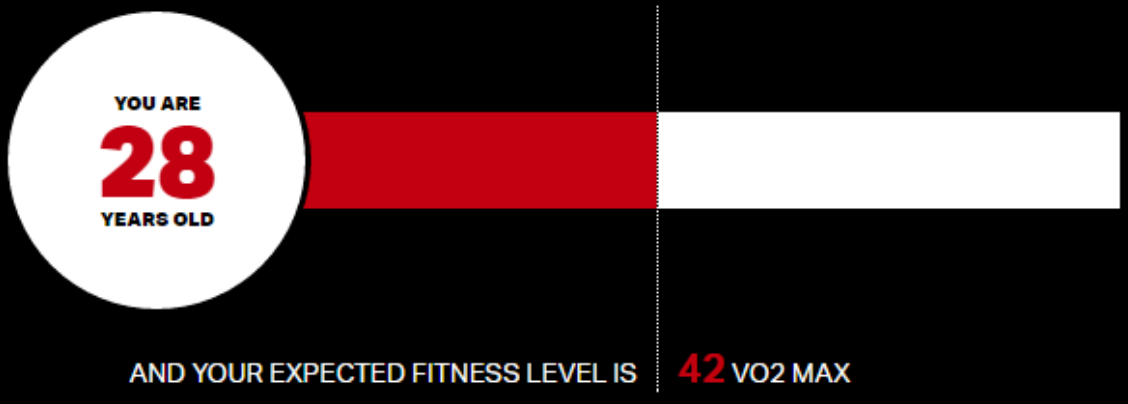
STEP 6 of 6

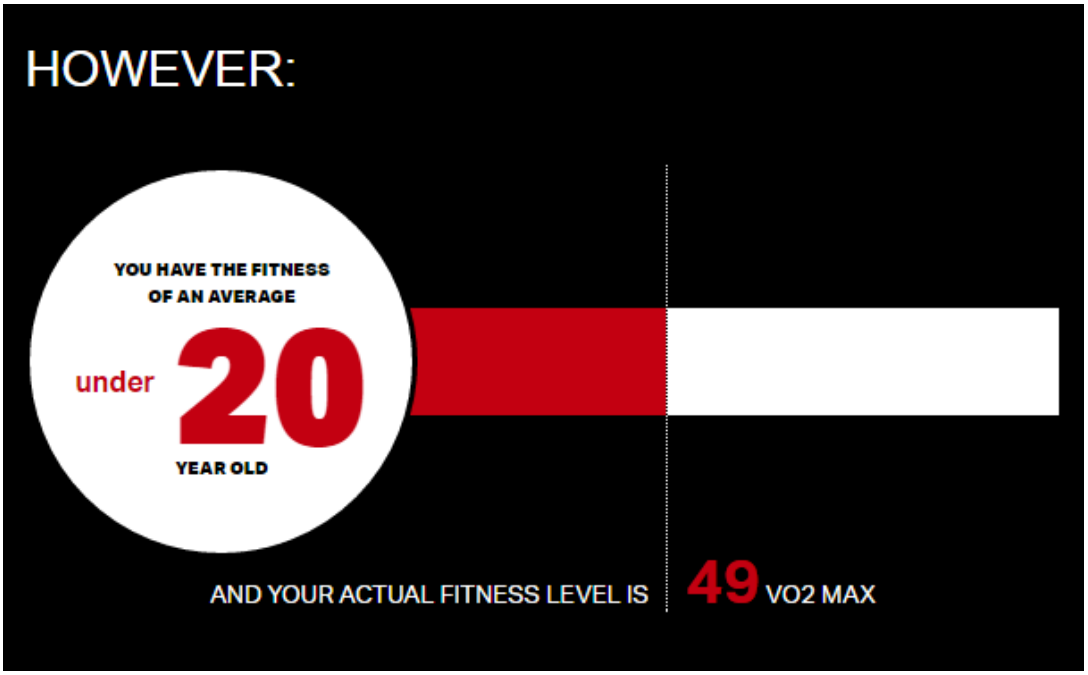
☑ I understand that CERG will use my data for research and that the information in no way can be traced back to me personally.

Help us to better understand the cardiovascular fitness of the worlds population by answering a few more questions before getting your results.

Yes, I'm happy to answer more questions | No, just take me to my results.

SO, THE TRUTH OF THE MATTER IS:




HOWEVER:
YOU HAVE THE FITNESS
OF AN AVERAGE
under
20
YEAR OLD
AND YOUR ACTUAL FITNESS LEVEL IS
49
VO2 MAX

APPENDIX E

WORKLOAD ESTIMATION

Workload Estimation Form

Participant ID: _____ Date_____

Termination Heart Rate: _____ bpm (70-75% of Age-Predicted Max Heart Rate)

Time (minutes)	Speed (mph)	% Grade	Heart Rate (bpm)
0:00 - 1:00	2.4	0.0%	
1:01 - 2:00	2.6	0.0%	
2:01 - 3:00	2.8	0.0%	
3:01 - 4:00	3.0	0.0%	
4:01 - 5:00	3.2	0.0%	
5:01 - 6:00	3.4	0.0%	
6:01 - 7:00	3.4	0.5%	
7:01 - 8:00	3.4	1.0%	
8:01 - 9:00	3.4	1.5%	
9:01 - 10:00	3.4	2.0%	
10:01 - 11:00	3.4	2.5%	
11:01 - 12:00	3.4	3.0%	
12:01 - 13:00	3.4	3.5%	
13:01 - 14:00	3.4	4.0%	
14:01 - 15:00	3.4	4.5%	
15:01 - 16:00	3.4	5.0%	
16:01 - 17:00	3.4	5.5%	
17:01 - 18:00	3.4	6.0%	
18:01 - 19:00	3.4	6.5%	
19:01 - 20:00	3.4	7.0%	
20:01 - 21:00	3.4	7.5%	
21:01 - 22:00	3.4	8.0%	
22:01 - 23:00	3.4	8.5%	
23:01 - 24:00	3.4	9.0%	
24:01 - 25:00	3.4	9.5%	
25:01 - 26:00	3.4	10.0%	
26:01 - 27:00	3.4	10.5%	
27:01 - 28:00	3.4	11.0%	
28:01 - 29:00	3.4	11.5%	
29:01 - 30:00	3.4	12.0%	
Termination Time: ___:___			

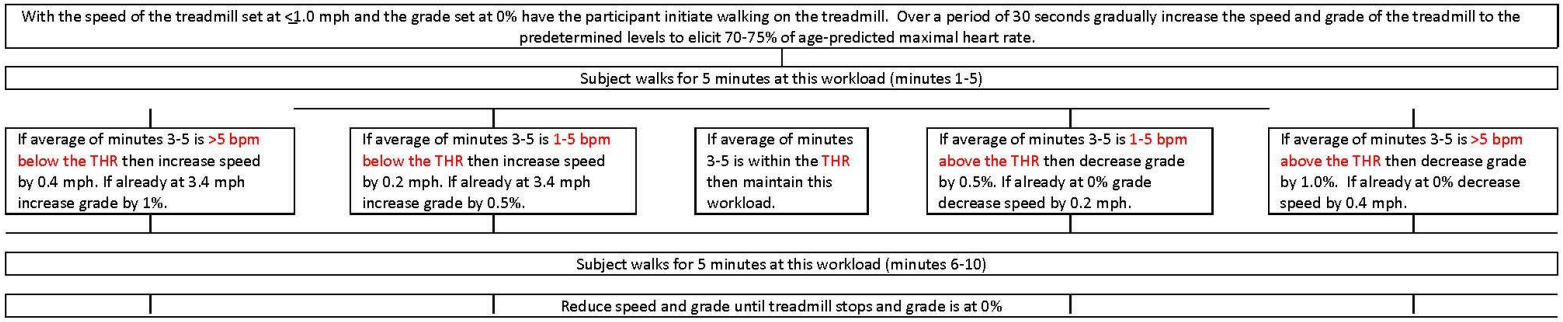
Starting Speed (mph) and % Grade: _____

APPENDIX F

ALGORITHM FOR 10-MINUTE EXERCISE SESSION (EX10)

FLOW CHART FOR 10-MINUTE (EX 10) WALKING SESSION

PARTICIPANT ID: _____ DATE OF EXERCISE SESSION: _____ Target Heart Rate: _____ bpm to _____ bpm



APPENDIX G

ALGORITHM FOR 30-MINUTE EXERCISE SESSION (EX30)

FLOW CHART FOR 30-MINUTE (EX 30) WALKING SESSION

PARTICIPANT ID: _____ DATE OF EXERCISE SESSION: _____ Target Heart Rate: _____bpm to _____ bpm

APPENDIX H

EXAMPLE OF EXPERIMENTAL SESSION TIMELINE

Example Experimental Session Timeline

0:00	**Participant is greeted** **Participant is walked to room 224** **Adherence to instructions (abstaining from exercise 24hr, nicotine and caffeine 12 hrs and a 4hr fast) is confirmed and recorded** **Participant is prompted to use restroom** **Participant puts on heart rate monitor**
0:05	Supine rest begins
0:15	Duplicate supine blood pressures are measured 1 min apart (**baseline**)
0:18	TCD
0:28	PWV
0:33	Transition from supine position to computer
0:36	Begin watching documentary series
1:06	End watching documentary series/Transition to supine position
1:09	Participant lays in supine position
1:19	Supine rest begins
1:29	Duplicate supine blood pressures are measured 1 min apart (**30-minute post**)
1:31	TCD
1:41	PWV
1:42	Participant remains supine
1:49	Supine rest begins
1:59	Duplicate supine blood pressures are measured 1 min apart (**60-minute post**)
2:01	TCD
2:11	PWV
2:21	Participant is finished Participant is prompted to use restroom Participant removes heart rate monitor

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