A Comparison of Exercise Training and Heat Therapy for Improving Blood Pressure in Adults with Untreated Hypertension

by

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DISSERTATION ABSTRACT

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Title: A Comparison of Exercise Training and Heat Therapy for Improving Blood Pressure in Adults with Untreated Hypertension

Hypertension, or high blood pressure, represents a primary yet preventable risk factor for cardiovascular disease, kidney dysfunction, and cognitive impairment that impacts nearly 50% of United States adults. Physical activity is the primary lifestyle recommendation to lower blood pressure, but many people are unwilling or unable to engage in exercise training. Heat therapy, in the form of hot water immersion, dry sauna, or far-infrared sauna, has gained attention in recent years as a potential therapeutic alternative to exercise for improving blood pressure and cardiovascular disease risk, with secondary benefits for multiple organ systems, including the renal and cerebral circulation. While both exercise and heat therapy have been demonstrated as effective in a variety of populations, there are no studies that have directly compared these interventions for lowering blood pressure in adults with untreated hypertension. Therefore, the purpose of this study was to compare the efficacy of heat therapy and exercise training for improving blood pressure, renal function, and biomarkers of Alzheimer's disease among adults with untreated hypertension. 41 adults were randomized to complete either 30 sessions of aerobic exercise training (n=20) or hot water immersion (n=21) over 8-10 weeks, with a battery of in-clinic and ambulatory assessments at baseline (PRE), after 15 sessions (MID), and after 30 sessions (POST). The impact of both interventions in this population was equivocal among outcome variables of interest, including both ambulatory and in-clinic blood pressure, pulse wave velocity, as well as biomarkers of renal function, cognitive function, and Alzheimer's Disease. These findings help to inform future lifestyle interventions aimed at improving blood pressure and cardiovascular disease risk among adults with untreated hypertension.

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CHAPTER I
INTRODUCTION

Hypertension, or high blood pressure, impacts a staggeringly high percentage of the adult population in the United States as well as world-wide. In the United States alone, nearly 50% of all adults over the age of 18 have high blood pressure (1). Hypertension is a primary yet preventable risk factor for cardiovascular disease, kidney disease, as well as Alzheimer's Disease and related dementias. Through repeated insults to the vascular endothelium, as well as increases in oxidative stress and inflammation, hypertension precipitates a decline in vascular function with deleterious effects across multiple organ systems. Low-resistance vascular beds, such as the renal and cerebral circulation, are particularly vulnerable to the detrimental effects of chronically elevated blood pressure.

Under recently updated classifications, a greater percentage of United States adults are considered hypertensive (2, 3). Accordingly, more adults with high blood pressure are recommended for anti-hypertensive interventions, either lifestyle-based or through pharmacologic means. Currently, medication is recommended for individuals that present with a blood pressure of 140/90 or greater, while this same figure serves as the goal blood pressure for individuals receiving pharmacological treatment. For these individuals, approximately 80% of all United States adults with hypertension, recommended treatment includes both lifestyle interventions *and* pharmacological treatment (4). However, there remain nearly 25 million individuals for whom the recommended treatment is a lifestyle-based intervention such as physical activity.

Physical activity for improved cardiovascular health across the lifespan can take many forms, but the primary recommendation from the American College of Sports Medicine is either 150 minutes of moderate-intensity or 75 minutes of vigorous physical activity per week, ideally in combination with two muscle-strengthening resistance training sessions per week (5). Despite the widely espoused benefits of physical activity, there are those who are unable to engage in traditional exercise training, such as patient populations (peripheral arterial disease, chronic heart failure, chronic obstructive pulmonary disease, etc.) or individuals with spinal cord injury. For those that do not obtain the proposed benefits of exercise training, the literature suggests considering alternative means for improving health (6). One such alternative is heat therapy, an

ancient practice often with cultural roots that has emerged in recent years as a suitable and potentially more efficacious alternative to exercise training for improving cardiovascular health (7). These improvements appear to be driven by reductions in blood pressure (8, 9), arterial stiffness (8, 9), and intermittent increases in beneficial vascular shear stress (8–11), much the same as exercise training.

Finally, hypertension is an independent as well as synergistic risk factor for Alzheimer's disease (AD), along with advancing age. At present, 6 million Americans have AD, a figure that is projected increase to nearly 14 million by 2060 (12). Additionally, the total costs of AD treatment in 2022 were in excess of $320 billion, which is forecasted to increase to nearly $1 trillion by 2050 (12). With a larger aging population, interventions that identify alternative means for improving risk for neurodegenerative diseases are paramount, particularly in concert with improved cardiovascular health.

Essential hypertension is a multifaceted disease with diverse etiology. The progression of essential hypertension into cardiovascular disease is slow, occurring over decades, but often with deleterious consequences. For this reason, combined with more stringent ideal blood pressure classifications, there is an evolving need for lifestyle interventions that can improve blood pressure and long-term cardiovascular disease risk in individuals with elevated blood pressure as well as those with overt hypertension.

STATEMENT OF THE PROBLEM

There is a substantial body of evidence examining the effects of exercise training on blood pressure in individuals with hypertension. Additionally, there are an increasing number of studies examining the cardiovascular and metabolic benefits of heat therapy. The literature clearly demonstrates that both interventions are effective for lowering blood pressure; however, these interventions have yet to be directly compared. Accordingly, we conducted a randomized controlled trial to compare the efficacy of heat therapy versus exercise training for lowering blood pressure in adults with untreated hypertension. We elected to investigate the effect of either 30 sessions of upright cycling or hot water immersion on in-clinic blood pressure and arterial stiffness. Furthermore, we examined the effect of these interventions on 24-hour ambulatory blood pressure, as well as several biomarkers of kidney function. Lastly, in a subset of eligible participants, we compared the potential of heat therapy and exercise to improve

cognitive function and MRI-based markers of AD risk. We addressed these questions through the following aims:

1) To examine ambulatory blood pressure and kidney function following 30 sessions of either aerobic exercise training or hot water immersion. Ambulatory blood pressure was assessed over a 24-hour period in conjunction with a 24-hour urine collection. We examined 24-hour urine albumin excretion and estimated glomerular filtration rate calculated using serum cystatin C. We hypothesized that hot water immersion would elicit greater reductions in ambulatory blood pressure and urine albumin excretion and an improvement in estimated glomerular filtration rate compared to aerobic exercise. Furthermore, we hypothesized that improvements in the blood-based biomarkers Interleukin-6, Endothelin-1, and C-Reactive Protein would predict improvements in ambulatory blood pressure and renal function. The results of these studies are discussed in Chapter IV.
2) To examine hemodynamic and vascular function following 30 sessions of either aerobic exercise training or hot water immersion. We achieved this by measuring heart rate variability, cardiac output, blood pressure, and pulse wave velocity as an estimate of arterial stiffness. Lastly, we measured cardiorespiratory fitness. These are clinically relevant markers which have well-established links to cardiovascular as well as all-cause mortality. We hypothesized that blood pressure and arterial stiffness would be reduced to a greater extent following hot water immersion compared to aerobic exercise, but that there would be no difference between hot water immersion and aerobic exercise for improvements in heart rate variability, cardiac output, or cardiorespiratory fitness. The results of these studies are discussed in Chapter V.
3) To examine cognitive function as well as structural and functional measures of Alzheimer's Disease risk following 30 sessions of either aerobic exercise training or hot water immersion. Participants completed the NIH toolbox assessment of episodic and working memory, as well as magnetic resonance imaging-based assessments of cerebral perfusion and structure. Specifically, we examined hippocampal volume, as well as gray and white matter perfusion. We hypothesized that hot water immersion would result in greater improvements in cognitive function as well as structural and functional

biomarkers of Alzheimer's disease and related dementia risk. We hypothesized that reductions in systolic blood pressure and arterial stiffness and improvements in serum Brain-Derived Neurotrophic Factor would play a causal role in improved biomarker risk profiles. The results of these studies are discussed in Chapter VI.

SIGNIFICANCE

If these aims are supported, these data will demonstrate that heat therapy, in the form of hot water immersion, is an effective lifestyle-based intervention for improving blood pressure in adults with untreated hypertension. These data will be presented in conjunction with, and in comparison to, aerobic exercise training, the current gold-standard intervention for improving blood pressure and cardiovascular disease risk. To maximize the ecological validity of these findings, the exercise undertaken in these studies match recommendations from the American College of Sports Medicine, and are time matched the duration of hot water immersion.

CHAPTER II
REVIEW OF THE LITERATURE

INTRODUCTION

Cardiovascular disease remains the leading cause of death in the United States and worldwide (13, 14). Hypertension, or high blood pressure, accounts for more cardiovascular disease deaths than any other preventable risk factor (15) and impacts nearly 50% of all United States adults over the age of 18 (1, 16). The current definition of hypertension, as outlined by the 8th report of the Joint National Committee for the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC8), is a systolic blood pressure ≥130 mmHg and/or a diastolic blood pressure ≥80 mmHg, with a sub-classification of "elevated" blood pressure for systolic pressures between 120-129 mmHg (3). The increase in the prevalence of hypertension under revised guidelines is not congruent with a greater incidence of hypertension, but rather represents more stringent blood pressure classifications (3). Specifically, these classifications increased the prevalence of hypertension among United States adults from 32% under JNC7 blood pressure classifications to 46% under JNC8 classifications (3). Consequently, more individuals are recommended for lifestyle modifications to address blood pressure as a risk factor for cardiovascular disease, but only a small number are recommended for anti-hypertensive pharmacological treatment (3).

While age is an independent risk factor for cardiovascular disease and mortality, hypertension acts synergistically with advancing age to exacerbate the risk of major cardiovascular events and risk for cardiovascular disease (17). A meta-analysis of over 1 million middle-aged and older adults reports a log-linear increase in cardiovascular disease risk with each 1 mmHg increase in either systolic or diastolic blood pressure, beginning with a normotensive blood pressure of 115/75 (18). In middle-aged adults specifically, a 20 and 10 mmHg increase in systolic and diastolic blood pressure, respectively, above healthy levels are associated with double the risk of stroke and cardiovascular mortality (18).

Conversely, improvements in blood pressure to normotensive values at the population level is associated with a 7.3% and 3.8% reduction in mortality for women and men, respectively (19). Modest blood pressure reductions in individuals with hypertension carry significant risk reduction for cardiovascular disease, even if blood pressure does not reach normotensive levels

(20). Taken together, these data suggest that there are substantial benefits to be obtained from interventions to lower blood pressure at any classification of hypertension.

HYPERTENSION AND THE VASCULATURE

Essential hypertension is a multifaceted condition, with an etiology that is still not completely understood (21). There are many diverse pathologies that influence the development and manifestation of essential hypertension. It is likely that molecular and cellular perturbations potentiate maladaptation in the vasculature, with considerable negative consequences at the organismal level. The below sections correspond to the putative pathways that may be permissive, if not causal, in the development of hypertension. A summary of these molecular and cellular maladaptations can be found below in figure 2.1.

Reactive Oxygen Species and Oxidative Stress

Reactive oxygen species are cellular signaling molecules with a wide variety of impact on cellular function, the most pertinent of which include regulation of the extracellular matrix, modulation of nitric oxide activity, and activation of various kinases and proinflammatory genes (22–26). Through their actions along these pathways, reactive oxygen species play a critical role in the development and progression of hypertension, mediated by a production of superoxide anions and hydrogen peroxide that exceeds their removal. Superoxide, hydrogen peroxide, and other derivatives of molecular oxygen are generated in the mitochondria as byproducts of mitochondrial respiration. These molecules and their actions, termed, “oxidative stress” have been implicated in a causal role in the development of essential hypertension (27, 28). These derivatives oxidize biological molecules with numerous downstream consequences, such as reducing the bioavailability of nitric oxide (29, 30), downregulating the expression of endothelial nitric oxide synthase (31–34), and a host of deleterious impacts on the vasculature and the kidneys (24, 25). Furthermore, reactive oxygen species are associated with increased vascular cell proliferation and alterations in the extracellular matrix, which elicits vascular remodeling and hypertrophy commonly found in hypertension (35). Collectively, these events have important implications for future cardiovascular events (36, 37).

Reactive oxygen species exert a variety of deleterious effects on the vasculature, but perhaps their greatest impact is related to the production and bioavailability of nitric oxide, an

important anti-atherogenic and vasodilatory signaling molecule (38–40). The bioavailability of nitric oxide is influenced primarily by the expression of three isoforms of nitric oxide synthase, neuronal, inducible, and endothelial (41).

Paradoxically, nitric oxide synthase can be associated with increased reactive oxygen species production. Each of the three isoforms of nitric oxide synthase have been shown to uncouple and produce superoxide rather than nitric oxide (42, 43), with the eventual consequence of atherosclerosis initiation and progression (44). The uncoupling of endothelial nitric oxide synthase is precipitated by the absence of essential cofactors L-arginine and tetrahydrobiopterin (45). This uncoupling can be attenuated, as experimental rodent models of hypertension have shown that supplementation with tetrahydrobiopterin ameliorates high blood pressure via decreased production of reactive oxygen species and greater production of nitric oxide (46, 47). Unfortunately, the negative consequence of uncoupled endothelial nitric oxide synthase-mediated superoxide production is highly cyclical in nature. Specifically, increased superoxide production oxidizes tetrahydrobiopterin, interfering with its role as an essential cofactor in the production of nitric oxide as well as uncoupling endothelial nitric oxide synthase, resulting in still further production of reactive oxygen species (48). The production of superoxide and hydrogen peroxide has been demonstrated to upregulate the expression of endothelial nitric oxide synthase (49–51), which may transiently offset the increases in reactive oxygen species production but is ultimately antagonized by greater superoxide production as endothelial nitric oxide synthase is increasingly uncoupled.

Data from animal models of hypertension indicate that treatment with superoxide dismutase (an anti-oxidative enzyme) mimetics or an antioxidant rich diet can effectively lower blood pressure ostensibly caused by oxidative stress (52, 53), suggesting that attenuation of oxidative stress may promote improvements in vascular health as well as blood pressure. Regrettably, there are several critical shortcomings with antioxidant supplementation in human models of hypertension *in vivo*, which are discussed below.

Among individuals with high blood pressure, accentuating factors such as increased sympathetic neural activity, chronic activation of the renin-angiotensin-aldosterone system, and greater concentration of vasoactive molecules such as endothelin-1 can elicit increases in reactive oxygen species-generating enzymes such as NADPH oxidase (54). In healthy individuals with physiologically normal redox signaling, antioxidant enzymes such as superoxide

dismutase offset the negative impact of reactive oxygen species (55). Superoxide dismutase, which has isoforms that act within the cytosol, mitochondria, as well as extracellular matrix, prevents the formation of peroxynitrite that results from the interaction of nitric oxide and superoxide (55). Individuals with hypertension present not only with increased oxidative stress relative to their normotensive peers, but also impaired *in vivo* antioxidant mechanisms, such as reduced superoxide dismutase bioavailability (56). Indeed, there is compelling evidence that individuals with essential hypertension have reduced content of multiple antioxidative molecules, including glutathione, catalase, and glutathione-peroxidase-1 (56–58). NADPH oxidase-mediated oxidative stress leads to the oxidation of tetrahydrobiopterin, an essential cofactor for endothelial nitric oxide synthase. This cascade results in reduced nitric oxide bioavailability, ultimately resulting in endothelial dysfunction (46). Additionally, localization of reactive oxygen species to the tunica media and tunica adventitia, the layers of the vessel between the endothelium and vascular smooth muscle, may further impair the vasodilatory actions of nitric oxide (59, 60). Underscoring the negative organismal consequences of reactive oxygen species are data from Simic and colleagues, reporting a significant negative correlation between cellular superoxide dismutase content and both systolic and diastolic blood pressure among individuals with established essential hypertension (58).

Investigations of therapeutic targets to mitigate the impact of oxidative stress in hypertension have focused on increasing anti-oxidant capacity, increasing nitric oxide bioavailability, and reducing reactive oxygen species generation. To date, large clinical trials examining the effectiveness of antioxidant supplementation on oxidative stress have been equivocal. The major limitations of these interventions are that antioxidants may not be able to effectively scavenge intracellular free radicals (61), and that supplementation does not address the continued production of reactive oxygen species. Effective therapeutic interventions may include supplementation in a supporting role but must address the foundational issue of increased reactive oxygen species production and reduced antioxidant capacity in individuals with hypertension.

C-Reactive Protein

C-Reactive Protein (CRP) is a biomarker of systemic inflammation and is associated with incident cardiovascular disease and increased risk for adverse cardiovascular events (62–65).

Cross-sectional data strongly supports increased levels of CRP among individuals with hypertension (66–68), although the causal relationship between the two is dubious (69, 70). Perhaps more likely is that increased levels of CRP facilitate the initial stages of vascular endothelial dysfunction. Nitric oxide production and bioavailability in human aortic and umbilical vein endothelial cells is significantly reduced when cultured with physiologically relevant doses of CRP, evidenced by reduced endothelial nitric oxide synthase expression (71, 72). Furthermore, CRP-mediated impairments in endothelial function have been implicated in atherogenesis, precipitating declines in vascular function that may result in incident cardiovascular disease (73, 74). Lastly, CRP has been demonstrated to significantly increase the expression of endothelin-1, as vasoconstrictor peptide with independent and synergetic consequences for impaired vascular health (75).

Endothelin-1

Endothelin-1, a potent vasoactive peptide, undoubtedly plays a role in the development and progression of endothelial dysfunction in hypertension (76–78), primarily through its link to oxidative stress and vascular inflammation. In experimental models of hypertension, endothelin-1 has been implicated in NADPH-oxidase-mediated vascular dysfunction (79, 80). Data from older individuals with hypertension reports a significant negative relationship between plasma endothelin-1 concentration and plasma antioxidant content (81). Additionally, data suggests those with moderate to severe essential hypertension have an increased expression of prepro-ET-1 (an endothelin-1 precursor) mRNA in the endothelium of their resistance vessels (82). Molecules such as endothelin-1 act to downregulate the production and bioavailability of nitric oxide, as well as increase the production of superoxide (83, 84). Finally, the vasoconstriction and stimulation of vascular smooth muscle cell proliferation elicited by endothelin-1 may play a causal role in arterial stiffening with hypertension (85, 86).

Shear Stress and Endothelial Function

The endothelium is the innermost lining of blood vessels, comprised of a single layer of cells. It has many auto- and paracrine functions, including vasodilation and vasoconstriction elicited by a host of signaling molecules and mechanisms, with profound impact on blood pressure and flow. Investigations of endothelial function can inform clinicians and researchers regarding risk for future cardiovascular events, disease, and mortality (87). Experimentally, conduit artery endothelial function is often assessed using a technique known as flow-mediated dilation. Flow-mediated dilation occludes blood flow distal to a conduit artery such as the brachial or common femoral for approximately 5 minutes and, upon release of the occlusion, measures the change in artery diameter in response to the transient increase in vascular shear stress that initiates a signaling cascade resulting in the release of nitric oxide (88, 89). Flow-mediated dilation has significant prognostic value for future cardiovascular disease risk, and describes the endothelium-dependent dilatory capacity of the vessel (90, 91). The prognostic utility of this measurement comes from data that demonstrates that impaired brachial artery endothelial function is associated with significantly greater risk for impaired coronary artery endothelial function (92).

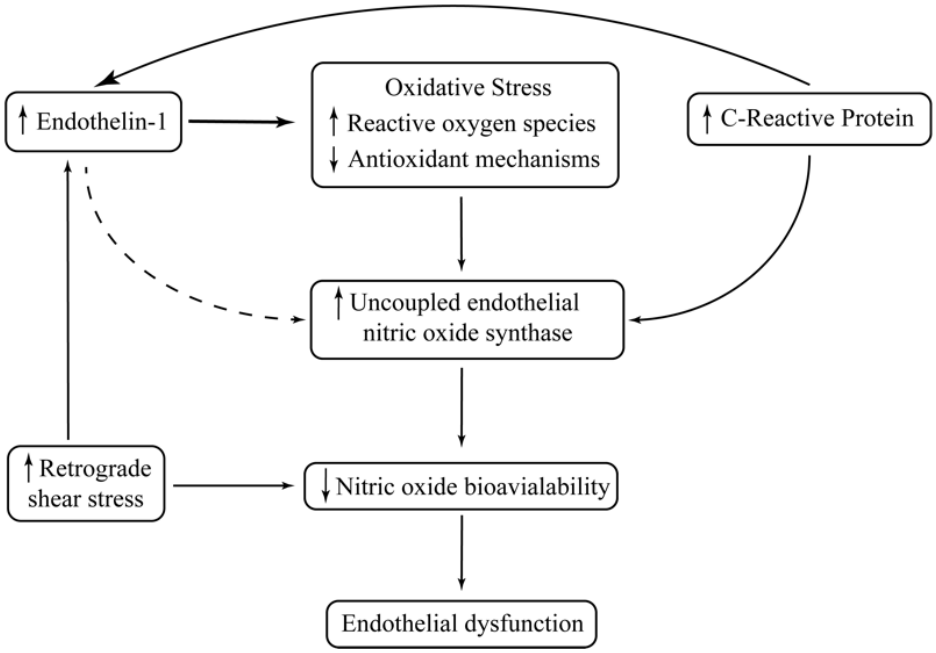

Figure 2.1. Graphic of the relationship between Endothelin-1, C-Reactive Protein, Oxidative stress, and endothelial dysfunction in individuals with essential hypertension.

Endothelial Dysfunction in Hypertension

Hypertension is associated with a maladaptive vascular remodeling that manifests as macrovascular and hemodynamic dysfunction, foremost among which is high blood pressure (93, 94). Maladaptive remodeling begins at the endothelium, or the innermost lining of blood vessels, which represents the interface between blood and the vasculature. Owing to the putative mechanisms described above, individuals with hypertension present with impaired endothelial function, measured via both acetylcholine infusion (95, 96) and reduced brachial artery flow-mediated dilation (97, 98). These impairments are indicative of reduced activity of nitric oxide mediated by accumulated oxidative stress as well as an imbalance of vasoactive molecules such as endothelin-1 (99). The vascular and hemodynamic consequences of these impaired responses to acetylcholine infusion and brachial artery Flow-mediated dilation are reduced vasodilatory capacity and increased systemic vascular resistance and blood pressure (100, 101).

Endothelial dysfunction in individuals with untreated hypertension also offers prognostic value, as demonstrated in a study quantifying the forearm blood flow response to acetylcholine infusion. Perticone and colleagues demonstrated that those with untreated hypertension with the worst blood flow response to acetylcholine infusion were at a twofold higher risk for major adverse cardiovascular events compared to those with the greatest blood flow response (87).

Damaging Effects of Oscillatory and Retrograde Shear Stress

Although there is a wealth of evidence for the role the molecular mechanisms described above play in endothelial dysfunction, blood flow patterns play an equally important role in the regulation and dysregulation of the vascular endothelium. In an idealized model with a perfect pump, blood flow through the vasculature is laminar and without interruption. Physiologically, blood flow throughout the arterial tree is a balance of anterograde (forward) and retrograde (backward) flow. The propagation of pressure and flow is achieved through the combined action of the heart and the pressure reservoir of the large elastic arteries, such as the aorta, known as the Windkessel effect. A third characteristic of blood flow, turbulent flow, is common at blood vessel bifurcations or in the presence of plaque in the vessel lumen. Both reduced mean flow and turbulent flow are proatherogenic or supporting formation of plaque (102–105). Moreover, turbulent flow decreases endothelial nitric oxide synthase expression in cultured endothelial cells, permissive in the development of atherosclerotic plaque (106). While there is an

established damaging role of turbulent flow, retrograde blood flow and shear stress do not appear to be obligatory for impairments in endothelial function in the presence of low flow (107). Despite this apparent dichotomy, increases in retrograde shear stress are associated with a dose-dependent decrement in flow-mediated dilation (108, 109) as well as increases in endothelin-1 expression *in vitro* (31). Retrograde and oscillatory shear stress undoubtedly contribute to the development and progression of endothelial dysfunction, which is implicated in the pathogenesis of hypertension. However, others suggest that some combination of anterograde and oscillatory, or a combination of forward and backward shear stress and the turbulent flow that results, may play an important role in vascular adaptations (110). Lastly, experimentally induced increases in sympathetic neural outflow were associated with increases in pro-atherogenic retrograde shear stress (111).

Autonomic Dysfunction and Sympathetic Overactivity in Hypertension

The autonomic nervous system plays a critical role in short-term regulation of blood pressure through the actions of its component branches, sympathetic and parasympathetic. Essential hypertension undoubtedly has a diverse pathology but may be influenced in part by autonomic dysfunction. The initial stages of hypertension can be characterized by an excess of sympathetic nervous system activity (112), as evidenced by decreased heart rate variability (113), increased plasma norepinephrine spillover (114), and elevated muscle sympathetic nerve activity (115) in individuals with hypertension compared to their normotensive peers.

In animal models of chronic renal hypertension, the baroreflex is reset to defend a higher blood pressure as the set point of the reflex arc (116). In humans, long-term fluid volume, electrolyte balance, and blood pressure control are highly integrated with the kidney, which complicates the interpretation of baroreflex resetting in hypertension (117). Despite this, there is compelling data in humans demonstrating that baroreflex sensitivity, assessed using the modified Oxford technique, was inversely correlated with 24-hour ambulatory mean arterial pressure and positively correlated with heart rate variability (118). This data was collected in young, healthy, individuals with normotensive blood pressure, which limits the generalizability of these findings to middle-aged adults with essential hypertension.

To date, there has been limited study of baroreflex sensitivity in adults with hypertension. Bristow and colleagues report significantly lower baroreflex sensitivity in individuals with

hypertension compared to age-matched normotensive controls (119). Additionally, baroreflex sensitivity was reduced in those with a family history of hypertension, regardless of current blood pressure classification (120). Specifically, among both normotensive and hypertensive individuals with a family history of high blood pressure, baroreflex sensitivity was significantly reduced compared to normotensive and hypertensive individuals without a family history of hypertension (120). Moreover, baroreflex sensitivity in a large cohort of individuals with hypertension was significantly inversely related to 24h ambulatory systolic blood pressure, and, interestingly, inversely related to pulse wave velocity. Collectively these data suggest that baroreflex sensitivity as a descriptor of autonomic function may influence the progression of hypertension and cardiovascular disease risk factors (121).

Essential hypertension is characterized by in part by exaggerated sympathetic nerve activity, relative to normotensive individuals (122, 123). Grassi and colleagues demonstrate significant stepwise increases in muscle sympathetic nerve activity burst incidence when studying normotensive, essential hypertensive, and severe essential hypertensive individuals (124). Interestingly, those with secondary hypertension (high blood pressure resulting from renovascular disease in this investigation) did not present with increased sympathetic neural outflow relative to normotensive individuals (124). This data serves as further evidence of a neurogenic component in essential hypertension that is central to the disease pathology. Among those with advanced cardiovascular disease (heart failure, heart failure with concomitant end-stage renal disease), excessive sympathetic nerve activity is associated with increased cardiovascular morbidity and mortality (125, 126). As such, interventions that are capable of attenuating elevated sympathetic nerve activity in individuals with hypertension are critical for improving and maintaining long-term cardiovascular health.

Large Artery Stiffening in Hypertension

During systole, the left ventricle contracts and ejects blood into the aorta. Systolic pressure achieved during contraction stretches the aorta, creating a pressure reservoir owing to the elastic nature of the aorta. During diastole, blood travels through the systemic arterial tree, driven by the pressure reservoir of the aorta, now propagating distally. This pressure waveform can be described as a “pulse wave” which travels throughout the arterial tree at a given velocity, or a pulse wave velocity. Bramwell and Hill were the first to speculate on the nature of the pulse

wave in an intact vasculature, inferred from a series of experiments in an isolated carotid artery (127). They presciently noted that the velocity of the pulse wave throughout the vascular tree is dependent upon the integrity and composition of the vessel in both health and disease (127).

Pulse wave velocity is incongruent with blood velocity, but, because of the relative ease of non-invasive measurement, is an accepted representative of arterial stiffness. Applanation tonometry is capable of providing a non-invasive estimate of arterial stiffness (128–130). Plainly, the distance between two anatomical locations (*i.e.*, carotid and common femoral arteries; brachial and pedal arteries) is measured. Next, using applanation tonometry, researchers may non-invasively generate pressure waveforms during each cardiac cycle. Dividing the anatomical distance between the measurement site on each artery by the time delay between the upswing of the systolic waveform of both anatomical locations of choice yields pulse wave velocity.

Pulse wave velocity is a predictor of cardiovascular disease risk independent of age, co-morbidities, and previous cardiovascular disease, and has particular utility for forecasting cardiovascular events in individuals with hypertension (128, 131, 132). Results from the Rotterdam Study, a large prospective cohort study, indicate that aortic pulse wave velocity is strongly correlated with coronary heart disease and stroke, and provides added benefits above traditional risk factors when forecasting cardiovascular disease risk (133). Furthermore, aortic pulse wave velocity greater than the 25th percentile is associated with a twofold increase in the risk of cardiovascular disease, a two- to threefold increase in the risk of stroke, and a >50% risk of coronary heart disease-related events (134). Furthermore, the efficacy of aortic pulse wave velocity in predicting cardiovascular events is maintained after adjusting for 24-hour ambulatory blood pressure as opposed to in-clinic measurements. Importantly, these findings are substantiated regardless of age, race, or biological sex (134). It is likely that increased arterial stiffness is permissive in the development of hypertension and is associated with worsening cardiovascular disease risk (135). Considering that central elastic arteries such as the aorta and the carotid have been demonstrated to be stiffer in individuals with hypertension across the lifespan (136–140), the impact of increased arterial stiffness in this population cannot be understated. Indeed, data from the Framingham Offspring study report that higher aortic stiffness is associated with a higher risk for incident hypertension (141).

Maladaptive vascular remodeling and dysfunction may appear as a precursor to overt cardiovascular disease, particularly among individuals with hypertension (142). In addition to

increase in stiffness that is related to pathological conditions, arterial stiffness increases as a function of age (143). Unfortunately for long-term vascular health, these two elements may act synergistically with a host of adverse effects and serve as a prognostic indicator of cardiovascular mortality risk (131). Increases in arterial thickness occur with aging, with the greatest increases in thickness located primarily in the intima (144).

HYPERTENSION AND THE KIDNEY

The kidney is intricately linked to long-term cardiovascular regulation in both health and disease, such as the development and progression of cardiovascular and chronic kidney disease. Essential hypertension is multifaceted in nature, with multiple axes of dysregulation ultimately culminating in high blood pressure. Kidney dysfunction is unique in that it can serve as both a cause and consequence of high blood pressure. There is a plethora of factors that potentially contribute to increased cardiovascular disease risk, often with shared commonalities between hypertension and kidney dysfunction. The same problems that plague the vasculature have been implicated in kidney dysfunction including oxidative stress, inflammation, endothelial dysfunction, increased arterial stiffness, and elevations in vascular resistance. The subsections below are clinically relevant to kidney function and dysfunction, and pertinent to the development and progression of hypertension and cardiovascular disease risk.

Renin-Angiotensin-Aldosterone System

The kidney works in concert with the cardiovascular system in service of long-term blood pressure regulation. The heart and vasculature generate and modulate the perfusion pressure of the kidney, an organ that plays a foundational role in electrolyte and fluid balance. Two primary mechanisms through which the kidney integrates into cardiovascular regulation of blood pressure are sympathetic activity and the renin-angiotensin-aldosterone system. While nominally distinct, it will become evident that sympathetic outflow and the renin-angiotensin-aldosterone system are intricately linked in the regulation of fluid volume and blood pressure.

Granular cells in the juxtaglomerular apparatus of the late afferent arteriole produce and secrete renin. Renin is a proteolytic enzyme that cleaves angiotensinogen, a protein produced in the liver but found abundantly in plasma, into a 10-amino acid peptide Angiotensin I. Angiotensin I is then converted into Angiotensin II by the aptly named angiotensin-converting

enzyme. Angiotensin-converting enzyme is expressed throughout the vasculature, with a particularly high concentration in the pulmonary vasculature. Often, the amount of renin available to catalyze angiotensinogen into angiotensin I is the limiting factor for the creation and concentration of angiotensin II in circulation.

Once converted, angiotensin II has several direct impacts on blood pressure. Primarily, angiotensin II is a potent vasoconstrictor in peripheral tissues with the aim of increasing mean arterial pressure. Secondly, angiotensin II stimulates the production and secretion of aldosterone from the adrenal cortex. Aldosterone acts on the distal nephron to increase sodium reabsorption, thereby increasing total blood volume, with the goal of increasing mean arterial pressure. The primary regulator of aldosterone secretion from the adrenal cortex is the amount of circulating angiotensin II. Recall, angiotensin II is controlled upstream by the release of renin from the juxtaglomerular apparatus. The half-life of renin and aldosterone are short (~15 minutes) while the half-life of angiotensin II is very short (~1 minute).

The integral role of renin in this elegant long-term regulation of blood pressure cannot be understated. There are three primary regulators of renin secretion. The first is sympathetic activity. Norepinephrine released from postganglionic sympathetic neurons onto β_1 adrenergic receptors on granular cells in the juxtaglomerular apparatus initiate a cyclic adenosine monophosphate-mediated pathway that results in the release of renin. Basal sympathetic tone of the renal efferent arterioles and vasculature is low, despite a rich density of α_1 and β_1 adrenergic receptors. As such, the kidney responds robustly to increases in sympathetic outflow, which accounts for the effectiveness of sympathetic-mediated release of renin from granular cells. This mechanism of renin release is usually secondary to a systemic increase in sympathetic neural outflow, elicited by a reduction in mean arterial pressure.

Distending, or transmural, pressure of the renal afferent arteriole is also a primary mediator of renin release. A decrease in afferent arteriolar pressure, which parallels systemic arterial pressure, stimulates the production of renin. Despite a lack of neural innervation to the cardiovascular control centers, granular cells can be classified as baroreceptors owing to their ability to evoke a physiological action in response to a change in pressure. The release of renin in response to a decrease in both local as well as systemic arterial pressure is an elegant example of redundant mechanisms in the regulation of arterial pressure. A decrease in systemic arterial pressure is sensed by the carotid baroreceptors, eliciting an increase in sympathetic outflow. This

increase in sympathetic outflow acts on β_1 adrenergic receptors on the granular cells to increase the release of renin. Simultaneously, transmural pressure in the renal afferent arteriole decreases, also stimulating the release of renin. This combined release of renin is an important initial step in the cascade of long-term blood pressure regulation as discussed above. Clinical data suggests that individuals with hypertension present with an increase in sympathetic outflow, precipitating dysregulation along the renin-angiotensin-aldosterone axis (112, 122, 123, 145, 146).

Estimated Glomerular Filtration Rate

Glomerular filtration rate is an assessment of the function of our greatest biological filter, the kidney. Despite the widespread utility of this measurement and its ability to inform baseline kidney function, pharmacological impacts on the kidney, as well as progression of chronic kidney disease, it is not easily measured in clinical settings. Ideally, glomerular filtration is measured using the clearance of exogenous molecules such as inulin or iohexol, but this procedure is costly and rarely used clinically (147). Accordingly, researchers and clinicians employ estimated glomerular filtration rate as an index of kidney function (148–150). Glomerular filtration rate can be estimated via equations accounting for serum creatinine or cystatin C, age, biological sex, and body size (151). Despite the widespread use of this estimated glomerular filtration rate clinically, creatinine clearance is impacted by renal tubular creatinine secretion, resulting in two-fold limitation at the extremes of physiological ranges (147). Namely, at the low and high end of estimated glomerular filtration rate, creatinine clearance tends to over- and under-estimate glomerular filtration rate, respectively (147). Furthermore, creatinine excretion is impacted by patient muscle mass (152). Cystatin C is a protein that is removed from circulation exclusively through glomerular filtration and is not impacted by factors such as muscle mass. For this reason, serum cystatin C is often used independently or as a modifier in the calculation of estimated glomerular filtration rate (153). Furthermore, cystatin C has been demonstrated to have a significantly more linear relationship with risk for chronic kidney disease compared to creatinine, in both healthy as well as clinical populations (154–156). Clinically, baseline blood pressure, particularly when elevated or high, is associated with a more rapid decline in kidney function, quantified as estimated glomerular filtration rate (157).

Glomerular hyperfiltration

Glomerular hyperfiltration represents an increase in glomerular filtration above baseline values, typically two standard deviations above resting in healthy individuals (149, 158), although this definition is complicated by age-related decline in glomerular filtration rate. Hyperfiltration can occur at the single nephron level which compounds at the level of the whole kidney. While there remains to be a consensus, the most compelling data suggests that single-nephron hyperfiltration precipitates compensatory renal hemodynamic changes that ultimately results in global impairments in glomerular filtration rate and the inexorable progression towards chronic kidney disease (159–161). Single nephron hyperfiltration, sometimes referred to as relative hyperfiltration, results in glomerular damage that impairs the filtering efficacy of the nephron in question (162). As the proportion of impacted nephrons increases, the remaining unaffected nephrons subsequently increase their glomerular filtration rate to offset the decreases wrought by the impaired nephrons. Despite large quantities of nephrons per kidney, eventually this progressive glomerular damage exhausts the supply of healthy filtration units, resulting in impairments in kidney function and, eventually, chronic kidney disease (160, 161). In healthy individuals, the resilience of the kidney to a transient state of absolute hyperfiltration is termed "renal functional reserve" and represents the ability of the kidney to respond to a stressor that evokes an increase in filtration (163). The inability of the kidney to increase glomerular filtration rate in response to a stimulus such as an oral protein load suggests glomerular hyperfiltration at rest, a hallmark of impairment and putative indicator of early-stage renal disease (164).

Glomerular hyperfiltration is a complex pathological state, as it is both a cause and consequence of kidney injury, either acute or chronically. As it relates to essential hypertension, the primary drivers of hyperfiltration are likely increased renal afferent arteriolar pressure and increased renal efferent arteriolar vasoconstriction (158). Renal afferent arteriolar pressure parallels systemic arterial pressure and renal efferent arteriolar vasoconstriction is most commonly elicited via the renin-angiotensin-aldosterone system (158). In individuals with elevated and high blood pressure, these pathologies would be met by increased arterial pressure as well as sympathetic overactivity, respectively. As such, interventions aimed at improving the pathogenesis of glomerular hyperfiltration would likely improve long-term renal functional outcomes and reduced the risk of incident chronic kidney disease (165–167).

Despite renal autoregulation, it has been postulated that systemic hypertension evokes repeated increases in glomerular pressure, resulting in endothelial cell damage and loss of nephron functionality (168, 169). The progressive loss of nephrons is hypothesized as a primary driver of essential hypertension, demonstrative of the vicious cycle of hypertension and impaired kidney function (170). Repeated endothelial insult by elevated blood pressure compromises the number of functional nephrons (168). Autopsy data from middle-aged healthy individuals report that those with elevated and high blood pressure present with lower absolute numbers of nephrons compared to their normotensive counterparts (171).

Hypertension and Albuminuria

Albumin is a protein produced by the liver that is found in blood and helps to generate oncotic pressures within blood for long-term capillary fluid balance. As established, persistent elevations in single nephron glomerular filtration, driven in large part by increased blood pressure, result in glomerular damage. The clinical manifestation of this damage is albuminuria, wherein damaged glomeruli permit albumin to be excreted into the urine. Microalbuminuria, classified as urine albumin excretion of 30-300 mg/24h, was initially associated with the risk of developing proteinuria and premature mortality in individuals with type II diabetes mellitus (172). In non-diabetic populations, sub-clinical albumin excretion also predicts the development of both cardiovascular disease as well as chronic kidney disease (173–175). In a 10-year population-based cohort study, albuminuria was shown to be an independent predictor of ischemic heart disease, and is associated with a significant reduction in disease-free survival during the follow-up period (173). Similarly, cross-sectional data suggests that microalbuminuria is associated with an increased blood pressure and cardiovascular disease risk (176–178). Perhaps most compellingly is data that suggests elevated urine albumin excretion and urinary albumin:creatinine ratio in the highest quartile of participants was associated with a twofold higher risk for hypertension, even among presently normotensive individuals (174, 179). Collectively, these population-based analyses indicate that microalbuminuria may represent incipient cardiovascular disease as readily as it does chronic kidney disease. To that end, 24h urine albumin excretion as low as 5mg/g of creatinine are associated with an increased risk of hypertension, major cardiovascular events, as well as all-cause mortality in healthy individuals (180–182).

The suggested mechanisms underlying urine albumin excretion are impairments in glomerular endothelial function, ostensibly indicative of increased permeability (183, 184). Among adults with untreated hypertension but with normal serum creatinine levels, impaired vasodilatory response to acetylcholine infusion is significantly associated with compromised renal function as well as reductions in estimated glomerular filtration rate (185, 186). Furthermore, cross-transplantation data from animals with experimental hypertension to those with normal blood pressure evokes a hypertensive profile in otherwise healthy animals, further underscoring the central role of the kidney in cardiovascular dysfunction and hypertension (187). Data in individuals with type II diabetes has shown that Thiazolidinediones, a class of drugs that improve insulin sensitivity, elicited improvements renal hemodynamic and endothelial function, mediated through improvements in nitric oxide bioavailability (188). Furthermore, renovascular hypertension is associated with impairments in endothelium-dependent dilation, demonstrably due to renin-angiotensin-aldosterone activation and subsequent increases in oxidative stress (189). Additionally, impairments in endothelial function as well as increased reactive oxygen species are associated with increased ambulatory blood pressure as well as blood pressure variability (190).

Arterial Stiffness and the Kidney

The kidney, much like the brain, receives a staggeringly high percentage of total resting cardiac output relative to its size. The consequence of being a low resistance, high flow organ is that the kidney is particularly susceptible to increased arterial stiffness, high blood pressure, and pulsatile changes in blood flow (191, 192). Increased arterial stiffness is significantly correlated with increased urine albumin excretion and impaired kidney function, as measured by estimated glomerular filtration rate (193–195). Importantly, both previous occur in the absence of "classic" cardiovascular and metabolic disease risk factors such as hypertension, dyslipidemia, and diabetes.

The relationship between increased arterial stiffness and impaired kidney function is highly integrative. Depicted below in Figure 2.2. is a schematic of the vicious cycle precipitated by long-term unchecked high blood pressure. The cellular signature that precipitates the cycle in Figure 2.2 is endothelial dysfunction, characterized by a reduced production of nitric oxide mediated by endothelin-1 and oxidative stress. Hypertension and endothelial dysfunction

contribute to increased conduit artery stiffness. Increased arterial stiffness causes increases in pulsatile pressure in vulnerable vascular beds, such as the kidney, which ultimately results in glomerular damage (191). Additional contributors include neurogenic and humoral axes such as excessive sympathetic nervous system activity and chronically active renin-angiotensin-aldosterone system activity.

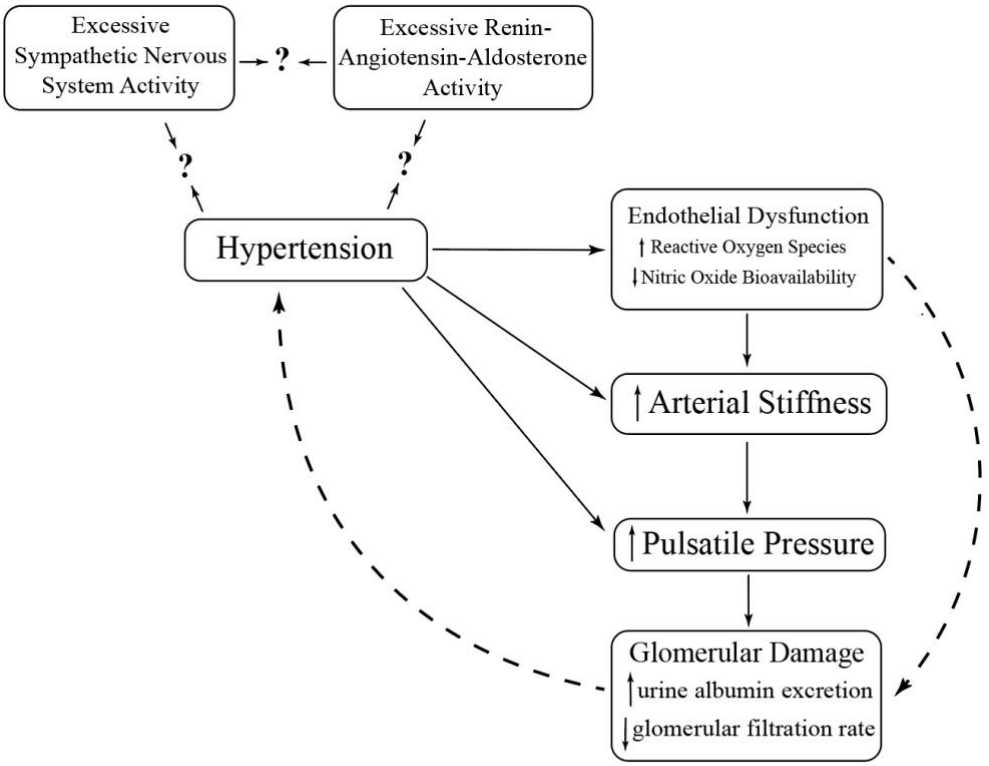

Figure 2.2. Relationship between microvascular and macrovascular changes with hypertension and kidney function. Chronic dysregulation of the sympathetic nervous system and the renin-angiotensin-aldosterone system may potentiate further impairment in kidney function mediated by hypertension.

HYPERTENSION AND ALZHEIMER'S DISEASE

A decline in cognitive function that occurs with aging often represents the first salient symptom of neurodegenerative diseases such as Alzheimer's Disease (AD). Alzheimer's Disease is the most common neurodegenerative disease, impacting over 6 million Americans, a figure that is projected to nearly triple by 2050 in light of an aging population (196). Aging also represents the primary risk factor for cardiovascular disease, and age-associated increases in systolic blood pressure and vascular dysfunction are undoubtedly involved in cognitive decline (197). Hypertension acts synergistically with age to increase the risk of Alzheimer's Disease.

The subsections below detail common vascular and hemodynamic changes in hypertension and their role in the development and progression of AD.

Hypertension and Alzheimer's Disease

Aging represents a primary risk factor for Alzheimer's Disease (198), which is exacerbated by cardiovascular disease (199). High blood pressure is a modifiable risk factor for cardiovascular and cerebrovascular disease and an important therapeutic target for attenuating Alzheimer's Disease risk (200–203). Indeed, even in the absence of overt neurodegenerative disease, there is evidence for impaired cognitive function in individuals with hypertension (204–206). Furthermore, midlife hypertension is associated with an increased incidence of dementia in later life in a dose-dependent fashion (207–211). Collectively, these data demonstrate that blood pressure control in mid-life is critical for maintaining optimal cerebrovascular health and maintaining cognitive function.

Among the first studies published regarding blood pressure and cognitive function was from Wilkie and Eisdorfer, demonstrating that diastolic hypertension was related to significant declines in cognitive performance when compared with normotensive individuals (212). Recently updated blood pressure classifications have increased the prevalence of hypertension in the United States, meaning are a greater percentage of individuals that are at increased risk for cognitive impairment and Alzheimer's Disease, exacerbated by a large aging population and predicted increases in cardiovascular disease risk in this population (201, 202, 213).

Hypertension has been shown to be the only risk factor for AD where a reduction in blood pressure elicited a reduction in the incidence of dementia, both among adults with a history of hypertension (214, 215), as well as cerebrovascular disease (216). Importantly, strategies aimed at improving blood pressure carry multi-fold benefits through midlife and with advancing age. Lastly, younger individuals with high blood pressure are more susceptible to neuroanatomical changes later in life (204, 205). For these reasons, it is of critical importance to establish further therapeutic strategies for improving blood pressure and cardiovascular disease risk in the prodromal period before overt cardiovascular disease manifests.

Ambulatory Blood Pressure and the Brain

Ambulatory blood pressure is a non-invasive assessment of blood pressure during daily life, which is to say outside of the clinic. Ambulatory blood pressure is more predictive of target organ damage when compared with clinic blood pressure and bears particular relevance for individuals with elevated or mild hypertension (217). Ambulatory blood pressure is generally assessed for 24-48 hours, with a frequency of measurement ranging from every 15 minutes to once per hour. Because blood pressure is measured for an entire 24-hour period, clinicians and researchers can more completely characterize blood pressure in waking and sleeping hours. Individuals who experience a nocturnal decrease in blood pressure of ≤ 10% are termed "non-dippers," while their counterparts who experience a fall between 10-20%, "dippers," and those with a fall >20%, "extreme dippers" (218). Among individuals with hypertension, failure to achieve a nocturnal reduction in blood pressure of ≥ 10%, termed non-dipping, is associated with an increased risk of future cardio- and cerebrovascular events, as well as target organ damage (*e.g.*, the brain and kidneys) (219–225).

White matter hyperintensities (WMH), which are putative markers of small vessel disease and damage, represent a biomarker of the interaction between cardiovascular and Alzheimer's disease risk during neuroimaging. Hypertension in mid- and late-life is associated with an increase in WMH, although this risk can be reduced with blood pressure treatment (226). Others have reported that higher ambulatory blood pressure irrespective of time (total, awake, or asleep) was associated with significantly greater volume of total, periventricular, and deep WMH (227). Furthermore, greater ambulatory systolic blood pressure, but not clinic systolic blood pressure, is associated with greater WMH accrual among mid-life and older adults (228). These reports, and others (229), suggest that ambulatory blood pressure is more predictive of WMH accumulation and risk for cerebrovascular disease and dementia, particularly among individuals with hypertension. 24-hour ambulatory blood pressure profiles may predict the location and nature of white matter lesions, inasmuch as greater 24h and daytime pulse pressure is associated with a greater volume of WMH, while higher 24h mean arterial pressure was more closely associated with lacunar infarcts (230).

Paradoxically, extreme dipping (nocturnal reduction in blood pressure ≥20%) was also associated with silent cerebrovascular disease and an increased risk for cerebrovascular events (218). Individuals with extreme nocturnal dipping are most likely to experience isolated systolic

hypertension, although the progression of silent cerebrovascular disease may also be explained in part by cerebral hypoperfusion owing to the dramatic fall in nocturnal blood pressure (218). Furthermore, this alleged hypoperfusion may be exacerbated by pharmacological control of blood pressure (231), although others have reported that this reduction in perfusion is transient in nature and cerebral vascular promotes adaptation to normalize perfusion (232). It is unknown if this relationship persists in individuals who experience an improvement in blood pressure as the result of lifestyle interventions such as physical activity, nutrition, or sleep.

Lastly, high blood pressure is associated with increased risk of total brain atrophy (233–237). Interestingly, nocturnal ambulatory systolic blood pressure is most strongly negatively correlated with total brain volume and may serve as a link between blood pressure and brain atrophy (226, 236).

Hypertension and Cerebral Blood Flow

Oftentimes, studies of cerebral blood flow and hypertension examine the synergistic relationship of aging and high blood pressure. For this reason, it can be challenging to elucidate the isolated effects of hypertension on cerebral blood flow, both regionally or globally, refined to white matter or gray matter. Fortunately, the strongest of these studies includes a healthy, age-matched control, and perhaps a young hypertensive group. From these, we can impute with reasonable success the impact of hypertension on cerebral blood flow and perfusion. Cerebral blood flow refers to the total volume of blood that supplies the brain, while cerebral perfusion is blood flow that has been normalized to tissue mass or volume, particularly white and gray matter. Previous data has suggested that small vessel disease may play a causal role in the impairment in cerebral blood flow seen with hypertension (238–240). Indeed, among individuals with hypertension, regional and global cerebral perfusion is reduced relative to their age-matched counterparts (238, 241), although this reduction can be ameliorated through pharmacological interventions to lower blood pressure (242, 243). At present, there is a dearth of research examining the role of lifestyle interventions such as exercise or heat therapy for improving cerebral blood flow in this population.

White Matter Hyperintensities

Clinically, white matter hyperintensities (WMH) have been suggested to be a cause and a consequence of cerebral small vessel disease (244, 245). However, because of the non-specific etiology of the increased signal intensity (*e.g.*, T2-weighted imaging reports increased water at a given location within the brain irrespective of cause rather than in response to a discreet physiological event), as well as the heterogeneity of location, WMH lack strong diagnostic capabilities (246–248).

Longitudinal data among normo- and hypertensive individuals suggests that higher blood pressure at baseline is significantly associated with risk for severe WMH, and that incident hypertension during the follow-up period was associated with greater WMH volume (249). These findings were subsequently replicated among longer follow up intervals in the Rotterdam scan study (250). Collectively, these findings indicate that higher blood pressure in mid-life are associated with increased WMH, and by extension cerebral small vessel disease later in life (244, 249, 251). While the exact pathology is unclear, some have postulated that increased arterial stiffness may be permissive in the development of WMH (252). Indeed, among adults with untreated hypertension, higher arterial stiffness, as measured by pulse wave velocity, was associated with a greater volume of white matter lesions (252).

Arterial Stiffness and the Brain

Large artery stiffening, which occurs in normal aging, can be accelerated by hypertension (253). In recent years, greater arterial stiffness and the subsequent increases in blood pressure and pulse wave velocity have been demonstrated as risk factors for dementias such as Alzheimer's Disease (254). The brain, like the kidney, is a high-flow, low-resistance organ, which renders it particularly susceptible to high pulsatile pressure. These pulsatile pressures ultimately result in microvascular and structural damage (191, 255). Current understanding suggests that large artery stiffening and the concurrent increase in pulse pressure plays an integral role in the manifestation and progression of microvascular disease in the brain and the kidney (131, 191, 256). Furthermore, small vessel damage related to increased pulse wave velocity is undoubtedly related to cerebral hypoperfusion, a contributing factor to Alzheimer's Disease risk (257). As is common with multiple pathologies of cardiovascular disease, elevated systolic blood pressure and the subsequent widening of pulse pressure, or the difference between

systolic and diastolic pressure, may precipitate a decline in endothelial function that is causal in the development of Alzheimer's Disease (256).

There is an increasing body of evidence from large longitudinal clinical trials in support of the role that increased arterial stiffness plays in AD risk. In support of this putative progression, data from the Baltimore Longitudinal Study of Aging report declines in performance on tests of verbal learning, delayed recall, and nonverbal memory with increasing pulse wave velocity (258). Triantafyllidi and colleagues demonstrated a link between cognitive function and large artery stiffness in individuals with never-treated hypertensive individuals with mild-to-moderate essential hypertension (259). Specifically, the authors report that carotid-femoral pulse wave velocity was the only predictor of performance on the Mini Mental State Examination in this population (259). These findings have been supported by Hajjar and colleagues, who demonstrated that individuals with both hypertension and elevated pulse wave velocity presented with the greatest decline in executive score (260). Additionally, the authors argue that carotid-femoral pulse wave velocity is superior to blood pressure in its ability to predict cognitive decline and represents a key outcome target for preventing cognitive decline, particularly in individuals with hypertension (260).

Interventions aimed at improving blood pressure in middle-aged adults have the potential to improve both cardiovascular as well as neurodegenerative disease risk to a greater extent than those implemented later in life (261–263). At present, aerobic exercise is the gold standard for both improving cardiovascular disease risk (264, 265), as well as neurodegenerative disease risk (266), but there is emerging evidence that heat therapy may carry benefits for lifelong and aging brain health (267–270). Lifestyle interventions can improve cerebrovascular health and prevent age- and cardiovascular disease-related declines in cognitive function through putative mechanisms such as: improvements in endothelial function and reductions in inflammation, large artery stiffness, and blood pressure.

EXERCISE AS A LIFESTYLE MODIFICATION FOR CARDIOVASCULAR HEALTH:

Background

The recommendation of physical activity as a primary lifestyle modification for reducing blood pressure originated with statements from governing bodies such as the United States Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the World Health Organization and International Society of Hypertension, and the American College of Sports Medicine (271–273). These position stances were further expanded upon in future statements, such as each subsequent Joint National Committee publication (197, 274). In a now-seminal paper, Morris and colleagues were the first to effectively demonstrate a link between increased levels of physical activity and a reduced risk for coronary heart disease (275). In the intervening years between its publication in 1953 and today, countless studies, both original research and epidemiological investigations, have demonstrated the link between physical activity and improved cardiovascular health. In the interest of brevity, only studies relevant to hypertension will be discussed here.

Physical activity has been long appreciated for its beneficial role in improving cardiovascular, cognitive, and metabolic health. The American College of Sports Medicine recommends that adults should aim for a minimum of 150 minute per week of moderate-intensity aerobic activity each week (276). Additional health benefits may be obtained for physical activity totaling over 150 minutes per week, including greater reductions in the risk for cardiovascular disease such as hypertension. Slightly over half (~53%) of all United States adults over the age of 18 meet the American College of Sports Medicine guidelines for recommended weekly physical activity (5). The inability to meet physical activity guidelines may in part explain the prevalence of hypertension within the United States, as current data suggests that even small amounts of physical activity (less than 30 minutes per day) are capable of significantly reducing premature mortality in individuals that are at risk for coronary heart disease (277, 278). There is a high ceiling for the potential benefits obtained through regular exercise, as vigorous physical activity is strongly inversely associated with coronary heart disease in both men and women (279). The subsections below discuss the data demonstrating the benefits of exercise on the established contributors to vascular dysfunction in essential hypertension.

Exercise and Oxidative Stress

Mild-to-moderate exercise is capable of mitigating the damage of reactive oxygen species on the intrinsic anti-oxidative molecules superoxide dismutase and glutathione (280), as well as increasing antioxidant content (281). In an example of hormesis, endurance exercise training offers protective benefit from the oxidative stress insult of future bouts of heavy exercise (282). Improved antioxidant efficacy is beneficial for mitigating future oxidative stress, but effectively combating the detrimental effects of oxidative stress also requires an intervention that reduces reactive oxygen species production.

In clinical populations, previous data has demonstrated the benefits of exercise training for decreasing the content of pro-oxidative proteins and increasing the content of anti-oxidative proteins (283). In older men with stable coronary artery disease, moderate-intensity endurance-exercise training elicited an increase in superoxide dismutase content as well as increased endothelium-dependent dilation, suggesting a superoxide dismutase-mediated increase in vascular endothelial nitric oxide synthase. While chronically high levels of hydrogen peroxide cause cellular damage, normal physiological concentrations, which result from the neutralization of superoxide by superoxide dismutase during exercise, potentially mediate improved vascular health following exercise training (284). Hydrogen peroxide has been implicated in increased concentration of endothelial nitric oxide synthase both *in vitro* (51, 285) and *in vivo* (286). Taken together, these data suggest that regular physical activity is capable of evoking antioxidative benefits among healthy individuals as well as populations that are characterized by increased oxidative stress, such as those with hypertension and cardiovascular disease (284).

Exercise and Endothelial Function

During exercise, increases in heart rate, cardiac output, and blood flow elicit increases in vascular shear stress, which is both a physiological stimulus for endothelial nitric oxide production, as well as a mediator of increased vascular endothelial nitric oxide synthase expression (287). In cultured human umbilical vein endothelial cells, laminar shear stress is associated with a dose-dependent upregulation of nitric oxide synthase, compared to no increase in nitric oxide synthase with turbulent flow (288). Data from animal models supports the conclusion that chronic intermittent increases in blood flow and, subsequently, shear stress, are associated with an upregulation of vascular nitric oxide synthase activity (289–291).

A series of elegant studies from Green and colleagues were among the first to demonstrate that the improvement in endothelial function with exercise training is a shear-stress- and nitric oxide-dependent phenomenon. Initial studies examined the role of nitric oxide for controlling blood flow to non-active vascular beds in the upper body during lower-body exercise. Using the nitric oxide synthase inhibitor L-NMMA, the authors report that nitric oxide activity is increased in the forearm during lower body exercise (292). These results serve as the foundation for future studies interrogating the role of nitric oxide in improvements in global vascular health following exercise training. Additional investigations demonstrated the importance of shear patterns (either antegrade or retrograde) for the release of nitric oxide during exercise (293). Indeed, endothelial responsiveness to a shear stimulus, such as flow-mediated dilation, is dependent on nitric oxide in conduit vessels (294). Tinken and colleagues demonstrated, using bilateral arm exercise with unilateral vessel occlusion, that shear stress is obligatory for improvements in endothelium-dependent dilation following exercise training (295). Intermittent increases in shear stress with exercise training are requisite for improvements in endothelial function and health with exercise training, both in response to local small muscle mass (296), and whole body dynamic exercise (297, 298).

Endothelial function declines with age, but lifelong exercise as well as exercise training in mid- and late-life have been shown to significantly increase nitric oxide-dependent microvascular function, relative to sedentary controls (299). With regular exercise training, endothelium-dependent dilation increases initially but returns to baseline, while vessel dilatory capacity increases throughout training, indicative of favorable vessel remodeling and increased lumen diameter (300). Regular physical activity has been demonstrated to improve endothelial function among healthy individuals across the lifespan (301–304), those with early-stage cardiovascular disease (305), and populations with established cardiovascular disease (278, 306–309).

Finally, and perhaps most importantly, improvements in endothelial function can occur following exercise training without concurrent improvement in other traditional cardiovascular disease risk factors, such as blood pressure, plasma lipids, or body mass index (310). The previous data set was highly homogenized across the spectrum of health and disease, including individuals with treated and untreated hypercholesterolemia, coronary artery disease, chronic heart failure, type 2 diabetes, and healthy controls (310).

Exercise Interventions to Improve Arterial Stiffness

There is a sizable body of evidence in support of exercise-mediated improvements in arterial stiffness in healthy individuals (311–314), ostensibly through beneficial vascular remodeling, improved endothelial function, or reduced sympathetic outflow (315, 316). The data surrounding the benefits of exercise on arterial stiffness among individuals with high blood pressure, however, is mixed (317). Some reports suggest that exercise can effectively reduce arterial stiffness in those with elevated blood pressure (318–321), while others report no change (317, 322, 323), and still others that initial reductions in arterial stiffness revert to baseline values after cessation of training (324). The results of a recent meta-analysis suggest that aerobic exercise is consistently capable of eliciting reductions in arterial stiffness when accompanied by significant reductions in blood pressure, or when training is prolonged (>6 months) (317).

Aging remains the greatest risk factor for increased arterial stiffness. In the absence of overt cardiovascular disease and a low burden of risk factors, age was the strongest correlate of carotid-femoral pulse wave velocity (325). Exercise is known to mitigate age-related increases in arterial stiffness, wherein more active individuals present with lower arterial stiffness than their sedentary, age-matched counterparts (326–331). Furthermore, exercise training has been shown to improve arterial stiffness in a linear fashion related to the improvements in cardiorespiratory fitness (332).

Life-long aerobic exercise has been shown to slow age-related increases in arterial stiffness, maintaining biologically "younger" aortic age in highly active and aerobically fit older individuals (326–331, 333). Despite these well-established benefits of life-long aerobic exercise, it does not appear that exercise interventions later in life are capable of improving the biological aortic age of formerly sedentary individuals to their fit counterparts, despite improvements in most other metrics (*e.g.*, body mass, cardiorespiratory fitness, diastolic blood pressure) (334).

Exercise and Sympathetic Nervous System Activity

The beneficial effects of regular physical activity on the cardiovascular system may be mediated in part by reductions in sympathetic nerve activity, which exerts potent effects on resting and exercising blood pressure, in both healthy and diseased populations (264, 335–339). To date, there have been several extensive reviews examining the impact of exercise training on sympathetic nervous system activity, with equivocal findings (340, 341). Often, investigations

utilize plasma norepinephrine, local norepinephrine spillover, or direct muscle sympathetic nerve activity recordings to assess changes in sympathetic nervous system activity following exercise training. It is possible that the technique used to quantify sympathetic nervous system activity may influence the interpretation of the impact of exercise on sympathetic neural outflow. On the whole and across all techniques, exercise may have minimal impact on sympathetic neural outflow, but specific investigations suggest that exercise training may reduce plasma norepinephrine levels by up to 40% (264). This is corroborated in part by data suggesting that local renal norepinephrine spillover, but not cardiac norepinephrine spillover, is reduced following exercise training (336). Although this is a single investigation with limited scope, the kidney plays a highly integrative role in long-term blood pressure regulation. Taken together, these data still compellingly demonstrate that exercise training can evoke improvements in sympathetic outflow that may be causal in improvements in cardiovascular health.

To date, there has been one well-controlled study examining reductions in sympathetic neural activity in adults with untreated essential hypertension. Adults with never-before-treated hypertension were randomized into either a 4-month exercise training program or a time-matched sedentary control condition. The authors report that regular aerobic exercise training was capable of significantly reducing resting blood pressure and muscle sympathetic nerve activity (342). Furthermore, exercise training improved baroreflex control of heart rate and blood pressure during the modified oxford technique such that it was significantly different than the non-exercising group and comparable to the endurance-trained normotensive control subjects (342).

In individuals with metabolic syndrome, which is defined in part by hypertension, a combined physical activity and weight loss program reduced both resting muscle sympathetic nerve activity as well as resting whole-body norepinephrine spillover (343). Unfortunately, the authors report that individuals in the structured weight loss without exercise group saw similar reductions in sympathetic neural outflow and norepinephrine spillover (343).

In an additional clinical population, those with a history of uncomplicated myocardial infarction, exercise training significantly reduced systolic blood pressure and muscle sympathetic nerve activity, as well as normalized baroreflex sensitivity compared to non-exercising individuals with a history of myocardial infarction (344). Regular physical activity maintained

these improvements at a 3-month follow-up, such that the experimental group was not different from healthy controls with no history of adverse cardiovascular events (344).

While the fidelity of its representation of cardiac autonomic modulation is contested (345–347), heart rate variability is often utilized experimentally as a non-invasive and easy estimate of cardiac sympathetic and parasympathetic activity. Among individuals with elevated blood pressure and stage 1 hypertension, both aerobic and resistance exercise elicited reductions in low frequency and increases in high frequency power, which represent a decrease in sympathetic and increase in vagal tone, respectively (348). Furthermore, both experimental groups experienced a significant reduction in both systolic and diastolic blood pressure following the intervention (348).

Randomized Controlled Trials: Exercise and Hypertension

A recent systematic review and meta-analysis examining the role of physical activity to prevent and treat hypertension underscores the importance of an active lifestyle for improving blood pressure (349). The studies examined found that the magnitude of change in blood pressure following traditional exercise interventions ranged from 2-7 mmHg and 1-4 mmHg for systolic and diastolic pressure, respectively (264, 265, 350–353). The aforementioned studies were conducted in individuals with pre-hypertension, or more accurately termed "elevated" blood pressure according to the JNC8 guidelines (274). Several other intervention of individuals with established hypertension also support the beneficial effects of exercise on blood pressure (264, 265, 354)

Exercise remains the most impactful lifestyle modification for improving cardiovascular disease risk in individuals with hypertension, (355, 356), comparable to reductions in blood pressure that are seen with initiation of anti-hypertensive treatment (357). There is an inverse relationship between physical activity and cardiovascular disease risk (358), which remains true for both men and women after adjusting for other cardiovascular disease risk factors (359).

Cardiorespiratory Fitness and Cardiovascular Morbidity and Mortality

High cardiorespiratory fitness is associated with reductions in cardiovascular as well as all-cause mortality. In a cohort study of over 250,000 individuals in Sweden, Ekblom-Bak and colleagues reported that with each 1 mL/kg/min increase in VO_{2max}, there is a 2.3% and 2.6%

reduction in cardiovascular disease risk in men and women, respectively (360). For men, this relationship did not plateau over the range of VO_2 values reported, indicating that further increases in cardiorespiratory fitness would correspond to decreases in cardiovascular disease risk and mortality (360). These findings have been substantiated elsewhere, as the results of the Aerobics Center Longitudinal Study report that each 1-MET improvement in during maximal exercise is associated with a 15% lower risk of all-cause mortality, and a 19% reduction in cardiovascular disease mortality (361). More specifically, increases in cardiorespiratory fitness are associated with reductions in the risk of incident hypertension. Juraschek and colleagues reported that greater achieved metabolic equivalents during graded maximal exercise testing corresponds to development of incident hypertension during a 5-year follow-up period (362). Specifically, individuals that reached MET values ≥12 had a 20% lower risk of developing hypertension during the follow-up period compared to those that achieved ≤6 METs (362). Farrell and colleagues examined the potential for a gradient of risk across the range of cardiorespiratory fitness in a large cohort of men studied over a mean follow-up period of 19 years. They report that men in the lowest quintile of cardiorespiratory fitness present with the highest all-cause mortality rates (363)

Hypertension specifically is also intimately linked with cardiorespiratory fitness in both men and women, as reported from a large cohort study of Swedish adults (364), as well as others (365). Holmlund and colleagues report that even after adjusting for changes in smoking, body mass, diet, stress, and exercise habits, a large increase in cardiorespiratory fitness (defined as a ≥3% annual change) is associated with an 11% lower risk of incident hypertension compared to individuals that maintained cardiorespiratory fitness (defined as ±1% annual change). Conversely, a small (-1% to -3%) and large (≥-3%) decrease in cardiorespiratory fitness was associated with a 21% and 25% increase in the risk of incident hypertension, respectively (364). It is clear from these data that maintaining or increasing cardiorespiratory fitness is imperative in reducing both cardiovascular disease risk, as well as all-cause mortality.

Exercise and Improvements in Kidney Function

Due to its tight relationship with the systemic vasculature and integral role in long-term fluid balance and blood pressure, improvements in kidney function are often considered secondary to improvements in blood pressure. Furthermore, much of the existing literature

surrounding exercise training and kidney function has been conducted in individuals with overt chronic kidney disease, type II diabetes, or both. Owing to the relationship between the kidney and blood pressure, these data are often challenging to extricate and apply to other populations, such as middle-aged adults with high blood pressure but without overt cardiometabolic disease other than hypertension.

Albuminuria

Microalbuminuria (30-300 mg albumin excretion/day) is associated with impaired renal endothelial health and higher blood pressure (169). There is compelling data from individuals with type II diabetes that regular physical activity is associated with a reduction in albuminuria (366, 367), while the data from non-diabetic healthy individuals is somewhat equivocal (368, 369). Data from the Nurses' Health Study I and II conducted in the United States from 1976-1997 and 1989-2000, respectively, report a significant negative correlation between albuminuria and regular physical activity (370). This relationship was valid for both low-intensity exercise (quantified as walking time) as well as strenuous activity of any modality (370). Still others report no effect of regular physical activity on albuminuria (369).

Exercise to Improve Cognitive Function and Brain Health

Brain-Derived Neurotrophic Factor

Brain-derived neurotropic factor (BDNF) is released primarily (~70%) from the brain, with supporting contributions from vascular endothelial and smooth muscle cells, BDNF formerly bound to platelets, as well as immune cells (371, 372). Shear stress was an effective stimulus for BDNF release in cultured coronary endothelial cells and possibly from platelets, a response that may potentiate the upregulation of BDNF expression with exercise training (373, 374). BDNF released from the brain is primary concentrated within the hippocampus and cortex, and supports neuron survival and growth, as well as synaptic plasticity (375–379). Various animal models of exercise have demonstrated that exercise reliably and reproducibly increases BDNF concentrations (380–384), while human data demonstrate that BDNF concentration in serum increases following exercise in an intensity-dependent manner (385–387). In an animal model, both daily as well as alternate day exercise were capable of inducing BDNF protein

expression within multiple subregions of the hippocampus, an expression that remained elevated for several days following cessation of exercise (388). The transient, exercise-mediated increases in concentration appear to be a primary mediator of attenuated hippocampal atrophy as well as improved memory function with aging (389, 390). Furthermore, among animal models, BDNF receptor blockade results in attenuated improvements in cognitive function and hippocampal-dependent learning (383).

Hippocampal Volume

The hippocampus is a primary locus of memory in the brain that unfortunately experiences a reduction in volume that is associated with advanced aging and as well as Alzheimer's Disease risk (391, 392). Hippocampal atrophy is accelerated by hypertension, a reduction that is both progressive and cumulative, wherein a longer period of high blood pressure is associated with a greater reduction in volume (234, 393). To date, there have been several studies examining the impact of aerobic exercise training on hippocampal volume, often with equivocal results. Data from randomized controlled trials of humans are largely in support of the benefit of exercise on hippocampal volume. Among healthy as well as patient populations, exercise training has been demonstrated to increase hippocampal volume, with the increase in volume significantly correlated with increases in maximal oxygen consumption (394). Furthermore, aerobic exercise has been demonstrated to increase hippocampal volume among adults following interventions lasting as short as 7 weeks up to 2 years in length (395–397). Moreover, sedentary older adults exhibit improvements in cognitive performance following aerobic exercise, and that the change in aerobic fitness were positively correlated with the change in hippocampal volume (398), replicating previous studies (395, 399). It has been postulated that the increases in volume are the result of neurogenesis (400, 401), or increased angiogenesis (402, 403). Lastly, these improvements are not limited to healthy older adults, but have been demonstrated in adults with mild cognitive impairment (404), as well as probable Alzheimer's Disease (405). Despite compelling data regarding the beneficial impact of aerobic exercise training, hippocampal volume does not always increase in response to exercise training (406) and may regress to pre-intervention baseline in the absence of regular physical activity (399).

White Matter Hyperintensities

White matter hyperintensities, termed because of their high signal density assessed using a T2-weighted or fluid attenuated inversion recovery sequences, are frequently seen with advancing age (244, 245, 407, 408). Additionally, WMH have been implicated in the progression of cognitive decline ultimately resulting in dementia (244, 409), or at the very least are permissive in the manifestation and expression of an overt clinical diagnosis of dementia (410–412). Data from lifelong athletes indicates that compared to their age-matched sedentary peers, master's athletes have significantly lower WMH volume, and that VO_{2max} was significantly negatively correlated with WMH volume (413). The previous study was conducted in a relatively small sample size, but these findings were supported in a larger cohort of adults in their 7^{th} decade, wherein physical activity was associated with reduced cerebral atrophy and less white matter lesions (414). Furthermore, greater physical activity and physical fitness is associated with greater white matter volume (415, 416). The causal link for this relationship is not fully understood, but limited data from meta-analyses suggests that greater physical activity is associated with reduced white matter hyperintensity volume (416).

Cerebral Blood Flow

While the cognitive benefits of exercise are multifactorial, Radak and colleagues suggest that regular increases in cerebral blood flow during exercise is linked to angiogenesis and improved handling of glucose, as well as the upregulation of neurotrophins involved in memory and brain plasticity (417). Indeed, in a large prospective cohort study examining adults aged 65 years old and greater reported that among individuals that perform physical activity ≥3 sessions/week, the rate of incident hypertension was 13.0 per 1000 person-years, as compared to a rate of 19.7 per 1000 person years for individuals that exercise ≤3 sessions/week (418). Additionally, results of the FINE study, examining cognitive decline in individuals aged at least 70 or greater, report that the rate of cognitive decline was proportional to the decline in physical activity later in life (419).

There is an abundance of evidence demonstrating the benefits of exercise on brain health (420–422). Murrell and colleagues demonstrated improved cerebrovascular reactivity and middle cerebral artery blood velocity at rest in both young and older adults following 12 weeks of aerobic exercise training (423). Others have demonstrated improvements in regional cerebral

blood flow and executive function following exercise in both older men (424) and women (425). Data from animal models suggests that increases in vascular endothelial growth factor as well as endothelial nitric oxide synthase may be partially responsible for the improvements in cerebral blood flow following exercise training (426).

Physical activity leads to an increase in cardiac output, which in turn leads to an increase in cerebral blood flow, permitting greater oxygen and nutrient delivery to the cerebral circulation. The effect of this is two-fold: there is (1) an increase in antioxidant enzymes, such as superoxide dismutase, as well as (2) an improvement in glucose handling and metabolism. Collectively, these changes result in angiogenesis, improvements in nitric oxide and endothelial nitric oxide synthase pathways, as well as liberation of neurotrophic factors, such as BDNF (427). The primary responses to these hemodynamic and vascular improvements are neurogenesis, synaptogenesis, as well as neurotransmitter synthesis. These changes have the potential to elicit positive alterations in cerebral structures involved in cognition and memorization, as well as the neural circuits involved in cognition and movement (428, 429).

Despite the compelling nature of these studies, there are still foundational differences between total cerebral blood flow and cerebral perfusion, which refers to blood flow to a specific region, often gray matter and white matter. Substantial pharmacologically-induced reductions in blood pressure in the elderly were associated with significant improvements in gray matter perfusion compared to age matched peers undergoing conventional blood pressure-lowering treatment (242). These data are encouraging insofar as suggesting that ameliorating elevated or high blood pressure has the potential to improve cerebral perfusion.

Cognitive Function

There is a wealth of evidence that suggest an association between regular physical activity and improvements in cognitive and executive function. It is beyond the scope of this review of the literature to discuss the putative mechanisms underlying these improvements in cognitive function. However, a brief discussion of the impact of exercise training on specific, clinically relevant, validated assessments of cognitive function is warranted. Previous data suggests that regular physical activity is correlated with improved scores on the Mini-Mental State Examination (430, 431). Furthermore, in individuals with overt Alzheimer's disease, regular physical activity prevented a reduction in mini mental state examination score, although

exercise was conducted in a group setting which may influence these results (432). In older adults, an acute bout of exercise was associated with improved performance on the mini-mental state examination, a global test of cognitive impairment (433).

HISTORICAL PERSPECTIVES OF HEAT THERAPY

Heat therapy is an ancient practice, often with cultural roots (7). Despite a long history of repeated bouts of heat stress as a mechanism for improved health, relaxation, or greater sense of community, only recently has there been an increase in investigations into the efficacy of heat therapy to improve cardiovascular, metabolic, and cognitive health. There are a host of recent review articles examining heat therapy and potential overlapping mechanisms between exercise and heat therapy (434–437). These reviews highlight the importance of therapeutic alternatives to exercise training for improving cardiovascular health, particularly for populations that are unable or unwilling to engage in traditional exercise training. This overview of the literature is organized according to modality of passive heating, with a principal focus on methods that may be employed chronically for improved cardiovascular, cerebrovascular, and metabolic health. These interventions include Finnish sauna, far-infrared sauna (also known as Waon therapy), Japanese Onsen bathing, Bikram Yoga, and Hot Water Immersion.

Perhaps the greatest limitation to research surrounding chronic hot water immersion for improving cardiovascular health is gaps in our collective understanding regarding the most appropriate “dose” of heat stress to improve outcome measures. To date, there have been several studies that have examined the impact of heat therapy on cardiovascular health across a wide variety of modalities and populations. However, there is a critical need for long term studies examining the impact of heat therapy on cardiovascular health in a controlled research setting. The primary focus of these controlled longitudinal investigations is the elucidation of the ideal timing, dose, and modality of passive heating for improved cardiovascular health.

MODALITIES OF PASSIVE HEATING

Finnish Sauna

Much of our knowledge regarding the cardiovascular health benefits of heat therapy comes from a prospective cohort study of data from the Finnish Kuopio Ischemic Heart Disease Risk Factor Study (7). These data provide insight into the benefits of life-long sauna use regarding cardiovascular health, but are limited in their translatability to other populations, given how common sauna use is in Finland, where sauna bathing has strong cultural roots. Despite the value of these insights, experimental data examining the effects of traditional sauna bathing on vascular and hemodynamic parameters are limited.

Laukkanen and colleagues conducted a prospective cohort study of 2,315 middle-aged men, with annual follow-ups over a median length of nearly 21 years. The authors report that the cumulative hazard ratio for sudden cardiac death was lowest in individuals that used sauna 4-7 times per week, for greater than 19 minutes with each duration (7). There were similarly associated reductions in risk for fatal coronary heart disease, fatal cardiovascular disease, as well as all-cause mortality among men that utilized the sauna with the greatest frequency and longest duration. These findings were validated in a second cohort that included women, further underscoring the efficacy of life-long heat therapy for promoting and maintaining cardiovascular health (438). Subsequent investigations report that among individuals with normal blood pressure at baseline, sauna use is inversely associated with incident hypertension in a dose-dependent manner (439). Among individuals with heart failure, traditional sauna bathing daily for 4 weeks is capable of reducing systolic blood pressure and plasma catecholamines, as well as increasing 6-minute walk test distance and peak oxygen consumption (440).

A recent study from Lee and colleagues has addressed the dearth of long-term clinical trials comparing exercise and heat therapy with each other and with a time-matched control (441). The authors compared supervised exercise training, supervised exercise training with post-exercise sauna, and a control condition for their impact on key outcome variables of blood pressure and cardiorespiratory fitness. The authors reported that compared to the control group, individuals in the supervised exercise group saw significant increases in cardiorespiratory fitness, as well as significant decrease in fat mass (441). When compared with individuals in the supervised exercise group, individuals in the combined exercise and sauna group saw significant

reductions in systolic blood pressure and total cholesterol, as well as significantly greater increases in cardiorespiratory fitness (441). The exercise protocol was created to mirror Finnish national recommendations for physical activity, which in turn are based on those of the American College of Sports Medicine (*i.e.*, 150 minutes per week of aerobic physical activity, two 30-minute resistance training sessions). Despite the efficacy of this physical activity intervention to improve body composition and cardiorespiratory fitness, the authors report no differences in cardiovascular outcomes such as blood pressure or pulse wave velocity (441). The addition of 15 minutes of sauna after exercise three times per week for 8 weeks, however, yielded significant reductions in both systolic blood pressure and total cholesterol, in addition to a significant increase in cardiorespiratory fitness (441). An important caveat is that the study design lacked a sauna-only group, which precluded the authors from comparing the effects of sauna alone with exercise and sauna. This study demonstrates the cardiovascular health benefits of combined sauna and heat therapy, but there is still a critical need to compare heat therapy with supervised exercise training in those with one primary modifiable cardiovascular disease risk factor.

The acute effects of sauna bathing on cardiovascular and hemodynamic function are mixed. Laukkanen and colleagues reported that in individuals with at least one cardiovascular disease risk factor, a single 30-minute sauna bathing sessions elicited significant reductions in carotid-femoral pulse wave velocity, mean systolic blood pressure, as well as mean diastolic blood pressure, but these reductions are limited in duration (442). Further evidence supports this data that a single traditional Finnish sauna session is capable of eliciting desirable hemodynamic and vascular responses, namely reductions in blood pressure and pulse wave velocity (443). Still others have reported that systolic and mean arterial pressure are reduced during, but not following, sauna bathing (444). In the same study, Gravel and colleagues demonstrated that an acute session of sauna bathing did not improve brachial artery Flow-mediated dilation or reactive hyperemia in healthy middle-aged or older adults (444). However, in a follow up study, Gravel et al. reported that a single session of sauna bathing *does* acutely improve peripheral flow-mediated dilation in middle-aged and older adults with stable coronary artery disease (445). Finally, in comparison to a time-matched control, only a combination of aerobic exercise and sauna bathing elicited reductions in recovery and 24h ambulatory systolic blood pressure, while sauna bathing alone elicited no effects on hemodynamic variables (446).

Far-Infrared Sauna and Waon Therapy

Waon therapy, developed in 1989 as a therapeutic alternative to traditional sauna bathing for individuals with heart failure, relies on a lower dry temperature (~60°C) than traditional Finnish sauna (~80-90°C). "Waon" is a combination of words meaning "soothing" and "warmth." The protocol of Waon therapy involves 15 minutes in a dry sauna at 60°C, during which time the anticipated rise in core temperature is between 1.2 and 1.5°C. Following this, individuals rest for an additional 30 minutes covered with blankets to maintain the rise in core temperature during this period (447).

The efficacy of this intervention for individuals with heart failure is evident. Indeed, during a retrospective study following individuals with heart failure during a 5-year period, those who completed Waon therapy presented with a significantly lower incidence of re-hospitalization due to heart failure or cardiac death compared to those who did not partake in Waon therapy (31.3% vs 68.7%, respectively) (448). Individuals with heart failure often present with concurrent vascular dysfunction, as evidenced by reductions in peripheral blood flow and shear stress, which result in reduced release of nitric oxide from the endothelium. Waon therapy has been demonstrated to improve vascular endothelial function, as measured by flow-mediated dilation, in both individuals with coronary disease risk factors (449), as well as those with chronic heart failure (450, 451). The putative mechanisms underlying these improvements in flow-mediated dilation is a shear stress-mediated upregulation of endothelial nitric oxide synthase expression, which has been demonstrated in animal models in response to Waon therapy (452, 453).

Furthermore, endothelial nitric oxide synthase has been implicated in angiogenesis in a mouse model of hindlimb ischemia following Waon therapy. Briefly, the authors report that systemic L-NAME (an endothelial nitric oxide synthase inhibitor) administration abolished angiogenesis in mice undergoing Waon therapy, compared to mice undergoing the same thermal therapy regimen without L-NAME (454). In the same model of hindlimb ischemia in mice, Waon therapy has been demonstrated to upregulate heat shock protein 90 (HSP90), phosphorylated Akt, and phosphorylated endothelial nitric oxide synthase compared to control animals that did not undergo Waon therapy (455). Additionally, HSP90 inhibition prevented this upregulation in mice undergoing Waon therapy, as well as abolishing the attendant angiogenesis (455). Collectively, these data suggest that Waon therapy is a robust stimulus for improved

vascular health, mediated by improvements in endothelial function, with a cellular signature of increased HSP90. Much the same as exercise, shear stress remains an integral component of improvements in vascular health.

Acutely, far-infrared sauna has been shown to increase flow-mediated dilation and antioxidant markers in individuals with chronic heart failure (456). In the same population, four weeks of chronic Waon therapy has been shown to decrease markers of oxidative stress and increase nitric oxide metabolites compared to controls (457). Furthermore, in a comparative animal model undergoing Waon therapy, the authors report a significant upregulation in cardiac expression of HSP27, HSP32, and superoxide manganese dismutase, each of which reduce oxidative stress, compared to animals that did not engage in Waon therapy (457). Additionally, Waon therapy has been shown to increase flow-mediated dilation in individuals with chronic heart failure (451), as well as improving exercise capacity (451, 458).

In isolated human umbilical vein endothelial cells, FIR therapy has been shown to induce Heme-Oxygenase-1 dependent on the NRF2/ARE complex and may also inhibit the expression of endothelial cell adhesion molecules and the adhesion of monocytes to the vascular endothelium (459). Moreover, in bovine aortic endothelial cells, FIR increases endothelial nitric oxide synthase serine 1179 phosphorylation, which results in increases in nitric oxide concentrations as well as intracellular calcium and calmodulin protein kinase II, mechanisms that may underlie the improvements in endothelial function following FIR (460). Others have demonstrated that Waon therapy may result in increased circulation of endothelial progenitor cells, namely CD34+, ostensibly increasing neovascularization (461, 462), although others have reported a decrease in CD34+ cells following Waon therapy (458). In individuals with chronic heart failure, short duration (4 weeks) of Waon therapy has been shown to significantly increase cardiac output and left ventricular ejection fraction compared to conventional chronic heart failure treatment (463). Furthermore, Waon therapy is associated with an increase in high frequency heart rate variability, as well as a reduced LF/HF ratio, both of which are associated with more favorable cardiac sympathovagal balance (463).

Onsen Bathing

In Japan, an Onsen is a naturally occurring hot spring that is often developed into a community-oriented location for hot water immersion. Despite widespread use of Onsen bathing, there are limited population-based studies of hemodynamic and vascular health outcomes among practitioners. One available survey of individuals reports reduced brachial-ankle pulse wave velocity and central pulse pressure in individuals that engaged in Onsen bathing at least 5 times per week (464). Another study examining incidence of cardiovascular disease in men and women who regularly engage in Onsen bathing reports that habitual heat therapy in the form of a hot water bath may reduce the risk of hypertension in women, as well all-cause cardiovascular disease risk in men (465).

Bikram Yoga

Bikram yoga is a style of *hatha* yoga popularized by its eponymous founder, Bikram Choudhury, where practitioners complete 90 minutes of yoga in a hot environment with low relative humidity (40°C and 40%, respectively) (466). There have been limited investigations into the efficacy of Bikram Yoga to improve cardiovascular health in individuals with cardiovascular disease, but there is limited cross sectional evidence to suggests that regular Bikram yoga practitioners present with lower blood pressure than their age-matched counterparts (467). Furthermore, pilot investigations report that a single session of Bikram yoga is capable of eliciting a reduction in diastolic blood pressure that may potentiate long-term changes with regular practice (468).

Longitudinal and controlled investigations present mixed findings. Following an 8-week intervention where a heterogeneous group of middle-aged experienced and naïve practitioners completed between 2 and 7 sessions per week, mean arterial pressure tended to be reduced on the order of 4 mmHg. Although underpowered to draw conclusions from the data, the authors report substantial improvements in systolic blood pressure in heat stress-naïve individuals following the intervention (469). In a separate intention-to-treat intervention lasting 16 weeks, middle-aged individuals with a greater adherence to the experimental protocol of 3-5 Bikram Yoga sessions per week presented with significantly greater reductions in diastolic blood pressure than their less adherent counterparts (470). Conversely, investigations examining the effects of Bikram

yoga on measures of cardiovascular function and disease risk in young, healthy, sedentary individuals demonstrate no greater efficacy compared to a control group (471).

Hunter and colleagues have conducted the greatest number of investigations into the putative mechanisms by which Bikram Yoga can improve cardiovascular health. Following 8 weeks of Bikram yoga, middle-aged and older adults demonstrated significantly improved endothelium-dependent dilation, assessed non-invasively using brachial artery flow-mediated dilation, compared to no improvement among young adults (472). However, subsequent investigations which included a thermoneutral (23°C) control condition that still involved 90 minutes of *hatha* yoga report comparable improvements in endothelium-dependent dilation to the Bikram (40°C) group (473).

Further investigations of the benefits of hot yoga on macrovascular function have focused on arterial stiffness. Following 8 weeks of Bikram yoga, carotid artery compliance and beta-stiffness index was significantly improved in young, but not middle-aged or older adults (474). Additional investigations have demonstrated that longitudinal Bikram yoga interventions can improve brachial-ankle pulse wave velocity in overweight and obese adults, irrespective of changes in blood pressure (475). Finally, limited investigations have demonstrated the efficacy of Bikram yoga for improving metabolic health. Hunter and colleagues report that 8 weeks of 3 sessions per week of Bikram yoga can reduce area under the glucose curve during an oral glucose tolerance test in middle-aged obese adults, but not younger, lean individuals (476).

Collectively, these data demonstrate that regular physical activity in the form of *hatha* yoga can improve endothelium-dependent dilation, and that these results may be potentiated by the presence of hyperthermic conditions that are characteristic of Bikram yoga. As is such with interventions combining physical activity and heat stress, it can be challenging to elucidate whether exercise or heat stress is driving beneficial adaptations. Despite this, compared to thermoneutral control conditions, Bikram yoga elicits improvements in arterial stiffness, endothelial function, and in some cases blood pressure and metabolic health. Taken together, these results suggest that regular practice may evoke more favorable cardiovascular health and cardiovascular disease risk profiles.

Acute Hot Water Immersion

Hot water immersion, often in the form of a hot bath or commercially available hot tub, has recently emerged as a popular modality for research models of passive heat stress for cardiovascular health as well as heat acclimation. Due to the high thermal conductivity of water and high percentage of body surface area submerged, hot water immersion elicits substantial increases in core temperature and sweat rate, both requisite conditions for beneficial adaptations to repeated heat stress (477–480). Research has demonstrated that isolated limb as well as whole-body hot water immersion is capable of eliciting acute and chronic beneficial physiological adaptations at the macro- and microvascular level. The data surrounding these studies is presented below.

Brunt and colleagues report that acute hot water immersion protects against impaired brachial artery vascular function following 20 minutes of forearm ischemia as compared to a time-matched control (481), a finding that was supported in older individuals by Hemingway and colleagues (482). Additionally, hot water immersion preserved microvascular peak reactive hyperemia, suggesting preserved endothelial function (481). Lower limb immersion has also been demonstrated to be effective at maintaining macrovascular function following a simulated ischemia-reperfusion insult. Engalland and colleagues reports that, compared to a sham thermoneutral immersion, lower leg hot water immersion is protective against the damages of ischemia-reperfusion injury in the brachial artery (483). These findings are reminiscent of upper-limb improvements in endothelial function following lower-limb exercise (297).

Akin to exercise, hot water immersion is capable of evoking responses across several organ systems. Leicht and colleagues have demonstrated that an acute bout of hot water immersion is capable of eliciting an increase in IL-6, IL-8, and IL-1ra (484). Similarly, Hoekstra and colleagues report a significant increase in IL-6 following acute hot water immersion in overweight and sedentary men (485). Transient increases in IL-6 are reported to increase anti-inflammatory cytokines, which is suggested to be the mechanism through which chronic heat therapy reduces systemic inflammation.

Thomas and colleagues report that in older individuals with and without peripheral artery disease, systolic, diastolic, and mean arterial pressure are reduced following an acute 30-min bout of hot water immersion (11). Recent data demonstrates that the blood pressure response to an acute bout of hot water immersion is moderately correlated with the change in resting systolic

and diastolic blood pressure (486). The magnitude of this acute blood pressure response was greater than the exercising condition, which completed a high-intensity interval training (486). These results differ from those of Francisco and colleagues, who report similar magnitudes of reductions in blood pressure following exercise and hot water immersion (487). Notably, the individuals in the study by Francisco and colleagues were young, healthy individuals, while those studied by Roxburgh and colleagues were individuals with osteoarthritis and predominantly hypertensive (486).

Combined Exercise and Heat Therapy

There are limited data examining the combined effects of exercise and sauna, either acutely or following training. Acutely, combined aerobic exercise and sauna has been reported to significantly decrease systolic blood pressure for up to 24 hours following the intervention, while diastolic blood pressure was decreased only immediately following (488). Additionally, there is limited data to suggest that with training, a combination of exercise and traditional sauna elicits significantly greater reductions in systolic blood pressure and total cholesterol, as well as increases in cardiorespiratory fitness, compared to either exercise or sauna alone (441). Finally, among adults with untreated hypertension, exercise followed by sauna elicited significant reductions in ambulatory systolic blood pressure, while sauna alone had no effect compared to a control condition (446).

Chronic Hot Water Immersion

Initial studies from Fox and colleagues demonstrate that chronic hot water immersion is capable of eliciting beneficial adaptations to sweat rate, blood flow, and core temperature when undertaken in a controlled laboratory setting (477, 480). These studies were primarily concerned with adaptation to repeated heat stress for physiological resilience, rather than improvements in cardiovascular health. More recently, and in an intervention aimed at improving cardiovascular health, Brunt and colleagues demonstrated that chronic hot water immersion can reduce mean arterial pressure in young, healthy, but otherwise sedentary adults compared to a thermoneutral control, in addition to robust improvements in cardiovascular health as assessed using multiple indices of cardiovascular function. (8). Brachial artery flow-mediated dilation, a measure of nitric-oxide-dependent endothelial function, was significantly improved relative to baseline as

well as the thermoneutral control following chronic hot water immersion. Lastly, individuals within the hot water immersion group demonstrated improved (*i.e.*, reduced) aortic pulse wave velocity, a measure of arterial stiffness (8). These findings were later substantiated by Ely and colleagues, who quantified the effects of chronic hot water immersion on cardiometabolic health in women with polycystic ovary syndrome, a population characterized in part by autonomic dysfunction and cardiovascular disease risk factors, including high blood pressure (9). The authors report substantial reductions in systolic and diastolic blood pressure, as well as improvements in flow-mediated dilation and arterial stiffness in the hot water immersion group compared to the sham immersion group (9). These macrovascular improvements are mediated through cellular and molecular adaptations that are evident with heat therapy. Brunt and colleagues demonstrated improvements in microvascular function through nitric oxide-mediated mechanisms following 8 weeks of passive heat therapy (489). As described above, hypertension is characterized by reduced bioavailability of nitric oxide, and therefore interventions that can improve endogenous endothelial nitric oxide are critical for attenuating cardiovascular disease risk and progression.

A particularly relevant study in furthering our understanding of the benefits of heat therapy in comparison with exercise training examines the role of supervised exercise training compared to passive heat therapy in individuals with peripheral arterial disease (10). Exercise is a primary lifestyle intervention for improving quality of life and reducing cardiovascular disease risk in this population, similar to recommendations for individuals with hypertension (490). Akerman and colleagues compared a traditional exercise intervention with chronic hot water immersion to determine which more effectively increased total walking distance, a key clinical outcome in individuals with peripheral arterial disease. The authors hypothesized that hot water immersion would elicit more robust hemodynamic and cardiovascular improvements than exercise, the functional result of which would be an improvement in walking distance compared to exercise training (10). Both exercise and heat therapy resulted in significant reductions in systolic, diastolic, and mean arterial pressure, but systolic blood pressure was reduced to a greater extent in the heat therapy group (10). These documented improvements in blood pressure bear particular importance for lifestyle modifications for improving cardiovascular disease risk. Many individuals are unable or unwilling to engage in traditional exercise training interventions.

Akerman and colleagues demonstrated greater adherence within the hot water immersion group (10).

MECHANISMS OF IMPROVED CARDIOVASCULAR HEALTH FOLLOWING HEAT THERAPY

The associated benefits of heat therapy range across a spectrum of organ systems, including cardiovascular, cerebrovascular, and metabolic. There are several common mechanisms that underlie improvements in each of these organ systems, including expression of molecular chaperone heat shock proteins, reductions in oxidative stress and inflammation, improvements in vascular function, as well as improvements in autonomic function. The evidence for heat therapy-related improvements in each of these pathways is discussed below.

Improvements in Cellular and Molecular Pathways

Heat Shock Proteins

Originally discovered in response to heat stress in drosophila cells (491, 492), heat shock proteins (HSPs) are highly conserved stress proteins and function as molecular chaperones, serving to help with protein folding or as cellular scaffolding (493). They are classified according to their molecular weight in kilodaltons, with the most studied isoforms being HSP70 and HSP90. As the name suggests, these molecular chaperones are induced following heat stress. As such, HSPs may be induced during repeated heat therapy, by which this pre-conditioning protects against subsequent cellular insult (493). Importantly, there is a wealth of evidence to suggest that intracellular heat shock proteins increase following both heat acclimation as well as chronic heat therapy (494–501).

HSP90

Heat Shock Protein 90 is an essential cofactor in the production of nitric oxide via endothelial nitric oxide synthase, exerting direct effects on the endothelium, and by extension, the vasculature (502–504). HSP90 facilitates the dissociation of endothelial nitric oxide synthase from caveolin, initiating a cascade that results in nitric oxide production and release (502, 505). Beyond the endothelium, HSP90 also has a critical role in the regulation of other nitric oxide

synthase isoforms, such as neuronal nitric oxide synthase (506). The important interdependence of HSP90 and nitric oxide has been demonstrated across multiple vascular beds, including the cerebral (507), mesenteric (508), and pulmonary (509) circulations.

HSP90 is essential for the proper folding of immature endothelial nitric oxide synthase (510), in addition to an central role in signal transduction cascades. Indeed, conformational changes to HSP90 reduce production of nitric oxide and result in greater superoxide production (502). Furthermore, the binding of endothelial nitric oxide synthase to HSP90 prevents the degradation of both proteins (511).

Despite the well-evidenced role of nitric oxide bioavailability for vascular health over the course of the lifespan, data supporting the efficacy of chronic heat stress on nitric oxide and its cofactors is lacking. Our current limited understanding comes in part from work from Brunt and colleagues. In response to a single bout of hot water immersion, protein abundance of HSP90 in peripheral blood mononuclear cells was significantly increased, although this increase was not maintained following 8 weeks of chronic hot water immersion (512). Endothelial tubule formation was significantly increased in cells cultured with serum from individuals after a single bout of hot water immersion as well as 8 weeks of heat therapy (512). Importantly, this tubule formation was prevented when cells were incubated with L-NNA, a nitric oxide synthase inhibitor, demonstrating that increased endothelial tubule formation is a nitric oxide-dependent phenomenon (512). Perhaps explaining this increase in tubule formation is a significantly increased concentration in endothelial nitric oxide synthase abundance in cells cultured with serum from individuals following 8 weeks of passive heat therapy (512).

HSP70

Among the earliest studies to quantify the cytoprotective nature of HSP70 reported that an animal model of whole body hyperthermia induced HSP70 within cardiomyocytes, which in turn was correlated with improved muscle function, as well as reduced markers of muscle damage, following ischemia-reperfusion injury (513). This finding was subsequently supported through a series of studies reporting that heat stress is associated with an increase in HSP70 mRNA (514), as well improved cardiomyocyte viability following ischemia reperfusion insult (515–517). Despite the evidence of protection to insult, some studies have reported that prior hyperthermia does not confer protection to ischemia-reperfusion injury (518–520). It is likely

that the protective effects of hyperthermia are explained by overlapping redundant mechanisms in response to a stressor as potent as ischemia reperfusion. Indeed, multiple studies have suggested that the concurrent upregulation of catalase or manganese superoxide dismutase, both potent antioxidative proteins, is obligatory for induced HSP70 to provide cytoprotection in response to ischemia reperfusion insult (514, 521, 522).

Initial work from Maloyan and colleagues demonstrated that heat acclimation resulted in a greater constitutive expression and concentration of HSP72 and more readily inducible HSP72 gene transcription (523). These adaptations potentiate the response of the organism to a subsequent heat shock. Moreover, the resilience to subsequent heat stress is correlated with the length of heat acclimation (the authors compared the heat shock response after 1, 2, and 30 days of consecutive heat exposure) (523). Notably, following the greatest length heat acclimation, greater cumulative heat strain is required to induce a comparable or greater HSP72 response, indicating improved resilience (523). More recently and in humans, Cheng and colleagues examined the minimum effective dose of lower limb heating needed to elicit acute changes in upper limb micro- and macrovascular health, as well as circulating levels of HSP72 (524). While increases in extracellular HSP72 are not necessarily representative of intracellular concentrations of HSP72, this finding demonstrates support for the efficacy of passive heating for increasing concentrations of heat shock proteins, which are well-demonstrated cellular chaperones with potential to confer physiological benefits.

There is a substantial amount of research examining the small heat shock proteins in response to heat stress in animal models, which is beyond the scope of this review. In an animal model, heat stress upregulated HSP27 and HSP70, the former of which is associated with attenuating vascular smooth muscle cell proliferation following vessel wall injury. HSP70 has also been shown to exhibit similar protective mechanisms against vascular smooth muscle cell hypertrophy induced by angiotensin II (525).

Reductions in Oxidative Stress

There is compelling evidence of a strong interplay between inducible heat shock proteins and increased concentration of manganese superoxide dismutase, offering a putative link between heat therapy and increased resilience to superoxide and hydrogen peroxide. Data from Suzuki and colleagues report that the cellular chaperone HSP72 mediates an increase in

superoxide dismutase activity, which serves to scavenge free radicals and reactive oxygen species following ischemia-reperfusion injury (526). Additionally, in an animal model, both acute and chronic heat stress upregulate HSP70 and superoxide dismutase concentration. Ultimately, HSP27 and HPS70 have been shown to attenuate heat stressed-induced increases in reactive oxygen species (527, 528).

Despite compelling data from isolated cell culture and pre-clinical animal models of acute and chronic heat stress and their impact of reactive oxygen species, there is a dearth of evidence for the efficacy of chronic passive heat therapy on similar benefits in humans. Data from Brunt and colleagues provides insight into the putative mechanisms that mediate these improvements. Both isolated heat treatment of cells (warming to 39°C) as well as exposure of cells to serum from humans who had completed 8 weeks of passive heat therapy reduced basal oxidative stress relative to thermoneutral control cells (37°C) and culture with serum from individuals who did not participate in heat therapy (499). Furthermore, heat treatment and cell culture with serum from those that had completed heat therapy elicited lower concentrations of superoxide anions in response to hypoxia-reoxygenation insult (499). Heme oygenase-1, otherwise known as HSP32, plays a primary role in the regulation of vascular inflammation, protecting the vasculature from oxidative stress and inflammation (529). Brunt and colleagues demonstrated that heat pre-treatment, as well as cell culture with serum from individuals who have completed 8 weeks of heat therapy prevents the suppression of heme oxygenase-1 induction following hypoxia-reoxygenation insult (499). Lastly, HSP70 was increased in response to acute heating, both in isolated cell culture as well as in peripheral blood mononuclear cells from individuals undergoing heat therapy (499). Notably, this increase in HSP70 concentration in PBMCs is maintained following 8 weeks of chronic hot water immersion in young, healthy, individuals (499). Collectively, these beneficial acute and chronic responses to heat stress and heat therapy demonstrate strong benefits for improved vascular function and cellular resilience to stress with passive heating.

Markers of Inflammation and Metabolism

Hoekstra and colleagues examined the acute and chronic effects of hot water immersion on markers of inflammation and metabolism in overweight sedentary men, demonstrating that an acute bout of hot water immersion is capable of increasing plasma IL-6 concentrations (485).

The authors report no significant change in plasma IL-6 after chronic hot water immersion, but despite this there is potential for the acute change in IL-6 to act along a similar pathway to that recognized in exercise to stimulate anti-inflammatory pathways. Namely, increase in concentration of IL-6 has been shown to increase concentrations of IL-10 and IL-1ra, which both act in an anti-inflammatory manner (530).

Improvements in metabolic and inflammatory profiles following chronic hot water immersion have been demonstrated across a diverse subject population, beginning with initial work from Hooper studying hot water immersion in adults with type II diabetes (531). Ely and colleagues examined the ameliorative effects of chronic hot water immersion on both metabolic and inflammatory profiles of women with Polycystic Ovary Syndrome. They report that serum IL-6 and tumor necrosis factor is reduced following chronic hot water immersion (494). Furthermore, IL-1β as well as IL-8, assessed in Stromal Vascular Fraction as measured via adipose biopsy, were reduced following heat therapy (494). In this same population, Ely and colleagues demonstrated a significant reduction in area under the curve for both glucose and insulin during an oral glucose tolerance test (494). Additionally, Hesketh and colleagues report that both heat therapy significantly improves whole-body insulin sensitivity (532), while Hoekstra and colleagues report significant reductions in fasting glucose and insulin following heat therapy (485). Collectively, these improvements in metabolic as well as inflammatory profiles support the therapeutic efficacy of hot water immersion as compared to exercise.

Endothelin-1

Neff and colleagues report that 90 minutes of leg heating in individuals with peripheral artery disease is capable of acutely reducing circulating concentrations of endothelin-1 (533), a finding substantiated by Engelland and colleagues (534). Endothelin-1 represents the most abundant vasoconstrictor in the endothelium, therefore the ability of isolated limb heating to evoke transient reductions may partly explain the associated reductions in blood pressure following acute as well as chronic heating (535).

C-Reactive Protein

Released from the liver in response to inflammatory cytokines, C-reactive protein is a global marker of inflammation and has been identified as a reliable biomarker for stratifying risk for cardiovascular events, with particular utility in the initial stages of disease manifestation and progression (536, 537). Ely and colleagues report that in women with Polycystic Ovary Syndrome, 30 sessions of hot water immersion significantly reduced C-Reactive Protein, indicating reduced global inflammation (9).

Reductions in inflammation may mediate some of the reported benefits of life-long sauna use on cardiovascular and all-cause mortality as described in a series of papers from the Kuopio Ischemic Heart Disease Risk Factor Study (538, 539). Amongst the men included in the prospective cohort study, there was a significant inverse relationship between frequency of sauna bathing and measured C-Reactive Protein, a relationship that persisted after multivariate analysis accounting for age, body mass index, systolic blood pressure, smoking status, type 2 diabetes, history of myocardial infarction, and serum low-density lipoprotein (538).

Improvements in Endothelial Function

Shear Stress and Endothelial Function

Shear stress, or the frictional force exerted by blood on vessel walls, is central to several molecular cascades, such as the production of nitric oxide via endothelial nitric oxide synthase (540–542). Corson and colleagues were the first to demonstrate fluid shear stress-dependent phosphorylation of endothelial nitric oxide synthase (541), a finding that ultimately bears significant relevance on the intact circulation during physical stressors. Shear stress represents the principle physiological stimulus for beneficial vascular remodeling and improved endothelial function following exercise training, as well as isolated increases in shear (296, 543). Indeed, greater increases in antegrade shear stress elicited through different physiological stressors resulted in greater endothelial vasodilator function (295). To that end, when increases in blood flow and shear stress in response to a stimulus such as exercise or heat stress are attenuated, the attendant increases in microvascular and endothelial function are abolished (295, 544).

Shear Stress during Heat Stress

Repeated increases in core temperature, such as those that are seen with chronic heat therapy, have been shown to elicit beneficial increases in conduit artery function, contingent upon vascular shear stress (545). Heat stress-induced increases in blood flow and shear stress are valid in scenarios of lower-limb heating (514) and isolated limb heating (515). Green and colleagues demonstrated that, akin to exercise training, intermittent increases in vascular shear stress are obligatory for improvements in microvascular vasodilatory function following forearm heating via hot water immersion (544). A follow-up study demonstrated that local forearm heating via hot water immersion elicited necessary increases in blood flow to evoke improvements in endothelial function, and that these adaptations are shear-stress dependent (546).

In support of the notion that heat stress is a global cardiovascular stressor, lower body passive heating resulted in a significant increase in upper body conduit artery endothelial function, as determined through brachial artery flow-mediated dilation (524, 545). During isolated limb heating, Chiesa and colleagues demonstrated a reduction in retrograde and oscillatory shear in each of the 3 major arteries of the leg (547), which bears significant therapeutic benefit for endothelial health (31, 105, 107, 108). Furthermore, hot water immersion-induced increases in peripheral artery shear stress are robust in both elderly and clinical populations (447).

Comparisons of Shear Stress during Exercise and Hot Water Immersion

Both exercise and heat stress have been reported to elicit increases in conduit artery shear stress (487, 548, 549). Thomas and colleagues demonstrated that compared with treadmill running, lower body hot water immersion elicits greater increases in total and antegrade superficial femoral artery shear, but only hot water immersion resulted in a decrease in mean arterial pressure (548). Francisco and colleagues demonstrated that both time-matched upright cycling and hot water immersion resulted in an increase in brachial and femoral artery shear stress up to 20 minutes following each intervention (487). Expectedly, upright cycling elicited a significant increase in superficial femoral artery antegrade shear stress for 60 minutes following cessation of exercise (487). Interestingly, hot water immersion resulted in a significant decrease in brachial artery retrograde shear for 60 minutes following exercise compared to both exercise

and baseline (487). Amin and colleagues also report significant increases in brachial and common femoral artery shear stress during passive hot water immersion (549). Amin and colleagues compared change in conduit artery shear during heating with two conditions of exercise: cardiac output- and heart rate-matched (to end heating values). Of these exercise intensities, only heart rate-matched elicited greater increases in both total and anterograde common femoral artery shear stress compared with passive hot water immersion (549). However, hot water immersion resulted in significantly greater brachial artery shear stress compared to both exercise intensities (549). Given the importance of conduit artery shear on potentiating endothelial and vascular adaptations following training (295, 296, 544), repeated bouts of hot water immersion undoubtedly confer cardiovascular benefits much the same as exercise training. These beneficial cardiovascular adaptations are due to shared commonalities between the two stressors, one of which is a marked rise in core temperature. Coombs and colleagues reduced the influence of shear stress via cuff inflation distal to the brachial artery during passive heating. Despite minimizing vascular shear stress, the authors did not abolish improvements in brachial artery Flow-mediated dilation, demonstrating that a rise in core and skin temperature during passive heating contributes to the vascular adaptations following heat stress (550).

Improvements in Nitric Oxide Bioavailability Following Heat Therapy

The above data indicates that hypertension is characterized in part by endothelial dysfunction, mediated through increases in oxidative stress as well as impaired release of nitric oxide from vascular endothelial cells (551). There is convincing evidence that both exercise training and hot water immersion can improve endothelial health, mediated by improvements in nitric oxide bioavailability. The support for these conclusions following heat therapy comes from Brunt et al., who demonstrated that serum from individuals who have undergone acute as well as chronic passive heating elicits nitric oxide-mediated improvements in endothelial cell angiogenesis (512). Furthermore, endothelial nitric oxide synthase abundance was improved by culturing human umbilical vein endothelial cells with serum from individuals who have undergone 8 weeks of passive heat therapy, an improvement that is implicated in greater endothelial tubule formation (512).

Improvements in Arterial Stiffness

Following Acute Heating

Changes in arterial stiffness, often assessed as Ankle-Brachial Pulse Index, Carotid-Ankle Vascular Index, or pulse wave velocity, may reflect changes in blood pressure or vascular tone following an acute bout of heat stress (435). Variability in the method of assessment has led to mixed results within previous literature. Some have reported a decrease in arterial stiffness following heat stress (11, 443, 552), while others (553, 554) have not. Of note, despite no mean difference in arterial stiffness following heat stress, Ganio and colleagues reported that baseline pulse wave velocity is significantly correlated with the magnitude of reduction following heat stress (553), a finding that was subsequently confirmed by Schlader and colleagues (555). Following passive heating, but not exercise in either a hot or cool environment, or a thermoneutral control, Caldwell and colleagues reported a significant decrease in peripheral (radial/carotid) pulse wave velocity (556). It is possible that reductions in retrograde shear stress, as demonstrated by others (557), are permissive in an improvement in arterial compliance leading to a reduction in peripheral pulse wave velocity. Lastly, a single session of lower leg heating was demonstrated to significantly reduce lower limb (femoral-foot) arterial stiffness in young healthy individuals (524).

Following Chronic Heat Therapy

Chronic heat therapy in many forms is associated with an improvement in conduit artery vascular health, undoubtedly contributing to reduced blood pressure following heat therapy interventions. Following an 8-week course of Bikram Yoga, performed in a hot (40.5°C) room, younger adults demonstrated an increase in carotid artery compliance and a reduction in β-stiffness index, while older adults did not (474). Follow-up investigations also failed to reduce arterial stiffness following a 12-week Bikram yoga intervention (558). Brunt and colleagues reported a significant decrease in carotid-femoral pulse wave velocity following 8 weeks of chronic heat therapy in young, sedentary, but otherwise healthy adults (8). Ely and colleagues report that common carotid and femoral wall thickness was significantly reduced following 30 sessions of passive heat therapy in women with Polycystic Ovary Syndrome, accompanying a significant reduction in brachial-ankle pulse wave velocity (9). Others have found that chronic

hot water immersion resulted in reduced carotid intima-media thickness, but without changes to femoral artery intima thickness (8).

Mechanisms of Improved Arterial Stiffness

Joannides and colleagues demonstrated that shear stress is directly related to arterial wall stiffness and compliance *in vivo*. Through blood flow occlusion and hand heating, the authors determined that a decrease and increase in shear stress is associated with an increase or a decrease in radial artery wall stiffness, respectively (559). Mechanistically, modulation of wall stiffness and vascular tone achieved via changes in shear stress during heating is mediated through the actions of nitric oxide, with contributions from endothelium-derived hyperpolarizing factor (560). Bellien and colleagues demonstrated that concurrent inhibition of nitric oxide synthase (via L-NMMA) and endothelium-derived hyperpolarizing factor (via TEA) impaired the decrease in wall stiffness and vascular smooth muscle tone in the radial artery during local hand heating (560). Others (561) have demonstrated a central role of nitric oxide in arterial compliance and pulse wave velocity, suggesting that a loss of nitric oxide, as is associated with the progression of cardiovascular disease, plays a central role in large artery stiffening and disease progression. Therefore, interventions such as heat therapy that improve the bioavailability of nitric oxide may also elicit attendant reductions in arterial stiffness.

Implications For Improved Renal Function

Data from the Health ABC study reports that higher arterial pulse wave velocity is associated with rapid kidney function decline and incident chronic kidney disease (562). Importantly, this relationship was attenuated when adjusted for blood pressure, suggesting that interventions aimed at decreasing arterial stiffness and blood pressure would be beneficial for improved renal function (562). Indeed, in individuals with end-stage renal disease, higher arterial stiffness is associated with an increased risk of all-cause, but primarily cardiovascular mortality (131).

Improved Autonomic Function

During acute passive heat stress, increases in sympathetic nervous system activity promote several cardiovascular and thermoregulatory adjustments that aid in the dissipation of heat. The threshold for these adjustments in humans is as low as a 0.3°C increase in core temperature (563, 564). Among the necessary components to these responses are sympathetically mediated increases in heart rate and cardiac output, reductions in blood flow to the splanchnic and renal vascular beds, and sympathetically-mediated cutaneous vasodilation and blood flow to promote sweating and heat loss (565–569). Akin to exercise, passive heating promotes a distinct recovery period which may be characterized in part by changes in sympathetic nervous systemic activity and autonomic modulation. Furthermore, it is possible that these acute changes in autonomic nervous system activity may inform reductions in resting sympathetic nervous system activity with heat therapy. Despite these compelling hypotheses, there is a dearth of evidence investigating alterations in autonomic function following chronic heating in a controlled laboratory setting. Ecologically, previous data suggests that muscle sympathetic nervous system activity is reduced in warmer summer months compared to winter months (570). Additionally, both short-term (14 days) and long-term (≥30 days) heat acclimation in rodents have demonstrated significant alterations in cardiac autonomic activity compared to pre-acclimation baseline measurements (571). The studies discussed below represent the limited but compelling data from controlled laboratory studies in humans demonstrating more favorable autonomic modulation following passive heat stress.

Following Acute Heat Stress

Romero and colleagues demonstrated that a single bout of lower-leg heating elicited a significant reduction in blood pressure in older, but not younger, adults (572), ostensibly mediated through alterations in neural control of blood pressure or the local release of vasoactive substances, such as endothelin-1 (533). Utilizing muscle sympathetic nerve activity recordings, Engelland and colleagues demonstrated that following 45 minutes of lower leg heating, older adults presented with a significantly lower burst incidence compared to pre-heating baseline values, while younger adults were unchanged (534). Additionally, older adults presented with a significant (~7 mmHg) reduction in mean arterial pressure. Normally, this hypotension would be offset by increased sympathetic nerve activity, but following lower-leg heating there is clearly an

alteration to the neural control of blood pressure among older adults. Moreover, in both young and older adults, plasma endothelin-1 was significantly increased following heating (534). In older adults, despite an increase in endothelin-1, blood pressure remained reduced following lower leg heating, suggesting altered endothelin-1-mediated vasoconstriction in recovery from heat stress. Finally, the authors suggest that the alterations in neurovascular control of blood pressure in older adults following heating may be due in part to the increases in sympathetic nerve activity and blood pressure that occur with advancing age (534). Individuals with essential hypertension are often characterized by increased sympathetic activity relative to their normotensive peers (146), which suggests that passive heating offers therapeutic potential in this population.

Spectral analysis of heart rate variability offers a non-invasive estimate of cardiac autonomic modulation, which may offer some prognostic utility in the absence of techniques such as direct nerve recordings. Gayda and colleagues compared the ability of sauna alone with a combination of sauna and exercise training to impact cardiac autonomic modulation in adults with untreated hypertension. The authors report significant increases in low frequency heart rate variability and reductions in high frequency heart rate variability, ostensibly indicating an increase in sympathetic and a decrease in parasympathetic activity (347, 446, 573). Similarly, others have reported a significant increase in the LF/HF ratio following sauna with post-heating cold-water immersion among both healthy controls as well as individuals with coronary artery disease, suggesting increased cardiac sympathetic activity (574). Conversely, Laukkanen and colleagues report favorable autonomic modulation following a single sauna session, notably an increase in high frequency variability and a decrease in low frequency variability, in the 30 minutes following recovery from sauna bathing (575). The authors suggest that these acute favorable modulations may underpin long-term cardiovascular changes following chronic sauna use.

Following Chronic Heat Stress

Ely and colleagues investigated the potential cardiometabolic therapeutic benefits of heat therapy in women with polycystic ovary syndrome, a population characterized in part by autonomic dysfunction and impaired cardiovascular health (576–578). Following just 5 weeks of chronic hot water immersion, Ely and colleagues report a ~40% reduction in muscle

sympathetic nerve activity burst incidence compared to pre-intervention values (9), a reduction that is comparable to 16 weeks of moderate-intensity exercise training or electroacupuncture in the same population (579). It is likely that this substantial reduction in burst incidence, which was maintained after the completion of all 30 sessions of hot water immersion, play a critical role in the significant reductions in systolic and diastolic blood pressure reported by Ely and colleagues (9). These data are compelling and suggest that heat therapy is capable of eliciting reductions in muscle sympathetic nervous activity among individuals with increased risk for cardiovascular disease, including hypertension.

While not the gold standard, heat acclimation protocols, either active or passive, may offer some utility for inferring the benefits of chronic heat stress on cardiac autonomic activity. Flouris and colleagues report significant increases in frequency domains associated with parasympathetic activity following 14 days of heat acclimation, but that these beneficial changes decay after 2 weeks to baseline levels without further heat exposure (580). Others report a significant increase in cardiac sympathetic activity, with modest increases in vagal tone following acclimation (581). Lastly, short-term passive heat acclimation has no impact on the changes in heart rate variability during a subsequent bout heat stress (582).

Improved Cardiorespiratory Fitness

There is limited, yet compelling, evidence that passive heat therapy may be sufficient to induce improvements in cardiorespiratory fitness. Miyamoto and colleagues report that 4 weeks of daily sauna bathing among individuals with heart failure resulted in significant improvements in 6-minute walk test distance, as well as peak oxygen consumption (440). This finding has been subsequently replicated in young, healthy individuals, with reports of significant increases in maximal oxygen consumption following 8 weeks of chronic warm-water immersion, comparable to increases seen with exercise (583). Hesketh and colleagues report that passive heat therapy, completed as time spent in an environmental chamber at 40°C, can increase maximal oxygen consumption to the same degree as moderate intensity continuous exercise (532). Despite differences in modality, frequency, and duration, the above data suggest that passive heat therapy is capable of increasing cardiorespiratory fitness, an important correlate of all-cause mortality (584, 585).

Benefits of Heat Therapy on Alzheimer's Disease Biomarkers:

Heat therapy in any form is in its infancy as a widely employed therapeutic modality for improving cardiovascular, metabolic, and cerebrovascular health. As such, there is limited data regarding the benefits of heat therapy specifically on improvements in cognitive function and brain health, although this is an exciting area of future research (270). The literature discussed below represent the published data investigating this area, comprised mostly of prospective cohort studies, with controlled laboratory investigations included where appropriate.

A series of papers from Laukkanen and colleagues presenting data from the Finnish Kuopio Ischemic Heart Disease Risk Factor Study examine the relationship between heat therapy, aging, cardiovascular disease, and cognitive function (7, 267, 439, 538). The relationship between cardiovascular and cerebrovascular health is intimately linked, and as such it is unsurprising that improvements in cardiovascular function are permissive to improvements in cerebrovascular function. To that end, Laukkanen and colleagues demonstrated that increased frequency and duration of sauna use is associated with reduced hazard ratios of sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, as well as all-cause mortality (7). The authors suggest several putative mechanisms accounting for these improvements, including improvements in endothelial function, left ventricular ejection fraction, and lower blood pressure following repeated sauna use (7).

A successive study from Laukkanen and colleagues demonstrated a strong inverse relationship between sauna bathing and incidence of Alzheimer's Disease (267). The authors acknowledge that risk for AD is undoubtedly multifactorial, with contributions from aging, vascular and hemodynamic dysfunction, and inflammation. However, sauna bathing has been associated with improvements in cardiovascular disease risk and inflammation (393, 508, 556). Improvements in endothelial function and inflammation with heat therapy are undeniably linked, and regardless of origin, improvements in both contribute to reduced risk for Alzheimer's Disease with advancing age. Additionally, greater frequency of sauna bathing is associated with reduced systemic inflammation (538, 539). While systemic inflammation has been implicated in multiple disease pathologies, it has a principal role in the development and progression of AD (586).

As noted above, sauna bathing is associated with decreased hazard ratios for sudden cardiac death, coronary heart disease, cardiovascular disease, all-cause mortality, and

Alzheimer's disease (7, 267, 587). Hypertension represents a primary risk factor for adverse cardiovascular outcomes, and reductions in blood pressure with sauna bathing may underpin improvements in the above associated risk factors. Indeed, control of blood pressure to normotensive levels is fundamental in improving and maintaining low cardiovascular disease risk. Zaccardi and colleagues demonstrate that frequency of sauna bathing is associated with decreased risk of incident hypertension in the cohort of the Finnish Kuopio Ischemic Heart Disease Risk Factor Study (439). Therefore, the cardioprotective effects of sauna bathing may occur via reductions in blood pressure (399).

Mechanisms of Heat Stress Mediated Improvements in AD Biomarkers

Brain-derived neurotrophic factor has also been shown to increase in response to whole-body passive heating, measured in both serum and plasma (588). In the previous study, both whole-body and lower-body passive heating were effective at increasing serum BDNF, possibly subsequent to significant increase in femoral artery shear rate during heating (588). S100β, a marker of blood-brain barrier disruption, has been demonstrated to increase following exercise in an intensity- and duration-dependent fashion (589), which may be augmented following exercise in a warm environment (590). Collectively, these results suggest that a disruption to the blood-brain barrier following exertion in a warm environment that may be permissive to increases in serum BDNF, although others have reported an increase in serum BDNF following passive heating without a concomitant increase in S100β (591). Still others have reported no significant increase in S100β following passive heating (592), further underscoring the importance of non-cerebral contributions to circulating levels of BDNF following exertion (372).

In a manner like that of exercise, it appears BDNF increases following passive heating in a dose-dependent manner. Following just 20 minutes of acute hot water immersion, BDNF has been demonstrated to increase in healthy men (591). Furthermore, both short-duration (2 weeks) and medium-duration (10 week) passive heating interventions elicited significant increases in serum BDNF (593, 594). Exercise, as described above, has been shown to robustly increase BDNF, and exercise in hyperthermic conditions (30°C) has been shown to elicit significantly greater concentrations of BDNF compared to thermoneutral conditions (18°C) (595). Taken together, these data suggest that heat stress has the capability to increase BDNF above baseline levels, although perhaps not to the same extent as aerobic exercise. Despite this, when coupled with the

alleged vascular benefits of heat therapy, chronic passive heat stress may elicit comparable improvements in AD biomarker risk. Furthermore, heat therapy has been demonstrated to improve endothelial function. Carter and colleagues were among the first to demonstrate that repeated lower limb heating can elicit increases in conduit artery function, dependent on transient increases in vascular shear stress caused by heating (545). These data agree with many previous studies that have shown improved endothelial function following acute (255, 447, 493, 517, 518, 541) and chronic (451, 457, 553) heat stress. To date, two studies that have used chronic hot water immersion for 8-10 weeks have reported significant reductions in arterial stiffness and carotid wall thickness (451, 457). While limited, these data compellingly suggest that heat therapy is capable of eliciting vascular improvements that offer protective benefits for the microvasculature. Finally, the hydrostatic effect of euthermic water immersion has been shown to increase cerebral blood flow velocity (596). It is possible that the thermoregulatory challenge of hot water immersion would further increase shear stress and induce beneficial adaptation integral to improved cerebrovascular health.

CHRONIC HEAT THERAPY – STRENGTH OF THE EVIDENCE

A recent review and meta-analysis from Pizzey et al. examined the strength of evidence for improved health outcomes following chronic heat therapy across several modalities; data in this meta-analysis included both Waon therapy and hot water immersion (436). Using the Cochrane Risk of Bias 2 tool, studies were classified according to their risk of bias, either low, moderate, or high. The authors suggest that there is moderate-to-high risk of bias of overall results among all studies included, driven in large part with concerns over randomization practices and issues with adherence to the prescribed heat therapy intervention (436). Furthermore, the authors classified several outcome variables according to the certainty of evidence. In brief, high certainty suggests that future research is unlikely to alter the certainty of evidence, moderate certainty could possibly be influenced by future studies, while low certainty outcomes are highly likely to be influenced by future data collection. In the present meta-analysis, systolic and diastolic blood pressure as well as resting heart rate were deemed to have moderate certainty of evidence, while mean arterial pressure and flow-mediated dilation were classified as low certainty of evidence (436).

Interestingly, brachial artery flow-mediated dilation was identified as having low certainty of evidence. A consequence of the relatively few studies examining the health benefits of heat therapy is considerable heterogeneity in the populations that have been studied. In reference to brachial artery flow-mediated dilation, even marginal improvements are associated with reductions in the risk of cardiovascular events (90, 597), so while the certainty of this evidence may be low, there is promising upside to the improvements reported by previous authors (8, 9).

In summary, this review highlights the discordance in modality, number of sessions, session duration, and different populations as the primary reasons for limited certainty of evidence among certain outcome variables, further underscoring the need for additional studies to identify the ideal "dose" of heating for therapeutic benefits in healthy as well as diseased populations.

CHAPTER III
EXPLANATION OF THE METHODOLOGY

Overview of the Project

The goal of this dissertation was to compare the efficacy of heat therapy with aerobic exercise training for lowering blood pressure in adults with untreated hypertension. Heat therapy, in the form of hot water immersion, has been shown to improve blood pressure and vascular function in healthy populations as well as those with risk factors for cardiovascular disease (8, 9). Despite this, heat therapy has never been directly compared to the current gold standard for improving blood pressure, aerobic exercise, among adults with untreated hypertension. To investigate this question, we conducted a randomized controlled trial in 41 adults with untreated hypertension, but free from other cardiorespiratory or metabolic disease. Subjects were randomized to complete either 30 sessions of hot water immersion or aerobic exercise training over 8-10 weeks, completing 3-4 sessions per week. At baseline, after 15 sessions, and after 30 sessions, participants completed 24-hour ambulatory blood pressure monitoring and urine collection and resting hemodynamic and vascular function testing. Pre- and post-intervention, all participants completed a cardiorespiratory fitness assessment as well as a battery of cognitive function testing. Lastly, in a subset of eligible participants, we also acquired magnetic resonance-based imaging metrics of Alzheimer's Disease risk. A figure depicting the protocol overview can be found below in Figure 3.1.

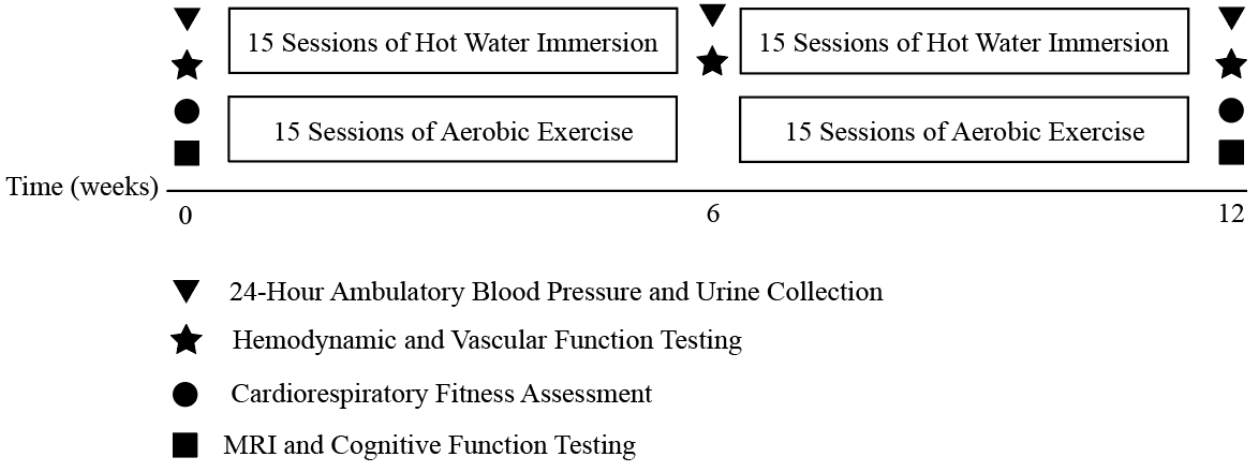

Figure 3.1. Timeline of study. 24-hour data collection was performed at minimum 24 hours following the final bout of hot water immersion or exercise.

Human Participants

All protocols involving human subjects were approved by the Institutional Review Board at the University of Oregon (Protocol # 05042018.006). All subjects gave oral and written informed consent as set forth in the Declaration of Helsinki. The study was registered as a clinical trial on clinicaltrials.gov (Identifier: NCT03557502). The progression of subjects through the study is summarized on the following page. Inclusion criteria included individuals aged 35-60, systolic blood pressure between 120 and 180 mmHg, and diastolic blood pressure between 80 and 120 mmHg. Exclusion criteria included systolic blood pressure >180 mmHg or diastolic blood pressure >120 mmHg; chronic cardiorespiratory or metabolic disease other than hypertension; abnormal resting or exercise electrocardiogram; currently taking antihypertensive medication; BMI ≥ 35 kg/m^2; fasting glucose ≥126 mg/dL; fasting hemoglobin A1c ≥7%; LDL ≥160 mg/dL; high levels of physical activity assessed according to the International Physical Activity Questionnaire (IPAQ); persons who are pregnant, nursing, or currently trying to conceive, aged 34 years or younger, or 61 years and older. Anthropometric data for the individuals that participated in this research can be found below in Table 3.1.

	Aerobic Exercise (EX)	Hot Water Immersion (HWI)
n	20 (7F)	21 (8F)
Age (years)	49 ± 7	47 ± 7
Body Mass (kg • m^{-2})	30 ± 3	30 ± 3
Systolic Blood Pressure (mmHg)	134 ± 11	127 ± 9
Diastolic Blood Pressure (mmHg)	89 ± 7	84 ± 10
# Elevated	4	4
# Stage 1	7	12
# Stage 2	9	5

Table 3.1. Subject Characteristics. Data are mean ± SD.

Screening and Hypertension Classification

All participants were screened by the Research Coordinator (Lindan Comrada) to ensure eligibility for their participation in this research. Interested individuals completed a pre-screening survey to determine their eligibility to participate in this research. A progression of subject involvement in this research can be found below in Figure 3.2.

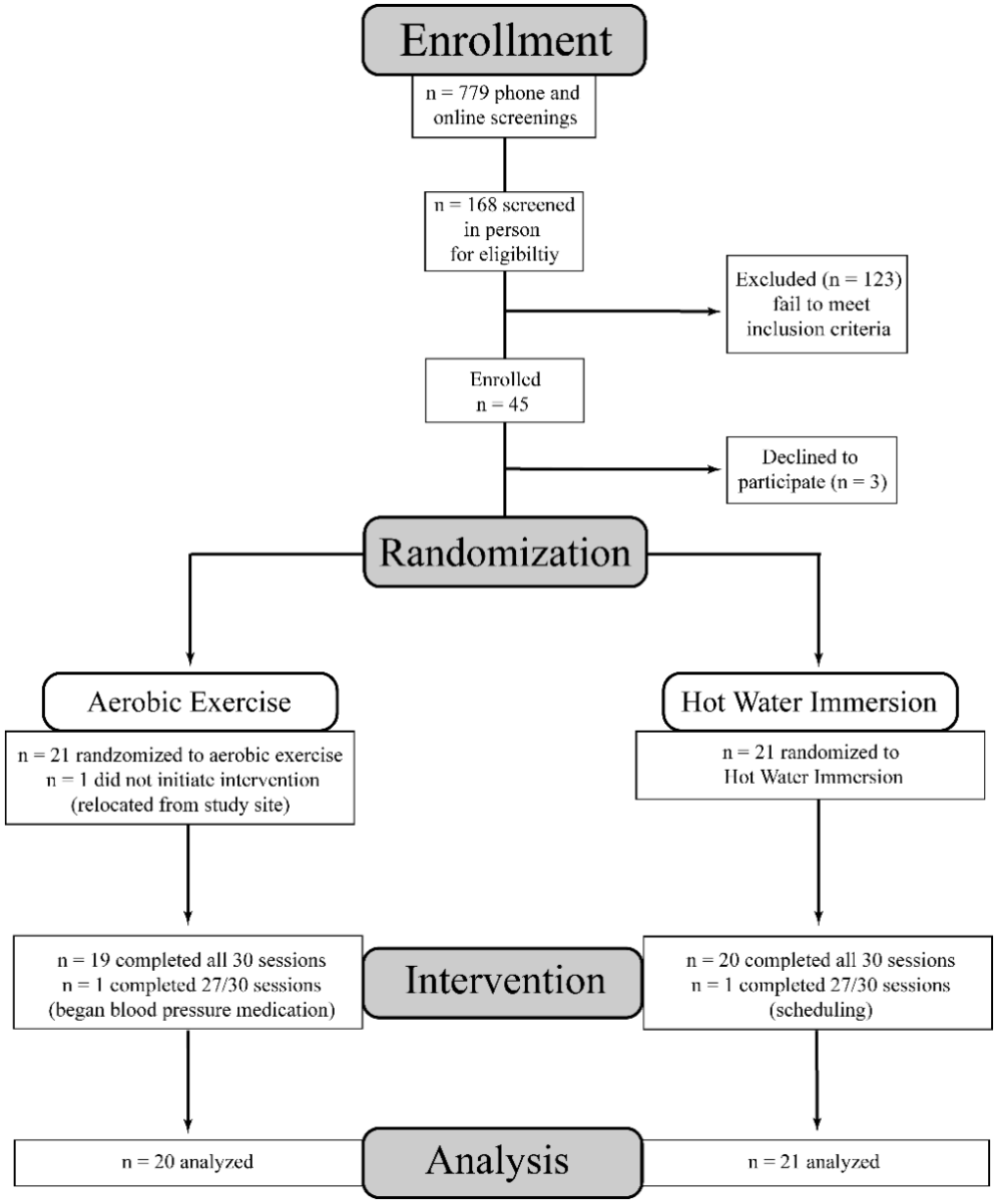

Figure 3.2. Progression of research volunteer involvement.

Volunteers reported to the Bowerman Sports Science Center at Hayward Field for two separate days of screening. Blood pressure was assessed according to current American College of Cardiology and American Heart Association guidelines (3) which consisted of ≥2 readings on ≥2 days, to prevent the mis-representation of blood pressure and the mis-diagnosis of hypertension that is common with a single clinic assessment of blood pressure (598, 599). On the

initial screening day, volunteers were fitted with an oscillometric blood pressure cuff on their upper arm (CARESCAPE Vital Sign Monitor V100, GE Healthcare, Chicago, IL, USA). Blood pressure was measured after 5 minutes of quiet rest in a seated position with legs uncrossed. After 1 minute, the cuff was switched to the opposite arm for an additional measurement of blood pressure. On a separate day, volunteers returned to lab for two additional measurements of blood pressure on whichever arm presented with a higher blood pressure on the initial screening visit. All four systolic and diastolic blood pressures, respectively, were averaged to determine mean systolic and mean diastolic blood pressure. Individuals were eligible to participate if their systolic or diastolic blood pressure was elevated, stage 1, or stage 2 hypertensive, according to the most recent 8th Joint National Committee Report on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Specifically, qualifying blood pressure included systolic pressure between 120-180 mmHg and diastolic pressure between 80-120 mmHg. Additionally, point-of-care assessments of fasting glucose, hemoglobin A1c, and cholesterol were completed (Cholestech LDX Analyzer, Cholestech, San Diego, CA, USA). These point-of-care measurements were used to ensure that prospective participants did not present with type 2 diabetes mellitus or dyslipidemia, as described by the parameters listed above in the exclusion criteria.

During the initial screening visit, all participants conducted an MRI pre-screening form to assess their eligibility to participate in this supplemental arm of the protocol. Individuals were unable to participate in this secondary protocol if they presented with current psychiatric conditions, have ever been unconscious for more than 15 minutes, have had 3 or more losses of consciousness of 5 minutes or greater, have brain or spinal cord injuries, are unable to lie flat for ≥60 minutes, have any artificial or metal devices implanted, have been exposed to loose metal shavings or scraps, or are left handed. Upon arrival to the Lewis Center for Neuroimaging, participants completed a second safety questionnaire to ensure adequate compliance with the necessary safety protocols prior to magnetic resonance imaging. All testing, including screening procedures, occurred at the Bowerman Sports Science Center at Hayward Field at the University of Oregon. Magnetic Resonance Imaging occurred at the Lewis Center for Neuroimaging at the University of Oregon. Prior to randomization, all participants completed baseline testing as depicted in Figure 3.1 and described below.

24h Data Collection

24h Urine Collection

At least 24 hours prior to the resting hemodynamic and vascular function testing, subjects arrived at the lab to receive instruction and materials for 24-hour urine collection. Upon arrival, participants were instructed to void their bladder. Subjects were then provided with two light-resistant 3L containers to collect their urine for the following 24 hours. Upon return to the lab the following day, subjects were instructed to perform one final void into the 24h collection containers prior to investigators drawing a venous blood sample. 24h urine collection was initiated at the same time of day within each subject to account for diurnal fluctuations in urine concentration. 24h urine collection represents the gold-standard technique for assessing urine albumin excretion, although morning first-void samples may be used (600, 601). Thoroughly mixed 24h urine samples were aliquoted into cryogenic tubes and frozen at –80°C until later analysis of urinary biomarkers of interest, described below.

24-Hour Ambulatory Blood Pressure

Ambulatory blood pressure offers substantial prognostic value above blood pressure values acquired in clinic (217, 602–605). Furthermore, ambulatory blood pressure is capable of identifying individuals with white coat hypertension, which is to say those with normal blood pressure that rises during routine, in-clinic assessments of blood pressure (606, 607). Lastly, ambulatory blood pressure provides a more complete diagnostic profile of nighttime blood pressure. This is critical in identifying individuals at greater risk for cardiovascular events owing to their lack of a reduction (≤ 10% reduction from daytime values) in blood pressure during nighttime hours, termed “non-dippers” (608, 609). Non-dipping is prevalent in approximately 35% of adults with essential untreated hypertension (610) and is associated with impaired endothelium-dependent dilation (611). At present, there is limited data examining the effectiveness of interventions such as hot water immersion for improving ambulatory and nocturnal blood pressure profiles in this population.

Subjects arrived at lab at least 24 hours prior to hemodynamic and vascular function testing to be fitted with the ambulatory blood pressure monitor. For measurements of ambulatory blood pressure, subjects were fitted with an oscillometric blood pressure cuff on the upper

portion of their non-dominant arm. The ambulatory blood pressure monitor (Oscar 2, SunTech Medical, Durham, NC, USA) was programmed to inflate and record blood pressure once every 20 minutes during waking, and once every hour during sleeping. In the event of an unsuccessful blood pressure measurement, the Oscar 2 monitor initiates a second attempt 5 minutes following the first attempt to maximize the number of valid readings within the 24-hour recording period. Programmed awake and asleep times were noted and replicated during subsequent MID- and POST-testing visits. During 24-hour ambulatory blood pressure assessment, participants were asked to abstain from all over-the-counter vitamins, medications, and supplements; alcohol; caffeine; exercise and heat therapy; and prolonged time in a motorized vehicle.

24-Hour Dietary Recall and Activity Log

Participants were instructed to complete a 24-hour dietary recall form. From this voluntary dietary recall, researchers were able to estimate 24-hour dietary sodium consumption. Participants were provided with a copy of their initial baseline dietary log at MID- and POST-intervention testing and asked to replicate their diet as faithfully as possible. This was done in an effort to mitigate the effects of fluctuations in dietary sodium intake on blood pressure (612, 613), as well as urinary albumin excretion (614, 615). Additionally, volunteers were asked to provide an hourly summary of their physical activity level during the ambulatory blood pressure measurement period.

Venous Blood Sampling

Venous blood was sampled using venipuncture following a minimum fast of 12 hours. Venous blood sampling occurred at the same time of day under identical conditions to account for circadian fluctuations in blood-borne biomarkers of interest. After disinfection, a 21g needle was inserted into a suitable vein in the antecubital space or dorsal hand. Blood was collected into serum separating vacutainers (BD Vacutainers SST #367988, Becton Dickinson, Franklin Lakes, NJ, USA) Ethylenediaminetetraacetic acid-treated (EDTA #366643) plasma vacutainers, and Sodium Fluoride-treated (NaF #367001) plasma vacutainers. EDTA and NaF plasma samples were processed, aliquoted, and frozen immediately, while serum samples were allowed to clot upright for 30 minutes prior to centrifugation. All samples were centrifuged at 4°C and 1300

RCF for 10 minutes. Samples were then aliquoted into cryogenic tubes and frozen at –80°C until later analysis.

Hemodynamic and Vascular Testing

Volunteers arrived at the lab having abstained from heavy exercise and heat therapy for 48 hours; alcohol for 24 hours; over-the-counter vitamins, medications, and supplements; caffeine; and food for 12 hours. After obtaining a semi-nude height and weight, subjects were instrumented with 3-lead electrocardiogram for the assessment of heart rate and rhythm (CardioCap; Datex Ohmeda, Madison, WI, USA). Blood pressure was assessed via automated auscultation of the brachial artery using a cuff fitted on the upper left arm (Tango M2, SunTech Medical, Durham, NC, USA). Blood pressure was assessed following 20 minutes of quiet supine rest in conjunction with measures of resting cardiac output for the calculation of total peripheral resistance.

Heart Rate Variability

Heart rate variability is a widely used and non-invasive index of autonomic modulation of heart rate. Following 20 minutes of quiet rest in a thermoneutral room (22-24°C), participants were instructed to breathe with a metronome at a frequency of 0.25 Hz, corresponding to 15 breaths per minute. This frequency was selected to minimize potential confounding influence by spontaneous respiration. Respiratory sinus arrythmia, a central respiratory controlled/mediated phenomenon, is directly related to respiratory drive and influences cardiac sympathovagal activity at rest (616). Breathing frequency has been demonstrated to have profound effects on the measured power of cardiovagal rhythms; thus, paced breathing is recommended to improve the reliability and reproducibility of heart rate variability data (617–619).

Heart rate and rhythm were collected for 8 minutes while subjects continued to breathe at the prescribed frequency. Electrocardiogram data were collected at a frequency of 250 Hz and digitized using Windaq Data Acquisition Software (DATAQ Instruments, Akron, OH, USA). Comparisons of 100 Hz and 500 Hz demonstrated significant differences in parameters of heart rate variability, but these differences were not found when comparing 200Hz with 500Hz (620). The utility of heart rate variability to reliably inform researchers about sympathovagal balance requires that the ECG waveform be edited such that it is free of ectopic beats and irregular

rhythms. Ectopic beats originate in locations other than the sinoatrial node and therefore are unable to inform researchers as to the relative influence of either sympathetic or parasympathetic innervation. ECG waveforms were processed by automated marking the peak of each R wave of electrocardiogram waveforms originating at the sinoatrial node, with visual inspection to ensure appropriate and accurate waveform identification.

After each R wave was successfully marked, the initial and final 90 seconds of each recording were removed so that only the interim 5 minutes remained. A recording duration of 5 minutes has been demonstrated to be the optimal duration for detection of high, low, and very low-frequency fluctuations on heart rate (621). Following, a time series data file of R-R interval duration was generated using Advanced CODAS Analysis Software (DATAQ Instruments, Akron, OH, USA). The time series data were then input into a custom LabView program for the analysis of frequency domain measures of heart rate variability (UO HRV_G1, University of Oregon, Eugene, OR, USA). Imported data were first interpolated to 4 Hz to obtain equidistant time intervals and then divided into five equal length overlapping segments of 256 samples each. Each segment was de-trended, Hanning-filtered, and fast-Fourier-transformed to derive a periodogram. The five periodograms were averaged to produce the spectrum estimate for the entire time series. For this procedure, the frequency resolution was 0.016 Hz. We defined the high or respiratory frequency band as 0.15-0.40 Hz, a range inclusive of all respiratory power, and the low frequency band as 0.04-0.15 Hz. Total power was reported as the band $\leq$ 0.40 Hz. Mathematically, very low frequency fluctuations (< 0.04 Hz) in heart rate variability are considered non-harmonic, and therefore do not contribute to the overall variability in recordings of such a short duration (621). To avoid erroneous interpretation of low- and high-frequency components of heart rate variability, very low-frequency power was excluded from this analysis. Data are presented and interpreted as absolute low- and high-frequency power, as well as total power.

Arterial Blood Pressure

Arterial blood pressure was assessed in the supine position using automated auscultation of the brachial artery using a cuff fitted on the upper left arm (Tango M2, SunTech Medical, Durham, NC, USA). Each blood pressure was paired with an assessment of cardiac output, as described below. Blood pressure was assessed a minimum of three times via automated

auscultation. If subsequent values differed by ≥10 mmHg, blood pressure measurement was repeated after 1 minute until values agreed (e.g., ≤ 10 mmHg difference). Mean arterial pressure was used for the calculation of total peripheral resistance and systemic vascular conductance. The equations for mean arterial pressure and total peripheral resistance (Equation 2) are found below:

Equation 3.1 *Mean Arterial Pressure*

$$\text{Diastolic Blood Pressure} + \frac{(\text{Systolic Blood Pressure-Diastolic Blood Pressure})}{3}$$

Equation 3.2 *Total Peripheral Resistance*

$$\frac{\text{Mean Arterial Pressure (mmHg)}}{\text{Cardiac Output (L/min)}}$$

Cardiac Output

Resting cardiac output was assessed via the open circuit acetylene wash-in method, as described previously (487, 622, 623). Initially developed in 1975 (624), and subsequently modified in 1993 (625), this method allows for non-invasive determinations of cardiac output at rest and during exercise and has been validated against direct Fick measurements of cardiac output (626). Resting in a supine position, subjects were instructed to breathe with a metronome at a frequency of 0.25 Hz, or 15 breaths per minute, while fitted with nose clips to prevent mixed nasal and mouth breathing. During expiration, an automatic sliding valve transitioned the inspiratory port from room air to a customized lung diffusion blend, which subjects breathed for approximately 8-10 breaths. The lung diffusion gas blend consisted of 0.6% acetylene, 9% helium, 20.9% oxygen, and balance nitrogen. Acetylene readily diffuses into circulation, while helium is insoluble in blood; therefore, the disappearance rate of acetylene is proportional to pulmonary capillary blood volume, providing an estimate of cardiac output (627). Tidal volume was measured on a breath-by-breath basis using a pneumotachograph (model 4700, Hans Rudolph, Shawnee, KS, USA) connected to a two-way Y-shaped non-rebreathing valve (Series 1420, Hans Rudolph, Shawnee, KS, USA). Tidal volume determinations were performed using the linearized method, as previously described (628). As all cardiac output measurement were conducted at rest, we utilized a pediatric pneumotachograph and two-way non-rebreathing valve

to minimize dead space, which measured 36mL. Breath-by-breath helium and acetylene were measured using a mass spectrometer (MGA 1100, MA Tech Services, St. Louis, MO, USA). Cardiac output calculations were performed using customized software (Beck Integrated Physiological Testing Systems, St. Paul, MN, USA) based on previously published methods (626). Using heart rate measured via electrocardiography during the period where inspired and expired gases are analyzed, stroke volume was calculated as cardiac output (L/min)/heart rate (beats/min) and presented as (mL/beat) (Equation 3.3) at all time points.

Equation 3.3. Stroke Volume

$$\text{Stroke Volume (mL/beat)} = \frac{\text{Cardiac Output } (\frac{\text{mL}}{\text{min}})}{\text{Heart Rate } (\frac{\text{beats}}{\text{min}})}$$

Pulse Wave Velocity

Pulse wave velocity is a robust and reproducible measure of arterial stiffness, with strong predictive value for cardiovascular disease risk (132, 629, 630). Pulse wave velocity is often measured at the common carotid and femoral arteries, for both ease of measurement and prognostic utility for estimating aortic stiffness (131, 132, 631). In the present study, pulse wave velocity was measured using the SphygmoCor XCel system (AtCOR Medical, Naperville, IL, USA). Subjects rested in a supine position for >10 minutes without speaking, and having avoided prior caffeine, food, alcohol, and smoking for at least 3 hours, as set forth in recommended guidelines (632). Subjects were fitted with a blood pressure cuff on their upper left arm. Brachial blood pressure was assessed in duplicate via the oscillometric method prior to initial measurement of pulse wave velocity. Following the measurement of brachial blood pressure, subjects were fitted with an inflatable cuff on the upper portion of their ipsilateral leg. The inflatable cuff on the ipsilateral leg produces a femoral artery pulse waveform using air displacement.

Prior to determination of pulse wave velocity, anatomical measures were taken by two researchers. Researchers measured the distance from the location of the carotid tonometer to the sternal notch, from the sternal notch to the top of the femoral artery cuff, from the location of the strongest femoral pulse to the top of the femoral artery cuff, and lastly from the location of the

carotid tonometer to the top of the femoral artery cuff. Assessments of pulse wave velocity were completed until two values were recorded that were within 0.3 m/s.

The SphygmoCor XCel device calculates carotid-femoral pulse wave velocity according to the estimated distance the pulse wave travels divided by the duration between the foot of the pulse wave of the carotid pulse waveform and the foot of the femoral pulse waveform. Equation 4 describes the calculation of carotid-femoral pulse wave velocity using the SphygmoCor XCel system.

Equation 3.4 *Carotid-Femoral Pulse Wave Velocity*

$$\text{Carotid-Femoral}_{\text{PWV}} = \frac{d_{sfC} - d_{sc} - d_{fTfC}}{tt_{cfC} - k_1 - k_2 * d_{fTfC}}$$

d_{sfC} represents the distance from the suprasternal notch to the top of the femoral cuff. d_{sc} represents the distance from the location of the carotid tonometer to the suprasternal notch. d_{fTfC} represents the distance from the location of the strongest femoral pulse to the top of the femoral cuff. Tt_{cfC} represents the time difference between the foot of the carotid artery waveform, generated using the carotid artery applanation tonometer, and the foot of the femoral artery waveform, generated using the inflatable cuff placed on the upper leg. K_1 is a time constant to adjust for the delay from when air is displaced by the femoral pulse until when the waveform is transmitted to the pressure transducer, compared to the carotid waveform in which the transducer is placed directly against the skin. K_2 represents the time constant proportional to the distance from the location of the strongest femoral pulse to the top of the femoral artery cuff. K_2 accounts for discrepancies in the rate of stiffening with age between the femoral artery and the aorta (633).

The SphygmoCor XCel system has been validated against traditional measurements of carotid-femoral pulse wave velocity, wherein two applanation tonometers are applied sequentially on the carotid and femoral arteries, respectively (634). The SphygmoCor XCel system has been used in clinical populations as well, including individuals with hypertension (635), kidney disease (636), diabetes (637), and coronary artery disease (638, 639). While the device was not used for this purpose during this investigation, the SphygmoCor XCel system is the only device validated by the Food and Drug Administration for non-invasive assessment of central aortic pressure waveforms, demonstrating clinical relevance.

Cardiorespiratory Fitness Assessment

Cardiorespiratory fitness was assessed using an incremental ramp test to exhaustion performed on a stationary bike (Lode Excalibur Sport, Lode BV, Groningen, Netherlands). Whole body oxygen uptake was assessed at baseline prior to randomization and following all 30 sessions of exercise training or heat therapy.

Prior to instrumentation, participants provided a partially nude bodyweight. Participants were instrumented with a heart rate monitor (Polar Electro Inc., Lake Success, NY, USA), a two-way non-re-breathing T-valve to allow for the inspiration of room air and the collection of expired air (Series 2700, Hans Rudolph, Shawnee, KS, USA), and nose clips. Expired air was collected in a mixing chamber for fractional analysis of expired concentrations of O_2 (paramagnetic) and CO_2 (infrared) at 15s intervals (TrueOne 2400, ParvoMedics, Sandy, UT, USA).

Following instrumentation, subjects rested quietly for 2 minutes while seated on the stationary cycle ergometer on which the test was performed. Subjects began exercise with a 4-minute warm-up at a resistance of 30 watts at a self-selected cadence. Ramp rate was either 15, 20, 25, or 30 watts per minute, depending on subject age, sex, and current fitness, with the goal of eliciting volitional exhaustion in 8-12 minutes. Incremental work rate was subsequently replicated at the post-intervention assessment of peak oxygen consumption. Work rate was increased according to the defined ramp protocol until the participant reached volitional fatigue. Criteria for ending the test included: rating of perceived exertion 17-20 on the Borg Scale (640), achievement of age-predicted maximum heart rate (220-age), respiratory exchange ratio $\geq$1.1, a plateau in oxygen consumption despite increasing work rate, and cadence $\leq$60 revolutions per minute for $\geq$10 seconds despite strong verbal encouragement. An acceptable figure constituting a plateau in oxygen consumption is 2.1 ml/kg/min with increasing work rate (641), although this increase is debated by some (642), as it may be influenced by sampling interval of VO_2 (643, 644). Following volitional fatigue and cessation of cycling, subjects completed a cool-down for 10 minutes prior to a supramaximal validation of peak oxygen consumption. In brief, participants pedaled at $\geq$ 60 rpm until volitional fatigue at a constant work rate corresponding to 110% of peak ramp power, to ensure no further increases in peak oxygen consumption at a greater work rate than that which elicited a plateau in oxygen consumption (642). Following supramaximal verification, participants completed a second cool down. Peak oxygen consumption was

measured as the highest 30s average oxygen consumption from either the initial ramp protocol, or the supramaximal verification bout, whichever was greater. Maximal heart rate was determined as the highest heart rate achieved during either the initial ramp protocol or the supramaximal verification bout. This value was then used to calculate heart rate reserve percentages for exercise training sessions.

Intervention Overview

Regardless of group, our intervention included 3-4 sessions per week for a total of 8-10 weeks. Owing to scheduling constraints, illness, and unanticipated travel, multiple individuals required more than 8-10 weeks to complete all 30 sessions. This study was designed as intention-to-treat, wherein all individuals who are randomized and undertook treatment are included in the analysis regardless of the duration of their involvement. This contrasts with a per-protocol design, where only those with strict protocol adherence are included in the final analysis. Fortunately, despite an occasionally protracted duration, all individuals bar one in each group (who each completed 27/30 sessions), completed all 30 sessions of their prescribed intervention.

Hot Water Immersion

The aim of the hot water immersion protocol was to elicit repeated increases in core temperature, a requisite response for adaptation to heat stress (477, 479, 480). Hot water immersion elicits a similar or greater rise in core temperature than moderate-intensity dynamic exercise, owing to its unique thermoregulatory challenges (i.e., reduced effectiveness of sweating under water) (645). The cardiovascular, thermoregulatory, and molecular responses to elevated core temperature, such as increases in cardiac output, vascular shear stress, sweat rate, and heat shock protein expression, have been identified as mediators of the beneficial adaptations following heat therapy (434, 435, 478, 545). The protocol utilized in this dissertation was designed based upon previous research within our laboratory reporting significant improvements in micro- and macrovascular function in healthy individuals as well as women with polycystic ovary syndrome following chronic hot water immersion (8, 9, 489). Lastly, the duration of hot water immersion was time-matched to the duration of the aerobic exercise intervention, described below.

Upon arrival at lab, individuals in the hot water immersion group provided a urine sample for the assessment of urine specific gravity to ensure adequate hydration prior to immersion. If urine specific gravity was ≥1.024, subjects were instructed to drink 5 mL of water/kg of body weight prior to immersion. After providing a urine sample, subjects provided a dry nude bodyweight prior to changing into suitable clothing for hot water immersion. Subjects were instrumented with a heart rate monitor (Polar Electro Inc., Lake Success, NY, USA) prior to immersion.

During hot water immersion, subjects were immersed to the level of the sternum in a 40.5°C water bath. Upon achieving a core temperature of 38.5°C, a fan was turned on, although subjects remained immersed. Previous research has demonstrated that core temperature in the range of 38.0-38.5°C is obligatory for the increased expression of heat shock proteins, cellular mediators of the benefits of heat therapy (434, 479), as well as thermoregulatory adaptations to heat stress (479, 480). While immersed, water was provided *ad libitum.* Immersion lasted 45 minutes, with a 5-minute recovery period outside of the hot tub. As this duration is shorter than previous research that reports beneficial adaptations following heat therapy, participants remained immersed once core temperature reached 38.5°C, rather than move to a more partially immersed position as was done in some prior studies (8, 9). A representative core temperature tracing can be found in Figure 3.3.

After the 5-minute seated recovery following immersion, subjects toweled dry and provided a second dry nude body weight. If body weight loss via sweat was not compensated via water intake such that body weight loss was <1%, additional fluids were provided prior to subjects leaving the lab. For the first 5 and 30th sessions, individuals in the hot water immersion were provided with an ingestible core temperature pill (HQ Inc., Palmetto, FL, USA). Subjects were asked to ingest the pill at least 6 hours prior to arriving at lab. Core temperature was measured at baseline and every 5 minutes during immersion and recovery.

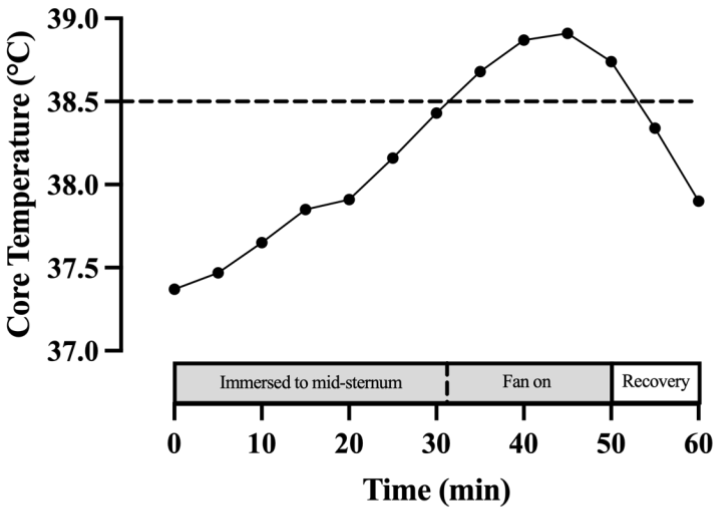

Figure 3.3. Representative core temperature tracing from one participant during hot water immersion. Baseline (time = 0 min) and recovery is in a seated position in a thermoneutral (22-24°C) room.

Aerobic Exercise Training

The target duration of exercise sessions was selected to meet the 150 minutes/week of moderate-intensity aerobic exercise per guidelines from the American College of Sports Medicine. All exercise training sessions were conducted on commercially available stationary bikes (Precor UBK 635, Woodinville, WA, USA). Individuals in the exercise group were instrumented with a heart rate monitor (Polar Electro Inc., Lake Success, NY, USA) prior to exercise. Subjects completed a 5-minute warm-up at 30% of heart rate reserve, calculated according to the Karvonen Method in Equation 3.5 (646).

Equation 3.5 *Heart Rate Reserve*

%HRR (Maximum Heart Rate – Resting Heart Rate) + Resting Heart Rate

Following the 5-minute warm-up at 30% of heart rate reserve, subjects completed 40 minutes at 60% of heart rate reserve, followed by a 5-minute cool-down at 30% of heart rate reserve. Water was provided during exercise *ad libitum*. Prior to the first and last exercise sessions, subjects in the exercise group were provided with an ingestible core temperature pill (HQ Inc., Palmetto, FL, USA) for non-invasive measurement of core temperature. Subjects were asked to ingest the

pill at least 6 hours prior to arriving at lab. For comparison purposes, a representative tracing of core temperature during aerobic exercise is included below in Figure 3.4.

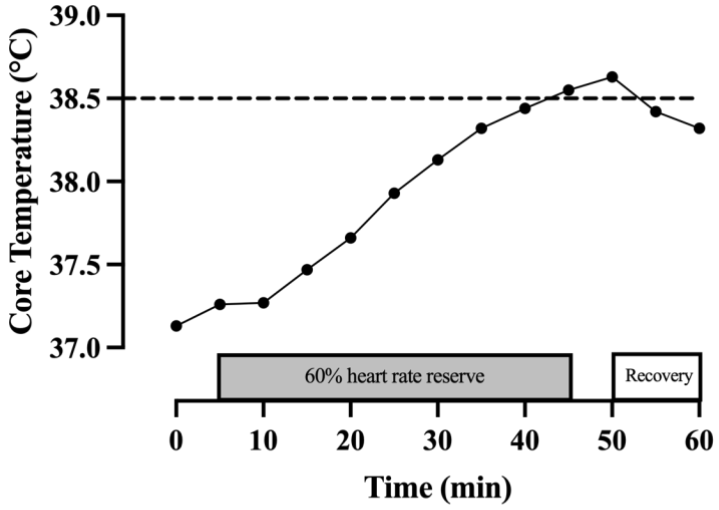

Figure 3.4. Representative core temperature tracing from one participant during aerobic exercise on an upright stationary cycle ergometer. Warm-up and cool-down period are 5 minutes each at 30% of heart rate reserve.

Post-Exercise and Post-Heating Hypotension

Passive heating evokes a distinct recovery period, similar to aerobic exercise. At present, there is compelling evidence that the blood pressure response to an acute bout of exercise may predict the blood pressure response to chronic exercise training (647–650). Furthermore, recent data from our laboratory suggests that hot water immersion elicits a reduction in mean arterial pressure that is comparable to aerobic exercise in young, healthy individuals (487). Taken together, it is possible that the blood pressure response to a single bout of passive heating among adults with untreated hypertension may predict the blood pressure response to chronic passive heating. To investigate this potential relationship, we measured the blood pressure response to a single bout of aerobic exercise or hot water immersion in stress-naïve participants.

Subjects were instructed to avoid all over-the-counter vitamins, medications, and supplements for >12 hours; heavy exercise and heat therapy for >12 hours; caffeine for >6 hours; alcohol for >12 hours; and food for >2 hours prior to arrival at lab. After ensuring adequate hydration status as described above and changing into clothing for either exercise or hot water immersion, subjects were instrumented with 3-lead electrocardiogram for the measurement of heart rate and rhythm (CardioCap; Datex Ohmeda, Madison, WI, USA), as well as an

auscultatory blood pressure cuff fitted on the upper left arm (Tango M2, SunTech Medical, Durham, NC, USA). Following 20 minutes of quiet supine rest in a temperature-controlled room (22-24°C), blood pressure, heart rate, and core temperature were measured in triplicate, each separated by ≥1 minute. Following these measurements, subjects completed either 50 minutes of exercise, or 45 minutes of hot water immersion with a 5-minute recovery. Immediately following, subjects returned to a supine position and were instrumented with 3-lead electrocardiogram and a blood pressure cuff on their upper left arm. Blood pressure, heart rate, and core temperature were measured every 5 minutes thereafter for 60 minutes to determine the blood pressure response to an acute bout of either exercise or hot water immersion in adults with untreated hypertension. We determined the mean reduction in blood pressure as well as the area under the curve of blood pressure for post-intervention values vs. pre-intervention values to further describe the predictive value of the magnitude of post-exercise and post-heating hypotension.

Biochemical Blood and Urine Analysis

Urine Albumin. 24-hour urine albumin excretion was quantified via colorimetric assay (Human Albumin ELISA kit, ab108788, abcam, Waltham, MA, USA) using samples from a mixed aliquot of 24-hour urine sample. Samples were diluted 400-fold to ensure optimal assay performance against recombinant urine albumin standards. Urine albumin excretion at high concentrations is correlated with cardiovascular and well as chronic kidney disease (173–175). Microalbuminuria, defined as a range 30-300mg of albumin excreted in urine during a 24h collection period, is associated with high blood pressure as well as the risk for future cardiovascular disease progression (176–178). However, urine albumin excretion as low as 5mg/24h is associated with increased risk of developing hypertension and cardiovascular disease (180–182).

Urine Creatinine. 24-hour urine creatinine excretion was quantified via enzyme-linked immunosorbent assay (catalog no. KGE005, R&D systems, Minneapolis, MN, USA) using samples from a mixed aliquot of 24-hour urine sample. The gold-standard assessment of urine albumin excretion is performed using a 24h urine collection; however, urine creatinine excretion is often used clinically to normalize single-void or limited time-frame urine collections (651, 652).

Serum Creatinine. Serum creatinine was quantified via colorimetric assay (Item no. 700460, Cayman Chemical, Ann Arbor, MI, USA). Serum creatinine concentration was used to calculate estimated glomerular filtration rate using the chronic kidney disease-epidemiological equation without adjustment for race, which has been suggested to have greater prognostic utility (151, 653). Despite its widespread use clinically, serum creatinine-based calculations of estimated glomerular filtration rate (Equations 3.6a and 3.6b) are not without their limitations. Serum creatinine is influenced by renal tubular creatinine secretion (147) as well as patient lean body mass (152), which often results in erroneous calculations of estimated glomerular filtration rate.

Equation 3.6a. *Estimated Glomerular Filtration Rate when Serum Creatinine >0.905 mg/dL*

$$eGFR_{cr} = 142 \times (S_{cr}/\kappa)^{-1.200} \times 0.9938^{Age} \times 1.012 \text{ [if female]}$$

Equation 3.6b. *Estimated Glomerular Filtration Rate when Serum Creatinine <0.905 mg/dL*

$$eGFR_{cr} = 142 \times (S_{cr}/\kappa)^{\alpha} \times 0.9938^{Age} \times 1.012 \text{ [if female]}$$

where S_{cr} is serum creatinine concentration, and α is a constant that accounts for biological sex (0.9 for biological male, 0.7 for biological female).

Serum Cystatin C. Serum Cystatin C was quantified via enzyme-linked immunosorbent assay (R&D Systems, catalog # DSCTC0, Minneapolis MN, USA). Serum samples were diluted 30-fold to ensure optimal assay performance against recombinant cystatin C standards. Serum Cystatin C was used in the calculation of estimated glomerular filtration rate at all time points. Serum Cystatin C can be used to estimate glomerular filtration rate, as described below in Equations 3.7a and 3.7b (151). Compared to serum creatinine, serum cystatin C is more linearly related to declining kidney function and incident chronic kidney disease (153, 155, 156).

Equation 3.7a. *Estimated Glomerular Filtration Rate when Cystatin C <0.8 mg/L*

$$133 \times (S_{cys}/0.8, 1)^{-0.499} \times 0.996^{Age} \times 0.932 \text{ [if female]}$$

Equation 3.7b. *Estimated Glomerular Filtration Rate when Cystatin C >0.8 mg/L*

$$133 \times (S_{cys}/0.8, 1)^{-1.328} \times 0.996^{Age} \times 0.932 \text{ [if female]}$$

Serum Brain-Derived Neurotrophic Factor. Serum Brain-Derived Neurotrophic Factor was quantified via enzyme-linked immunosorbent assay (Biosensis catalog # BEK-2211, Thebarton, Australia). Samples were diluted 100-fold to ensure optimal assay performance against recombinant human BDNF standards. Serum BDNF is a significant mediator of reduced hippocampal volume and poor memory performance in mid- and late-life, even when account for age-related declines in hippocampal volume and cognitive function (654). Lower hippocampal volume is intimately related to incident dementia in later life (655), and is significantly correlated with worse memory function assessments (656).

Endothelin-1. Endothelin-1 was quantified using enzyme-linked immunosorbent assay of EDTA-treated plasma samples (R&D Systems, Catalog #: DET100, Minneapolis, MN, USA). Endothelin-1 has been identified as a contributor to increased oxidative stress and endothelial dysfunction (54), mediating the development and progression of hypertension (76–78). Furthermore, endothelin-1 may play a causal role in increased arterial stiffness, which is associated with worse target organ damage (e.g., brain and kidneys) (85, 86).

Interleukin-6. Interleukin-6 was quantified using a high-sensitivity enzyme-linked immunosorbent assay (R&D Systems, catalog #: HS600C, Minneapolis, MN, USA). IL-6 is a pro-inflammatory cytokine that has been identified as a potential mediator of inflammatory dysregulation that may underlie essential hypertension (657). Transient and repeated increases in IL-6 may be responsible for decreases in chronic inflammation that have been reported with heat therapy (485, 494).

C-Reactive Protein. C-Reactive Protein was quantified using an enzyme-linked immunosorbent assay (R&D Systems, catalog # DCRP00B, Minneapolis, MN, USA). EDTA-treated plasma samples were diluted 100-fold to ensure optimal assay performance against recombinant CRP standards, according to manufacturer instructions. CRP offers diagnostic value for predicting future cardiovascular event risk (536, 537), and has been suggested as an independent risk factor for essential hypertension (70).

Cognitive Function Testing

Cognitive function was assessed at baseline and after completion of all 30 sessions using the freely available NIH toolbox. This cognitive function battery has been implemented in a wide variety of investigations and was designed to create a standard assessment of cognitive function across differing populations (658). Of particular interest were working memory and episodic memory, assessed using a list sorting task and a picture sequence task, respectively. Both working memory (659–661) and episodic memory assessments (662–664) have been validated for use in adults as well as across the lifespan.

At baseline, all participants completed the mini mental state examination (MMSE). The MMSE was initially conceived as a rudimentary assessment of cognitive function, with an emphasis on identifying mild-to-moderate cognitive impairment (665). The MMSE can be influenced by factors such as age (666), language (667), education (668), socioeconomic status (669), among others. While the MMSE is not the most complete assessment of mild-to-moderate cognitive decline (670), it offers the ability for a rudimentary assessment of cognitive function (671–673).

Finally, all participants completed the Pittsburgh Sleep Quality Index (PSQI) at baseline and after the intervention. Previous data has indicated that poor sleep quality, quantified using the Pittsburgh Sleep Quality Index, is an independent predictor of hypertension (674–676). Indeed, a higher PSQI score, indicating worse sleep, was associated with over a two-fold higher risk for hypertension compared to those with higher quality sleep (675). Poor sleep quality, particularly in midlife, is associated with worse episodic memory task performance as well as increased risk for cognitive decline in later life (677, 678).

Magnetic Resonance Imaging

In a subset of eligible participants, a series of magnetic resonance imaging metrics were acquired to quantify Alzheimer's disease and related dementia risk factors. For the present study, we conducted a scan sequence lasting approximately 1 hour. All scans were conducted at the Lewis Center for Neuroimaging at the University of Oregon using a 3T MRI machine using a Siemens 32-channel head coil with optimized sequences (Siemens Skyra, Erlangen, Germany). Prior to entering the scanning facility, participants changed into clothing free of ferrous metals.

High-resolution (1.0 x 1.0 x 1.0 mm) T1-weighted brain images were acquired using a 3D magnetization-prepared rapid gradient echo imaging protocol (679), TR/TE/TI = 2500ms/3.43ms/1100ms, FOV= 256mm, matrix size = 256x256, with 176 contiguous slices in ascending fashion. Furthermore, T2 sagittal turbo spin echo images (1.0 x 1.0 x 1.0 mm isotropic voxel resolution) were acquired with 192 slices per slab in ascending fashion. To assess white matter hyperintensities, we employed Fluid Attenuated Inversion Recovery in the axial plane, TR/TI/TE = 9000ms/113ms/2500ms, 1.0mm x 1.0mm x 2.5mm resolution, parallel imaging with a flip angle of 120°. Diffusion imaging was conducted in 4 runs, lasting 5 minutes each, TR/TE/ = 3222ms/89.2ms, 1.5mm isotropic voxels with whole brain coverage (92 contiguous slices), FOV = 210mm, with optimal directions at each b-values based on previously published literature (680). Arterial spin labelling was acquired using simultaneous multi-slice 2D echo-planar imaging for high spatial resolution whole-brain pseudo-continuous arterial spin labelling (pcASL) imaging. Images were acquired with 2.5mm in-plane resolution with 60 2.27mm thick slices with a 10% slice gap, and 5 post labelling delays (PLDs: 0.2, 0.7, 1.2, 1.7, 2.2). Hippocampal volume was assessed using T2- oblique coronal turbo spin echo imaging with 0.39mm x 0.39mm x 2.0 mm voxel resolution, TR/TE = 13520ms/88ms, echo train length = 15ms, bandwidth = 222Hz/pixel, echo spacing = 11.1ms, FOV = 220mm, 65 slices, with a flip angle = 150°. Hippocampal volume was segmented and corrected for total intracranial volume for comparisons before and following each intervention.

Magnetic Resonance Image Analysis

Functional magnetic resonance image BOLD was analyzed using a modified paradigm of the Human Connectome Project – Aging consortium (681), with anterior-to-posterior phase encoding. White matter hyperintensities were assessed using Schelten's Scale applied to T2 and FLAIR images (682, 683). From this, whole-brain white matter scores were calculated, and log transformed for subsequent analyses. Arterial spin labelling was analyzed according to the most recently released system parameters of the Human Connectome Project – Aging (681). To most accurately account for changes in arterial transit time documented with aging (684), multiband echo planar imaging was used in conjunction with 5 post-label delays. Anatomical images were pre-processed using Free Surfer (7.4.1., Harvard University, Cambridge, MA, USA). DICOM files corresponding to each post-label delay were converted to NIfTI and merged prior to

analysis using BASIL (Bayesian Inference for Arterial Spin Labelling; v.6.0.1, University of Oxford, Oxford, UK). BASIL allows researchers to quantify resting gray and white matter perfusion, with corrections for bolus duration, post-label delay, and partial volume correction. Using anatomical calibration images, we analyzed merged NIfTI files for mean gray matter and white matter perfusion, mean arrival time in gray matter and white matter, as well as the perfusion calibrated values for both gray and white matter. Lastly, volumetric segmentation of the hippocampus and estimates of total intracranial volume were performed using FreeSurfer (7.4.1., Harvard University, Cambridge, MA, USA). Hippocampal volume was normalized to estimated total intracranial volume for pre- to post-intervention and between-subjects comparisons.

Statistical Analysis

Subject characteristics throughout are presented as means ± SD and were compared using an unpaired two-tailed Student's *t-test*. Primary outcome variables for each specific aim were analyzed using linear mixed-effects models, completed in collaboration with a biostatistician from Oregon Health & Science University. The linear mixed-effects models included group and timepoint main effects with an interaction effect to determine changes over time within groups. Random effects were included to account for correlation of measurements within patients. Mixed effect models were performed using the lme4 package (v1.1-33). All analyses were performed in R (v4.3.0) with an alpha of 0.05 used to determine significance. Secondary outcome variables for each specific aim were analyzed using a two-way repeated-measures analysis of variance with main effects of group and time. In the event of a significant main effect, multiple comparisons were completed using the Sidak analysis. Significance was accepted as $p<0.05$. Secondary outcome variable statistical analysis was completed in GraphPad Prism 10.1.0 (GraphPad, Dotmatics, San Diego, CA, USA).

CHAPTER IV
KIDNEY FUNCTION AND AMBULATORY BLOOD PRESSURE

INTRODUCTION

The kidney plays a central role in long-term blood pressure regulation, through coordination of fluid volume and electrolyte balance, as well as hormonal axes such as the renin-angiotensin-aldosterone system. As a low-resistance, high-flow organ, the kidney is highly susceptible to damage from high blood pressure, which may be exacerbated by common vascular complications of cardiovascular disease, such as increased arterial stiffness (192, 194). Impaired renal function is intricately linked to poor cardiovascular health, as well as increased mortality among individuals with hypertension (191, 685).

Albumin, an abundant protein found in blood, is commonly measured in the urine to estimate glomerular damage. Elevated arterial blood pressure causes damage to the basement membrane of the glomerulus, the filtration unit of the nephron within the kidney, allowing increased albumin excretion in urine (686, 687). Individuals with essential hypertension consistently present with greater urine albumin excretion than their normotensive peers (688–691). Elevated urine albumin excretion is also significantly associated with the development of hypertension in normotensive individuals (174). The predictive relationship between albumin excretion and risk of hypertension is evident at values as low at 5mg/24h, well below the clinical cut-off for macroalbuminuria, 300 mg/24h (174). Clinically, 24h urine albumin:creatinine ratio offers the strongest predictive value for the development of future hypertension among adults with elevated blood pressure according to the most recent JNC8 guidelines (692). The relationship between reduced blood pressure and reduced urine albumin excretion has been demonstrated using pharmacological intervention to lower blood pressure (693, 694), but not through lifestyle interventions. Among populations with overt impairments in kidney function, reductions in urine albumin excretion are associated with improvements in renal function as assessed by estimated glomerular filtration rate (695). Taken together, these data suggest that interventions capable of reducing urine albumin excretion carry long-term cardiovascular and renal benefits. Causally related to albumin excretion, glomerular endothelial dysfunction and the subsequent renal impairment is a precursor to the development of essential hypertension (185,

186). Reductions in blood pressure and improvements in endothelial function may protect the glomerulus from damage that precipitates a decline in function and renal compromise (165, 168).

Aerobic exercise training has been demonstrated to improve endothelial function in young healthy (301) and pre-hypertensive (305) individuals, as well as individuals with cardiovascular disease (306, 307, 696). Heat therapy has emerged in recent years as a potential alternative to exercise training for improving endothelial health, mediated by intermittent increases in vascular shear stress, similar to exercise (8, 9, 434, 435). Secondarily, or perhaps synergistically, with these improvements in endothelial function is an improvement in blood pressure. Regular physical activity is well-documented and recommended for its cardiovascular health benefits (5). Heat therapy in the form of chronic hot water immersion has been reported to have similar benefits across healthy populations, as well as those characterized by elevated risk for cardiovascular disease (8–10). Through improvements in endothelial function and blood pressure, both interventions carry secondary benefits for kidney function, although this has not yet been explored among individuals with untreated essential hypertension.

Ambulatory blood pressure offers a more complete blood pressure profile than traditional in-office blood pressure measurements and is better correlated with cardiovascular disease risk (217, 602, 603, 697, 698). Ambulatory blood pressure measurements allow researchers to identify individuals with white coat hypertension, meaning elevated in-clinic blood pressure but otherwise normal blood pressure during daily living, as well as masked hypertension, or apparently normal blood pressure that is revealed to be elevated or high when measured out of the clinic. Furthermore, a complete 24-hour blood pressure profile allows researchers to identify the presence or absence of a nocturnal fall in blood pressure. A ≥10% decrease in blood pressure qualifies an individual as a “dipper”, referring to the nocturnal decrease in blood pressure, while a reduction of <10% qualifies an individual as a “non-dipper”, referring to the absence of a nocturnal decrease in blood pressure. Among individuals with renal impairment and chronic kidney disease, nighttime systolic blood pressure is significantly correlated with cardiovascular disease risk, compared to no predictive value for clinic blood pressure (699).

The purpose of this study was to examine the effect of 30 sessions of either aerobic exercise training or hot water immersion on 24h ambulatory blood pressure, urine albumin excretion, and estimated glomerular filtration rate among adults with untreated hypertension. We hypothesized that heat therapy would be more effective than exercise training for improving

ambulatory blood pressure as well as reducing urine albumin excretion and increasing estimated glomerular filtration rate.

METHODS

Participants. Before participation, each volunteer gave written informed consent as set forth in the Declaration of Helsinki. All protocols were approved by the Institutional Review Board of the University of Oregon. This trial was registered on clinicaltrials.gov (NCT03557502). The data presented here represents a subset of data from the parent clinical trial examining the efficacy of hot water immersion versus aerobic exercise training for improving blood pressure in adults with untreated hypertension. Elements of the protocol have been abbreviated for conciseness.

Inclusion criteria included individuals aged 35-60, systolic blood pressure between 120 and 180 mmHg, and diastolic blood pressure between 80 and 120 mmHg. Exclusion criteria included systolic blood pressure greater than 180 mmHg or diastolic blood pressure greater than 120 mmHg; chronic cardiorespiratory or metabolic disease other than hypertension; abnormal resting or exercise electrocardiogram; currently taking antihypertensive medication; BMI ≥35 kg/m^2; fasting glucose ≥126 mg/dL; fasting hemoglobin A1c ≥7%; LDL ≥160 mg/dL; high levels of physical activity assessed according to the International Physical Activity Questionnaire (IPAQ); persons who are pregnant, nursing, or currently trying to conceive, aged 34 years or younger, or 61 years and older. Participant characteristics including age, sex, body mass index, and baseline systolic and diastolic blood pressure can be found in Table 4.1.

	Aerobic Exercise (EX)	Hot Water Immersion (HWI)
n	20 (7F)	21 (8F)
Age (years)	49 ± 7	47 ± 7
Body Mass ($kg \cdot m^{-2}$)	30 ± 3	30 ± 3
Systolic Blood Pressure (mmHg)	134 ± 11	127 ± 9
Diastolic Blood Pressure (mmHg)	89 ± 7	84 ± 10

Table 4.1. Subject characteristics. Data are means ± SD.

24-hour Ambulatory Blood Pressure. Participants completed 24-hour ambulatory blood pressure measurements at baseline (PRE), after completion of 15 sessions (MID), and after

completion of all 30 sessions (POST). 2 individuals performed post-intervention testing after completing only 27 sessions (1 EX and 1 HT). Participants arrived at lab and were fitted with an oscillometric blood pressure cuff attached to an ambulatory blood pressure monitor (Oscar2, SunTech Medical, Morrisville, NC, USA). The cuff was programmed to inflate every 20 minutes during self-reported waking hours and every 60 minutes during self-reported sleeping hours. Waking and sleeping times were replicated at MID and POST within each participant. During 24-hour ambulatory blood pressure assessments, subjects were instructed to avoid all over-the-counter vitamins, medications, and supplements; alcohol; caffeine; exercise and heat therapy; and prolonged time in a motorized vehicle. Participants were also instructed to complete an hourly log where they self-reported activity. Lastly, participants completed a dietary recall form for the 24-hour period of data collection. Dietary recall was completed to ensure that dietary sodium consumption did not differ between conditions.

Ambulatory blood pressure data is presented as 24-hour total for both systolic and diastolic blood pressure, as well as mean daytime (self-reported waking hours) and nighttime (self-reported sleeping hours) systolic and diastolic blood pressure. 24-hour total systolic and diastolic pressure were averaged such that daytime and nighttime values were weighted equally despite a disparity in measurement period (20min vs 60min). Percent reduction in nocturnal blood pressure (*e.g.*, "dipper" vs. "non-dipper") was calculated as the percent reduction in blood pressure during self-reported sleeping hours compared to self-reported waking hours.

24h Urine Collection. Participants performed gold-standard 24-hour urine collection for the determination of 24-hour urine albumin excretion. Participants reported to lab at the same time of day for PRE, MID and POST-intervention testing. After a forced void that was not collected, participants were instructed to collect all urine in light-protected 3000mL containers for the following 24 hours. Upon return to lab the following day, participants were asked to force a final void into the 24-hour collection container. Researchers measured total urine volume as well as urine specific gravity (Atago master manual urine refractometer, ATAGO CO. LTD., Tokyo, Japan). Mixed aliquots from the 24-hour collection container were frozen in cryogenic tubes at –80°C until analysis.

To ensure that there was no undue influence of dietary sodium on kidney biomarkers of interest, we instructed participants to complete a 24h dietary recall form during the pre-intervention 24h urine collection. Subjects were provided with a copy of this dietary log and

asked to replicate their food and beverage consumption as faithfully as possible during mid- and post-intervention data collection.

Venous Blood Sampling. We obtained a venous serum sample via venipuncture and centrifugation. A fasted blood draw was performed from a suitable antecubital or dorsal hand vein into a serum separating vacutainer with a clot activator (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were allowed to clot upright at room temperature for 30 minutes. Samples were then centrifuged at 4°C and 1300 RCF for 10 minutes. The serum was then aliquoted into cryogenic tubes and frozen at –80°C until later analysis.

Intervention. Following screening and consent to participate in research, subjects completed baseline 24-hour urine collection. Subjects returned to lab for a fasted blood draw, as well as resting measurements of blood pressure. 24h data collection at the mid- and post-intervention time points was completed at least 24 hours following the end of the 15^{th} and 30^{th} exercise or hot water immersion session, respectively.

Randomization. Following completion of pre-intervention 24h ambulatory blood pressure assessment and 24h urine collection, volunteers were randomized using a computer-generated block randomization module in REDCap (REDCap, Vanderbilt University, Nashville, TN, USA). Blocks consisted of 4 participants, counter-balanced for biological sex and age.

Exercise Training. Participants randomized into the exercise training group completed 30 sessions of aerobic exercise training on a stationary bike in a temperature-controlled room (22-24°C). Participants completed a 5-minute warm-up at 30% of heart rate reserve (HRR), 40 minutes at target intensity of 60% HRR, followed by a 5-minute cool down at 30% HRR. During exercise, heart rate was monitored via telemetry (Polar Team Pro, Polar Electro, Lake Success, NY) and workload was recorded every 5 minutes.

Heat Therapy. Participants randomized into heat therapy completed 30 sessions of hot water immersion to mid-sternum in 40°C water for 45 minutes with a 5-minute seated recovery period no longer immersed. During hot water immersion, heart rate monitored via telemetry and was recorded every 5 minutes using a commercially available heart rate monitor (Polar Electro Inc., Lake Success, NY, USA).

Biochemical Analysis. We assessed serum cystatin C, 24-hour urine creatinine excretion, and 24-hour urine albumin excretion in duplicate for all subjects at PRE, MID, and POST-intervention. In limited collection windows, (*e.g.,* 6-, 12-, or 18-hour urine collection) urine

creatinine is used to normalize urine albumin excretion, although 24h urine albumin:creatinine ratio carries significant prognostic utility (651, 700). 24-hour urine creatinine excretion was assessed via enzyme-linked immunosorbent assay (catalog no. KGE005, R&D systems, Minneapolis, MN, USA). 24-hour urine albumin excretion was quantified via colorimetric assay (Human Albumin ELISA kit, ab108788, Abcam, Waltham, MA, USA). Serum cystatin C was quantified via commercially available ELISA (Quantikine Catalog # DCSTC0, R&D systems, Minneapolis, MN, USA).

To predict potential changes in ambulatory blood pressure and kidney function, we measured several relevant biomarkers of oxidative stress and inflammation, including Endothelin-1, C-Reactive Protein, and IL-6. Endothelin-1 was analyzed in EDTA-treated plasma using a commercially available ELISA (R&D Systems, Catalog # DET100, Minneapolis, MN, USA). C-Reactive Protein was measured in EDTA-treated plasma using a commercially available ELISA (R&D Systems, Catalog # DCRP00B, Minneapolis, MN, USA). IL-6 was quantified using a high-sensitivity commercially available ELISA (R&D Systems, Catalog # HS600C, Minneapolis, MN, USA).

Estimated glomerular filtration rate was calculated using the CKD-EPI equation without adjustment for race, utilizing serum Cystatin C (151, 653). Calculation of eGFR using serum cystatin C has been shown to more faithfully represent glomerular filtration rate, and has less potential confounders than serum creatinine (153, 701). The CKD-EPI formula for eGFR can be found below in Equations 4.1a and 4.1b.

Equation 4.1a. *Estimated Glomerular Filtration Rate when Cystatin C <0.8 mg/L*

$$133 \times (S_{cys}/0.8, 1)^{-0.499} \times 0.996^{Age} \times 0.932 \text{ [if female]}$$

Equation 4.1b. *Estimated Glomerular Filtration Rate when Cystatin C >0.8 mg/L*

$$133 \times (S_{cys}/0.8, 1)^{-1.328} \times 0.996^{Age} \times 0.932 \text{ [if female]}$$

Statistical Analysis. Participant characteristics were analyzed using an unpaired, two-tailed Student's *t*-test and are presented as means ± SD. Primary outcome variables, ambulatory blood pressure and biomarkers of oxidative stress and inflammation, were analyzed using linear mixed-effects model with group and timepoint main effects and an interaction effect to

determine changes over time within groups. These analyses were completed in collaboration with biostatisticians from Oregon Health & Science University. Prior to inclusion in analyses, measures of inflammation and oxidative stress, IL-6, CRP, ET-1, and urine albumin, had observations below or above the limit of detection replaced with assay limits of detection and were log2 transformed for normalcy. Mixed effect models were performed using the lme4 package (v1.1-33). All analyses were performed in R (v4.3.0) with an alpha of 0.05 used to determine significance. Secondary outcome variables, including percent change in blood pressure during nighttime, estimated glomerular filtration rate, and urinary albumin:creatinine ratio, are presented as the mean difference from PRE to POST with 95% CI. Secondary outcome variables were analyzed using a two-way repeated measures analysis of variance with main effects of group and time. In the event of a significant main effect, post-hoc testing was conducted using the Sidak analysis for multiple comparisons. Statistical analysis was performed in GraphPad Prism 10.1.0 (GraphPad, Dotmatics, San Diego, CA, USA). Significance was accepted as $p<0.05$.

RESULTS

Participant anthropometric and demographic data are presented in Table 4.1. Primary outcome variables are presented as mean of the difference from PRE to POST with 95% CI in the text. Secondary outcome variables are presented as the mean of the difference from PRE to POST with 95% CI, with the associated *p*-value from statistical comparison. Mean data for all outcome variable for both groups at all time points can be found in Tables 4.4 and 4.6.

24-hour Ambulatory Blood Pressure. Neither 24-hour total ambulatory SBP was changed following either exercise training (0 [-4, 5]) or hot water immersion (0, [-5, 4]), nor was 24-hour DBP changed following either exercise (1 [-3, 5]) or hot water immersion (0 [-4, 3]). Statistical analysis of 24h SBP and DBP can be found in table 4.2. Daytime ambulatory SBP was not changed following either exercise (0 [-6, 6]) or hot water immersion (-1 [-6, 5]). Statistical analysis of daytime SBP and DBP can be found in table 4.3. Likewise, daytime ambulatory DBP was not different following exercise training (0 [-4, 5]) or hot water immersion (-1 [-5, 3]). Nighttime ambulatory SBP was not impacted by either exercise training (4 [-2, 10]) or hot water immersion (2 [-5, 9]). Similarly, nighttime ambulatory diastolic blood pressure was not different

following exercise training (3 [-1, 7]) or hot water immersion (1 [-3, 5]). Statistical analysis of nighttime SBP and DBP can be found in table 4.4. Graphical representation of all ambulatory BP data can be found in figure 4.1. Percent reduction in ambulatory SBP and DBP during nighttime was not impacted by either exercise (2 [-2, 7], p=0.44) or heat therapy (2 [-2, 6], p=0.45) (Figure 4.2.). Mean [95% CI] for all variables associated with ambulatory blood pressure can be found in table 4.5.

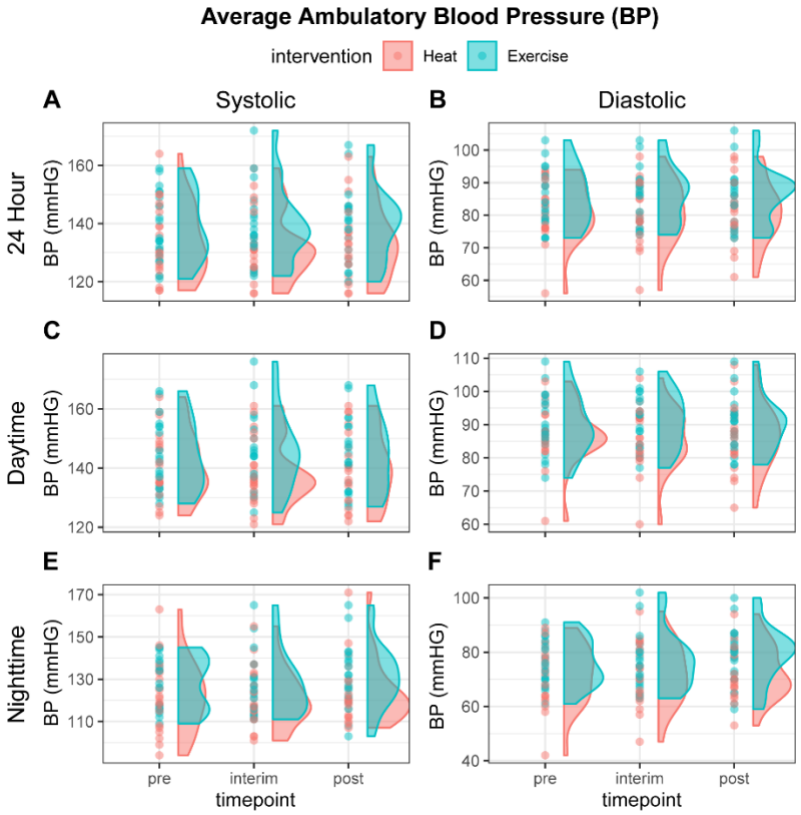

Figure 4.1. Group and individual data for dot and violin plots for Ambulatory Blood Pressure. 1 missing observation for EX PRE, 2 missing observations for EX MID, and 1 missing observation for EX POST.

	Systolic Blood Pressure		**Diastolic Blood Pressure**	
Fixed Effect*	**Estimate (95% CI)**	***P* value****	**Estimate (95% CI)**	***P* value****
24H Ambulatory				
Intercept	138.80 (133.37, 144.23)	-	85.33 (81.26, 89.14)	-
Heat Intervention	-4.90 (-12.48, 2.69)	.10	-3.72 (-9.24, 1.788)	.09
Timepoint		.84		.82
interim	0.76 (-2.36, 3.88)		0.77 (-1.33, 2.87)	
post	0.85 (-2.21, 3.91)		1.05 (-1.01, 3.11)	
Interaction		.61		.57
Heat:interim	-2.19 (-6.50, 2.13)		-1.44 (-4.34, 1.47)	
Heat:post	-1.14 (-5.41, 3.14)		-1.24 (-4.12, 1.64)	

Table 4.2. Results of linear mixed-effect model analysis of 24h total ambulatory blood pressure for EX and HT.

	Systolic Blood Pressure		Diastolic Blood Pressure	
Fixed Effect*	**Estimate (95% CI)**	***P* value****	**Estimate (95% CI)**	***P* value****
Daytime				
Intercept	144.15 (138.93, 149.37)	-	89.70 (85.78, 93.61)	-
Heat Intervention	-3.29 (-10.58, 4.00)	.15	-2.56 (-8.03, 2.91)	.17
Timepoint		.89		.98
interim	1.46 (-1.91, 4.84)		1.01 (-1.47, 3.49)	
post	0.25 (-3.06, 3.56)		0.40 (-2.03, 2.83)	
Interaction		.25		.48
Heat:interim	-3.89 (-8.56, 0.78)		-2.11 (-5.54, 1.33)	
Heat:post	-1.20 (-5.83, 3.43)		-1.11 (-4.51, 2.28)	

Table 4.3. Results of linear mixed-effect model analysis of daytime ambulatory blood pressure for EX and HT

	Systolic Blood Pressure		Diastolic Blood Pressure	
Fixed Effect*	**Estimate (95% CI)**	***P* value****	**Estimate (95% CI)**	***P* value****
Nighttime				
Intercept	127.68 (121.18, 134.18)	-	76.21 (71.63, 80.80)	-
Heat Intervention	-5.91 (-14.97, 3.13)	.11	-4.74 (-11.12, 1.64)	.08
Timepoint		.13		.10
interim	0.58 (-3.97, 5.13)		0.34 (-2.56, 3.26)	
post	3.52 (-0.95, 7.99)		2.89 (0.03, 5.74)	
Interaction		.89		.62
Heat:interim	-1.01 (-7.25, 5.24)		-0.01 (-4.01, 3.98)	
Heat:post	-1.47 (-7.66, 4.71)		-1.69 (-5.65, 2.26)	

Table 4.4. Results of linear mixed-effect model analysis of nighttime ambulatory blood pressure for EX and HT

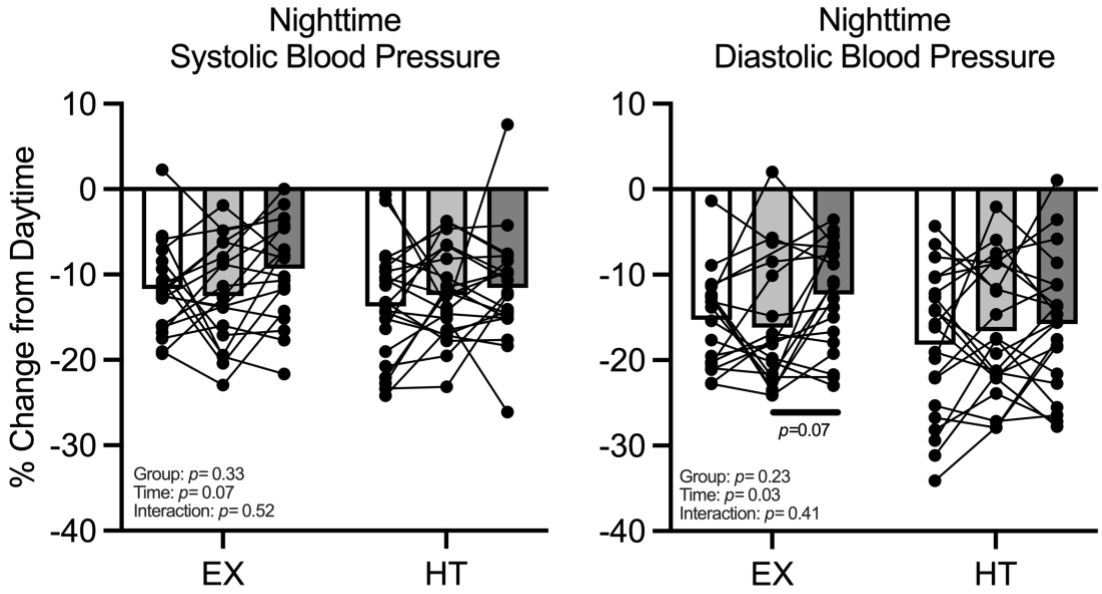

Figure 4.2. Percent change in ambulatory systolic and diastolic blood pressure from daytime to nighttime. Data are reported as mean and individual values for both groups at all time points. 1 missing observation at EX PRE, 2 missing observations at EX MID, and 1 missing observation at EX POST.

Variable	*Group*	*PRE*	*MID*	*POST*
24h Total SBP (mmHg)	EX	139 [133, 145]	139 [133, 146]	140 [134, 146]
	HT	134 [128, 140]	132 [127, 138]	133 [128, 140]
24h Total DBP (mmHg)	EX	85 [81, 89]	86 [81, 90]	86 [82, 90]
	HT	81 [77, 86]	81 [76, 85]	81 [76, 86]
Awake SBP (mmHg)	EX	145 [139, 150]	146 [139, 152]	145 [139, 151]
	HT	141 [136, 146]	138 [133, 143]	140 [134, 146]
Awake DBP (mmHg)	EX	90 [85, 94]	91 [87, 95]	90 [86, 94]
	HT	87 [83, 91]	86 [82, 90]	86 [82, 91]
Asleep SBP (mmHg)	EX	128 [122, 134]	128 [120, 135]	131 [124, 138]
	HT	122 [114, 129]	121 [115, 128]	124 [117, 131]
Asleep DBP (mmHg)	EX	76 [72, 80]	76 [71, 82]	79 [74, 84]
	HT	71 [66, 77]	72 [67, 77]	73 [68, 77]
% Change Daytime to Nighttime aSBP	EX	-12 [-14, -9]	-13 [-15, -10]	-9 [-12, -6]
	HT	-14 [-17, -10]	-12 [-15, -10]	-12 [-15, -9]
% Change Daytime to Nighttime aDBP	EX	-15 [-18, -13]	-16 [-20, -12]	-12 [-15, -9]
	HT	-18 [-22, -14]	-18 [-20, -13]	-16 [-19, -12]

Table 4.5. 24h total, awake, and asleep ambulatory blood pressure data for both groups at all time points. Also shown are % change from daytime to nighttime for both ambulatory systolic and diastolic blood pressure for both groups at all time points. Data are presented as means with [95% CI]. 1 missing observation from EX PRE, 2 missing observations from EX MID, and 1 missing observation from EX POST.

Biomarkers of Inflammation and Oxidative Stress

Biomarkers of inflammation and oxidative stress including interleukin-6, C-Reactive Protein, Endothelin-1 were log transformed for normality and subsequently analyzed. Interleukin-6 was not impacted by either aerobic exercise training (-0.24 pg/mL [-1.86, 1.34]; p=0.97) or heat therapy (-0.47 [-1.22, 0.28]; p=0.31). C-Reactive Protein was not impacted by either exercise training (-0.15 mg/L [-0.63, 0.33]: p=0.79) or heat therapy (-0.26 [-1.06, 0.55]; p=0.80). Endothelin-1 was not reduced following either exercise (0.02 pg/mL [-0.33, 0.38]: p=0.99) or heat therapy (-0.07 [-0.34, 0.25]; p=0.94). biomarkers of inflammation and oxidative stress are graphically represented in figure 4.3. Results of the linear-mixed effects model analysis of these data can be found in table 4.6.

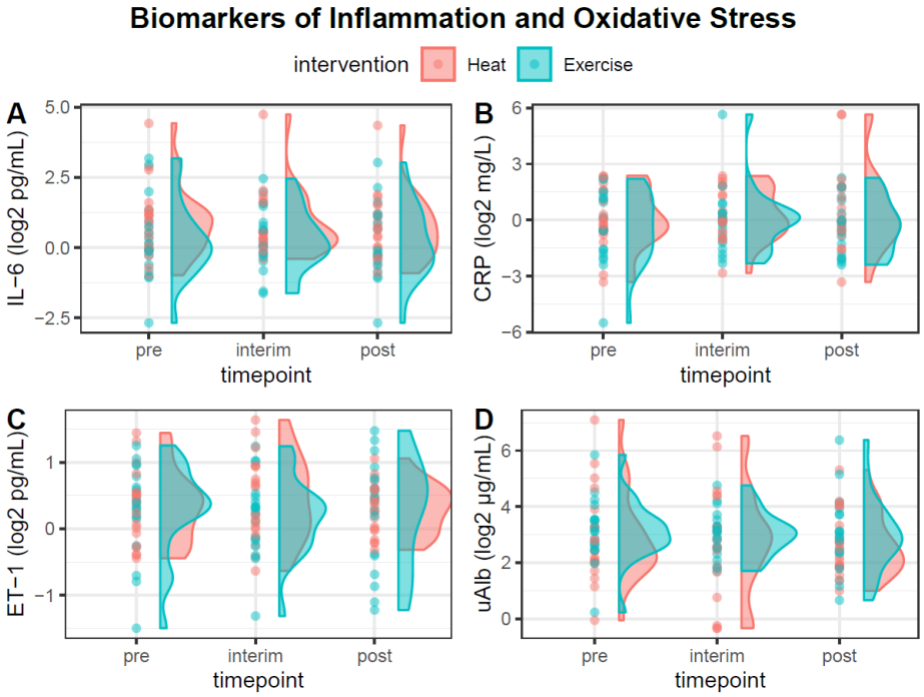

Figure 4.3. Group and individual data dot and violin plots for biomarkers of inflammation, oxidative stress, and glomerular damage. Missing data points were interpolated as equivalent to either the high or low standard of the respective assay.

Fixed Effect*	IL-6, log2 (95% CI)	CRP, log2 (95% CI)	ET-1, log2 (95% CI)	uAlb, log2 (95% CI)
Intercept	0.28 (-0.28, 0.83)	-0.42 (-1.12, 0.37)	0.29 (0.01, 0.57)	3.15 (2.54, 3.75)
Heat Intervention	0.64 (-0.13, 1.41)	0.52 (-0.56, 1.60)	0.03 (-0.35, 0.41)	-0.24 (-0.35, 0.41)
Timepoint				
interim	0.01 (-0.40, 0.42)	0.41 (-0.26, 1.09)	-0.11 (-0.34, 0.12)	-0.03 (-0.56, 0.50)
post	-0.07 (-0.48, 0.35)	0.12 (-0.55, 0.80)	-0.04 (-0.27, 0.19)	-0.17 (-0.69, 0.36)
Interaction				
Heat:interim	-0.07 (-0.65, 0.50)	-0.25 (-1.19, 0.69)	0.25 (-0.07, 0.57)	-0.13 (-0.86, 0.61)
Heat:post	-0.18 (-0.75, 0.39)	0.21 (-0.73, 1.14)	0.03 (-0.29, 0.34)	-0.07 (-0.80, 0.66)

Table 4.6. Results of linear mixed-effect model analysis of biomarkers of inflammation and kidney function, including IL-6, C-Reactive Protein, Endothelin-1, and urine Albumin. Data are log transformed and presented relative to a fixed intercept.

Renal Function

Renal function mean data with 95% CI can be found in Table 4.6. Estimated glomerular filtration rate was quantified using serum cystatin C. Cystatin C may be used to supplement eGFR findings as determined by serum creatinine, as it more closely reflects glomerular filtration rate and has fewer potential confounders (153, 701). eGFR, as calculated with serum cystatin C, was not different following either exercise (-1.66 mL/min/1.73m^2 [-7.9, 4.59], p = 0.97) or hot water immersion (1.7 [-8, 11.5], p = 0.99) (Figure 4.4). 24h urine albumin: creatinine ratio, while highly variable, was not different following either aerobic exercise training (2 mg/g [-14, 17]; p=0.96) or heat therapy (-9 [-24, 6]; p=0.31) (Figure 4.5). Dietary sodium consumption was not different throughout either exercise training (147mg [-563, 858]; p=0.857) or heat therapy (185mg [-842, 472]; p=0.785). Self-reported mean dietary sodium consumption can be found in Table 4.7.

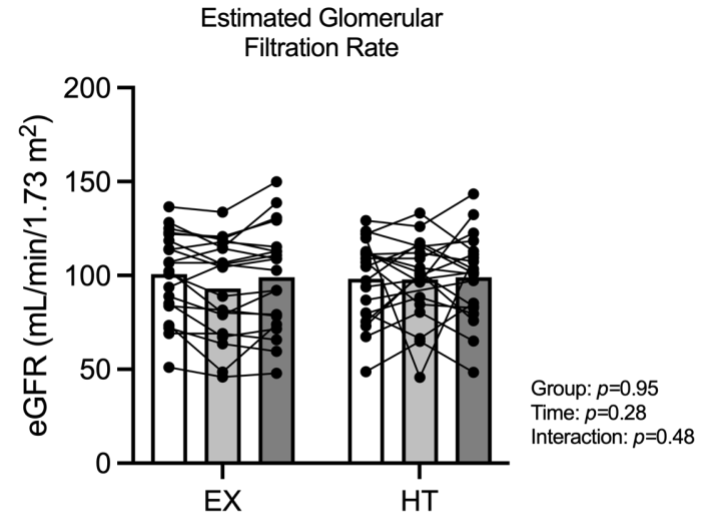

Figure 4.4. Estimated Glomerular Filtration Rate. Data are presented as means and individual values for both groups at pre-, mid-, and post-intervention

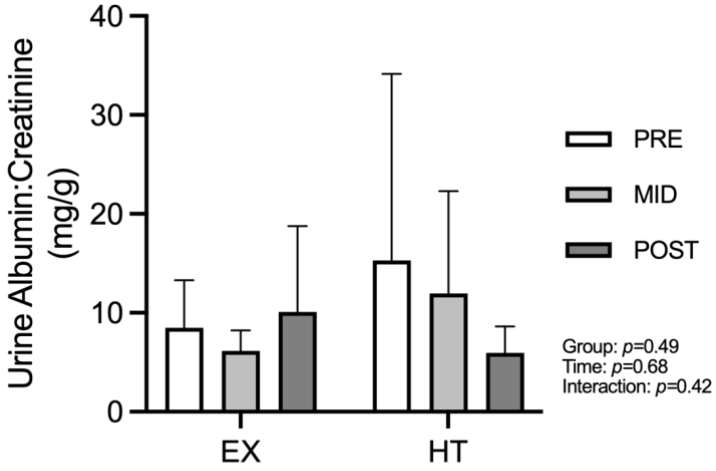

Figure 4.5. 24h urine albumin:creatinine ratio. Data are presented as means with 95% CI

Variable	*Group*	*PRE*	*MID*	*POST*
Urine Albumin Excretion (mg/24h)	EX	11.0 [6.3,17.5]	9.6 [6.7, 12.5]	12.9 [4.4, 21.4]
	HT	16.2 [2.6, 29.7]	16.4 [4.8, 27.9]	8.8 [4.8, 12.8]
eGFR (mL/min/1.73m^2)	EX	101 [90, 112]	93* [81, 106]	99 [86, 112]
	HT	98 [88, 109]	98 [87, 109]	99 [88, 110]
Urine Albumin: Creatinine (mg/g)	EX	8.49 [3.69, 13.3]	6.16 [4.09, 8.23]	10.9 [1.4, 18.78]
	HT	15.3 [-3.6, 34.1]	11.95 [1.6, 22.3]	5.964 [3.3, 8.625]
Endothelin-1 (pg/mL)	EX	1.343 [1.09, 1.6]	1.260 [1.03, 1.5]	1.363 [1.03, 1.7]
	HT	1.336 [1.09, 1.6]	1.504 [1.2, 1.8]	1.274 [1.1, 1.5]
C-Reactive Protein (pg/mL)	EX	1.489 [0.78, 2.19]	1.06 [0.63, 1.5]	1.334 [0.67, 2]
	HT	1.67 [0.86, 2.5]	1.51 [0.85, 2.18]	1.41 [0.78, 2.05]
Interleukin-6 (pg/mL)	EX	1.98 [0.81, 3.2]	1.61 [0.97, 2.3]	1.74 [0.9, 5.3]
	HT	3.1 [0.9, 5.3]	3.14 0.35, 5.93]	2.63 [0.63, 4.64]
Dietary Sodium (mg)	EX	2252 [1792, 2792]	2706 [2207, 3205]	2399 [1809, 2988]
	HT	2613 [2073, 3151]	2243 [1719, 2768]	2428 [1812, 3043]

Table 4.7. Biomarkers of kidney function and inflammation. Data are mean and [95% CI] for both groups at all time points. *$p<0.01$ compared to PRE.

DISCUSSION

The primary finding of this study is that neither 30 sessions of aerobic exercise training nor chronic hot water immersion were sufficient to reduce total, daytime, or nighttime parameters of ambulatory blood pressure. Additionally, we report no significant differences in 24h urine albumin excretion or glomerular filtration rate estimated using serum Cystatin C.

Regular aerobic exercise is a primary lifestyle modification to lower blood pressure. To date, there have been a number of systematic reviews and meta-analyses that have reported the benefits of exercise training on ambulatory blood pressure among adults with untreated hypertension, controlled hypertension, as well as resistant hypertension (702–705). We conducted this trial due to compelling data that suggest heat therapy may be more effective than exercise training for improving blood pressure (8, 10). Unfortunately, we fail to report any significant changes in ambulatory blood pressure following either heat therapy or the current gold standard, aerobic exercise training. We are not the first to demonstrate that moderate-intensity aerobic exercise training is not sufficient to improve blood pressure among adults with untreated hypertension (706–709). Among each of the previous interventions, exercise training either had equivocal or no impact on ambulatory blood pressure. There are, however, several compelling systematic reviews and meta-analyses to suggest that regular dynamic aerobic exercise is capable of eliciting significant reductions in blood pressure (264, 265, 710). The primary difference between interventions that report no impact of exercise training on blood pressure and those that do is the duration of the training period. The range of training duration varied from as short as 1 month to a long as one year among studies included in a systematic review and meta-analysis of the beneficial effects of exercise on blood pressure published by Cornelissen & Fagard (264). It is possible that our intervention and others are not sufficiently long enough to elicit a meaningful and significant reduction in blood pressure.

Additionally, data regarding the impact of exercise intensity on the hypotensive effects of physical activity are equally mixed (711–713). While we assessed cardiorespiratory fitness prior to and following both interventions, exercise prescription was formulated according to heart rate reserve. This allowed researchers to titrate work rate according to improved cardiorespiratory fitness during the intervention, with a target working intensity of 60% of heart rate reserve. It is possible that with a greater exercise intensity, we would report reductions in ambulatory blood pressure in this population. Changes in ambulatory blood pressure following heat therapy are presented relative to changes in the gold standard, aerobic exercise. As we report no changes in ambulatory blood pressure following exercise, perhaps it should be expected that we also report no changes following heat therapy. There is a dearth of evidence to suggest that heat therapy elicits a reduction in ambulatory blood pressure, despite compelling evidence for a reduction in clinic blood pressure (9, 10).

To explore the integrative relationship between the kidney and long-term blood pressure regulation, we pursued several exploratory biomarkers of kidney function prior to and following both interventions, including 24h urine albumin excretion, urine albumin:creatinine ratio, and glomerular filtration rate estimated using serum cystatin C. Contrary to our hypothesis, heat therapy did not elicit reductions in urine albumin excretion or urine albumin:creatinine ratio. It is likely that the lack of improvement in urine albumin excretion and urine albumin:creatinine ratio is due to a lack of a change in blood pressure following either intervention. Multiple previous investigations have reported a reduction in urine albumin secondary to a reduction in blood pressure across a variety of populations and comorbidities (714–716). These reductions in blood pressure were achieved through reductions in dietary sodium, which has been shown to have a direct impact on urine albumin excretion (614, 717). Dietary sodium-mediated increases in arterial stiffness amplify the effects of pulsatile pressure on the kidney, which manifests as increased urine albumin excretion, representative of endothelial dysfunction and kidney damage (718–720). Through reductions in dietary sodium and attendant reductions in arterial stiffness, urine albumin excretion is reduced, suggesting that albuminuria is a pressure-dependent phenomenon, in spite of renal autoregulation to prevent the transmission of systemic pressures to the renal vasculature (721). In the present intervention, we report no differences in dietary sodium between conditions, although this was assessed using a self-reported dietary recall (722).

Albuminuria and, by extension, the albumin:creatinine ratio, can be indicative of vascular endothelial dysfunction (687) as well as risk for hypertension and cardiovascular disease (174, 700, 723). We do not report any changes to 24h urine albumin excretion or 24h urine albumin: creatinine ratio. Unfortunately, we did not collect any measurements of endothelial function, although previous data have demonstrated that endothelial function is impaired in essential hypertension through a combination of vascular, inflammatory, and vasoactive molecules (85, 186, 687). Improvements in endothelial function following repeated bouts of hyperthermia have been demonstrated primarily in young, healthy, individuals (8, 545, 546), although Ely and colleagues report significant improvements in flow-mediated dilation in individuals with Polycystic Ovary Syndrome, a population characterized as pre-diabetic, pre-hypertensive, and often presenting with autonomic dysfunction (9). Among other clinical populations, Gravel and colleagues demonstrated that a single Finnish sauna session was capable of acutely improving peripheral endothelial function among adults with stable coronary artery disease (445), but not in

healthy older adults (444). However, following chronic Finnish sauna use, Debray and colleagues report no improvements in flow-mediated dilation among adults with stable coronary artery disease (724). Following chronic hot water immersion, Akerman and colleagues report no significant improvement in brachial artery flow-mediated dilation in adults with peripheral arterial disease (10). Conversely, following Waon therapy, both Imamura et al. and Kihara et al. demonstrated improvements in endothelial function among adults with chronic heart failure (449, 450).

We report no significant impact of either aerobic exercise training or heat therapy on glomerular filtration rate estimated using serum cystatin C. The impact of blood pressure on estimated glomerular filtration rate is a continuous relationship and not limited to a single insult, which complicates the interpretation of the effect of a short-term interaction such as the present study (725). Previous interventions have explored the impact of intensive pharmacological interventions to lower blood pressure (726), or changes in eGFR among individuals with established chronic kidney disease (727), but there is limited data examining individuals with untreated essential hypertension without impairments in kidney function. Estimated glomerular filtration rate is in part a pressure-dependent phenomenon, and substantial reductions in blood pressure can elicit short-term reductions in eGFR which are not indicative of a decline in kidney function but are hemodynamic in nature. It is possible that this explains the transient reduction in eGFR at the mid-intervention assessment, despite no difference in ambulatory blood pressure at this same timepoint. Conversely, elevated blood pressure, despite being a risk factor for impaired kidney function long-term, is associated with elevated eGFR. Systemic arterial pressure-mediated glomerular hyperfiltration is common in the initial stages of essential hypertension, and is highly predictive of future cardiovascular events and kidney decline (162, 728, 729). Nascent hyperfiltration represents a promising biomarker for identifying individuals with a high likelihood of benefit from anti-hypertensive medication, or lifestyle interventions to improve blood pressure (729). There is limited data to suggest that lifestyle interventions such as aerobic exercise can reduce blood pressure while maintaining estimated glomerular filtration rate (727). Despite results that are contrary to our hypothesis, previous reports of the compelling hemodynamic benefits of exercise training and heat therapy warrant future study of the impact of lifestyle interventions on eGFR.

Limitations

The present study did not assess kidney function at baseline using urine albumin/creatinine ratio. Accordingly, it is possible that some individuals present with impaired kidney function at baseline. The kidney plays a central role in long-term blood pressure regulation, possibly precluding these individuals from experiencing aerobic exercise training or heat therapy on kidney function.

Furthermore, it is possible that our interventional timeline influenced these results. The study was designed with an intention-to-treat protocol, wherein all individuals that were randomized and initiated either aerobic exercise or heat therapy were included in the intervention. Our projected and ideal time to complete the intervention was 8-10 weeks, with an average frequency of 3-4 sessions per week. Unfortunately, not all participants completed the intervention within this timeframe. Several individuals within both groups required over 10 weeks to complete the intervention, which may have influenced our results. It is possible that with stricter adherence guidelines, our results would more closely match those previously reported (349, 702, 704). Despite this protracted timeline, all participants except one in each group completed all 30 sessions of their assigned intervention. Among those that did not complete all 30 sessions, both completed 27/30.

CONCLUSION

These data indicate that the design of the current study, wherein middle-aged individuals with untreated hypertension completed 30 sessions of either aerobic exercise training or hot water immersion, is not sufficient to improve ambulatory blood pressure or relevant biomarkers of kidney function.

CHAPTER V

CARDIOVASCULAR FUNCTION

INTRODUCTION

Cardiovascular disease represents the leading cause of death both in the United States as well as world-wide (2, 3, 201). Hypertension is among the leading primary, yet preventable, risk factors for cardiovascular disease, including smoking and physical inactivity. Exercise training represents a crucial lifestyle intervention for improving both blood pressure as well as cardiovascular disease risk, yet approximately half of all united states adults do not meet the American College of Sports Medicine recommendations for physical activity (5). Despite low adherence, exercise training remains a crucial non-pharmacological intervention for improving blood pressure and cardiovascular disease risk.

Heat therapy has been a cultural practice for thousands of years, including Finnish Saunas, Japanese Onsens, and Native American sweat lodges. These cultural practices have evolved into a potential therapeutic alternative to exercise for improving cardiovascular (8) and metabolic (485, 494) risk factors, as well as risk for incident hypertension (439), Alzheimer's disease (267), and all-cause mortality (7). There is a particularly strong body of evidence for the efficacy of heat therapy, in the form of Waon therapy (far-infrared sauna bathing with subsequent maintenance of core temperature using a tightly wrapped blanket), particularly among individuals with heart failure (447, 448, 451, 457, 463). Similarly, hot water immersion also represents a suitable physiological stressor to elicit improvements in cardiovascular health among a variety of populations (8–10). Hot water immersion elicits a similar increase in core temperature as moderate-intensity exercise (645), which has been identified as mediating key improvements in vascular health with heating interventions (546, 730).

Despite the independent efficacy of each intervention, there have been relatively few comparisons of heat therapy with the gold standard, aerobic exercise training, for improving blood pressure and cardiovascular disease risk factors. One intervention demonstrated greater reductions in blood pressure following heating compared to exercise among individuals with peripheral arterial disease, but no change in arterial stiffness or endothelial function (10). Two previous trials (10, 731) utilized a combination of exercise training and heat stress compared to exercise alone, which precludes authors from quantifying the potential effects of heat therapy on

physiological variables such as blood pressure. Lastly, these interventions have not been directly compared among adults with untreated hypertension but without other cardiovascular diseases.

Therefore, the purpose of this study is to compare the efficacy of chronic hot water immersion and aerobic exercise training for improving blood pressure among adults with untreated hypertension. We hypothesized that hot water immersion would be more effective than aerobic exercise training for lowering blood pressure and improving arterial stiffness.

METHODS

Subjects. Forty-one adults with elevated or high blood pressure (defined as systolic blood pressure ≥120 mmHg and/or diastolic blood pressure ≥80 mmHg) volunteered to participate in this research. Inclusion criteria included individuals aged 35-60, systolic blood pressure between 120 and 180 mmHg, diastolic blood pressure between 80 and 120 mmHg. Exclusion criteria included systolic blood pressure greater than 180 mmHg or diastolic blood pressure greater than 120 mmHg; chronic cardiorespiratory or metabolic disease other than hypertension; abnormal resting or exercise electrocardiogram; currently taking antihypertensive medication; BMI ≥ 35 kg/m^2; fasting glucose ≥126 mg/dL; fasting hemoglobin A1c ≥7%; LDL ≥160 mg/dL; high levels of physical activity assessed according to the International Physical Activity Questionnaire (IPAQ); persons who are pregnant, nursing, or currently trying to conceive, aged 34 years or younger, or 61 years and older. Blood pressure was assessed in accordance with current American College of Cardiology and American Heart Association guidelines to confirm elevated to hypertensive blood pressure. Briefly, blood pressure was assessed ≥2 times on ≥2 days and averaged to determine hypertension classification. Based on these screening assessments, seven participants were classified as elevated blood pressure, eleven participants were classified as stage one hypertensive, and nineteen participants were classified as stage 2 hypertensive. Subject characteristics can be found below in Table 5.1.

	Aerobic Exercise (EX)	Hot Water Immersion (HWI)
n	20 (7F)	21 (8F)
Age (years)	49 ± 7	47 ± 7
Body Mass (kg • m^{-2})	30 ± 3	30 ± 3
Systolic Blood Pressure (mmHg)	134 ± 11	127 ± 9
Diastolic Blood Pressure (mmHg)	89 ± 7	84 ± 10
# Elevated Blood Pressure	4	4
# Stage 1 Hypertension	7	12
# Stage 2 Hypertension	9	5

Table 5.1. Subject characteristics at baseline. Data are means ± SD.

Randomization. Following completion of all pre-intervention testing, volunteers were randomized using a computer-generated randomization module in REDCap (REDCap, Vanderbilt University, Nashville, TN, USA).

Vascular Function. Subjects reported to lab three times throughout the course of data collection for resting measurements of vascular function. Baseline pre-intervention testing was conducted prior to randomization, mid-point testing was conducted after 15 sessions, and post-intervention testing was conducted after the completion of all 30 sessions. Vascular function testing was conducted at a minimum of 48 hours following the final exercise or hot water immersion session, and a maximum of 1 week. Prior to vascular function testing, subjects were asked to abstain from over-the-counter vitamins, medications, and supplements for ≥12 hours; exercise and heat therapy for ≥48 hours; caffeine for ≥12 hours; food for ≥12 hours; and alcohol for ≥24 hours. Time of day was held constant for each visit within subjects to minimize the impact of circadian fluctuations on variables of interest. Height and semi-nude weight were recorded, as well as a urine sample to confirm a negative pregnancy test in female participants.

Following these anthropometric measurements, subjects rested quietly supine for 20 minutes prior to hemodynamic and vascular measurements. During this time, subjects were instrumented with 3-lead electrocardiogram for the measurement of heart rate and rhythm, as well as a brachial blood pressure cuff. Measurements of autonomic, hemodynamic, and vascular function were completed in the same order for all participants: heart rate variability, blood pressure and cardiac output, followed by pulse wave velocity.

Heart Rate Variability. Heart rate variability was assessed to quantify the impact of exercise training and heat therapy on autonomic modulation.

Subjects were instructed on how to breathe with a metronome to elicit a breathing frequency of 0.25Hz, or 15 breaths per minute for a total of 8 minutes. During analysis the first and last 90 seconds were excluded, while the middle 5 minutes were analyzed. 5 minutes has been shown to be an optimal duration for detection of low- and high-frequency fluctuations in heart rate variability (621). A time series data file of R-R interval duration was generated using Advanced CODAS Analysis Software (DATAQ Instruments, Akron, OH, USA). The time series data were then input into a custom LabView program for the analysis of frequency domain measures of heart rate variability (UO HRV_G1, University of Oregon, Eugene, OR, USA). Imported data were first interpolated to 4 Hz to obtain equidistant time intervals and then divided into five equal length overlapping segments of 256 samples each. Each segment was de-trended, Hanning-filtered, and fast-Fourier-transformed to derive a periodogram. The five periodograms were averaged to produce the spectrum estimate for the entire time series. For this procedure, the frequency resolution was 0.016 Hz. We defined the high or respiratory frequency band as 0.15-0.40 Hz, a range inclusive of all respiratory power, and the low frequency band as 0.04-0.15 Hz. Total power was reported as the band ≤ 0.40 Hz.

Arterial Blood Pressure. Blood pressure was assessed in triplicate using automated auscultation of the brachial artery (Tango M2, Suntech Medical, Morrisville, NC, USA). Mean arterial pressure (Equation 5.1) was calculated as:

Equation 5.1 *Mean Arterial Pressure*

$$\text{Diastolic Blood Pressure} + \frac{(\text{Systolic Blood Pressure-Diastolic Blood Pressure})}{3}$$

If systolic and diastolic values differed by ≥10 mmHg, the assessment was repeated until ≥3 values agreed.

Cardiac output. Resting cardiac output was assessed via the open circuit acetylene wash-in method, as described previously (487, 622, 623). Initially developed in 1975 (624), and subsequently modified in 1993 (625), this method allows for non-invasive determinations of

cardiac output at rest and during exercise and has been validated against direct Fick measurements of cardiac output (626). Resting in a supine position, subjects were instructed to breathe with a metronome at a frequency of 0.25 Hz, or 15 breaths per minute while fitted with nose clips to prevent mixed nasal and mouth breathing. During expiration, an automatic sliding valve transitioned the inspiratory port from room air to a customized lung diffusion blend, which subjects breathed for approximately 8-10 breaths. The lung diffusion gas blend consisted of 0.6% acetylene, 9% helium, 20.9% oxygen, and balance nitrogen. Acetylene readily diffuses into circulation, while helium is insoluble in blood, therefore the disappearance rate of acetylene is proportional to pulmonary capillary blood volume, providing an estimate of cardiac output (627).Tidal volume was measured on a breath-by-breath basis using a pneumotachograph (model 4700, Hans Rudolph, Shawnee, KS, USA) connected to a two-way Y-shaped non-rebreathing valve (Series 1420, Hans Rudolph, Shawnee, KS, USA). Tidal volume determinations were performed using the linearized method, as previously described (628). As all cardiac output measurement were conducted at rest, we utilized a pediatric pneumotachograph and two-way non-rebreathing valve to minimize dead space, which measured 36mL. Breath-by-breath helium and acetylene were measured using a mass spectrometer (MGA 1100, MA Tech Services, St. Louis, MO, USA). Cardiac output calculations were performed using customized software (Beck Integrated Physiological Testing Systems, St. Paul, MN, USA) based on previously published methods (626).

Using heart rate measured via electrocardiography during the period where inspired and expired gases are analyzed, stroke volume (Equation 5.2) was calculated as:

Equation 5.2. Stroke Volume

$$\text{Stroke Volume (mL/beat)} = \frac{\text{Cardiac Output } (\frac{\text{mL}}{\text{min}})}{\text{Heart Rate } (\frac{\text{beats}}{\text{min}})}$$

Using cardiac output and mean arterial pressure, total peripheral resistance (Equation 5.3) was calculated as:

Equation 5.3. Total Peripheral Resistance

$$\text{Total Peripheral Resistance} = \frac{\text{mean arterial pressure (mmHg)}}{\text{cardiac output } (\frac{\text{L}}{\text{min}})}.$$

Pulse Wave Velocity. Pulse wave velocity was measured using the SphygmoCor XCEL system (ATCOR Medical, Naperville, IL, USA). Subjects were fitted with a blood pressure cuff on their upper left arm. Brachial blood pressure was assessed in duplicate via the oscillometric method prior to initial measurement of pulse wave velocity. Following the measurement of brachial blood pressure, subjects were fitted with an inflatable cuff on the upper portion of their ipsilateral leg. The inflatable cuff on the ipsilateral leg produces a femoral artery pulse waveform using air displacement (732).

Prior to applanation tonometry of the carotid artery, anatomical measures were taken by two researchers. Investigators palpated the carotid artery and measured the distance to the sternal notch, from the sternal notch to the top of the femoral artery cuff, from the location of the strongest femoral pulse to the top of the femoral artery cuff, and from the location of the carotid tonometer to the top of the femoral artery cuff.

Following, a pressure transducing tonometer was applied to the common carotid artery. Upon achieving a satisfactory carotid artery pressure waveform, the upper leg cuff was automatically inflated until a femoral artery pulse waveform was detected. The SphygmoCor XCel device calculates carotid-femoral pulse wave velocity according to the estimated distance the pulse wave travels divided by the duration between the foot of the pulse wave of the carotid pulse waveform and the femoral pulse waveform. Following these concurrent measurements of carotid and femoral artery pressure waveforms, two investigators completed above measurements of anatomical distance for estimation of pulse wave distance travelled. Pulse wave velocity assessments were repeated until recording 2 measurements within 0.3 m/s were obtained. Measurements were completed by the same investigator within a subject for all time points.

Cardiorespiratory Fitness Assessment. VO_{2peak} was assessed using an incremental ramp test to exhaustion performed on a stationary bike (Lode Excalibur Sport, Lode BV, Groningen, Netherlands). Whole body oxygen uptake was assessed at baseline prior to randomization and following all 30 sessions of exercise training or heat therapy.

Participants were instrumented with a heart rate monitor (Polar Electro Inc., Lake Success, NY, USA), a two-way non-re-breathing valve to allow for the inspiration of room air and the collection of expired air (Series 2700, Hans Rudolph, Shawnee, KS, USA), and nose clips. Expired air was collected in a mixing chamber for fractional analysis of expired concentrations of O_2 (paramagnetic) and CO_2 (infrared) at 15s intervals (TrueOne 2400, ParvoMedics, Sandy, UT, USA).

Following instrumentation, subjects rested quietly for 2 minutes while seated on the stationary cycle ergometer on which the test was performed. Subjects began exercise with a 4-minute warm-up at a resistance of 30 watts at a self-selected cadence. Ramp rate was either 15, 20, 25, or 30 watts per minute, depending on subject age, sex, and current fitness, with the goal of eliciting volitional exhaustion in 8-12 minutes. Incremental work rate was subsequently replicated at the post-intervention assessment of peak oxygen consumption. Work rate was increased according to the defined ramp protocol until the participant reached volitional fatigue. Criteria for ending the test included: rating of perceived exertion 17-20 on the Borg Scale (640), achievement of age-predicted maximum heart rate (220-age), respiratory exchange ratio ≥1.1, a plateau in oxygen consumption despite increasing work rate, and cadence ≤60 revolutions per minute for ≥10 seconds despite strong verbal encouragement. An acceptable figure constituting a plateau in oxygen consumption is 2.1 ml/kg/min with increasing work rate (641), although this increase is debated by some (642), as it may be influenced by sampling interval of VO_2 (643, 644). Following volitional fatigue and cessation of pedaling, subjects completed a cool-down for 10 minutes prior to a supramaximal validation of peak oxygen consumption. In brief, participants pedaled at ≥60 rpm until volitional fatigue at a constant work rate corresponding to 110% of peak ramp power, to ensure no further increases in peak oxygen consumption at a greater work rate than that which elicited a plateau in oxygen consumption (642). Following supramaximal verification, participants completed a second cool down. Peak oxygen consumption was measured as the highest 30s average oxygen consumption from either the initial ramp protocol, or the supramaximal verification bout, whichever was greater. Maximal heart rate was determined as the highest heart rate achieved during either the initial ramp protocol or the supramaximal verification bout. This value was then used to calculate heart rate reserve percentages for exercise training sessions.

Heat Therapy. Heat therapy consisted of 30 bouts of hot water immersion over 8-10 weeks, at frequency of 3-4 sessions per week. Hot water immersion has been demonstrated to elicit increases in core temperature that are similar to moderate intensity exercise (645), which are requisite for thermoregulatory adaptations and drive vascular improvements (477, 478, 480, 546, 730). This dosage of heating was informed by previous chronic hot water immersion research within our laboratory. Compelling data from young, healthy, but otherwise sedentary individuals demonstrated that thirty-six 90-minute sessions of hot water immersion over 8 weeks was capable of eliciting promising improvements in blood pressure and vascular function (8, 481, 489). More recently, data from our lab has demonstrated that a truncated dosage of heat therapy, thirty 60-minute sessions, could elicit similar improvements in blood pressure and vascular function in women with polycystic ovary syndrome (9, 494). The hot water immersion protocol in the present study was designed with the intention of matching the duration of heating to the exercise training arm of this protocol, as well as satisfying the American College of Sports Medicine recommended weekly aerobic physical activity guidelines (three 50-minute sessions/week).

Upon arriving at lab, participants provided a urine sample to ensure euhydration (USG ≤1.024) prior to hot water immersion. If urine specific gravity was ≥1.024, subjects were given 5mL/kg of nude body mass to consume before immersion. After providing a urine sample, subjects provided a dry nude body mass prior to changing into suitable clothing for hot water immersion. Prior to immersion, subjects were instrumented with a commercially available heart rate monitor (Polar Team Pro, Polar Electro, Lake Success, NY, USA). Heart rate was measured at baseline in a temperature-controlled room (22-24°C), and every 5min during immersion. For the first 5 and 30th hot water immersion sessions, subjects were provided with an ingestible core temperature pill (HQ Inc, Palmetto, FL, USA). Subjects were instructed to ingest the pill at least 6h prior to arriving at lab.

Participants were immersed to mid-sternum in a commercially available hot tub set to 40°C for 45 minutes. If core temperature exceeded 38.5°C, participants were provided with a fan, but remained immersed. During immersion, water was provided *ad libitum.* After 45 minutes, subjects completed a 5min cool-down in a seated position outside of the tub. Following the cool-down, subjects toweled dry and provided a second dry nude body mass. If body weight

loss via sweat was not compensated via water intake such that body weight loss was <1%, additional fluids were provided prior to subjects leaving the lab.

Exercise Training. Aerobic exercise training consisted of 30 sessions on an upright stationary cycle ergometer (Precor UBK 600, Woodinville, WA, USA) over 8-10 weeks, at a frequency of 3-4 sessions per week. This frequency and duration were selected to achieve the recommended minimum physical activity guidelines from the American College of Sports Medicine. Subjects arrived at lab in suitable clothing for exercise and were instrumented with a commercially available heart rate monitor (Polar Team Pro, Polar Electro, Lake Success, NY, USA). Heart rate was measured at baseline seated quietly on the exercise bike, as well as every 5 minutes during exercise. Exercise sessions consisted of a 5-minute warm-up and cool-down at 30% of heart rate reserve, with 40 minutes at our target intensity of 60% of heart rate reserve. Water was provided *ad libitum* during exercise.

Statistics. Participant characteristics were analyzed using an unpaired, two-tailed Student's *t*-test and are presented as means ± SD. Primary outcome variables, including in-clinic blood pressure, hemodynamic variables, and pulse wave velocity, were analyzed using linear mixed-effects model with group and timepoint main effects and an interaction effect to determine changes over time within groups, completed in collaboration with the biostatistics department at Oregon Health & Science University. Reference levels for fixed effects were exercise training and pre-intervention timepoint. Heat Intervention Fixed effect represents group differences across all timepoints. Timepoint estimates represent differences in interim or post-interventions blood pressure compared to pre-intervention across all groups. Secondary outcome variables, including VO_{2peak} and heart rate variability, were compared using a mixed-model analysis of variance with main effects of group and time. In the event of a significant main effect, multiple comparisons were completed using the Sidak analysis. Significance was accepted as $p<0.05$. Statistical analysis was conducted in GraphPad Prism 10.1.0 (GraphPad, Dotmatics, San Diego, CA, USA).

RESULTS

Participant anthropometric and demographic data are presented in Table 5.1. Primary outcome variables are presented as mean of the difference from PRE to POST with 95% CI in the text. Secondary outcome variables are presented as the mean of the difference from PRE to

POST with 95% CI, with the associated *p*-value from statistical comparison. Mean data for all outcome variable for both groups at all time points can be found in Table 5.4.

Blood Pressure. In-clinic systolic blood pressure (SBP) was not different following exercise training (1 mmHg [-3, 5]) or hot water immersion (1 [-6, 7]), nor was in clinic diastolic blood pressure (DBP) following exercise training (2 mmHg [-2, 6]) or hot water immersion (1 [-4, 6]) (Figure 5.1.). Results of linear mixed effects analysis of in-clinic blood pressure can be found in table 5.2.

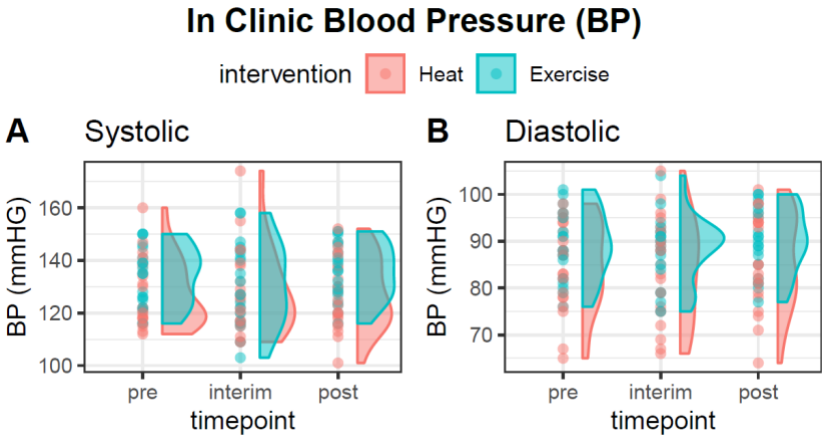

Figure 5.1. In-clinic systolic and diastolic blood pressure. Violin and dot plots show blood pressure assessments at pre, mid, and post-intervention for all participants.

	Systolic Blood Pressure		Diastolic Blood Pressure	
Fixed Effect	**Estimate (95% CI)**	**P Value**	**Estimate (95% CI)**	**P Value**
In clinic				
Intercept	134.30 (128.35, 140.25)	-	89.10 (85.22, 92.98)	-
Heat Intervention	-7.01 (-15.33, 1.31)	.12	-4.48 (-9.90, 0.94)	.07
Timepoint		.45		.17
interim	-3.15 (-7.11, 0.81)		-0.65 (-3.40, 2.10)	
post	1.15 (-2.81, 5.11)		1.25 (-1.50, 4.00)	
Interaction		.18		.99
Heat:interim	3.96 (-1.57, 9.49)		0.03 (-3.812, 3.87)	
Heat:post	-1.01 (-6.54, 4.53)		-0.11 (-3.95, 3.74)	

Table 5.2. Results of linear mixed-effects model for in-clinic systolic and diastolic blood pressure.

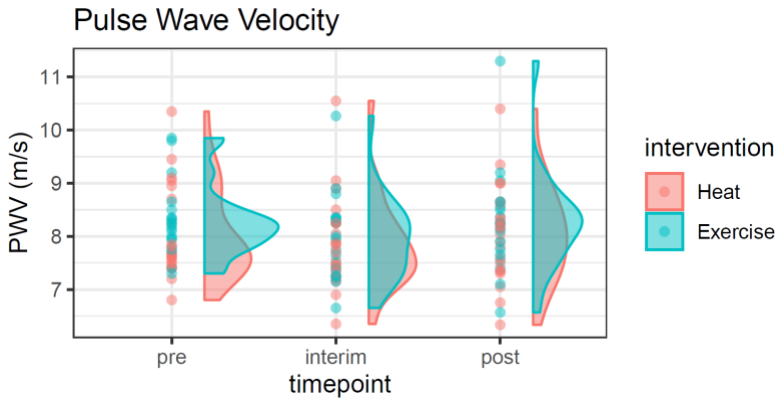

Figure 5.2. Pulse wave velocity. Violin and dot plots show blood pressure assessments at pre, mid, and post-intervention for all participants in both groups

Fixed Effect*	**Estimate (95% CI)**	***P* value****
Intercept	8.28 (7.90, 8.66)	-
Heat Intervention	-0.23 (-0.76, 0.30)	.44
Timepoint		.008
interim	-0.30 (-0.55, -0.04)	
post	-0.02 (-0.27, 0.23)	
Interaction		.84
Heat:interim	0.10 (-0.25, 0.45)	
Heat:post	0.017 (-0.34, 0.37)	

Table 5.3. Results of linear mixed-effects model for pulse wave velocity.

Carotid-femoral pulse wave velocity was significantly reduced in the exercise group at the mid-intervention assessment, but values returned to baseline by the post-intervention timepoint. Heat therapy was not different across all time points. These data are represented graphically in figure 5.2. The results of the linear mixed-effects model analysis of pulse wave velocity can be found in table 5.3. Cardiac output was not significantly different between or within groups following either aerobic exercise training (0.15 L/min [-0.3, 0.6]) or hot water immersion (-0.06 [-0.66, 0.53]). Likewise, total peripheral resistance was not changed for either exercise training (-0.25 mmHg/min/L [-2.3, 1.8]) or hot water immersion (0.12 [-1.9, 2.1]).

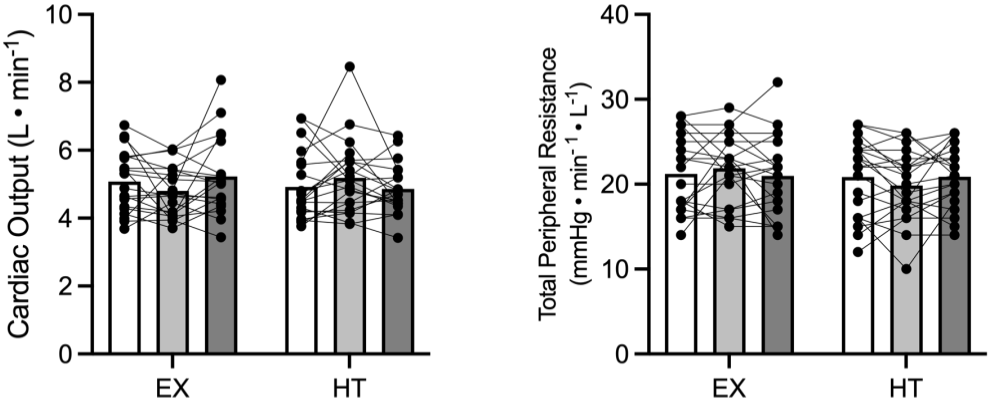

Figure 5.3. Resting cardiac output and total peripheral resistance. Data are presented as means and individual values for both groups at all time points

Resting hemodynamic data can be found in Figure 5.3.

Fixed Effect*	Heart Rate (95% CI)	Cardiac Output (95% CI)	Stroke Volume (95% CI)	TPR (95% CI)
Intercept	65.25 (60.62, 69.88)	5.03 (4.61, 5.44)	74.97 (67.00, 82.95)	21.46 (19.59, 23.32)
Heat Intervention	-3.44 (-9.91, 3.03)	-0.09 (-0.67, 0.49)	4.45 (-6.69, 15.60)	-0.67 (-3.27, 1.93)
Timepoint				
interim	-1.55 (-3.92, 0.82)	-0.23 (-0.61, 0.16)	0.17 (-6.55, 6.88)	0.48 (-0.89, 1.85)
post	-1.90 (-4.27, 0.47)	0.21 (-0.18, 0.59)	4.80 (-1.67, 11.26)	-0.45 (-1.83, 0.92)
Interaction				
Heat:interim	2.12 (-1.19, 5.43)	0.48 (-0.06, 1.02)	4.42 (-4.97, 13.81)	-1.55 (-3.47, 0.37)
Heat:post	1.71 (-1.60, 5.02)	-0.28 (-0.82, 0.26)	-7.08 (-16.49, 2.33)	0.58 (-1.35, 2.52)

Table 5.4. Resting hemodynamic data, including Cardiac Output, Heart Rate, Stroke Volume, and Total Peripheral Resistance.

Heart Rate variability is presented as the mean of the difference with 95% CI for absolute low and high frequency power, and total power. Low frequency power was not different following either aerobic exercise training (87 ms^2 [-71, 246]; p=0.42) or heat therapy (193 [-199, 586]; p=0.51). High frequency power was not different following either exercise (45 ms^2 [-45, 137]; p=0.49) or heat therapy (-10 [-235, 214]; p=0.99). Total power was not significantly

impacted by either exercise (155 ms^2 [-120, 432]; p=0.32) or heat therapy (315 [-439, 1069]; p=0.64). Heart rate variability data for both groups at all time points can be found in Table 5.3.

Peak oxygen consumption was significantly increased following exercise, (0.28 L/min [0.17, 0.397], p *<0.001*), but not heat therapy (0.01 [-0.1, 0.12], p = 0.90).

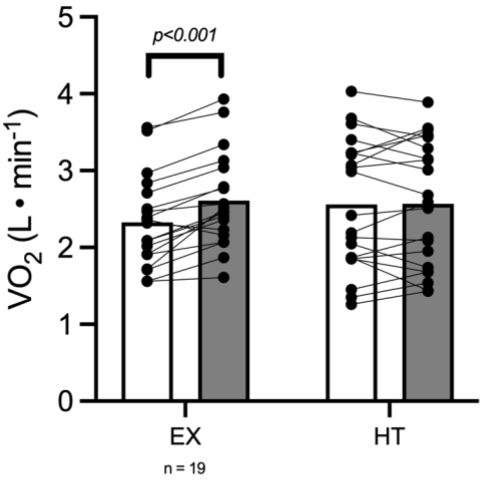

Figure 5.4. VO_{2peak} before and after both interventions. 1 individual was excluded from the exercise group because of failure to complete VO_{2peak} testing within 3 weeks of completing exercise training.

Variable	*Group*	*PRE*	*MID*	*POST*
In-clinic SBP (mmHg)	EX	135 [129, 140]	131 [124, 139]	135 [130, 140]
	HT	127 [121, 133]	128 [120, 135]	128 [121, 134]
In-clinic DBP (mmHg)	EX	89 [85, 92]	88 [85, 92]	90 [87, 94]
	HT	85 [80, 89]	84 [79, 89]	86 [81, 90]
PWV (m/s)	EX	8.2 [7.9, 8.6]	7.9 [7.6, 8.2]	8.1 [7.8, 8.4]
	HT	8.0 [7.6, 8.5]	7.9 [7.5, 8.3]	8.1 [7.7, 8.5]
VO_{2peak} (L/min)	EX	2.33 [2.04, 2.61]		2.61* [2.31, 2.9]
	HT	2.56 [2.17, 2.94]		2.57 [2.19, 2.94]

Variable	*Group*	*PRE*	*MID*	*POST*
Heart Rate Variability				
Low Frequency (0.04-0.15 Hz)	EX	80 [54, 106]	141 [60, 222]	167 [25, 310]
	HT	140 [61, 219]	220 [53, 386]	333 [13, 653]
High Frequency (0.15-0.40 Hz)	EX	79 [49, 106]	134 [58, 210]	125 [48, 202]
	HT	214 [83, 345]	173 [81, 266]	204 [61, 345]
Total Power (≤0.40 Hz)	EX	268 [203, 333]	421 [157, 685]	424 [175, 672]
	HT	503 [265, 741]	611 [283, 940]	818 [218, 1419]

Table 5.5. Mean [95% CI] at all timepoints for both intervention groups for outcome variables of interest. *$p<0.001$ compared to PRE.

DISCUSSION

Heat therapy in the form of chronic hot water immersion has gained attention in recent decades as a potential therapeutic alternative to exercise for improving cardiovascular health. Previous studies have reported compelling improvements in known risk factors for cardiovascular disease, such as blood pressure and arterial stiffness, as well as endothelial health. Previous investigations have focused on a variety of populations, but none have investigated the efficacy of heat therapy for improving blood pressure in adults with untreated hypertension in comparison with the gold standard, aerobic exercise training.

Contrary to our hypothesis, we do not report any significant reductions in blood pressure following chronic passive hot water immersion, nor do we report any differences with exercise training. Previous data from our lab that has reported significant reductions in blood pressure with chronic passive hot water immersion have utilized a greater heating stimulus, either 60 minutes (9) or 90 minutes (8) per session, compared to 45 minutes per session in the present intervention.

The data presented here are consistent with a growing body of literature that offers equivocal findings regarding the benefits of heat therapy on vascular health. A recent study investigating the impact of Finnish sauna on vascular health outcomes in individuals with stable coronary artery disease reported similar findings to those presented here, wherein heat therapy

did not elicit favorable improvements in cardiovascular health (724). Despite evidence of heat acclimation following 8 weeks of sauna use, namely lower resting core temperature and increased sweat rate, Debray and colleagues report no change in peripheral endothelial function, microvascular reactivity, vascular smooth muscle sensitivity, or blood pressure (724). These data are perhaps not surprising in light of data from a similar population that reported no impact of an acute bout of Finnish sauna on brachial artery Flow-mediated dilation (444).

An investigation comparing exercise training with hot water immersion for improving vascular health in individuals with peripheral artery disease offered similar findings. Akerman and colleagues report a significant reduction in systolic blood pressure following 12 weeks of hot water immersion, but no change in central or peripheral arterial stiffness, endothelial function, or diastolic blood pressure compared to pre-intervention values or the exercise group (10). Notably, rates of compliance differed between arms of the intervention. On average, individuals in the hot water immersion group completed 47 out of 57 possible sessions, while individuals in the exercise group completed 14 out of 24 possible sessions (10). It is very possible that the difference in accumulated stress played a role in the reduction in systolic blood pressure seen with heat therapy but not exercise training. To that end, we ensured nearly 100% compliance with both interventions in the present study. One individual in each arm of this dissertation failed to complete all 30 sessions, with each reaching 27 completed sessions. As such, we are confident that this dissertation was a fairer comparison of exercise training and heat therapy.

Additional investigations report no change in blood pressure following heat therapy. In an 8-week single-leg model of passive heating in young healthy females, McGarity-Shipley and colleagues report that systolic and mean arterial pressure were greater at week 4 and 6 compared to week 2 (733). The authors also report a substantial increase in vascular shear stress measured in the superficial femoral artery (~611% increase compared to baseline) during heating. This is critical, as repeated increases in shear stress are thought to be crucial in mediating the improvements in endothelial function and vascular health seen with heat therapy (8, 9, 546, 730). In light of consistent evidence for beneficial increases in antegrade shear stress with heat therapy, it is surprising that blood pressure was not reduced in this intervention.

Among individuals with heart failure, short duration warm water immersion (5 sessions per week for 2 weeks) failed to reduce blood pressure, despite improvements in cardiac function following the intervention (734). To that end, we report no significant differences between or

within groups for any of our hemodynamic variables: cardiac output, total peripheral resistance, or systemic vascular conductance. It is possible that the lack of change in these hemodynamic variables influenced our results. Previous data suggests that increased resting cardiac output and a shift towards increased sympathetic activity underlie the initial stages of neurogenic hypertension (735). In advanced stages of essential hypertension, cardiac output normalizes, but without an attendant reduction in blood pressure (735). In experimental animal models of hypertension, training-induced bradycardia and reductions in sympathetic outflow played a pivotal role in reducing resting cardiac output and blood pressure (736). Conversely, exercise training interventions among humans with essential hypertension report the primary hemodynamic adaptation is a reduction in total peripheral resistance, often accompanied by a significant *increase* in cardiac output, reflective of cardiac adaptation to prolonged training (737–739). These data suggest that there are hemodynamic adjustments that accompany improvements in neurogenic hypertension, although we do not report these changes in the present study.

There is a complex relationship between arterial stiffness, aging, and hypertension. Increases in blood pressure with aging cause greater stress to conduit arteries, resulting in elastin degradation and greater arterial stiffness (740). Among middle-aged and older adults, increased arterial stiffness is more often a consequence, not a cause, of essential hypertension, compared to younger individuals where vascular remodeling in resistance vessels elicits increased blood pressure (741). Finally, due to the synergistic nature of blood pressure and arterial stiffness and integrity, interventions that report improvements in arterial stiffness almost always report reductions in systemic blood pressure, particularly among middle-aged individuals (742). Brunt and colleagues report a significant reduction in arterial stiffness only at the conclusion of 8 weeks of hot water immersion among a young, healthy, but sedentary population (8). Utilizing a similar hot water immersion protocol in similarly young individuals who present with an increased cardiovascular disease risk profile, Ely and colleagues report no change in carotid-femoral pulse wave velocity following heat therapy (9). Furthermore, chronic heating interventions among older adults with cardiovascular disease offer equally mixed findings. Akerman and colleagues report no change in ankle-brachial index among adults with peripheral arterial disease, despite significant reductions in blood pressure (10), while Debray, et. al., report no change to either blood pressure or pulse wave velocity following 8 weeks of sauna use in

adults with stable coronary artery disease (724). Our findings are consistent with the current literature which suggests that arterial stiffness is malleable among younger individuals, particularly those that are otherwise healthy, but is less so among middle-aged and older adults with established cardiovascular disease. Finally, without a requisite reduction in blood pressure as suggested previously (742), perhaps it is unsurprising that we do not report any reductions in pulse wave velocity in the present study.

We report significant improvement in peak oxygen consumption following exercise training. Accordingly, we are confident that our exercise intervention was an effective training stimulus. The exercise training intervention was designed to meet the recommendations for weekly aerobic exercise from the American College of Sports Medicine (5). Previous reports that utilize a similar intervention (40 min of treadmill exercise at 60% HRR) reported a significant reduction in ambulatory blood pressure, but not carotid-femoral pulse wave velocity (743). Similarly, a large meta-analysis of studies investigating the impact of exercise on blood pressure report that dynamic exercise is most effective in individuals with established hypertension (265). Interestingly, the greatest reductions in aerobic exercise were reported after short duration (≤4 weeks) interventions with moderate-to-high intensity dynamic aerobic exercise (265). The present study utilized a longer (8-10 week) intervention, which may partially explain the lack of a change in blood pressure.

The hot water immersion intervention in the present study was designed with the intention of matching the time duration of the aerobic exercise training intervention. Perhaps contributing to the incongruence between our results and those previously published in our laboratory (8, 9) is the difference in heating load accumulated throughout the intervention. Brunt and colleagues utilized thirty-six 90min sessions over 8 weeks, while Ely and colleagues utilized thirty 60min sessions over the same time course. It is possible that with a greater frequency of heating, alone or in combination with a longer duration of each bout of heating, we would have demonstrated improvements in blood pressure as seen with these previous interventions.

Limitations

The present study was not without limitations and considerations. Primarily, the duration of time to complete the prescribed intervention. The target time to complete the intervention was 8-10 weeks, which corresponds to 3-4 sessions of either exercise or hot water immersion per

week. Several individuals did not complete their assigned intervention within this desired projected timeframe (maximum time to completion was 16 weeks). Despite this, all but two individuals (one exercise and one hot water immersion) completed all 30 sessions. Utilizing an intention-to-treat statistical model and study design, we have included all individuals in the data set for analysis, presented here.

CONCLUSIONS

These data suggest that an aerobic exercise training intervention that meets the guidelines from the American College of Sports Medicine, while an effective training intervention for improving cardiorespiratory fitness, is not capable of improving known risk factors for cardiovascular disease, such as blood pressure and arterial stiffness. Despite compelling evidence that heat therapy is an effective intervention for lowering blood pressure and improving cardiovascular health, we report no impact of 30 sessions of hot water immersion on blood pressure, arterial stiffness, or cardiorespiratory fitness.

CHAPTER VI
COGNITIVE FUNCTION AND ALZHEIMER'S DISEASE BIOMARKERS

INTRODUCTION

Alzheimer's Disease (AD) is the leading cause of dementia world-wide, with a diverse pathology that presents a challenge for identifying consistent and unifying biological signatures (744). In light of a larger aging population, the incidence of AD is projected to triple to approximately 14 million people in the United States alone by 2050, with a global financial burden in excess of $1 trillion (12, 745, 746). Amongst aging individuals, approximately 30 – 50% of AD cases can be mitigated by addressing modifiable risk factors in mid-life (747). Hypertension represents once such modifiable risk factor for cardiovascular and cerebrovascular disease (200–203), and accentuates the already heightened risk for AD with advancing age (198, 199). Indeed, even in the absence of AD, there is evidence for impaired cognitive function in individuals with hypertension (204–206), which is associated with an increased incidence of dementia in later life in a dose-dependent fashion (207–211).

As a low-resistance, high-flow organ, the brain is highly susceptible to chronic high blood pressure, which may manifest as small vessel disease and volumetric changes to gray matter or white matter within the brain. The hippocampus is the primary site for encoding new memories in the brain; this is the primary site of reduction in volume that is associated with advanced aging and as well as AD (391, 392). Hippocampal atrophy is accelerated by hypertension, a reduction that is both progressive and cumulative, such that advancing age and duration of hypertension is associated with a greater reduction in volume (234, 393, 748). There is compelling evidence to suggest that increased pulsatile pressure associated with hypertension is intricately linked to greater volume of white matter hyperintensities, which can be visualized using magnetic resonance imaging and represent small vessel damage within the brain (191, 255, 749). Accumulated volume of white matter hyperintensities are associated with the progression from mild cognitive impairment to overt dementia (750–752). Much the same as vascular and target organ damage in the periphery, large artery stiffness can exacerbate blood pressure-mediate small vessel damage within the brain (191, 256). Small vessel damage compounds the decline in cognitive function and brain health through impairments in cerebral blood flow seen with hypertension (238–240). Among individuals with hypertension, regional and global cerebral

perfusion is reduced relative to their age-matched normotensive counterparts (238, 241), which has been identified as a hallmark of the initial stages of AD progression (753, 754).

Interventions aimed at improving blood pressure in middle-aged adults have the potential to improve both cardiovascular as well as neurodegenerative disease risk to a greater extent than those implemented later in life (261–263). Previous investigations have demonstrated that pharmacological reductions in blood pressure are associated with an improvement in risk factors for AD in individuals with hypertension, such as normalization of cerebral hypoperfusion (242, 243). However, there are a dearth of lifestyle interventions among adults with high blood pressure but without documented mild cognitive impairment. There is a critical need to further explore lifestyle interventions that have the potential to delay the onset of cognitive decline and reduce Alzheimer's Disease risk.

Exercise training carries multifaceted benefits for not only cardiovascular disease risk, but also for brain health (266). Longitudinal exercise training interventions are demonstrated to significantly increase both gray matter and white matter in the prefrontal and temporal corticies among older adults compared to non-exercising age-matched controls (755), and are associated with increased hippocampal size and an attendant improvement in memory function (395). Furthermore, there is limited data to suggest that aerobic exercise training may attenuate white matter hyperintensity progression among individuals with mild cognitive impairment (756). In this investigation from Dao and colleagues, aerobic exercise training was effective at slowing white matter hyperintensity progression among males but not females (756).

At present, there is limited but compelling data that lifelong traditional Finnish sauna use is associated with a reduced risk for dementia, even after adjustment for potential confounding factors such as socioeconomic status, lifestyle, and metabolic risk factors (267–269, 587). Potentially contributing to the benefits of heat therapy on brain health and cognitive function are improvements in blood pressure and mitochondrial function, possibly mediated by heat shock proteins and centrally acting extracellular vesicles (270). Lastly, Bailey and colleagues report that 8 weeks of warm-water immersion can elicit similar improvements in cerebrovascular function as the same time course of exercise training (583).

The purpose of this study is to determine whether 30 sessions of either aerobic exercise training or chronic hot water immersion is more effective at improving cognitive function and MRI-based biomarkers of Alzheimer's Disease risk among middle-aged individuals with

untreated hypertension. We hypothesize that heat therapy will elicit greater improvements in cognitive function and biomarkers of Alzheimer's disease than aerobic exercise training.

METHODS

The data presented here are a subset of a parent clinical trial comparing the efficacy of heat therapy and aerobic exercise training on lowering blood pressure in adults with untreated hypertension (*clinicaltrials.gov* identifier: NCT03557502). Prior to participation in this research, all participants provided written informed consent according to the Declaration of Helsinki. This protocol approved by the University of Oregon Institutional Review Board (protocol # 05042018.006).

Participants. Twenty-seven adults with elevated or high blood pressure (defined as systolic blood pressure ≥ 120 and/or diastolic blood pressure ≥ 80) volunteered to participate in this research. Inclusion criteria included individuals aged 35-60, systolic blood pressure between 120 and 180 mmHg, diastolic blood pressure between 80 and 120 mmHg. Exclusion criteria included systolic blood pressure greater than 180 mmHg or diastolic blood pressure greater than 120 mmHg; chronic cardiorespiratory or metabolic disease other than hypertension; abnormal resting or exercise electrocardiogram; currently taking antihypertensive medication; BMI ≥ 35 kg/m^2; fasting glucose ≥126 mg/dL; fasting hemoglobin A1c ≥7%; LDL ≥160 mg/dL; high levels of physical activity assessed according to the International Physical Activity Questionnaire (IPAQ); persons who are pregnant, nursing, or currently trying to conceive, aged 34 years or younger, or 61 years and older. Blood pressure was assessed in accordance with current American College of Cardiology and American Heart Association guidelines to confirm elevated to hypertensive blood pressure. Briefly, blood pressure was assessed ≥2 times on ≥2 days and averaged to determine hypertension classification. Specific to this protocol, individuals were unable to participate if they presented with current psychiatric conditions, have been unconscious for more than 15 minutes, have had 3 or greater losses of consciousness of 5 minutes or greater, have brain or spinal cord injuries, are unable to lie flat for ≥60 minutes, have any artificial or metal devices implanted, have been exposed to loose metal shavings or scraps, or are left handed. Subject characteristics can be found in Table 6.1.

	Aerobic Exercise (EX)	Hot Water Immersion (HWI)
n	12 (5F)	15 (5F)
Age (years)	49 ± 7	47 ± 7
Body Mass (kg • m^{-2})	31 ± 3	30 ± 4
Systolic Blood Pressure (mmHg)	132 ± 12	128 ± 14
Diastolic Blood Pressure (mmHg)	87 ± 7	84 ± 10
Mini Mental State Exam Score	29 ± 1	29 ± 1

Table 6.1. Subject characteristics for individuals who completed MRI-based imaging measurements. Values are means ± SD

Exercise Training. Aerobic exercise training consisted of 30 sessions on an upright stationary cycle ergometer (Precor UBK 600, Woodinville, WA, USA) over 8-10 weeks, at a frequency of 3-4 sessions per week. This frequency and duration were selected to achieve the recommended minimum physical activity guidelines from the American College of Sports Medicine. Subjects arrived at lab in suitable clothing for exercise and were instrumented with a commercially available heart rate monitor (Polar Team Pro, Polar Electro, Lake Success, NY, USA). Heart rate was measured at baseline seated quietly on the exercise bike, as well as every 5 minutes during exercise. Exercise sessions consisted of a 5-minute warm-up and cool-down at 30% of heart rate reserve, with 40 minutes at our target intensity of 60% of heart rate reserve. Water was provided *ad libitum* during exercise.

Heat Therapy. Heat therapy consisted of 30 bouts of hot water immersion over 8-10 weeks, at frequency of 3-4 sessions per week. Prior to immersion, subjects were instrumented with a commercially available heart rate monitor (Polar Team Pro, Polar Electro, Lake Success, NY, USA). Participants were immersed to mid-sternum in a commercially available hot tub set to 40°C for 45 minutes, with a 5-min cool-down seated outside of the hot tub. This heating intervention was modeled on previous interventions in our lab that demonstrated significant improvements in blood pressure and vascular function among both healthy individuals and women with polycystic ovary syndrome (8, 9). Furthermore, the duration of heating was time-matched to the exercise intervention.

Venous Blood Sampling. We obtained a venous serum sample via venipuncture and centrifugation. A fasted blood draw was performed from a suitable antecubital or dorsal hand vein into a serum separating vacutainer with a clot activator (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were allowed to clot upright at room temperature for

30 minutes. Samples were then centrifuged at 4°C and 1300 RCF for 10 minutes. Serum was then aliquoted into cryogenic tubes and frozen at -80°C until later analysis.

Biochemical Analysis. We assessed serum Brain-Derived Neurotrophic Factor at baseline and following the intervention. Serum samples were diluted 100-fold so that samples fell within the range of the standard curve, as per manufacturer's instructions. Serum BDNF was quantified via *Rapid*™ enzyme-linked immunosorbent assay (Biosensis, catalog # BEK-2211, Thebarton, Australia).

Cognitive Function Testing. All participants completed the Mini Mental State Examination during the screening process and prior to randomization (757, 758). The MMSE was completed only at baseline to ensure subjects were not cognitively impaired prior to the intervention, and assess eligibility for participation in the imaging component of this research.

In addition to the MMSE, all participants completed the Blueprint-funded NIH Toolbox for Assessment of Neurological and Behavioral Function. We assessed both episodic memory and working memory using picture sequence and list sorting tasks, respectively. Both working memory (659–661) and episodic memory assessments (662–664) have been validated for use in adults as well as across the lifespan.

Lastly, all participants completed the Pittsburgh Sleep Quality Index (PSQI) at baseline as well as after the completion of all 30 sessions (759). The PSQI has been validated in healthy young as well as older adults (760). Furthermore, PSQI score has been shown to be strongly associated with prevalence as well as future development of hypertension (761). This index was included so that researchers may account for potentially confounding changes in sleep from the beginning to the end of the intervention.

Magnetic Resonance Imaging. Participants completed a baseline session of magnetic resonance imaging prior to randomization as well as after completion of all 30 sessions of either exercise or heat therapy. Post-intervention image acquisition was completed ≥48 hours following the final exercise or hot water immersion session to prevent any acute impact of these stressors on perfusion-based imaging metrics.

Upon arrival to the Lewis Center for Neuroimaging, participants completed a second safety questionnaire to ensure adequate compliance with the necessary safety protocols prior to magnetic resonance imaging. For the present study, we conducted an MRI scan sequence lasting approximately 1 hour. All scans were conducted at the Lewis Center for Neuroimaging at the

University of Oregon using a 3T MRI machine using a Siemens 32-channel head coil with optimized sequences (Siemens Skyra, Erlangen, Germany).

High-resolution (1.0 x 1.0 x 1.0 mm) T1-weighted brain images were acquired using a 3D magnetization-prepared rapid gradient echo imaging protocol (679), TR/TE/TI = 2500ms/3.43ms/1100ms, FOV= 256mm, matrix size = 256x256, with 176 contiguous slices in ascending fashion. Furthermore, T2 sagittal turbo spin echo images (1.0 x 1.0 x 1.0 mm isotropic voxel resolution) were acquired with 192 slices per slab in ascending fashion.

To assess white matter hyperintensities, we employed Fluid Attenuated Inversion Recovery in the axial plane, TR/TI/TE = 9000ms/113ms/2500ms, 1.0mm x 1.0mm x 2.5mm resolution, parallel imaging with a flip angle of 120°.

Arterial spin labelling was acquired using simultaneous multi-slice 2D echo-planar imaging for high spatial resolution whole-brain pseudo-continuous arterial spin labelling (pcASL) imaging. Images were acquired with 2.5mm in-plane resolution with 60 2.27mm thick slices with a 10% slice gap, and 5 post labelling delays (PLDs: 0.2, 0.7, 1.2, 1.7, 2.2).

Hippocampal volume was assessed using T2- oblique coronal turbo spin echo imaging with 0.39mm x 0.39mm x 2.0 mm voxel resolution, TR/TE = 13520ms/88ms, echo train length = 15ms, bandwidth = 222Hz/pixel, echo spacing = 11.1ms, FOV = 220mm, 65 slices, with a flip angle = 150°. Hippocampal volume was segmented and corrected for total intracranial volume for comparisons before and following each intervention. Structural MRI TSE sequences were analyzed using FreeSurfer (version 6.0.0; freely available at https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki) using automatic segmentation of hippocampal subregions (762–767). This analytical pipeline has been well-documented elsewhere (764, 768). Briefly, the cortical thickness of subregions in the hippocampus and volumetric assessments were calculated using this automated tool as part of the HC segmentation protocol. FreeSurfer derives a frequency histogram of possible structures (defined by the spatial template prior) and used this to compute the probability of a given anatomical label occurring at a given location (765). The probabilistic labels and corresponding predicted image intensities are priors, and the intensity similarity between the target image and the atlas is a likelihood term. The resulting subregional thickness values were used in statistical calculations. In order to calculate the intracranial head size for normalizing hippocampal thickness values, we used FreeSurfer on whole brain T1-weighted scans to calculate the estimated total intracranial volume

(ETIC). This software suite used tissue contrast to determine the boundary between GM, WM, and the pial surfaces to calculate the difference between vertices plotted as a mesh surface for each layer across the entire cortex. After the automated portion of the FreeSurfer pipeline was completed, each subject's scan was visually checked for accuracy.

Image Analysis. White matter hyperintensities were assessed using Schelten's Scale applied to T2 and FLAIR images (682, 683). From this, whole-brain white matter scores were calculated, and log transformed for subsequent analyses. Arterial spin labelling was analyzed according to the most recently released system parameters of the Human Connectome Project – Aging (681). In order to most accurately account for changes in arterial transit time documented with aging (684), multiband echo planar imaging was used in conjunction with 5 post-label delays. Anatomical images were pre-processed using Free Surfer (7.4.1., Harvard University, Cambridge, MA, USA). DICOM files corresponding to each post-label delay were converted to NIfTI and merged prior to analysis using BASIL (Bayesian Inference for Arterial Spin Labelling; v.6.0.1, University of Oxford, Oxford, UK). BASIL allows researchers to quantify resting gray and white matter perfusion, with corrections for bolus duration, post-label delay, and partial volume correction using dual-orientation anatomical calibration images.

Statistical Analysis. Participant characteristics were analyzed using an unpaired, two-tailed Student's *t*-test and are presented as means ± SD. Data are presented as mean of the difference from PRE to POST with 95% CI for both exercise (EX) and heat therapy (HT). Preliminary analysis of primary outcome variables was analyzed using a two-way repeated measures analysis of variance. Repeated measures were compared within EX and HT, and non-repeated measures were conducted between groups. In the event of a significant main effect, post-hoc testing was conducted using the Sidak analysis for multiple comparisons. Statistical analysis was performed in GraphPad Prism 10.1.0 (GraphPad, Dotmatics, San Diego, CA, USA). Significance was accepted as $p<0.05$.

RESULTS

A total of 27 participants from the parent clinical trial completed this protocol. Participant anthropometric, demographic, and group distribution data can be found in table 6.1.

Gray matter perfusion was not impacted by either aerobic exercise (5.5 [-3, 13]; $p=0.196$) or heat therapy (2 [-5, 9]; $p= 0.743$; Figure 6.1A). White Matter perfusion was not impacted by

either aerobic exercise (0 [-4, 5]; p=0.993) or heat therapy (-1.57 [-5, 3]; p=0.56; Figure 6.1B). Mean arrival time in gray matter was not impacted by either exercise training (0.03 [-0.03, 0.09]; p=0.37) or heat therapy (0.03 [-0.03, 0.08]; p=0.41; Figure 6.1C). Mean arrival time in white matter was not changed following either exercise training (0.01 [-0.05, 0.07]; p=0.89) or heat therapy (0.02 [-0.03, 0.08]; p=0.52; Figure 6.1D).

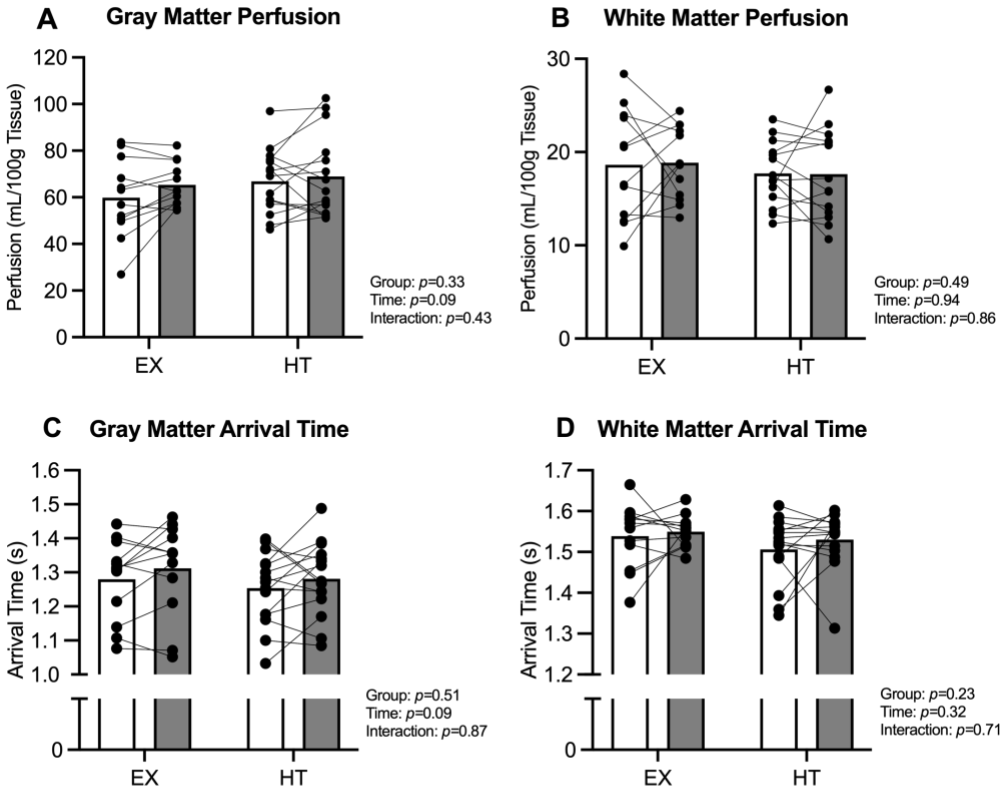

Figure 6.1. Mean Gray (1A) and White (1B) matter perfusion, as well as mean gray (1C) and white matter arrival time (1D) pre- and post-intervention for exercise (EX) and heat therapy (HT).

Hippocampal volume was not significantly impacted by either aerobic exercise training (-47 [-123, 30]; p=0.29) or Heat Therapy (-4 [-73, 64]; p=0.98; Figure 6.2). Serum brain-derived neurotrophic factor was not changed following either exercise training (-9 [-40, 23]; p=0.75) or heat therapy (1.3 [-29, 32]; p=0.99; Figure 6.3).

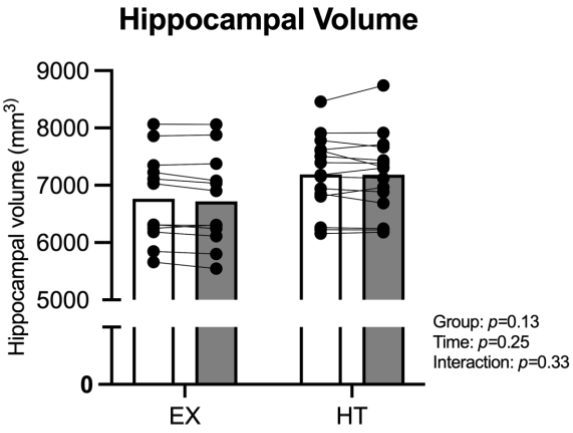

Figure 6.2. Total (Left and Right) Hippocampal Volume before and after exercise training (EX) and heat therapy (HT). Data are mean and individual values.

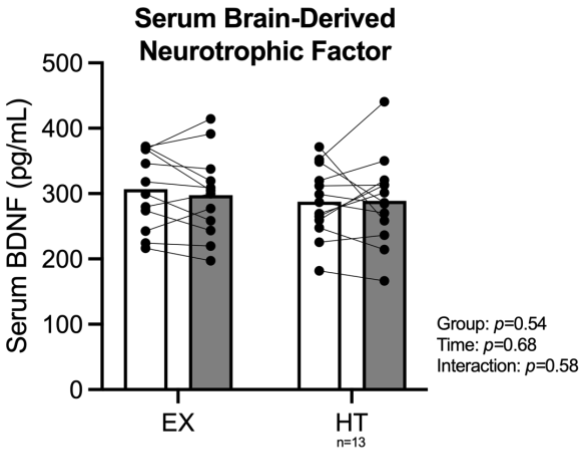

Figure 6.3. Serum BDNF before and after exercise training (EX) and Heat therapy (HT). Data are mean and individual values.

Absolute white matter hyperintensity volume was not impacted by aerobic exercise training (63 [-68, 194]; p=0.46), but was significantly greater following heat therapy (126 [9, 243] p=0.03; Figure 6.4A). Normalized white matter hyperintensity volume was not different following either exercise (-0.5 [-1.3, 0.4]; p=0.38) or heat therapy (-0.1 [-0.9, 0.7]; p=0.94; Figure 6.4B).

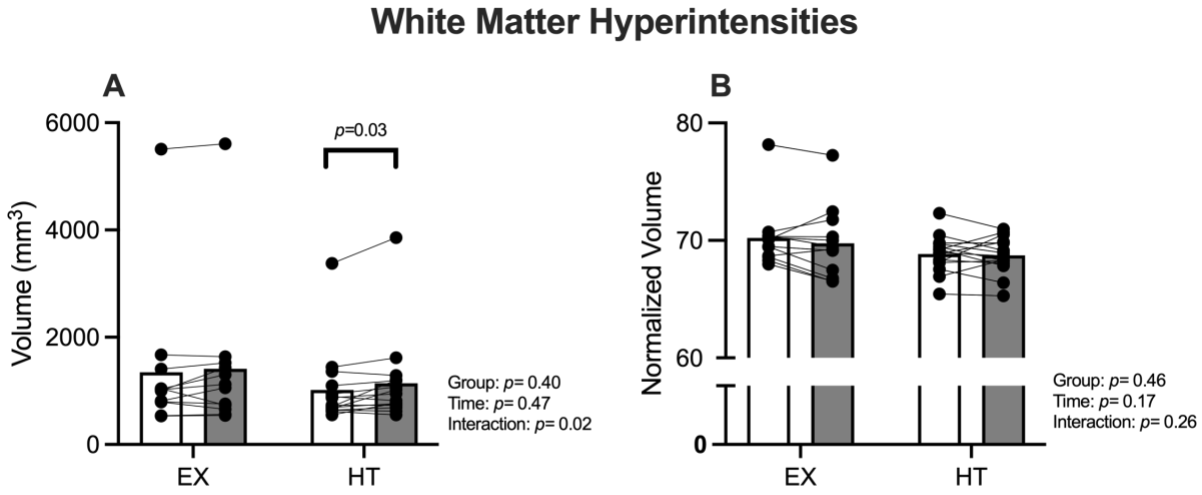

Figure 6.4. (A) Total white matter hyperintensity (WMH) volume. (B) normalized WMH volume. Total WMH volume was log transformed and normalized to whole brain WMH. Data are mean and individual values for both groups pre- and post-intervention.

Sleep quality, as assessed by the PSQI, was not different following either exercise (0 [-3, 2]; p=0.90) or heat therapy (0 [-2, 1]; p=0.84; Figure 6.5A). Working memory was not improved following either exercise (4 [-7, 16]; p=0.61) or heat therapy (1 [-9, 11]; p=0.98; Figure 6.5B).

Lastly, episodic memory was not improved following either exercise (7 [-11, 25]; p=0.57) or heat therapy (6 [-10, 22]; p=0.61; Figure 6.5C).

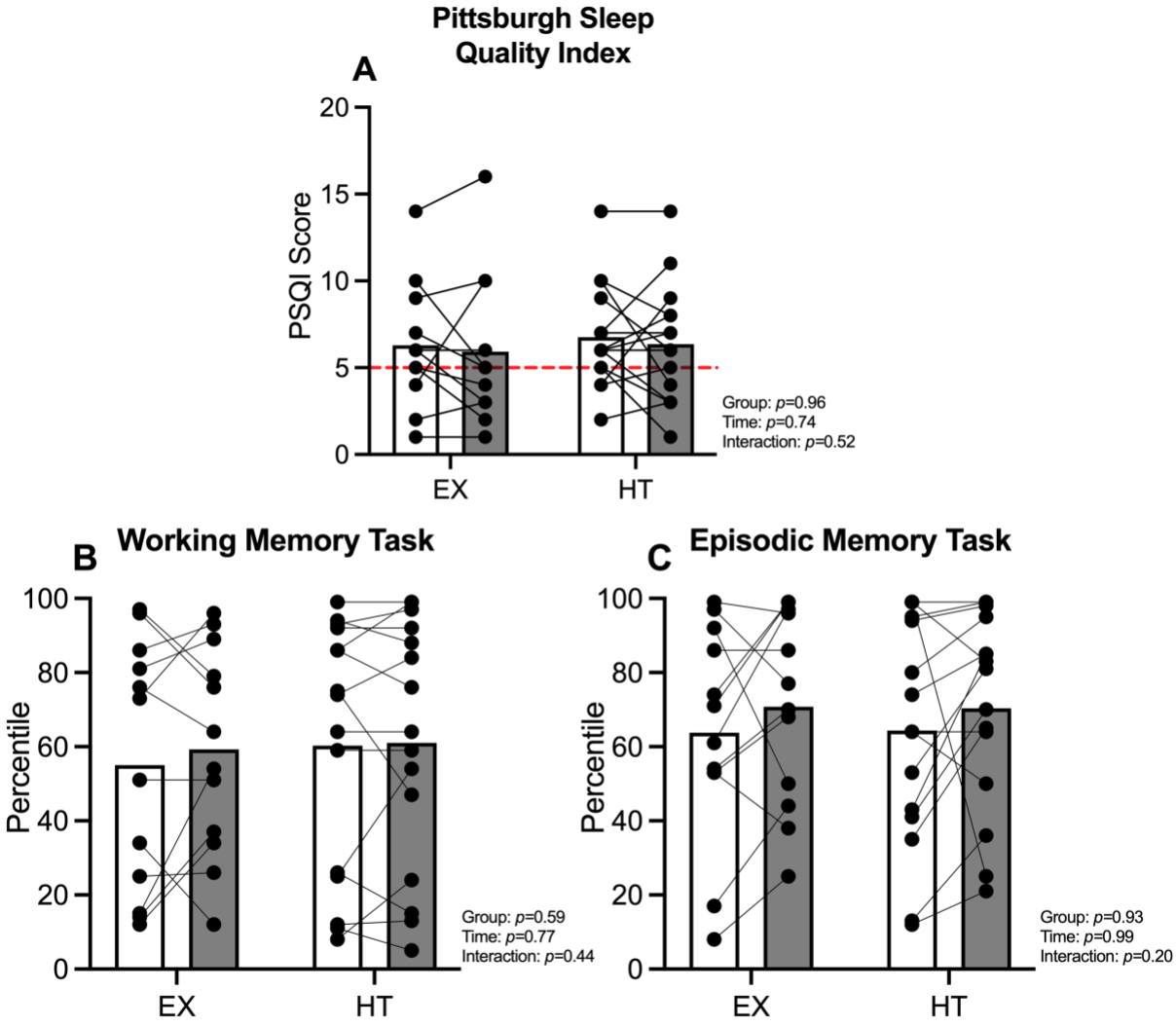

Figure 6.5. Pittsburgh Sleep Quality Index and cognitive function assessment results for both groups before and after exercise training (EX) and heat therapy (HT). Red dashed line on figure 6.5A represents the threshold for "poor sleep quality" as defined by PSQI survey.

Variable	*Group*	*PRE*	*POST*
Hippocampal Volume (mm^3)	EX	6767 [6270, 7264]	6720 [6213, 7227]
	HT	7189 [6822, 7557]	7185 [6799, 7572]
White Matter Hyperintensity Volume (mm^3)	EX	1348 [490, 2205]	1410 [536, 2285]
	HT	1017 [626, 1408]	1413 [697, 1589]
Normalized White Matter Hyperintensity volume	EX	70 [69, 72]	70 [68, 72]
	HT	69 [68, 70]	69 [68, 70]
Gray Matter Perfusion (mL/100g tissue)	EX	60 [49, 71]	65 [59, 71]
	HT	66 [59, 74]	68 [59, 79]
White Matter Perfusion (mL/100g tissue)	EX	18 [14, 22]	19 [16, 21]
	HT	19 [16, 22]	17 [15, 20]
Mean Arrival Time in Gray Matter (s)	EX	1.28 [1.2, 1.36]	1.31 [1.23, 1.39]
	HT	1.24 [1.2, 1.31]	1.28 [1.22, 1.34]
Mean Arrival Time in White Matter (s)	EX	1.54 [1.49, 1.59]	1.55 [1.52, 1.58]
	HT	1.51 [1.46, 1.55]	1.53 [1.49, 1.57]

Table 6.2. Structural and perfusion data for both groups pre- and post-intervention. Data are presented as mean [95% CI].

Variable	*Group*	*PRE*	*POST*
List Sorting Age Corrected Percentile	EX	55 [34, 76]	59 [41, 77]
	HT	60 [41, 79]	61 [42, 78]
Picture Sequence Age Corrected Percentile	EX	64 [41, 82]	71 [54, 87]
	HT	64 [47, 81]	70 [56, 85]
Pittsburgh Sleep Quality Index	EX	6 [4, 9]	6 [3, 9]
	HT	7 [5, 8]	6 [4, 8]

Table 6.3. Cognitive function data (list sorting and picture sequence from NIH toolbox). As well as Pittsburgh Sleep Quality Index scores for both groups pre- and post-intervention. Data are mean [95% CI].

DISCUSSION

Contrary to our hypothesis, neither 30 sessions of aerobic exercise training nor hot water immersion improve cognitive function or MRI-based biomarkers of Alzheimer's disease among adults with untreated hypertension. The putative mechanisms that underlie hypothesized improvements are reductions in systolic blood pressure and arterial stiffness, and improvements in vascular endothelial function. In the absence of these beneficial adaptations to either intervention, it is unsurprising that we do not report improvements among our key MRI-based biomarkers of AD risk. Furthermore, the duration of this intervention was relatively short (8-10 weeks) compared to other interventions aimed at improving brain health and cognitive function.

The macrovascular and hemodynamic changes associated with hypertension elicit a host of deleterious effects on the cerebral vasculature and cognitive function. Increased pulsatile pressure and subsequent increases in arterial stiffness are associated with small vessel damage in the brain, with implications for impaired cognitive function (191, 255, 749). Interventions aimed at lowering blood pressure and improving arterial stiffness have the potential to mitigate further declines in cerebrovascular health and cognitive function that are present with cardiovascular disease (205, 256, 260). Exercise is recognized as the primary lifestyle intervention for maintaining and improving brain health across the lifespan (266, 389, 769), but there is emerging experimental and prospective evidence to suggest that heat therapy may also promote beneficial adaptations for brain health (267, 270, 770).

Aerobic exercise training has been demonstrated to attenuate age-related declines in hippocampal volume (395). Maintenance of hippocampal volume with advancing age appears to be driven in large part by exercise-mediated increases in brain-derived neurotrophic factor (BDNF). Interventions that increase BDNF expression and concentration during mid-life have high probability of maintaining brain health and cognitive function in later life (771). There is a strong body of evidence to suggest that aerobic exercise training is capable of eliciting robust increases in BDNF concentrations (380, 383, 389), and that serum BDNF concentration is related to hippocampal volume as well as memory task performance (654). However, there is also emerging evidence that repeated bouts of hyperthermia can elicit increases in serum BDNF in young, healthy adults (591, 593, 594). Kojima and colleagues report a significant increase in serum BDNF immediately and 15 minutes following head-out hot water immersion, compared to baseline as well as a thermoneutral water immersion condition (591). Serum BDNF

concentrations assessed 30 minutes post-immersion do not differ from pre-intervention baseline values, however, suggesting that increased concentrations may be short lived, and that repeated bouts of hot water immersion may be necessary to elicit comparable effects to exercise (591). Glazachev and colleagues report that repeated bouts of hyperthermia is capable of eliciting increases in serum BDNF concentrations (594). However, this intervention conducted 10 sessions of whole-body far-infrared sauna condensed into 2 weeks (594). This is a considerably higher frequency of hyperthermia compared to our intervention and may partially explain the incongruence between our findings and those reported by Glazachev and colleagues. In a longer intervention, Glazachev and colleagues report that 24 sessions of far-infrared sauna is capable of increasing serum BDNF to a greater extent than 24 sessions of light-intensity exercise (593). While the timeline of this intervention more closely mirrors the intervention of the present study, the authors utilized a young, healthy population, compared to our population of adults with untreated hypertension. At present, there is a paucity of evidence regarding the efficacy of chronic passive heating to elicit improvements in serum BDNF in adults with untreated essential hypertension.

Erickson and colleagues report a significant relationship between the increase in cardiorespiratory fitness and the increase in hippocampal volume following exercise training, and that higher baseline fitness was associated with attenuated loss of hippocampal volume during a 1-year follow-up period (395). While these data are compelling and we do report a significant increase in cardiorespiratory fitness following our exercise intervention, the duration of our protocol (8-10 weeks) may not be of sufficient duration to elicit increases in hippocampal volume similar to those reported previously (395).

There appears to be a direct linear relationship between a history of high blood pressure and severity of white matter hyperintensities (249, 772, 773). Previous data suggests that hypertension at baseline is associated with significantly greater risk for severe white matter hyperintensities at a 4-year follow-up assessment compared to normotensive controls (249). Additionally, normotension and pharmacological control of blood pressure was associated with reduced WMH compared to established and incident hypertension during the follow-up period (249). Investigations of WMH volume and severity are often conducted among older adults, who have the potential for a greater number of years of accumulated high blood pressure, potentially influencing these previous findings. Our intervention was conducted among adults between 35

and 60 years old, with a varied duration of high blood pressure. We did not anticipate that either intervention would decrease white matter hyperintensity volume, but rather might prevent the further accumulation of white matter hyperintensities, insofar as previous research indicates that greater white matter hyperintensity volume is associated with progression from mild cognitive impairment to overt dementia (751, 752). To that end, while we report a significant increase in absolute WMH volume in the heat therapy group, this difference is not substantiated when WMH volume is log transformed and normalized to whole brain WMH score. Lastly, these data were collected from a relatively small cohort, which is more susceptible to influence by a single subject. It is likely that the finding of increased absolute white matter hyperintensity volume would not be substantiated in a larger cohort.

Long-term hypertension is associated with vessel damage that results in maladaptive hypertrophy, decreasing lumen diameter and resulting in a reduction in cerebral blood flow (774). Our hypothesis that heat therapy would result in greater improvements in MRI-based biomarkers of AD risk was formulated based on previous data to suggest that heat therapy can elicit improvements in blood pressure and endothelial function (8, 9, 434). In the absence of changes in our measurements of hemodynamic and vascular function, it is unsurprising that we report no changes in either gray matter or white matter perfusion following either intervention. Previous data reporting an increase in cerebral blood flow with pharmacological control of blood pressure measure total cerebral blood flow (242, 243), which is not necessarily congruent with gray and white matter perfusion. Despite no reported improvement in blood pressure with either intervention, there tended to be a significant main effect of time among measurements of gray matter perfusion, although the difference failed to reach significance. Beneficial increases in gray matter perfusion following both exercise and heat therapy among middle-aged adults with untreated hypertension remains an exciting area of future research, particularly among those that are at an increased risk for AD.

Poor sleep quality, quantified using the Pittsburgh Sleep Quality Index (PSQI), is an independent predictor of hypertension (674–676). A higher PSQI score, indicating worse sleep, was associated with over a two-fold higher risk for hypertension compared to those with higher quality sleep (675). Poor sleep quality, particularly in midlife, is associated with worse episodic memory task performance as well as increased risk for cognitive decline in later life (677, 678). The PSQI, our subjective assessment of sleep quality, also did not differ following either

intervention. There have been several reports that have explored the casual and correlative relationship between sleep quality and hypertension (674, 676, 761), with the conclusion that poorer quality sleep is significantly associated with worse blood pressure and cardiovascular health. The PSQI is a subjective survey of sleep quality, and perhaps the lack of change in PSQI score during this study should be expected considering no change in either ambulatory or clinic blood pressure. We similarly report no changes in performance on assessments of episodic or working memory, assessed using the NIH toolbox picture sequence and list-sorting tasks, respectively. Despite the reported validity of the NIH toolbox across the lifespan (660, 663, 664), there is considerable variability in the data (Figures 6.5B and 6.5C) which may speak to subject motivation to complete the task to the best of their ability, rather than a reflection in changes to working and episodic memory following either intervention.

Limitations

The present study is not without its limitations. Perhaps chiefly among them is that we did not collect any measurements of stress, subjective or otherwise, in conjunction with baseline and post-intervention magnetic resonance imaging and blood pressure assessments. Stress undoubtedly has a negative impact on cognitive function and memory tasks (775). Given the duration of this intervention, an assessment of stress would have potentially informed our findings regarding cognitive function testing and biomarkers of Alzheimer's Disease biomarkers.

The timeline of this intervention may have influenced these results. We selected the frequency and duration of exercise and heating sessions based on previous research within our lab that was sufficient to promote beneficial improvements in blood pressure and arterial health (8, 9). The most compelling longitudinal studies surrounding exercise and brain health often target interventions that are between 6 and 12 months, with multi-year follow-up periods (395, 755). Our hypothesis was well-founded in previous reports of the efficacy of heat therapy; however, those adaptations were not substantiated in the present study. It is possible that with a longer duration intervention, or perhaps a higher heating and exercise load, we may have seen improvements in cognitive function and biomarkers of AD risk in this population.

CONCLUSION

These data indicate that 30 sessions of either exercise training or heat therapy are not sufficient to improve common biomarkers of cognitive function and Alzheimer's disease among adults with untreated hypertension. Despite compelling data on the documented benefits of exercise (389, 769, 776) and suggested benefits of heat therapy (270, 583) for cognitive function and cerebrovascular health, the present intervention was not sufficient to impact measures such as hippocampal volume, white matter hyperintensity volume, gray and white matter perfusion, or assessments of working and episodic memory. Despite these findings, the use of heat therapy for improving cerebrovascular health and Alzheimer's disease risk remains an exciting area of research. Future studies should examine the role of a greater dose of heating, either longer duration of session or a longer course of heat treatment, to quantify the potential impact of heat therapy on these biomarkers.

CHAPTER VII

SUMMARY AND FUTURE DIRECTIONS

Heat therapy remains an emerging and exciting area of research, with a greater number of studies investigating the physiological responses and attendant adaptations to repeated bouts of hyperthermia. To date, there has been compelling data that demonstrates the vascular benefits of heat therapy among healthy populations (8), women with polycystic ovary syndrome (9), as well as individuals with peripheral arterial disease (10). This dissertation expanded upon these previous investigations by applying this intervention to individuals with essential hypertension. The impetus for this research was to compare the efficacy of heat therapy as a therapeutic tool for improving blood pressure compared to the current non-pharmacological gold standard, aerobic exercise.

The findings in Chapter IV indicate that neither aerobic exercise training nor chronic hot water immersion at the doses prescribed in the present study can improve 24-hour ambulatory blood pressure. Mechanistic studies of heat therapy in any form (e.g., traditional dry sauna, far infrared sauna, or hot water immersion) is in its infancy, and there has yet to be compelling data showing that these interventions reduce ambulatory blood pressure, despite promising reductions in clinic blood pressure assessments (9, 10). Is it possible that the lack of a reduction in ambulatory blood pressure is related to the disparity in heat load between our intervention and previous interventions that have reported significant reductions in blood pressure. Furthermore, we report no significant improvements in any markers of renal function following either intervention. The individuals included in this research met the inclusion criteria that stipulated no additional overt cardiometabolic disease other than hypertension (e.g., no diagnosed coronary artery disease, type II diabetes, or peripheral arterial disease), and were not currently taking medication to lower their blood pressure. Most interventions related to improvements in kidney function have studied individuals with known risk factors for or overt chronic kidney disease. We excluded individuals with these comorbidities, save hypertension. As such, it is possible that the lack of reduction in blood pressure obscured any measured improvements in albumin excretion or estimated glomerular filtration rate. Furthermore, improvements in biomarkers such as urine albumin excretion are often related to improvements in vascular endothelial function.

The ability of heat therapy to improve endothelial health and function is promising in younger individuals (8, 9, 583), but is less consistent among middle-aged and older individuals with cardiovascular disease (10, 724).

The findings in Chapter V are contrary to our hypothesis. We hypothesized that heat therapy would elicit greater reductions in blood pressure than aerobic exercise training in part because of the results of Ely, et. al., who reported significant reductions in systolic and diastolic blood pressure following chronic hot water immersion in women with polycystic ovary syndrome, a population characterized in part by high blood pressure and elevated cardiovascular disease risk (9). Furthermore, we report no significant differences between or within groups for pulse wave velocity, our measurement of arterial stiffness. Similar to improvements in endothelial function, there is an emerging body of literature to suggest that middle-aged and older individuals or those with cardiovascular disease, present with minimal to no change in arterial stiffness following heat therapy (9, 10, 724). Lastly, we report no significant differences between or within groups for the potential molecular and cellular mediators of essential hypertension assessed here, including C-reactive protein, endothelin-1, or IL-6. These biomarkers were selected for their role as molecular or cellular signature that underlies the improvement in hemodynamic and vascular health following heat therapy. Without changes in our key outcome variables of blood pressure or arterial stiffness, it is unsurprising that we report no changes in each of these biomarkers.

The findings in Chapter VI do not support our hypothesis that hot water immersion would result in greater improvements in cognitive function and known biomarkers of Alzheimer's Disease than aerobic exercise training. Analogous to the minimal improvements in kidney function, it is possible that the lack of improvement in cognitive function and brain health is due to the lack of change in blood pressure with either intervention. Previous reports have suggested that upregulation of molecular pathways may play a causal role in the prevention of AD with heat therapy (270). These molecular mechanisms may act synergistically with demonstrated improvements in vascular health following chronic passive heating, such as endothelial function (8, 545), arterial stiffness (8), and blood pressure (9, 10) to reduce the risk for AD among adults with untreated hypertension. Cardiovascular disease and Alzheimer’s Disease take decades to manifest. While the present study was a significant undertaking to conduct in a supervised

laboratory setting, its relatively short duration (8-10 weeks) may not be sufficient to impact the AD biomarkers of interest measured here.

While exposures to heat stress is an ancient practice with cultural roots, there remains to be a consensus on the minimum effective dose of heating among healthy as well as diseased populations. Although previous literature has shown success with regard to improving cardiovascular (8, 9, 448), as well as metabolic health (494, 531) following heat therapy, these findings have not been consistently replicated across all populations and heating modalities. Specifically, there appears to be a critical window within which heat therapy is most effective for imparting beneficial improvements in cardiovascular and metabolic health. The most profound reports of the benefits of heat therapy have been reported among younger and mostly healthy individuals (8, 9, 485, 524, 583). Laukkanen and colleagues, in a large prospective cohort analysis of the impact of Finnish Sauna on fatal cardiovascular events and all-cause mortality, report that the greatest risk reduction occurs with very frequent sauna use, with a prolonged duration for each session. Sauna use is a cultural phenomenon in Finland, which perhaps helps to inform the interpretation of the growing body of literature surrounding heat therapy. Heat therapy may be an effective tool for maintaining cardiovascular health among healthy persons and an effective intervention in the prodromal period of cardiovascular disease but is limited in its ability to combat established cardiovascular disease.

Like many patient populations (*e.g.,* peripheral arterial disease), there are varying degrees of hypertension. In the present study, there was a range of blood pressures represented, including elevated, stage 1, and stage 2. Logically, a higher resting blood pressure has a greater potential to be reduced compared to resting blood pressure closer to normotensive values. It is possible that individuals with stage 2 hypertension require a greater heat stress or exercise load than the protocol employed in the present study. To wit, sauna bathing 4-7 times per week or ≥19 minutes per session is associated with the greatest risk reduction for all-cause mortality and sudden cardiac death, as reported by Laukkanen and colleagues (7). Future investigations should examine the interaction between the degree of hypertension and heat stress required to evoke physiologically relevant improvements in cardiovascular health. For instance, perhaps individuals with stage 2 hypertension require a long duration or more frequent heat exposure to obtain previously reported benefits (8–10). This agrees with recently published data to suggest

that those with cardiovascular disease may present with limited or no cardiovascular benefits following chronic passive heating (10, 724).

Exercise training interventions, both experimentally and ecologically, are driven by the *F.I.T.T.* Principle, which refers to the frequency, intensity, time, and type of exercise. Permutations among each of these four parameters allow for tailored exercise programming as dictated by the population as well as desired outcomes. Heat therapy, while utilized culturally with empirical benefits for thousands of years, has only recently emerged as a therapeutic intervention for improving common cardiovascular and metabolic risk factors for chronic disease in a manner ostensibly like exercise. It is prudent to continue to examine the pillars of the *F.I.T.T.* principle as they apply to heat therapy to improve their utility across a variety of populations and modalities. By way of example, previous studies have demonstrated the benefits of traditional sauna (7, 267, 438), far-infrared sauna (448, 451, 457, 463), repeated short wave diathermy (500, 501), as well as hot water immersion (8–10). Despite this body of evidence, the data presented here do not support these previous reports, perhaps due to the present intervention failing to achieve some minimal effective dose of heating. For instance, Brunt and colleagues demonstrated a 5 mmHg decrease in mean arterial pressure against a cumulative heat load of 3,240 minutes over the course of the intervention (36 sessions at 90 minutes/session). Ely and colleagues demonstrated more profound reductions in systolic and diastolic blood pressure against a lower, but still substantial, heat load of 1,800 minutes (30 sessions at 60 minutes/session). From this perspective, our intervention was more similar to those of Akerman et al. (10) and Thomas et al. (11), each employing a modest heat load of 30 minutes for 3-5 sessions per week for a total of 12 weeks, albeit in a different population (individuals with peripheral arterial disease). Our intervention consisted of thirty 45-minute sessions with a target time-to-completion of 8-10 weeks, for a total heat load of 1,350 minutes.

Perhaps the most salient takeaway from these studies is that the minimum effective dose of heating is higher for individuals with overt cardiovascular disease or common risk factors. While these data did not support our hypotheses, they effectively help to address the pillar of "time" from the *F.I.T.T.* principle as adapted from exercise. Future permutations of heat therapy, varied in either population, modality, or physiological outcome, should consider the *F.I.T.T.* principle as a component of study design. The concept of physiological "non-responders" to exercise training is controversial (6, 777, 778). Previous research has argued that responders are

oftentimes the result of poor experimental design and the lack of a completely non-experimental control group (779). Perhaps more relevant to this study is the position that non-responders simply require a higher dosage of the prescribed intervention to elicit sufficiently relevant physiological change (778). Previous research that has demonstrated greater improvements in blood pressure following heat therapy in clinical populations achieved these results in part through greater compliance to heat therapy than exercise training (10). Greater compliance and subjective subject reports of improved quality of life are encouraging and suggest that heat therapy is an effective lifestyle intervention across a wide range of populations.

The design of this research was such that it achieved the aerobic physical activity recommendations from the American College of Sports Medicine, with a parallel arm of time-matched hot water immersion. Furthermore, we ensured near-complete compliance (1 subject per group completed 27/30 sessions). Despite these considerations, we fail to report any significant differences between or within groups for our primary outcome variables across our three aims. There is limited, moderately compelling data to suggest that a combined exercise and heat therapy intervention may offer benefits beyond either intervention independently (441). The combination of exercise and heat therapy is problematic as it allows for the accumulation of a higher "dose" of stress when compared to either intervention by itself. Despite this possible experimental design flaw, an investigation comparing exercise, hot water immersion, and a combined group would be a worthwhile undertaking.

REFERENCES CITED

1. **Ostchega Y**, **Fryar CD**, **Nwankwo T**, **Nguyen DT**. Hypertension prevalence among adults aged 18 and over: United States, 2017–2018. .

2. **Muntner P**, **Carey RM**, **Gidding S**, **Jones DW**, **Taler SJ**, **Wright JT**, **Whelton PK**. Potential US Population Impact of the 2017 ACC/AHA High Blood Pressure Guideline. *Circulation* 137: 109–118, 2018. doi: 10.1161/CIRCULATIONAHA.117.032582.

3. **Whelton Paul K.**, **Carey Robert M.**, **Aronow Wilbert S.**, **Casey Donald E.**, **Collins Karen J.**, **Dennison Himmelfarb Cheryl**, **DePalma Sondra M.**, **Gidding Samuel**, **Jamerson Kenneth A.**, **Jones Daniel W.**, **MacLaughlin Eric J.**, **Muntner Paul**, **Ovbiagele Bruce**, **Smith Sidney C.**, **Spencer Crystal C.**, **Stafford Randall S.**, **Taler Sandra J.**, **Thomas Randal J.**, **Williams Kim A.**, **Williamson Jeff D.**, **Wright Jackson T.** 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *Journal of the American College of Cardiology* 71: e127–e248, 2018. doi: 10.1016/j.jacc.2017.11.006.

4. **Ritchey MD**, **Gillespie C**, **Wozniak G**, **Shay CM**, **Thompson-Paul AM**, **Loustalot F**, **Hong Y**. Potential need for expanded pharmacologic treatment and lifestyle modification services under the 2017 ACC/AHA Hypertension Guideline. *The Journal of Clinical Hypertension* 20: 1377–1391, 2018. doi: 10.1111/jch.13364.

5. **American College of Sports Medicine**. *ACSM's guidelines for exercise testing and prescription*. Lippincott williams & wilkins, 2013.

6. **Pickering C**, **Kiely J**. Do Non-Responders to Exercise Exist—and If So, What Should We Do About Them? *Sports Medicine* 49: 1–7, 2019. doi: 10.1007/s40279-018-01041-1.

7. **Laukkanen T**, **Khan H**, **Zaccardi F**, **Laukkanen JA**. Association Between Sauna Bathing and Fatal Cardiovascular and All-Cause Mortality Events. *JAMA Internal Medicine* 175: 542–548, 2015. doi: 10.1001/jamainternmed.2014.8187.

8. **Brunt VE**, **Howard MJ**, **Francisco MA**, **Ely BR**, **Minson CT**. Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. *The Journal of Physiology* 594: 5329–5342, 2016. doi: 10.1113/JP272453.

9. **Ely BR**, **Francisco MA**, **Halliwill JR**, **Bryan SD**, **Comrada LN**, **Larson EA**, **Brunt VE**, **Minson CT**. Heat therapy reduces sympathetic activity and improves cardiovascular risk profile in women who are obese with polycystic ovary syndrome. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 317: R630–R640, 2019. doi: 10.1152/ajpregu.00078.2019.

10. **Akerman AP**, **Thomas KN**, **van Rij AM**, **Body ED**, **Alfadhel M**, **Cotter JD**. Heat therapy vs. supervised exercise therapy for peripheral arterial disease: a 12-wk

randomized, controlled trial. *American Journal of Physiology-Heart and Circulatory Physiology* 316: H1495–H1506, 2019. doi: 10.1152/ajpheart.00151.2019.

11. **Thomas KN**, **van Rij AM**, **Lucas SJE**, **Cotter JD**. Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 312: R281–R291, 2017. doi: 10.1152/ajpregu.00404.2016.

12. **Skaria AP**. The economic and societal burden of Alzheimer disease: managed care considerations. *The American Journal of Managed Care* 28: S188–S196, 2022.

13. **Lim SS**, **Vos T**, **Flaxman AD**, **Danaei G**, **Shibuya K**, **Adair-Rohani H**, **AlMazroa MA**, **Amann M**, **Anderson HR**, **Andrews KG**. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet* 380: 2224–2260, 2012.

14. **Forouzanfar MH**, **Liu P**, **Roth GA**, **Ng M**, **Biryukov S**, **Marczak L**, **Alexander L**, **Estep K**, **Hassen Abate K**, **Akinyemiju TF**, **Ali R**, **Alvis-Guzman N**, **Azzopardi P**, **Banerjee A**, **Bärnighausen T**, **Basu A**, **Bekele T**, **Bennett DA**, **Biadgilign S**, **Catalá-López F**, **Feigin VL**, **Fernandes JC**, **Fischer F**, **Gebru AA**, **Gona P**, **Gupta R**, **Hankey GJ**, **Jonas JB**, **Judd SE**, **Khang Y-H**, **Khosravi A**, **Kim YJ**, **Kimokoti RW**, **Kokubo Y**, **Kolte D**, **Lopez A**, **Lotufo PA**, **Malekzadeh R**, **Melaku YA**, **Mensah GA**, **Misganaw A**, **Mokdad AH**, **Moran AE**, **Nawaz H**, **Neal B**, **Ngalesoni FN**, **Ohkubo T**, **Pourmalek F**, **Rafay A**, **Rai RK**, **Rojas-Rueda D**, **Sampson UK**, **Santos IS**, **Sawhney M**, **Schutte AE**, **Sepanlou SG**, **Shifa GT**, **Shiue I**, **Tedla BA**, **Thrift AG**, **Tonelli M**, **Truelsen T**, **Tsilimparis N**, **Ukwaja KN**, **Uthman OA**, **Vasankari T**, **Venketasubramanian N**, **Vlassov VV**, **Vos T**, **Westerman R**, **Yan LL**, **Yano Y**, **Yonemoto N**, **Zaki MES**, **Murray CJL**. Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990-2015. *JAMA* 317: 165–182, 2017. doi: 10.1001/jama.2016.19043.

15. **Danaei G**, **Ding EL**, **Mozaffarian D**, **Taylor B**, **Rehm J**, **Murray CJL**, **Ezzati M**. The Preventable Causes of Death in the United States: Comparative Risk Assessment of Dietary, Lifestyle, and Metabolic Risk Factors. *PLOS Medicine* 6: e1000058, 2009. doi: 10.1371/journal.pmed.1000058.

16. **Chobufo MD**, **Gayam V**, **Soluny J**, **Rahman EU**, **Enoru S**, **Foryoung JB**, **Agbor VN**, **Dufresne A**, **Nfor T**. Prevalence and control rates of hypertension in the USA: 2017–2018. *International Journal of Cardiology Hypertension* 6: 100044, 2020.

17. **Rapsomaniki E**, **Timmis A**, **George J**, **Pujades-Rodriguez M**, **Shah AD**, **Denaxas S**, **White IR**, **Caulfield MJ**, **Deanfield JE**, **Smeeth L**, **Williams B**, **Hingorani A**, **Hemingway H**. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. *The Lancet* 383: 1899–1911, 2014. doi: 10.1016/S0140-6736(14)60685-1.

18. **LEWINGTON S**. Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality : a meta -analysis of individual data for one million adults in 61 prospective studies. *Lancet* 360: 1903–1913, 2002.

19. **Patel SA**, **Winkel M**, **Ali MK**, **Narayan KV**, **Mehta NK**. Cardiovascular mortality associated with 5 leading risk factors: national and state preventable fractions estimated from survey data. *Annals of internal medicine* 163: 245–253, 2015.

20. **McInnes GT**. Lowering blood pressure for cardiovascular risk reduction [Online]. *Journal of Hypertension* 23, 2005. https://journals.lww.com/jhypertension/fulltext/2005/04001/lowering_blood_pressure_for_cardiovascular_risk.2.aspx.

21. **Carretero OA**, **Oparil S**. Essential Hypertension. *Circulation* 101: 329–335, 2000. doi: 10.1161/01.CIR.101.3.329.

22. **Harrison D**, **Widder J**, **Grumbach I**, **Chen W**, **Weber M**, **Searles C**. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *Journal of internal medicine* 259: 351–363, 2006.

23. **Touyz RM**. Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II–editorial review. *Current opinion in nephrology and hypertension* 14: 125–131, 2005.

24. **Touyz RM**. Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Review of Cardiovascular Therapy* 1: 91–106, 2003. doi: 10.1586/14779072.1.1.91.

25. **Touyz RM**. Reactive Oxygen Species, Vascular Oxidative Stress, and Redox Signaling in Hypertension. *Hypertension* 44: 248–252, 2004. doi: 10.1161/01.HYP.0000138070.47616.9d.

26. **Griendling KK**. ATVB in focus: redox mechanisms in blood vessels. *Arteriosclerosis, thrombosis, and vascular biology* 25: 272–273, 2005.

27. **Abdilla N**, **Tormo M**, **Fabia M**, **Chaves F**, **Saez G**, **Redon J**. Impact of the components of metabolic syndrome on oxidative stress and enzymatic antioxidant activity in essential hypertension. *Journal of Human Hypertension* 21: 68–75, 2007.

28. **Rodrigo R**, **Prat H**, **Passalacqua W**, **Araya J**, **Guichard C**, **Bächler JP**. Relationship between oxidative stress and essential hypertension. *Hypertension Research* 30: 1159–1167, 2007.

29. **Harrison DG**. Endothelial Function and Oxidant Stress. *Clinical Cardiology* 20: II–11, 1997. doi: 10.1002/j.1932-8737.1997.tb00007.x.

30. **Rubanyi GM**, **Vanhoutte PM**. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *American Journal of Physiology-Heart and Circulatory Physiology* 250: H822–H827, 1986. doi: 10.1152/ajpheart.1986.250.5.H822.

31. **Ziegler T**, **Bouzourène K**, **Harrison VJ**, **Brunner HR**, **Hayoz D**. Influence of Oscillatory and Unidirectional Flow Environments on the Expression of Endothelin and Nitric Oxide Synthase in Cultured Endothelial Cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* 18: 686–692, 1998. doi: 10.1161/01.ATV.18.5.686.

32. **Hwang J**, **Ing MH**, **Salazar A**, **Lassègue B**, **Griendling K**, **Navab M**, **Sevanian A**, **Hsiai TK**. Pulsatile Versus Oscillatory Shear Stress Regulates NADPH Oxidase Subunit Expression. *Circulation Research* 93: 1225–1232, 2003. doi: 10.1161/01.RES.0000104087.29395.66.

33. **Beckman JS**, **Koppenol WH**. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *American Journal of Physiology-cell physiology* 271: C1424–C1437, 1996.

34. **Kissner R**, **Nauser T**, **Bugnon P**, **Lye PG**, **Koppenol WH**. Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. *Chemical research in toxicology* 10: 1285–1292, 1997.

35. **Schulz E**, **Gori T**, **Münzel T**. Oxidative stress and endothelial dysfunction in hypertension. *Hypertension Research* 34: 665–673, 2011. doi: 10.1038/hr.2011.39.

36. **Schächinger V**, **Britten MB**, **Zeiher AM**. Prognostic Impact of Coronary Vasodilator Dysfunction on Adverse Long-Term Outcome of Coronary Heart Disease. *Circulation* 101: 1899–1906, 2000. doi: 10.1161/01.CIR.101.16.1899.

37. **Suwaidi JA**, **Hamasaki S**, **Higano ST**, **Nishimura RA**, **Holmes Jr DR**, **Lerman A**. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101: 948–954, 2000.

38. **Cooke JP**, **Tsao PS**. Is NO an endogenous antiatherogenic molecule? *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology* 14: 653–655, 1994. doi: 10.1161/01.ATV.14.5.653.

39. **Kawashima S**, **Yokoyama M**. Dysfunction of Endothelial Nitric Oxide Synthase and Atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* 24: 998–1005, 2004. doi: 10.1161/01.ATV.0000125114.88079.96.

40. **Joyner MJ**, **Dietz NM**. Nitric oxide and vasodilation in human limbs. *Journal of Applied Physiology* 83: 1785–1796, 1997. doi: 10.1152/jappl.1997.83.6.1785.

41. **Förstermann U**, **Gath I**, **Schwarz P**, **Closs EI**, **Kleinert H**. Isoforms of nitric oxide synthase: Properties, cellular distribution and expressional control. *Biochemical Pharmacology* 50: 1321–1332, 1995. doi: 10.1016/0006-2952(95)00181-6.

42. **Andrew PJ**, **Mayer B**. Enzymatic function of nitric oxide synthases. *Cardiovascular research* 43: 521–531, 1999.

43. **Münzel T**, **Daiber A**, **Ullrich V**, **Mülsch A**. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arteriosclerosis, thrombosis, and vascular biology* 25: 1551–1557, 2005.

44. **Takaya T**, **Hirata K**, **Yamashita T**, **Shinohara M**, **Sasaki N**, **Inoue N**, **Yada T**, **Goto M**, **Fukatsu A**, **Hayashi T**, **Alp NJ**, **Channon KM**, **Yokoyama M**, **Kawashima S**. A Specific Role for eNOS-Derived Reactive Oxygen Species in Atherosclerosis Progression. *Arteriosclerosis, Thrombosis, and Vascular Biology* 27: 1632–1637, 2007. doi: 10.1161/ATVBAHA.107.142182.

45. **Vásquez-Vivar J**, **Kalyanaraman B**, **Martásek P**, **Hogg N**, **Masters BSS**, **Karoui H**, **Tordo P**, **Pritchard Jr KA**. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proceedings of the National Academy of Sciences* 95: 9220–9225, 1998.

46. **Landmesser U**, **Dikalov S**, **Price SR**, **McCann L**, **Fukai T**, **Holland SM**, **Mitch WE**, **Harrison DG**. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111: 1201–1209, 2003. doi: 10.1172/JCI14172.

47. **Hong H-J**, **Hsiao G**, **Cheng T-H**, **Yen M-H**. Supplemention with tetrahydrobiopterin suppresses the development of hypertension in spontaneously hypertensive rats. *Hypertension* 38: 1044–1048, 2001.

48. **Landmesser U**, **Harrison DG**, **Drexler H**. Oxidant stress—a major cause of reduced endothelial nitric oxide availability in cardiovascular disease. *European Journal of Clinical Pharmacology* 62: 13–19, 2006. doi: 10.1007/s00228-005-0012-z.

49. **Hink U**, **Li H**, **Mollnau H**, **Oelze M**, **Matheis E**, **Hartmann M**, **Skatchkov M**, **Thaiss F**, **Stahl RA**, **Warnholtz A**. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circulation research* 88: e14–e22, 2001.

50. **Guzik TJ**, **Mussa S**, **Gastaldi D**, **Sadowski J**, **Ratnatunga C**, **Pillai R**, **Channon KM**. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD (P) H oxidase and endothelial nitric oxide synthase. *Circulation* 105: 1656–1662, 2002.

51. **Drummond GR**, **Cai H**, **Davis ME**, **Ramasamy S**, **Harrison DG**. Transcriptional and Posttranscriptional Regulation of Endothelial Nitric Oxide Synthase Expression by Hydrogen Peroxide. *Circulation Research* 86: 347–354, 2000. doi: 10.1161/01.RES.86.3.347.

52. **Virdis A**, **Neves MF**, **Amiri F**, **Touyz RM**, **Schiffrin EL**. Role of NAD(P)H oxidase on vascular alterations in angiotensin II-infused mice [Online]. *Journal of Hypertension* 22, 2004. https://journals.lww.com/jhypertension/Fulltext/2004/03000/Role_of_NAD_P_H_oxidase_on_vascular_alterations_in.16.aspx.

53. **Rodriguez-Iturbe B**, **Zhan C-D**, **Quiroz Y**, **Sindhu RK**, **Vaziri ND**. Antioxidant-Rich Diet Relieves Hypertension and Reduces Renal Immune Infiltration in Spontaneously Hypertensive Rats. *Hypertension* 41: 341–346, 2003. doi: 10.1161/01.HYP.0000052833.20759.64.

54. **Montezano AC**, **Dulak-Lis M**, **Tsiropoulou S**, **Harvey A**, **Briones AM**, **Touyz RM**. Oxidative Stress and Human Hypertension: Vascular Mechanisms, Biomarkers, and Novel Therapies. *Canadian Journal of Cardiology* 31: 631–641, 2015. doi: 10.1016/j.cjca.2015.02.008.

55. **Fukai T**, **Ushio-Fukai M**. Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases. *Antioxidants & Redox Signaling* 15: 1583–1606, 2011. doi: 10.1089/ars.2011.3999.

56. **Redón J**, **Oliva MR**, **Tormos C**, **Giner V**, **Chaves J**, **Iradi A**, **Sáez GT**. Antioxidant Activities and Oxidative Stress Byproducts in Human Hypertension. *Hypertension* 41: 1096–1101, 2003. doi: 10.1161/01.HYP.0000068370.21009.38.

57. **Sáez GT**, **Tormos C**, **Giner V**, **Chaves J**, **Lozano JV**, **Iradi A**, **Redón J**. Factors related to the impact of antihypertensive treatment in antioxidant activities and oxidative stress by-products in human hypertension*: *American Journal of Hypertension* 17: 809–816, 2004. doi: 10.1016/j.amjhyper.2004.05.022.

58. **Simic DV**, **Mimic-Oka J**, **Pljesa-Ercegovac M**, **Savic-Radojevic A**, **Opacic M**, **Matic D**, **Ivanovic B**, **Simic T**. Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. *Journal of Human Hypertension* 20: 149–155, 2006. doi: 10.1038/sj.jhh.1001945.

59. **Sorescu D**, **Weiss D**, **Lassègue B**, **Clempus RE**, **Szöcs K**, **Sorescu GP**, **Valppu L**, **Quinn MT**, **Lambeth JD**, **Vega JD**. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation* 105: 1429–1435, 2002.

60. **Pagano PJ**, **Clark JK**, **Cifuentes-Pagano ME**, **Clark SM**, **Callis GM**, **Quinn MT**. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proceedings of the National Academy of Sciences* 94: 14483–14488, 1997.

61. **Wu R**, **Lamontagne D**, **de Champlain J**. Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. *Circulation* 105: 387–392, 2002.

62. **Ridker PM**, **Cushman M**, **Stampfer MJ**, **Tracy RP**, **Hennekens CH**. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. *N Engl J Med* 336: 973–979, 1997. doi: 10.1056/NEJM199704033361401.

63. **Ridker PM**, **Hennekens CH**, **Buring JE**, **Rifai N**. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England journal of medicine* 342: 836–843, 2000.

64. **Koenig W**, **Sund M**, **Fröhlich M**, **Fischer H-G**, **Löwel H**, **Döring A**, **Hutchinson WL**, **Pepys MB**. C-Reactive Protein, a Sensitive Marker of Inflammation, Predicts Future Risk of Coronary Heart Disease in Initially Healthy Middle-Aged Men. *Circulation* 99: 237–242, 1999. doi: 10.1161/01.CIR.99.2.237.

65. **Tracy RP**, **Lemaitre RN**, **Psaty BM**, **Ives DG**, **Evans RW**, **Cushman M**, **Meilahn EN**, **Kuller LH**. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arteriosclerosis, thrombosis, and vascular biology* 17: 1121–1127, 1997.

66. **Rohde LE**, **Hennekens CH**, **Ridker PM**. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *The American journal of cardiology* 84: 1018–1022, 1999.

67. **Chae CU**, **Lee RT**, **Rifai N**, **Ridker PM**. Blood pressure and inflammation in apparently healthy men. *Hypertension* 38: 399–403, 2001.

68. **Bermudez EA**, **Rifai N**, **Buring J**, **Manson JE**, **Ridker PM**. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arteriosclerosis, thrombosis, and vascular biology* 22: 1668–1673, 2002.

69. **Smith GD**, **Lawlor DA**, **Harbord R**, **Timpson N**, **Rumley A**, **Lowe GDO**, **Day INM**, **Ebrahim S**. Association of C-Reactive Protein With Blood Pressure and Hypertension. *Arteriosclerosis, Thrombosis, and Vascular Biology* 25: 1051–1056, 2005. doi: 10.1161/01.ATV.0000160351.95181.d0.

70. **Bautista LE**, **López-Jaramillo P**, **Vera LM**, **Casas JP**, **Otero AP**, **Guaracao AI**. Is C-reactive protein an independent risk factor for essential hypertension? [Online]. *Journal of Hypertension* 19, 2001. https://journals.lww.com/jhypertension/fulltext/2001/05000/is_c_reactive_protein_an_independent_risk_factor.4.aspx.

71. **Verma S**, **Wang C-H**, **Li S-H**, **Dumont AS**, **Fedak PW**, **Badiwala MV**, **Dhillon B**, **Weisel RD**, **Li R-K**, **Mickle DA**. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation* 106: 913–919, 2002.

72. **Venugopal SK**, **Devaraj S**, **Yuhanna I**, **Shaul P**, **Jialal I**. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 106: 1439–1441, 2002.

73. **Wever RMF**, **Lüscher TF**, **Cosentino F**, **Rabelink TJ**. Atherosclerosis and the Two Faces of Endothelial Nitric Oxide Synthase. *Circulation* 97: 108–112, 1998. doi: 10.1161/01.CIR.97.1.108.

74. **Pasceri V**, **Willerson JT**, **Yeh ETH**. Direct Proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. *Circulation* 102: 2165–2168, 2000. doi: 10.1161/01.CIR.102.18.2165.

75. **Verma S**, **Li S-H**, **Badiwala MV**, **Weisel RD**, **Fedak PW**, **Li R-K**, **Dhillon B**, **Mickle DA**. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 105: 1890–1896, 2002.

76. **Konukoglu D**, **Uzun H**. Endothelial Dysfunction and Hypertension. In: *Hypertension: from basic research to clinical practice*, edited by Islam MdS. Springer International Publishing, p. 511–540.

77. **Masaki T**, **Sawamura T**. Endothelin and endothelial dysfunction. *Proceedings of the Japan Academy, Series B* 82: 17–24, 2006.

78. **Yanagisawa M**, **Kurihara H**, **Kimura S**, **Tomobe Y**, **Kobayashi M**, **Mitsui Y**, **Yazaki Y**, **Goto K**, **Masaki T**. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *nature* 332: 411–415, 1988.

79. **Li L**, **Watts SW**, **Banes AK**, **Galligan JJ**, **Fink GD**, **Chen AF**. NADPH oxidase–derived superoxide augments endothelin-1–induced venoconstriction in mineralocorticoid hypertension. *Hypertension* 42: 316–321, 2003.

80. **Li L**, **Fink GD**, **Watts SW**, **Northcott CA**, **Galligan JJ**, **Pagano PJ**, **Chen AF**. Endothelin-1 increases vascular superoxide via endothelinA–NADPH oxidase pathway in low-renin hypertension. *Circulation* 107: 1053–1058, 2003.

81. **Skalska AB**, **Pietrzycka A**, **Stępniewski M**. Correlation of endothelin 1 plasma levels with plasma antioxidant capacity in elderly patients treated for hypertension. *Clinical Biochemistry* 42: 358–364, 2009. doi: 10.1016/j.clinbiochem.2008.11.002.

82. **Schiffrin EL**. Role of endothelin-1 in hypertension and vascular disease*. *American Journal of Hypertension* 14: 83S-89S, 2001. doi: 10.1016/S0895-7061(01)02074-X.

83. **Romero M**, **Jiménez R**, **Sánchez M**, **López-Sepúlveda R**, **Zarzuelo A**, **Tamargo J**, **Pérez-Vizcaíno F**, **Duarte J**. Vascular superoxide production by endothelin-1 requires Src non-receptor protein tyrosine kinase and MAPK activation. *Atherosclerosis* 212: 78–85, 2010. doi: 10.1016/j.atherosclerosis.2010.04.031.

84. **Romero M**, **Jiménez R**, **Sánchez M**, **López-Sepúlveda R**, **Zarzuelo MJ**, **O'Valle F**, **Zarzuelo A**, **Pérez-Vizcaíno F**, **Duarte J**. Quercetin inhibits vascular superoxide production induced by endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. *Atherosclerosis* 202: 58–67, 2009. doi: 10.1016/j.atherosclerosis.2008.03.007.

85. **Bautista L**. Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. *Journal of human hypertension* 17: 223–230, 2003.

86. **Wang C-H**, **Li S-H**, **Weisel RD**, **Fedak PW**, **Dumont AS**, **Szmitko P**, **Li R-K**, **Mickle DA**, **Verma S**. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* 107: 1783–1790, 2003.

87. **Perticone F**, **Ceravolo R**, **Pujia A**, **Ventura G**, **Iacopino S**, **Scozzafava A**, **Ferraro A**, **Chello M**, **Mastroroberto P**, **Verdecchia P**, **Schillaci G**. Prognostic Significance of Endothelial Dysfunction in Hypertensive Patients. *Circulation* 104: 191–196, 2001. doi: 10.1161/01.CIR.104.2.191.

88. **Thijssen DH**, **Black MA**, **Pyke KE**, **Padilla J**, **Atkinson G**, **Harris RA**, **Parker B**, **Widlansky ME**, **Tschakovsky ME**, **Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American Journal of Physiology-Heart and Circulatory Physiology* 300: H2–H12, 2011.

89. **Thijssen DH**, **Bruno RM**, **van Mil AC**, **Holder SM**, **Faita F**, **Greyling A**, **Zock PL**, **Taddei S**, **Deanfield JE**, **Luscher T**. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *European heart journal* 40: 2534–2547, 2019.

90. **Green DJ**, **Jones H**, **Thijssen D**, **Cable NT**, **Atkinson G**. Flow-Mediated Dilation and Cardiovascular Event Prediction. *Hypertension* 57: 363–369, 2011. doi: 10.1161/HYPERTENSIONAHA.110.167015.

91. **Ras RT**, **Streppel MT**, **Draijer R**, **Zock PL**. Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. *International Journal of Cardiology* 168: 344–351, 2013. doi: 10.1016/j.ijcard.2012.09.047.

92. **Gokce N**, **Keaney Jr JF**, **Hunter LM**, **Watkins MT**, **Menzoian JO**, **Vita JA**. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 105: 1567–1572, 2002.

93. **Humphrey JD**. Mechanisms of Arterial Remodeling in Hypertension. *Hypertension* 52: 195–200, 2008. doi: 10.1161/HYPERTENSIONAHA.107.103440.

94. **Zanchi A**, **Wiesel P**, **Aubert J-F**, **Brunner HR**, **Hayoz D**. Time Course Changes of the Mechanical Properties of the Carotid Artery in Renal Hypertensive Rats. *Hypertension* 29: 1199–1203, 1997. doi: 10.1161/01.HYP.29.5.1199.

95. **Panza JA**, **Quyyumi AA**, **Brush JE**, **Epstein SE**. Abnormal Endothelium-Dependent Vascular Relaxation in Patients with Essential Hypertension. *N Engl J Med* 323: 22–27, 1990. doi: 10.1056/NEJM199007053230105.

96. **Linder L**, **Kiowski W**, **Bühler FR**, **Lüscher TF**. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. *Circulation* 81: 1762–1767, 1990. doi: 10.1161/01.CIR.81.6.1762.

97. **Gokce N**, **Holbrook M**, **Duffy SJ**, **Demissie S**, **Cupples LA**, **Biegelsen E**, **Keaney Jr JF**, **Loscalzo J**, **Vita JA**. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension* 38: 1349–1354, 2001.

98. **Muiesan ML**, **Salvetti M**, **Paini A**, **Monteduro C**, **Galbassini G**, **Poisa P**, **Porteri E**, **Agabiti-Rosei C**, **Paderno V**, **Belotti E**. Prognostic role of flow-mediated dilatation of the brachial artery in hypertensive patients. *Journal of hypertension* 26: 1612–1618, 2008.

99. **Paniagua OA**, **Bryant MB**, **Panza JA**. Role of Endothelial Nitric Oxide in Shear Stress–Induced Vasodilation of Human Microvasculature. *Circulation* 103: 1752–1758, 2001. doi: 10.1161/01.CIR.103.13.1752.

100. **Panza JA**, **Casino PR**, **Kilcoyne CM**, **Quyyumi AA**. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation* 87: 1468–1474, 1993. doi: 10.1161/01.CIR.87.5.1468.

101. **Cardillo Carmine**, **Kilcoyne Crescence M**, **Cannon Richard O**, **Panza Julio A**. Impairment of the nitric oxide–mediated vasodilator response to mental stress in hypertensive but not in hypercholesterolemic patients. *Journal of the American College of Cardiology* 32: 1207–1213, 1998. doi: 10.1016/S0735-1097(98)00391-X.

102. **Asakura T**, **Karino T**. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circulation Research* 66: 1045–1066, 1990. doi: 10.1161/01.RES.66.4.1045.

103. **CARO CG**, **FITZ-GERALD JM**, **SCHROTER RC**. Arterial Wall Shear and Distribution of Early Atheroma in Man. *Nature* 223: 1159–1161, 1969. doi: 10.1038/2231159a0.

104. **Chatzizisis Yiannis S.**, **Coskun Ahmet Umit**, **Jonas Michael**, **Edelman Elazer R.**, **Feldman Charles L.**, **Stone Peter H.** Role of Endothelial Shear Stress in the Natural History of Coronary Atherosclerosis and Vascular Remodeling. *Journal of the American College of Cardiology* 49: 2379–2393, 2007. doi: 10.1016/j.jacc.2007.02.059.

105. **Moore JE**, **Xu C**, **Glagov S**, **Zarins CK**, **Ku DN**. Fluid wall shear stress measurements in a model of the human abdominal aorta: oscillatory behavior and relationship to atherosclerosis. *Atherosclerosis* 110: 225–240, 1994. doi: 10.1016/0021-9150(94)90207-0.

106. **Ziegler T**, **Silacci P**, **Harrison VJ**, **Hayoz D**. Nitric Oxide Synthase Expression in Endothelial Cells Exposed to Mechanical Forces. *Hypertension* 32: 351–355, 1998. doi: 10.1161/01.HYP.32.2.351.

107. **Tremblay JC**, **Grewal AS**, **Pyke KE**. Examining the acute effects of retrograde versus low mean shear rate on flow-mediated dilation. *Journal of Applied Physiology* 126: 1335–1342, 2019. doi: 10.1152/japplphysiol.01065.2018.

108. **Thijssen DHJ**, **Dawson EA**, **Tinken TM**, **Cable NT**, **Green DJ**. Retrograde Flow and Shear Rate Acutely Impair Endothelial Function in Humans. *Hypertension* 53: 986–992, 2009. doi: 10.1161/HYPERTENSIONAHA.109.131508.

109. **Schreuder THA**, **Green DJ**, **Hopman MTE**, **Thijssen DHJ**. Acute impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in humans. *Physiological Reports* 2: e00193, 2014. doi: 10.1002/phy2.193.

110. **Cheng JL**, **Au JS**, **MacDonald MJ**. Peripheral artery endothelial function responses to altered shear stress patterns in humans. *Experimental Physiology* 104: 1126–1135, 2019. doi: 10.1113/EP087597.

111. **Thijssen DHJ**, **Atkinson CL**, **Ono K**, **Sprung VS**, **Spence AL**, **Pugh CJA**, **Green DJ**. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. *Journal of Applied Physiology* 116: 1300–1307, 2014. doi: 10.1152/japplphysiol.00110.2014.

112. **Carthy ER**. Autonomic dysfunction in essential hypertension: A systematic review. *Annals of Medicine and Surgery* 3: 2–7, 2014. doi: 10.1016/j.amsu.2013.11.002.

113. **Singh JP**, **Larson MG**, **Tsuji H**, **Evans JC**, **O'Donnell CJ**, **Levy D**. Reduced Heart Rate Variability and New-Onset Hypertension. *Hypertension* 32: 293–297, 1998. doi: 10.1161/01.HYP.32.2.293.

114. **Masuo K**, **Kawaguchi H**, **Mikami H**, **Ogihara T**, **Tuck ML**. Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. *Hypertension* 42: 474–480, 2003.

115. **Smith PA**, **Graham LN**, **Mackintosh AF**, **Stoker JB**, **Mary DA**. Sympathetic neural mechanisms in white-coat hypertension. *Journal of the American College of Cardiology* 40: 126–132, 2002.

116. **McCubbin J**, **Green J**, **PAGE IH**. Baroceptor function in chronic renal hypertension. *Circulation Research* 4: 205–210, 1956.

117. **Coleman T**, **Guyton A**, **Cowley Jr A**, **Bower J**, **Norman Jr R**, **Manning Jr R**. Feedback mechanisms of arterial pressure control. *Contributions to nephrology* 8: 5–12, 1977.

118. **Hesse C**, **Charkoudian N**, **Liu Z**, **Joyner MJ**, **Eisenach JH**. Baroreflex sensitivity inversely correlates with ambulatory blood pressure in healthy normotensive humans. *Hypertension* 50: 41–46, 2007.

119. **Bristow JD**, **HONOUR AJ**, **Pickering GW**, **Sleight P**, **SMYTH HS**. Diminished baroreflex sensitivity in high blood pressure. *Circulation* 39: 48–54, 1969.

120. **Parmer RJ**, **Cervenka JH**, **Stone RA**. Baroreflex sensitivity and heredity in essential hypertension. *Circulation* 85: 497–503, 1992. doi: 10.1161/01.CIR.85.2.497.

121. **Lantelme P**, **Khettab F**, **Custaud M-A**, **Rial M-O**, **Joanny C**, **Gharib C**, **Milon H**. Spontaneous baroreflex sensitivity: toward an ideal index of cardiovascular risk in hypertension? [Online]. *Journal of Hypertension* 20, 2002. https://journals.lww.com/jhypertension/fulltext/2002/05000/spontaneous_baroreflex_sensitivity__toward_an.29.aspx.

122. **Julius S**, **Nesbitt S**. Sympathetic overactivity in hypertension: a moving target. *American journal of hypertension* 9: 113S-120S, 1996.

123. **Schlaich MP**, **Lambert E**, **Kaye DM**, **Krozowski Z**, **Campbell DJ**, **Lambert G**, **Hastings J**, **Aggarwal A**, **Esler MD**. Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation. *Hypertension* 43: 169–175, 2004.

124. **Grassi G**, **Cattaneo BM**, **Seravalle G**, **Lanfranchi A**, **Mancia G**. Baroreflex Control of Sympathetic Nerve Activity in Essential and Secondary Hypertension. *Hypertension* 31: 68–72, 1998. doi: 10.1161/01.HYP.31.1.68.

125. **Zoccali C**, **Mallamaci F**, **Parlongo S**, **Cutrupi S**, **Benedetto FA**, **Tripepi G**, **Bonanno G**, **Rapisarda F**, **Fatuzzo P**, **Seminara G**. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation* 105: 1354–1359, 2002.

126. **Benedict CR**, **Shelton B**, **Johnstone DE**, **Francis G**, **Greenberg B**, **Konstam M**, **Probstfield JL**, **Yusuf S**. Prognostic significance of plasma norepinephrine in patients with asymptomatic left ventricular dysfunction. *Circulation* 94: 690–697, 1996.

127. **Bramwell JC**, **Hill AV**. The velocity of pulse wave in man. *Proceedings of the Royal Society of London Series B, Containing Papers of a Biological Character* 93: 298–306, 1922. doi: 10.1098/rspb.1922.0022.

128. **Vlachopoulos C**, **Aznaouridis K**, **Stefanadis C**. Aortic Stiffness for Cardiovascular Risk Prediction. *Journal of the American College of Cardiology* 63: 647–649, 2014. doi: 10.1016/j.jacc.2013.10.040.

129. **Lehmann ED**. Noninvasive measurements of aortic stiffness: methodological considerations. *Pathol Biol (Paris)* 47: 716–730, 1999.

130. **Pannier BM**, **Avolio AP**, **Hoeks A**, **Mancia G**, **Takazawa K**. Methods and devices for measuring arterial compliance in humans*. *American Journal of Hypertension* 15: 743–753, 2002. doi: 10.1016/S0895-7061(02)02962-X.

131. **Blacher J**, **Asmar R**, **Djane S**, **London GM**, **Safar ME**. Aortic Pulse Wave Velocity as a Marker of Cardiovascular Risk in Hypertensive Patients. *Hypertension* 33: 1111–1117, 1999. doi: 10.1161/01.HYP.33.5.1111.

132. **Laurent S**, **Boutouyrie P**, **Asmar R**, **Gautier I**, **Laloux B**, **Guize L**, **Ducimetiere P**, **Benetos A**. Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients. *Hypertension* 37: 1236–1241, 2001. doi: 10.1161/01.HYP.37.5.1236.

133. **Mattace-Raso FUS**, **van der Cammen TJM**, **Hofman A**, **van Popele NM**, **Bos ML**, **Schalekamp MADH**, **Asmar R**, **Reneman RS**, **Hoeks APG**, **Breteler MMB**, **Witteman JCM**. Arterial Stiffness and Risk of Coronary Heart Disease and Stroke. *Circulation* 113: 657–663, 2006. doi: 10.1161/CIRCULATIONAHA.105.555235.

134. **Sutton-Tyrrell K**, **Najjar SS**, **Boudreau RM**, **Venkitachalam L**, **Kupelian V**, **Simonsick EM**, **Havlik R**, **Lakatta EG**, **Spurgeon H**, **Kritchevsky S**, **Pahor M**, **Bauer D**, **Newman A**. Elevated Aortic Pulse Wave Velocity, a Marker of Arterial Stiffness, Predicts Cardiovascular Events in Well-Functioning Older Adults. *Circulation* 111: 3384–3390, 2005. doi: 10.1161/CIRCULATIONAHA.104.483628.

135. **Schiffrin E**. Vascular stiffening and arterial complianceImplications for systolic blood pressure. *American Journal of Hypertension* 17: S39–S48, 2004. doi: 10.1016/j.amjhyper.2004.08.019.

136. **Haynes FW**, **Ellis LB**, **Weiss S**. Pulse wave velocity and arterial elasticity in arterial hypertension, arteriosclerosis, and related conditions. *American Heart Journal* 11: 385–401, 1936.

137. **Asmar R**, **Benetos A**, **London G**, **Hugue C**, **Weiss Y**, **Topouchian J**, **Laloux B**, **Safar M**. Aortic distensibility in normotensive, untreated and treated hypertensive patients. *Blood pressure* 4: 48–54, 1995.

138. **Ting C**, **Chang M**, **Wang S**, **Chiang B**, **Yin FC**. Regional pulse wave velocities in hypertensive and normotensive humans. *Cardiovascular research* 24: 865–872, 1990.

139. **Hasegawa M**, **Nagao K**, **Kinoshita Y**, **Rodbard D**, **Asahina A**. Increased pulse wave velocity and shortened pulse wave transmission time in hypertension and aging. *Cardiology* 88: 147–151, 1997.

140. **Me S**, **Laurent S**. Behaviour of conduit arteries in hypertension. *Clinical and experimental hypertension* 15: 1033–1045, 1993.

141. **Kaess BM**, **Rong J**, **Larson MG**, **Hamburg NM**, **Vita JA**, **Levy D**, **Benjamin EJ**, **Vasan RS**, **Mitchell GF**. Aortic Stiffness, Blood Pressure Progression, and Incident Hypertension. *JAMA* 308: 875–881, 2012. doi: 10.1001/2012.jama.10503.

142. **Gimbrone MA Jr**. Vascular Endothelium, Hemodynamic Forces, and Atherogenesis. *The American Journal of Pathology* 155: 1–5, 1999. doi: 10.1016/S0002-9440(10)65090-0.

143. **Avolio A**. Genetic and Environmental Factors in the Function and Structure of the Arterial Wall. *Hypertension* 26: 34–37, 1995. doi: 10.1161/01.HYP.26.1.34.

144. **Lakatta EG**. Cardiovascular regulatory mechanisms in advanced age. *Physiological Reviews* 73: 413–467, 1993. doi: 10.1152/physrev.1993.73.2.413.

145. **Esler M**, **Lambert G**, **Brunner-La Rocca HP**, **Vaddadi G**, **Kaye D**. Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. *Acta Physiologica Scandinavica* 177: 275–284, 2003. doi: 10.1046/j.1365-201X.2003.01089.x.

146. **AJ M**, **LE P**, **MS K**. Sympathetic overactivity in hypertension and cardiovascular disease. *Current vascular pharmacology* 12: 4–15, 2014.

147. **Stevens LA**, **Coresh J**, **Greene T**, **Levey AS**. Assessing Kidney Function — Measured and Estimated Glomerular Filtration Rate. *N Engl J Med* 354: 2473–2483, 2006. doi: 10.1056/NEJMra054415.

148. **Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group**. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney international Supplement* 76: S1-130, 2009.

149. **Levey AS**, **Coresh J**, **Balk E**, **Kausz AT**, **Levin A**, **Steffes MW**, **Hogg RJ**, **Perrone RD**, **Lau J**, **Eknoyan G**. National Kidney Foundation Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. *Ann Intern Med* 139: 137–147, 2003. doi: 10.7326/0003-4819-139-2-200307150-00013.

150. **National Collaborating Centre for Chronic Conditions (Great Britain)**. Chronic kidney disease: national clinical guideline for early identification and management in adults in primary and secondary care. Royal College of Physicians, 2008.

151. **Levey AS**, **Stevens LA**, **Schmid CH**, **Zhang Y (Lucy)**, **Castro AF**, **Feldman HI**, **Kusek JW**, **Eggers P**, **Van Lente F**, **Greene T**, **Coresh J**. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* 150: 604–612, 2009. doi: 10.7326/0003-4819-150-9-200905050-00006.

152. **Levey AS**, **de Jong PE**, **Coresh J**, **El Nahas M**, **Astor BC**, **Matsushita K**, **Gansevoort RT**, **Kasiske BL**, **Eckardt K-U**. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney International* 80: 17–28, 2011. doi: 10.1038/ki.2010.483.

153. **Shlipak MG**, **Matsushita K**, **Ärnlöv J**, **Inker LA**, **Katz R**, **Polkinghorne KR**, **Rothenbacher D**, **Sarnak MJ**, **Astor BC**, **Coresh J**, **Levey AS**, **Gansevoort RT**.

Cystatin C versus Creatinine in Determining Risk Based on Kidney Function. *N Engl J Med* 369: 932–943, 2013. doi: 10.1056/NEJMoa1214234.

154. **Shlipak MG**, **Sarnak MJ**, **Katz R**, **Fried LF**, **Seliger SL**, **Newman AB**, **Siscovick DS**, **Stehman-Breen C**. Cystatin C and the Risk of Death and Cardiovascular Events among Elderly Persons. *N Engl J Med* 352: 2049–2060, 2005. doi: 10.1056/NEJMoa043161.

155. **Waheed S**, **Matsushita K**, **Sang Y**, **Hoogeveen R**, **Ballantyne C**, **Coresh J**, **Astor BC**. Combined Association of Albuminuria and Cystatin C–Based Estimated GFR With Mortality, Coronary Heart Disease, and Heart Failure Outcomes: The Atherosclerosis Risk in Communities (ARIC) Study. *American Journal of Kidney Diseases* 60: 207–216, 2012. doi: 10.1053/j.ajkd.2012.03.011.

156. **Astor BC**, **Levey AS**, **Stevens LA**, **Van Lente F**, **Selvin E**, **Coresh J**. Method of Glomerular Filtration Rate Estimation Affects Prediction of Mortality Risk [Online]. *Journal of the American Society of Nephrology* 20, 2009. https://journals.lww.com/jasn/fulltext/2009/10000/method_of_glomerular_filtration_rate_estimation.19.aspx.

157. **Young JH**, **Klag MJ**, **Muntner P**, **Whyte JL**, **Pahor M**, **Coresh J**. Blood pressure and decline in kidney function: findings from the Systolic Hypertension in the Elderly Program (SHEP). *Journal of the American Society of Nephrology* 13: 2776–2782, 2002.

158. **Helal I**, **Fick-Brosnahan GM**, **Reed-Gitomer B**, **Schrier RW**. Glomerular hyperfiltration: definitions, mechanisms and clinical implications. *Nature Reviews Nephrology* 8: 293–300, 2012. doi: 10.1038/nrneph.2012.19.

159. **Brenner B**, **Hostetter T**, **Olson J**, **Rennke H**, **Venkatachalam M**. The role of glomerular hyperfiltration in the initiation and progression of diabetic nephropathy. *Acta Endocrinol Suppl (Copenh)* 242: 7–10, 1981.

160. **Hostetter TH**, **Olson JL**, **Rennke HG**, **Venkatachalam MA**, **Brenner BM**. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *American Journal of Physiology-Renal Physiology* 241: F85–F93, 1981. doi: 10.1152/ajprenal.1981.241.1.F85.

161. **Brenner BM**, **Lawler EV**, **Mackenzie HS**. The hyperfiltration theory: A paradigm shift in nephrology. *Kidney International* 49: 1774–1777, 1996. doi: 10.1038/ki.1996.265.

162. **Cortinovis M**, **Perico N**, **Ruggenenti P**, **Remuzzi A**, **Remuzzi G**. Glomerular hyperfiltration. *Nature Reviews Nephrology* 18: 435–451, 2022. doi: 10.1038/s41581-022-00559-y.

163. **BERGSTRÖM J**, **AHLBERG M**, **ALVESTRAND A**. Influence of Protein Intake on Renal Hemodynamics and Plasma Hormone Concentrations in Normal Subjects. *Acta Medica Scandinavica* 217: 189–196, 1985. doi: 10.1111/j.0954-6820.1985.tb01655.x.

164. **Bosch JP**, **Lauer A**, **Glabman S**. Short-term protein loading in assessment of patients with renal disease. *The American Journal of Medicine* 77: 873–879, 1984. doi: 10.1016/0002-9343(84)90529-1.

165. **Anderson S**, **Brenner B**. The role of intraglomerular pressure in the initiation and progression of renal disease. *J Hypertens Suppl* 4: S236-8, 1986.

166. **Neuringer JR**, **Brenner BM**. Glomerular hypertension: cause and consequence of renal injury [Online]. *Journal of Hypertension* 10, 1992. https://journals.lww.com/jhypertension/Fulltext/1992/12007/Glomerular_hypertension__cause_and_consequence_of.11.aspx.

167. **Neuringer JR**, **Brenner BM**. Hemodynamic Theory of Progressive Renal Disease: A 10-Year Update in Brief Review. *American Journal of Kidney Diseases* 22: 98–104, 1993. doi: 10.1016/S0272-6386(12)70174-9.

168. **Brenner BM**, **Garcia DL**, **Anderson S**. Glomeruli and Blood Pressure: Less of One, More the Other? *American Journal of Hypertension* 1: 335–347, 1988. doi: 10.1093/ajh/1.4.335.

169. **Ochodnicky P**, **Henning RH**, **van Dokkum RP**, **de Zeeuw D**. Microalbuminuria and endothelial dysfunction: emerging targets for primary prevention of end-organ damage. *Journal of cardiovascular pharmacology* 47: S151–S162, 2006.

170. **Ingelfinger JR**. Is Microanatomy Destiny? *N Engl J Med* 348: 99–100, 2003. doi: 10.1056/NEJMp020168.

171. **Keller G**, **Zimmer G**, **Mall G**, **Ritz E**, **Amann K**. Nephron Number in Patients with Primary Hypertension. *N Engl J Med* 348: 101–108, 2003. doi: 10.1056/NEJMoa020549.

172. **Mogensen CE**. Microalbuminuria Predicts Clinical Proteinuria and Early Mortality in Maturity-Onset Diabetes. *N Engl J Med* 310: 356–360, 1984. doi: 10.1056/NEJM198402093100605.

173. **Borch-Johnsen K**, **Feldt-Rasmussen B**, **Strandgaard S**, **Schroll M**, **Jensen JS**. Urinary Albumin Excretion. *Arteriosclerosis, Thrombosis, and Vascular Biology* 19: 1992–1997, 1999. doi: 10.1161/01.ATV.19.8.1992.

174. **Wang TJ**, **Evans JC**, **Meigs JB**, **Rifai N**, **Fox CS**, **D'Agostino RB**, **Levy D**, **Vasan RS**. Low-Grade Albuminuria and the Risks of Hypertension and Blood Pressure Progression. *Circulation* 111: 1370–1376, 2005. doi: 10.1161/01.CIR.0000158434.69180.2D.

175. **Yudkin JohnS**, **Forrest RichardD**, **Jackson CarolineA**. MICROALBUMINURIA AS PREDICTOR OF VASCULAR DISEASE IN NON-DIABETIC SUBJECTS: Islington Diabetes Survey. *The Lancet* 332: 530–533, 1988. doi: 10.1016/S0140-6736(88)92657-8.

176. **HÖmer D**, **Fliser D**, **Klimm HP**, **Ritz E**. Albuminuria in normotensive and hypertensive individuals attending offices of general practitioners [Online]. *Journal of Hypertension* 14,

1996. https://journals.lww.com/jhypertension/Fulltext/1996/05000/Albuminuria_in_normotensive_and_hypertensive.16.aspx.

177. **Winocour PH**, **Harland JOE**, **Millar JP**, **Laker MF**, **Alberti KGMM**. Microalbuminuria and associated cardiovascular risk factors in the community. *Atherosclerosis* 93: 71–81, 1992. doi: 10.1016/0021-9150(92)90201-Q.

178. **Cirillo M**, **Senigalliesi L**, **Laurenzi M**, **Alfieri R**, **Stamler J**, **Stamler R**, **Panarelli W**, **De Santo NG**. Microalbuminuria in Nondiabetic Adults: Relation of Blood Pressure, Body Mass Index, Plasma Cholesterol Levels, and Smoking: The Gubbio Population Study. *Archives of Internal Medicine* 158: 1933–1939, 1998. doi: 10.1001/archinte.158.17.1933.

179. **Knight EL**, **Kramer HM**, **Curhan GC**. High-normal blood pressure and microalbuminuria. *American Journal of Kidney Diseases* 41: 588–595, 2003. doi: 10.1053/ajkd.2003.50120.

180. **Gerstein HC**, **Mann JFE**, **Yi Q**, **Zinman B**, **Dinneen SF**, **Hoogwerf B**, **Hallé JP**, **Young J**, **Rashkow A**, **Joyce C**, **Nawaz S**, **Yusuf S**, **for the HOPE Study Investigators**. Albuminuria and Risk of Cardiovascular Events, Death, and Heart Failure in Diabetic and Nondiabetic Individuals. *JAMA* 286: 421–426, 2001. doi: 10.1001/jama.286.4.421.

181. **Forman JP**, **Fisher ND**, **Schopick EL**, **Curhan GC**. Higher levels of albuminuria within the normal range predict incident hypertension. *Journal of the American Society of Nephrology: JASN* 19: 1983, 2008.

182. **Klausen K**, **Borch-Johnsen K**, **Feldt-Rasmussen B**, **Jensen G**, **Clausen P**, **Scharling H**, **Appleyard M**, **Jensen JS**. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation* 110: 32–35, 2004.

183. **Stehouwer CA**, **Zeldenrust G**, **den Ottolander GH**, **Hackeng W**, **Donker A**, **Nauta J**. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *The lancet* 340: 319–323, 1992.

184. **Weir MR**. Microalbuminuria and cardiovascular disease. *Clinical Journal of the American Society of Nephrology* 2: 581–590, 2007.

185. **Perticone F**, **Maio R**, **Tripepi G**, **Zoccali C**. Endothelial Dysfunction and Mild Renal Insufficiency in Essential Hypertension. *Circulation* 110: 821–825, 2004. doi: 10.1161/01.CIR.0000138745.21879.27.

186. **Perticone F**, **Maio R**, **Perticone M**, **Sciacqua A**, **Shehaj E**, **Naccarato P**, **Sesti G**. Endothelial Dysfunction and Subsequent Decline in Glomerular Filtration Rate in Hypertensive Patients. *Circulation* 122: 379–384, 2010. doi: 10.1161/CIRCULATIONAHA.110.940932.

187. **Cowley AW Jr**, **Roman RJ**. The Role of the Kidney in Hypertension. *JAMA* 275: 1581–1589, 1996. doi: 10.1001/jama.1996.03530440061038.

188. **Pistrosch F**, **Herbrig K**, **Kindel B**, **Passauer J**, **Fischer S**, **Gross P**. Rosiglitazone Improves Glomerular Hyperfiltration, Renal Endothelial Dysfunction, and Microalbuminuria of Incipient Diabetic Nephropathy in Patients. *Diabetes* 54: 2206–2211, 2005. doi: 10.2337/diabetes.54.7.2206.

189. **Higashi Y**, **Sasaki S**, **Nakagawa K**, **Matsuura H**, **Oshima T**, **Chayama K**. Endothelial Function and Oxidative Stress in Renovascular Hypertension. *N Engl J Med* 346: 1954–1962, 2002. doi: 10.1056/NEJMoa013591.

190. **Ozkayar N**, **Altun B**, **Yildirim T**, **Yilmaz R**, **Dede F**, **Arik G**, **Turkmen E**, **Hayran M**, **Aki FT**, **Arici M**, **Erdem Y**. Blood pressure measurements, blood pressure variability and endothelial function in renal transplant recipients. *Clinical and Experimental Hypertension* 36: 392–397, 2014. doi: 10.3109/10641963.2013.827706.

191. **O'Rourke MF**, **Safar ME**. Relationship Between Aortic Stiffening and Microvascular Disease in Brain and Kidney. *Hypertension* 46: 200–204, 2005. doi: 10.1161/01.HYP.0000168052.00426.65.

192. **Sedaghat S**, **Mattace-Raso FUS**, **Hoorn EJ**, **Uitterlinden AG**, **Hofman A**, **Ikram MA**, **Franco OH**, **Dehghan A**. Arterial Stiffness and Decline in Kidney Function [Online]. *Clinical Journal of the American Society of Nephrology* 10, 2015. https://journals.lww.com/cjasn/Fulltext/2015/12000/Arterial_Stiffness_and_Decline_in_Kidney_Function.13.aspx.

193. **Upadhyay A**, **Hwang S-J**, **Mitchell GF**, **Vasan RS**, **Vita JA**, **Stantchev PI**, **Meigs JB**, **Larson MG**, **Levy D**, **Benjamin EJ**, **Fox CS**. Arterial Stiffness in Mild-to-Moderate CKD [Online]. *Journal of the American Society of Nephrology* 20, 2009. https://journals.lww.com/jasn/Fulltext/2009/09000/Arterial_Stiffness_in_Mild_to_Moderate_CKD.27.aspx.

194. **Safar ME**, **London GM**, **Plante GE**. Arterial Stiffness and Kidney Function. *Hypertension* 43: 163–168, 2004. doi: 10.1161/01.HYP.0000114571.75762.b0.

195. **Kim JH**, **Kim SS**, **Kim IJ**, **Kim BH**, **Park JY**, **Lee CW**, **Suk JH**, **Shin SH**, **Son SP**, **Kim MC**, **Ahn JH**, **Lee KJ**, **Kwon MJ**, **Lee SH**, **Park JH**. Arterial Stiffness Is More Associated with Albuminuria than Decreased Glomerular Filtration Rate in Patients with Type 2 Diabetes Mellitus: The REBOUND Study. *Journal of Diabetes Research* 2017: 7047909, 2017. doi: 10.1155/2017/7047909.

196. **Alzheimer's Association**. 2019 Alzheimer's disease facts and figures. *Alzheimer's & Dementia* 15: 321–387, 2019. doi: 10.1016/j.jalz.2019.01.010.

197. **Chobanian AV**, **Bakris GL**, **Black HR**, **Cushman WC**, **Green LA**, **Izzo J Joseph L**, **Jones DW**, **Materson BJ**, **Oparil S**, **Wright J Jackson T**, **Roccella EJ**, **and the**

National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood PressureThe JNC 7 Report. *JAMA* 289: 2560–2571, 2003. doi: 10.1001/jama.289.19.2560.

198. **Waldstein SR**, **Giggey PP**, **Thayer JF**, **Zonderman AB**. Nonlinear Relations of Blood Pressure to Cognitive Function: The Baltimore Longitudinal Study of Aging. *Hypertension* 45: 374–379, 2005. doi: 10.1161/01.HYP.0000156744.44218.74.

199. **Meyer JS**, **Rauch GM**, **Rauch RA**, **Haque A**, **Crawford K**. Cardiovascular and Other Risk Factors for Alzheimer's Disease and Vascular Dementia. *Annals of the New York Academy of Sciences* 903: 411–423, 2000. doi: 10.1111/j.1749-6632.2000.tb06393.x.

200. **Egan BM**, **Li J**, **Hutchison FN**, **Ferdinand KC**. Hypertension in the United States, 1999 to 2012: Progress Toward Healthy People 2020 Goals. *Circulation* 130: 1692–1699, 2014. doi: 10.1161/CIRCULATIONAHA.114.010676.

201. **Virani SS**, **Alonso A**, **Benjamin EJ**, **Bittencourt MS**, **Callaway CW**, **Carson AP**, **Chamberlain AM**, **Chang AR**, **Cheng S**, **Delling FN**, **Djousse L**, **Elkind MSV**, **Ferguson JF**, **Fornage M**, **Khan SS**, **Kissela BM**, **Knutson KL**, **Kwan TW**, **Lackland DT**, **Lewis TT**, **Lichtman JH**, **Longenecker CT**, **Loop MS**, **Lutsey PL**, **Martin SS**, **Matsushita K**, **Moran AE**, **Mussolino ME**, **Perak AM**, **Rosamond WD**, **Roth GA**, **Sampson UKA**, **Satou GM**, **Schroeder EB**, **Shah SH**, **Shay CM**, **Spartano NL**, **Stokes A**, **Tirschwell DL**, **VanWagner LB**, **Tsao CW**, **On behalf of the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee**. Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association. *Circulation* 141, 2020. doi: 10.1161/CIR.0000000000000757.

202. **Mozaffarian D**, **Benjamin EJ**, **Go AS**, **Arnett DK**, **Blaha MJ**, **Cushman M**, **de Ferranti S**, **Després J-P**, **Fullerton HJ**, **Howard VJ**, **Huffman MD**, **Judd SE**, **Kissela BM**, **Lackland DT**, **Lichtman JH**, **Lisabeth LD**, **Liu S**, **Mackey RH**, **Matchar DB**, **McGuire DK**, **Iii ERM**, **Moy CS**, **Muntner P**, **Mussolino ME**, **Nasir K**, **Neumar RW**, **Nichol G**, **Palaniappan L**, **Pandey DK**, **Reeves MJ**, **Rodriguez CJ**, **Sorlie PD**, **Stein J**, **Towfighi A**, **Turan TN**, **Virani SS**, **Willey JZ**, **Woo D**, **Yeh RW**, **Turner MB**. Heart Disease and Stroke Statistics—2015 Update. .

203. **Black HR**. The burden of cardiovascular disease: following the link from hypertension to myocardial infarction and heart failure: *American Journal of Hypertension* 16: 4S-6S, 2003. doi: 10.1016/S0895-7061(03)00969-5.

204. **Waldstein SR**, **Elias MF**. *Neuropsychology of Cardiovascular Disease*. Psychology Press, 2001.

205. **Elias MF**, **Wolf PA**, **D'Agostino RB**, **Cobb J**, **White LR**. Untreated Blood Pressure Level Is Inversely Related to Cognitive Functioning: The Framingham Study. *American Journal of Epidemiology* 138: 353–364, 1993. doi: 10.1093/oxfordjournals.aje.a116868.

206. **Elias MF**, **Robbins MA**, **Elias PK**, **Streeten DHP**. A longitudinal study of blood pressure in relation to performance on the Wechsler Adult Intelligence Scale. *Health Psychology* 17: 486, 19981201. doi: 10.1037/0278-6133.17.6.486.

207. **R. A. Whitmer**, **S. Sidney**, **J. Selby**, **S. Claiborne Johnston**, **K. Yaffe**. Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology* 64: 277, 2005. doi: 10.1212/01.WNL.0000149519.47454.F2.

208. **Yamada M**, **Kasagi F**, **Sasaki H**, **Masunari N**, **Mimori Y**, **Suzuki G**. Association Between Dementia and Midlife Risk Factors: the Radiation Effects Research Foundation Adult Health Study. *Journal of the American Geriatrics Society* 51: 410–414, 2003. doi: 10.1046/j.1532-5415.2003.51117.x.

209. **Kivipelto M**, **Helkala E-L**, **Laakso MP**, **Hänninen T**, **Hallikainen M**, **Alhainen K**, **Soininen H**, **Tuomilehto J**, **Nissinen A**. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ* 322: 1447, 2001. doi: 10.1136/bmj.322.7300.1447.

210. **Launer LJ**, **Ross GW**, **Petrovitch H**, **Masaki K**, **Foley D**, **White LR**, **Havlik RJ**. Midlife blood pressure and dementia: the Honolulu–Asia aging study☆. *Neurobiology of Aging* 21: 49–55, 2000. doi: 10.1016/S0197-4580(00)00096-8.

211. **Wu C**, **Zhou D**, **Wen C**, **Zhang L**, **Como P**, **Qiao Y**. Relationship between blood pressure and Alzheimer's disease in Linxian County, China. *Life Sciences* 72: 1125–1133, 2003. doi: 10.1016/S0024-3205(02)02367-6.

212. **Wilkie F**, **Eisdorfer C**. Intelligence and Blood Pressure in the Aged. *Science* 172: 959–962, 1971.

213. **Heidenreich PA**, **Trogdon JG**, **Khavjou OA**, **Butler J**, **Dracup K**, **Ezekowitz MD**, **Finkelstein EA**, **Hong Y**, **Johnston SC**, **Khera A**, **Lloyd-Jones DM**, **Nelson SA**, **Nichol G**, **Orenstein D**, **Wilson PWF**, **Woo YJ**. Forecasting the Future of Cardiovascular Disease in the United States: A Policy Statement From the American Heart Association. *Circulation* 123: 933–944, 2011. doi: 10.1161/CIR.0b013e31820a55f5.

214. **Forette F**, **Seux M-L**, **Staessen JA**, **Thijs L**, **Birkenhäger WH**, **Babarskiene M-R**, **Babeanu S**, **Bossini A**, **Gil-Extremera B**, **Girerd X**, **Laks T**, **Lilov E**, **Moisseyev V**, **Tuomilehto J**, **Vanhanen H**, **Webster J**, **Yodfat Y**, **Fagard R**. Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. *The Lancet* 352: 1347–1351, 1998. doi: 10.1016/S0140-6736(98)03086-4.

215. **Forette F**, **Seux M-L**, **Staessen JA**, **Thijs L**, **Babarskiene M-R**, **Babeanu S**, **Bossini A**, **Fagard R**, **Gil-Extremera B**, **Laks T**, **Kobalava Z**, **Sarti C**, **Tuomilehto J**, **Vanhanen H**, **Webster J**, **Yodfat Y**, **Birkenhäger WH**, **for the Syst-Eur Investigators**. The Prevention of Dementia With Antihypertensive Treatment: New Evidence From the Systolic Hypertension in Europe (Syst-Eur) Study. *Archives of Internal Medicine* 162: 2046–2052, 2002. doi: 10.1001/archinte.162.18.2046.

216. **The PROGRESS Collaborative Group***. Effects of Blood Pressure Lowering With Perindopril and Indapamide Therapy on Dementia and Cognitive Decline in Patients With Cerebrovascular Disease. *Archives of Internal Medicine* 163: 1069–1075, 2003. doi: 10.1001/archinte.163.9.1069.

217. **Pickering TG**, **Harshfield GA**, **Devereux RB**, **Laragh JH**. What is the role of ambulatory blood pressure monitoring in the management of hypertensive patients? *Hypertension* 7: 171–177, 1985. doi: 10.1161/01.HYP.7.2.171.

218. **Kario K**, **Matsuo T**, **Kobayashi H**, **Imiya M**, **Matsuo M**, **Shimada K**. Nocturnal Fall of Blood Pressure and Silent Cerebrovascular Damage in Elderly Hypertensive Patients. *Hypertension* 27: 130–135, 1996. doi: 10.1161/01.HYP.27.1.130.

219. **Fagard RH**, **Thijs L**, **Staessen JA**, **Clement DL**, **De Buyzere ML**, **De Bacquer DA**. Night–day blood pressure ratio and dipping pattern as predictors of death and cardiovascular events in hypertension. *Journal of Human Hypertension* 23: 645–653, 2009. doi: 10.1038/jhh.2009.9.

220. **Gavriilaki M**, **Anyfanti P**, **Nikolaidou B**, **Lazaridis A**, **Gavriilaki E**, **Douma S**, **Gkaliagkousi E**. Nighttime dipping status and risk of cardiovascular events in patients with untreated hypertension: A systematic review and meta-analysis. *The Journal of Clinical Hypertension* 22: 1951–1959, 2020. doi: 10.1111/jch.14039.

221. **O'Brien E**, **Sheridan J**, **O'Malley K**. DIPPERS AND NON-DIPPERS. *The Lancet* 332: 397, 1988. doi: 10.1016/S0140-6736(88)92867-X.

222. **Kobrin I**, **Oigman W**, **Kumar A**, **Ventura HO**, **Messerli FH**, **Frohlich ED**, **Dunn FG**. Diurnal Variation of Blood Pressure in Elderly Patients with Essential Hypertension. *Journal of the American Geriatrics Society* 32: 896–899, 1984. doi: 10.1111/j.1532-5415.1984.tb00890.x.

223. **Shimada K**, **Kawamoto A**, **Matsubayashi K**, **Nishinaga M**, **Kimura S**, **Ozawa T**. Diurnal blood pressure variations and silent cerebrovascular damage in elderly patients with hypertension. *J Hypertens* 10: 875–878, 1992.

224. **Baumgart P**, **Walger P**, **Gemen S**, **von Eiff M**, **Raidt H**, **Rahn KH**. Blood Pressure Elevation during the Night in Chronic Renal Failure, Hemodialysis and after Renal Transplantation. *Nephron* 57: 293–298, 2008. doi: 10.1159/000186278.

225. **Bianchi S**, **Bigazzi R**, **Baldari G**, **Sgherri G**, **Campese VM**. Diurnal Variations of Blood Pressure and Microalbuminuria in Essential Hypertension. *American Journal of Hypertension* 7: 23–29, 1994. doi: 10.1093/ajh/7.1.23.

226. **Firbank MJ**, **Wiseman RM**, **Burton EJ**, **Saxby BK**, **O'Brien JT**, **Ford GA**. Brain atrophy and white matter hyperintensity change in older adults and relationship to blood pressure. *Journal of Neurology* 254: 713–721, 2007. doi: 10.1007/s00415-006-0238-4.

227. **Henskens LH**, **Kroon AA**, **van Oostenbrugge RJ**, **Gronenschild EH**, **Hofman PA**, **Lodder J**, **de Leeuw PW**. Associations of ambulatory blood pressure levels with white matter hyperintensity volumes in hypertensive patients [Online]. *Journal of Hypertension* 27, 2009. https://journals.lww.com/jhypertension/Fulltext/2009/07000/Associations_of_ambulatory_blood_pressure_levels.18.aspx.

228. **Wolfson L**, **Wakefield DB**, **Moscufo N**, **Kaplan RF**, **Hall CB**, **Schmidt JA**, **Guttmann CRG**, **White WB**. Rapid Buildup of Brain White Matter Hyperintensities Over 4 Years Linked to Ambulatory Blood Pressure, Mobility, Cognition, and Depression in Old Persons. *The Journals of Gerontology: Series A* 68: 1387–1394, 2013. doi: 10.1093/gerona/glt072.

229. **Sierra C**. Associations between Ambulatory Blood Pressure Parameters and Cerebral White Matter Lesions. *International Journal of Hypertension* 2011: 478710, 2011. doi: 10.4061/2011/478710.

230. **van Boxtel MPJ**, **Henskens LHG**, **Kroon AA**, **Hofman PAM**, **Gronenschild EHBM**, **Jolles J**, **de Leeuw PW**. Ambulatory blood pressure, asymptomatic cerebrovascular damage and cognitive function in essential hypertension. *Journal of Human Hypertension* 20: 5–13, 2006. doi: 10.1038/sj.jhh.1001934.

231. **Warnert EAH**, **Rodrigues JCL**, **Burchell AE**, **Neumann S**, **Ratcliffe LEK**, **Manghat NE**, **Harris AD**, **Adams Z**, **Nightingale AK**, **Wise RG**, **Paton JFR**, **Hart EC**. Is High Blood Pressure Self-Protection for the Brain? *Circulation Research* 119: e140–e151, 2016. doi: 10.1161/CIRCRESAHA.116.309493.

232. **Zhang R**, **Witkowski S**, **Fu Q**, **Claassen JAHR**, **Levine BD**. Cerebral Hemodynamics After Short- and Long-Term Reduction in Blood Pressure in Mild and Moderate Hypertension. *Hypertension* 49: 1149–1155, 2007. doi: 10.1161/HYPERTENSIONAHA.106.084939.

233. **Gianaros PJ**, **Greer PJ**, **Ryan CM**, **Jennings JR**. Higher blood pressure predicts lower regional grey matter volume: Consequences on short-term information processing. *NeuroImage* 31: 754–765, 2006. doi: 10.1016/j.neuroimage.2006.01.003.

234. **Korf ESC**, **White LR**, **Scheltens P**, **Launer LJ**. Midlife Blood Pressure and the Risk of Hippocampal Atrophy. *Hypertension* 44: 29–34, 2004. doi: 10.1161/01.HYP.0000132475.32317.bb.

235. **Iris B. Goldstein**, **George Bartzokis**, **Donald Guthrie**, **David Shapiro**. Ambulatory blood pressure and brain atrophy in the healthy elderly. *Neurology* 59: 713, 2002. doi: 10.1212/WNL.59.5.713.

236. **Nagai M**, **Hoshide S**, **Ishikawa J**, **Shimada K**, **Kario K**. Ambulatory blood pressure as an independent determinant of brain atrophy and cognitive function in elderly hypertension [Online]. *Journal of Hypertension* 26, 2008.

https://journals.lww.com/jhypertension/Fulltext/2008/08000/Ambulatory_blood_pressure_as_an_independent.21.aspx.

237. **Heijer T den**, **Skoog I**, **Oudkerk M**, **de Leeuw F-E**, **de Groot JC**, **Hofman A**, **Breteler MMB**. Association between blood pressure levels over time and brain atrophy in the elderly. *Neurobiology of Aging* 24: 307–313, 2003. doi: 10.1016/S0197-4580(02)00088-X.

238. **Wang T**, **Li Y**, **Guo X**, **Huang D**, **Ma L**, **Wang DJJ**, **Lou X**. Reduced perfusion in normal-appearing white matter in mild to moderate hypertension as revealed by 3D pseudocontinuous arterial spin labeling. *Journal of Magnetic Resonance Imaging* 43: 635–643, 2016. doi: 10.1002/jmri.25023.

239. **Kashgarian M**. Pathology of Small Blood Vessel Disease in Hypertension. *American Journal of Kidney Diseases* 5: A104–A110, 1985. doi: 10.1016/S0272-6386(85)80072-X.

240. **Cohen RA**. Hypertension and Cerebral Blood Flow. *Stroke* 38: 1715–1717, 2007. doi: 10.1161/STROKEAHA.107.487165.

241. **Fujishima M**, **Ibayashi S**, **Fujii K**, **Mori S**. Cerebral Blood Flow and Brain Function in Hypertension. *Hypertension Research* 18: 111–117, 1995. doi: 10.1291/hypres.18.111.

242. **Tryambake D**, **He J**, **Firbank MJ**, **O'Brien JT**, **Blamire AM**, **Ford GA**. Intensive Blood Pressure Lowering Increases Cerebral Blood Flow in Older Subjects With Hypertension. *Hypertension* 61: 1309–1315, 2013. doi: 10.1161/HYPERTENSIONAHA.112.200972.

243. **Muller M**, **van der Graaf Y**, **Visseren FL**, **Mali WPThM**, **Geerlings MI**, **for the SMART Study Group**. Hypertension and longitudinal changes in cerebral blood flow: The SMART-MR study. *Annals of Neurology* 71: 825–833, 2012. doi: 10.1002/ana.23554.

244. **Prins ND**, **Scheltens P**. White matter hyperintensities, cognitive impairment and dementia: an update. *Nature Reviews Neurology* 11: 157–165, 2015. doi: 10.1038/nrneurol.2015.10.

245. **Debette S**, **Markus HS**. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ* 341: c3666, 2010. doi: 10.1136/bmj.c3666.

246. **Alber J**, **Alladi S**, **Bae H-J**, **Barton DA**, **Beckett LA**, **Bell JM**, **Berman SE**, **Biessels GJ**, **Black SE**, **Bos I**, **Bowman GL**, **Brai E**, **Brickman AM**, **Callahan BL**, **Corriveau RA**, **Fossati S**, **Gottesman RF**, **Gustafson DR**, **Hachinski V**, **Hayden KM**, **Helman AM**, **Hughes TM**, **Isaacs JD**, **Jefferson AL**, **Johnson SC**, **Kapasi A**, **Kern S**, **Kwon JC**, **Kukolja J**, **Lee A**, **Lockhart SN**, **Murray A**, **Osborn KE**, **Power MC**, **Price BR**, **Rhodius-Meester HFM**, **Rondeau JA**, **Rosen AC**, **Rosene DL**, **Schneider JA**, **Scholtzova H**, **Shaaban CE**, **Silva NCBS**, **Snyder HM**, **Swardfager W**, **Troen AM**, **van Veluw SJ**, **Vemuri P**, **Wallin A**, **Wellington C**, **Wilcock DM**, **Xie SX**, **Hainsworth AH**. White matter hyperintensities in vascular contributions to cognitive impairment and dementia (VCID): Knowledge gaps and opportunities. *Alzheimer's & Dementia:*

Translational Research & Clinical Interventions 5: 107–117, 2019. doi: 10.1016/j.trci.2019.02.001.

247. **Schmidt R**, **Schmidt H**, **Haybaeck J**, **Loitfelder M**, **Weis S**, **Cavalieri M**, **Seiler S**, **Enzinger C**, **Ropele S**, **Erkinjuntti T**, **Pantoni L**, **Scheltens P**, **Fazekas F**, **Jellinger K**. Heterogeneity in age-related white matter changes. *Acta Neuropathologica* 122: 171–185, 2011. doi: 10.1007/s00401-011-0851-x.

248. **Alida A Gouw**, **Alexandra Seewann**, **Wiesje M van der Flier**, **Frederik Barkhof**, **Annemieke M Rozemuller**, **Philip Scheltens**, **Jeroen J G Geurts**. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *J Neurol Neurosurg Psychiatry* 82: 126, 2011. doi: 10.1136/jnnp.2009.204685.

249. **C. Dufouil**, **A. de Kersaint–Gilly**, **V. Besançon**, **C. Levy**, **E. Auffray**, **L. Brunnereau**, **A. Alpérovitch**, **C. Tzourio**. Longitudinal study of blood pressure and white matter hyperintensities. *Neurology* 56: 921, 2001. doi: 10.1212/WNL.56.7.921.

250. **van Dijk EJ**, **Prins ND**, **Vrooman HA**, **Hofman A**, **Koudstaal PJ**, **Breteler MMB**. Progression of Cerebral Small Vessel Disease in Relation to Risk Factors and Cognitive Consequences. *Stroke* 39: 2712–2719, 2008. doi: 10.1161/STROKEAHA.107.513176.

251. **Vuorinen M**, **Spulber G**, **Damangir S**, **Niskanen E**, **Ngandu T**, **Soininen H**, **Kivipelto M**, **Solomon A**. Midlife CAIDE Dementia Risk Score and Dementia-Related Brain Changes up to 30 Years Later on Magnetic Resonance Imaging. *Journal of Alzheimer's Disease* 44: 93–101, 2015. doi: 10.3233/JAD-140924.

252. **Poels MMF**, **Zaccai K**, **Verwoert GC**, **Vernooij MW**, **Hofman A**, **van der Lugt A**, **Witteman JCM**, **Breteler MMB**, **Mattace-Raso FUS**, **Ikram MA**. Arterial Stiffness and Cerebral Small Vessel Disease. *Stroke* 43: 2637–2642, 2012. doi: 10.1161/STROKEAHA.111.642264.

253. **Chirinos JA**, **Segers P**, **Hughes T**, **Townsend R**. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. *Journal of the American College of Cardiology* 74: 1237–1263, 2019. doi: 10.1016/j.jacc.2019.07.012.

254. **Iulita MF**, **Noriega de la Colina A**, **Girouard H**. Arterial stiffness, cognitive impairment and dementia: confounding factor or real risk? *Journal of Neurochemistry* 144: 527–548, 2018. doi: 10.1111/jnc.14235.

255. **Mitchell GF**, **van Buchem MA**, **Sigurdsson S**, **Gotal JD**, **Jonsdottir MK**, **Kjartansson Ó**, **Garcia M**, **Aspelund T**, **Harris TB**, **Gudnason V**, **Launer LJ**. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility – Reykjavik Study. *Brain* 134: 3398–3407, 2011. doi: 10.1093/brain/awr253.

256. **de Montgolfier O**, **Pinçon A**, **Pouliot P**, **Gillis M-A**, **Bishop J**, **Sled JG**, **Villeneuve L**, **Ferland G**, **Lévy BI**, **Lesage F**, **Thorin-Trescases N**, **Thorin É**. High Systolic Blood

Pressure Induces Cerebral Microvascular Endothelial Dysfunction, Neurovascular Unit Damage, and Cognitive Decline in Mice. *Hypertension* 73: 217–228, 2019. doi: 10.1161/HYPERTENSIONAHA.118.12048.

257. **Zeki Al Hazzouri A**, **Yaffe K**. Arterial Stiffness and Cognitive Function in the Elderly. *Journal of Alzheimer's Disease* 42: S503–S514, 2014. doi: 10.3233/JAD-141563.

258. **Waldstein SR**, **Rice SC**, **Thayer JF**, **Najjar SS**, **Scuteri A**, **Zonderman AB**. Pulse Pressure and Pulse Wave Velocity Are Related to Cognitive Decline in the Baltimore Longitudinal Study of Aging. *Hypertension* 51: 99–104, 2008. doi: 10.1161/HYPERTENSIONAHA.107.093674.

259. **Triantafyllidi H**, **Arvaniti C**, **Lekakis J**, **Ikonomidis I**, **Siafakas N**, **Tzortzis S**, **Trivilou P**, **Zerva L**, **Stamboulis E**, **Kremastinos DT**. Cognitive Impairment Is Related to Increased Arterial Stiffness and Microvascular Damage in Patients With Never-Treated Essential Hypertension. *American Journal of Hypertension* 22: 525–530, 2009. doi: 10.1038/ajh.2009.35.

260. **Hajjar I**, **Goldstein FC**, **Martin GS**, **Quyyumi AA**. Roles of Arterial Stiffness and Blood Pressure in Hypertension-Associated Cognitive Decline in Healthy Adults. *Hypertension* 67: 171–175, 2016. doi: 10.1161/HYPERTENSIONAHA.115.06277.

261. **Qiu C**, **Winblad B**, **Fratiglioni L**. The age-dependent relation of blood pressure to cognitive function and dementia. *The Lancet Neurology* 4: 487–499, 2005. doi: 10.1016/S1474-4422(05)70141-1.

262. **Rouch L**, **Cestac P**, **Hanon O**, **Cool C**, **Helmer C**, **Bouhanick B**, **Chamontin B**, **Dartigues J-F**, **Vellas B**, **Andrieu S**. Antihypertensive Drugs, Prevention of Cognitive Decline and Dementia: A Systematic Review of Observational Studies, Randomized Controlled Trials and Meta-Analyses, with Discussion of Potential Mechanisms. *CNS Drugs* 29: 113–130, 2015. doi: 10.1007/s40263-015-0230-6.

263. **Andrieu S**, **Coley N**, **Lovestone S**, **Aisen PS**, **Vellas B**. Prevention of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. *The Lancet Neurology* 14: 926–944, 2015. doi: 10.1016/S1474-4422(15)00153-2.

264. **Cornelissen VA**, **Fagard RH**. Effects of Endurance Training on Blood Pressure, Blood Pressure–Regulating Mechanisms, and Cardiovascular Risk Factors. *Hypertension* 46: 667–675, 2005. doi: 10.1161/01.HYP.0000184225.05629.51.

265. **Cornelissen VA**, **Smart NA**. Exercise Training for Blood Pressure: A Systematic Review and Meta-analysis. *Journal of the American Heart Association* 2: e004473, [date unknown]. doi: 10.1161/JAHA.112.004473.

266. **Hillman CH**, **Erickson KI**, **Kramer AF**. Be smart, exercise your heart: exercise effects on brain and cognition. *Nature Reviews Neuroscience* 9: 58–65, 2008. doi: 10.1038/nrn2298.

267. **Laukkanen T**, **Kunutsor S**, **Kauhanen J**, **Laukkanen JA**. Sauna bathing is inversely associated with dementia and Alzheimer's disease in middle-aged Finnish men. *Age and Ageing* 46: 245–249, 2017. doi: 10.1093/ageing/afw212.

268. **Heinonen I**, **Laukkanen JA**. Effects of heat and cold on health, with special reference to Finnish sauna bathing. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 314: R629–R638, 2018. doi: 10.1152/ajpregu.00115.2017.

269. **Laukkanen JA**, **Laukkanen T**, **Kunutsor SK**. Cardiovascular and Other Health Benefits of Sauna Bathing: A Review of the Evidence. *Mayo Clinic Proceedings* 93: 1111–1121, 2018. doi: 10.1016/j.mayocp.2018.04.008.

270. **Von Schulze AT**, **Deng F**, **Morris JK**, **Geiger PC**. Heat therapy: possible benefits for cognitive function and the aging brain. *Journal of Applied Physiology* 129: 1468–1476, 2020. doi: 10.1152/japplphysiol.00168.2020.

271. The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Archives of Internal Medicine* 157: 2413–2446, 1997. doi: 10.1001/archinte.1997.00440420033005.

272. **Zanchetti A**, **Chalmers JP**, **Gyafras I**, **Hansson L**, **Mancia G**, **Strasser T**. Prevention of Hypertension and Associated Cardiovascular Disease: A 1991 Statement Conclusions from a joint WHO/ISH Meeting. *null* 14: 333–341, 1992. doi: 10.3109/10641969209036191.

273. American College of Sports Medicine. Position Stand. Physical activity, physical fitness, and hypertension. *Medicine and science in sports and exercise* 25: i–x, 1993.

274. **James PA**, **Oparil S**, **Carter BL**, **Cushman WC**, **Dennison-Himmelfarb C**, **Handler J**, **Lackland DT**, **LeFevre ML**, **MacKenzie TD**, **Ogedegbe O**, **Smith SC**, **Svetkey LP**, **Taler SJ**, **Townsend RR**, **Wright JT**, **Narva AS**, **Ortiz E**. 2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults: Report From the Panel Members Appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 311: 507, 2014. doi: 10.1001/jama.2013.284427.

275. **Morris JN**, **Heady JA**, **Raffle PAB**, **Roberts CG**, **Parks JW**. CORONARY HEART-DISEASE AND PHYSICAL ACTIVITY OF WORK. *The Lancet* 262: 1111–1120, 1953. doi: 10.1016/S0140-6736(53)91495-0.

276. Physical Activity Guidelines for Americans, 2nd edition. .

277. **Leon A**, **Myers M**, **Connett J**. Leisure time physical activity and the 16-year risks of mortality from coronary heart disease and all-causes in the Multiple Risk Factor Intervention Trial (MRFIT). *International Journal of Sports Medicine* 18: S208–S215, 1997.

278. **Leon AS**, **Connett J**, **Jacobs DR**, **Rauramaa R**. Leisure-time physical activity levels and risk of coronary heart disease and death: the Multiple Risk Factor Intervention Trial. *Jama* 258: 2388–2395, 1987.

279. **FOLSOM AR**, **ARNETT DK**, **HUTCHINSON RG**, **LIAO F**, **CLEGG LX**, **COOPER LS**. Physical activity and incidence of coronary heart disease in middle-aged women and men [Online]. *Medicine & Science in Sports & Exercise* 29, 1997. https://journals.lww.com/acsm-msse/Fulltext/1997/07000/Physical_activity_and_incidence_of_coronary_heart.9.aspx.

280. **Wang J-S**, **Lee T**, **Chow S-E**. Role of exercise intensities in oxidized low-density lipoprotein-mediated redox status of monocyte in men. *Journal of Applied Physiology* 101: 740–744, 2006. doi: 10.1152/japplphysiol.00144.2006.

281. **Banerjee AK**, **Mandal A**, **Chanda D**, **Chakraborti S**. Oxidant, antioxidant and physical exercise. *Molecular and Cellular Biochemistry* 253: 307–312, 2003. doi: 10.1023/A:1026032404105.

282. **Miyazaki H**, **Oh-ishi S**, **Ookawara T**, **Kizaki T**, **Toshinai K**, **Ha S**, **Haga S**, **Ji LL**, **Ohno H**. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *European journal of applied physiology* 84: 1–6, 2001.

283. **Adams V**, **Linke A**, **Kränkel N**, **Erbs S**, **Gielen S**, **Möbius-Winkler S**, **Gummert JF**, **Mohr FW**, **Schuler G**, **Hambrecht R**. Impact of regular physical activity on the NAD (P) H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111: 555–562, 2005.

284. **Kojda G**, **Harrison D**. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. *Cardiovascular research* 43: 652–671, 1999.

285. **Cai H**, **Davis ME**, **Drummond GR**, **Harrison DG**. Induction of endothelial NO synthase by hydrogen peroxide via a Ca2+/calmodulin-dependent protein kinase II/janus kinase 2–dependent pathway. *Arteriosclerosis, thrombosis, and vascular biology* 21: 1571–1576, 2001.

286. **Lauer N**, **Suvorava T**, **Rüther U**, **Jacob R**, **Meyer W**, **Harrison DG**, **Kojda G**. Critical involvement of hydrogen peroxide in exercise-induced up-regulation of endothelial NO synthase. *Cardiovascular research* 65: 254–262, 2005.

287. **Fleming I**, **Busse R**. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 284: R1–R12, 2003.

288. **Noris M**, **Morigi M**, **Donadelli R**, **Aiello S**, **Foppolo M**, **Todeschini M**, **Orisio S**, **Remuzzi G**, **Remuzzi A**. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circulation research* 76: 536–543, 1995.

289. **Sessa WC**, **Pritchard K**, **Seyedi N**, **Wang J**, **Hintze TH**. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circulation research* 74: 349–353, 1994.

290. **Koller A**, **Huang A**, **Sun D**, **Kaley G**. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles: role of endothelial nitric oxide and prostaglandins. *Circulation research* 76: 544–550, 1995.

291. **Wang J**, **Wolin MS**, **Hintze TH**. Chronic exercise enhances endothelium-mediated dilation of epicardial coronary artery in conscious dogs. *Circulation research* 73: 829–838, 1993.

292. **Green D**, **Cheetham C**, **Mavaddat L**, **Watts K**, **Best M**, **Taylor R**, **O'Driscoll G**. Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *American Journal of Physiology-Heart and Circulatory Physiology* 283: H899–H907, 2002. doi: 10.1152/ajpheart.00049.2002.

293. **Green DJ**, **Bilsborough W**, **Naylor LH**, **Reed C**, **Wright J**, **O'Driscoll G**, **Walsh JH**. Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *The Journal of Physiology* 562: 617–628, 2005. doi: 10.1113/jphysiol.2004.075929.

294. **Kooijman M**, **Thijssen DHJ**, **De Groot PCE**, **Bleeker MWP**, **Van Kuppevelt HJM**, **Green DJ**, **Rongen GA**, **Smits P**, **Hopman MTE**. Flow-mediated dilatation in the superficial femoral artery is nitric oxide mediated in humans. *The Journal of Physiology* 586: 1137–1145, 2008. doi: 10.1113/jphysiol.2007.145722.

295. **Tinken TM**, **Thijssen DHJ**, **Hopkins N**, **Black MA**, **Dawson EA**, **Minson CT**, **Newcomer SC**, **Laughlin MH**, **Cable NT**, **Green DJ**. Impact of Shear Rate Modulation on Vascular Function in Humans. *Hypertension* 54: 278–285, 2009. doi: 10.1161/HYPERTENSIONAHA.109.134361.

296. **Tinken TM**, **Thijssen DHJ**, **Hopkins N**, **Dawson EA**, **Cable NT**, **Green DJ**. Shear Stress Mediates Endothelial Adaptations to Exercise Training in Humans. *Hypertension* 55: 312–318, 2010. doi: 10.1161/HYPERTENSIONAHA.109.146282.

297. **Birk GK**, **Dawson EA**, **Atkinson C**, **Haynes A**, **Cable NT**, **Thijssen DHJ**, **Green DJ**. Brachial artery adaptation to lower limb exercise training: role of shear stress. *Journal of Applied Physiology* 112: 1653–1658, 2012. doi: 10.1152/japplphysiol.01489.2011.

298. **Spence AL**, **Carter HH**, **Naylor LH**, **Green DJ**. A prospective randomized longitudinal study involving 6 months of endurance or resistance exercise. Conduit artery adaptation in humans. *The Journal of Physiology* 591: 1265–1275, 2013. doi: 10.1113/jphysiol.2012.247387.

299. **Black MA**, **Green DJ**, **Cable NT**. Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *The Journal of Physiology* 586: 3511–3524, 2008. doi: 10.1113/jphysiol.2008.153742.

300. **Tinken TM**, **Thijssen DHJ**, **Black MA**, **Cable NT**, **Green DJ**. Time course of change in vasodilator function and capacity in response to exercise training in humans. *The Journal of Physiology* 586: 5003–5012, 2008. doi: 10.1113/jphysiol.2008.158014.

301. **Clarkson Peter**, **Montgomery Hugh E**, **Mullen Michael J**, **Donald Ann E**, **Powe Amanda J**, **Bull Teresa**, **Jubb Michael**, **World Michael**, **Deanfield John E**. Exercise training enhances endothelial function in young men. *Journal of the American College of Cardiology* 33: 1379–1385, 1999. doi: 10.1016/S0735-1097(99)00036-4.

302. **Seals DR**, **Nagy EE**, **Moreau KL**. Aerobic exercise training and vascular function with ageing in healthy men and women. *The Journal of Physiology* 597: 4901–4914, 2019. doi: 10.1113/JP277764.

303. **Akazawa N**, **Choi Y**, **Miyaki A**, **Tanabe Y**, **Sugawara J**, **Ajisaka R**, **Maeda S**. Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutrition research* 32: 795–799, 2012.

304. **O'Sullivan S**. The effects of exercise training on markers of endothelial function in young healthy men. *International journal of sports medicine* 24: 404–409, 2003.

305. **Beck DT**, **Casey DP**, **Martin JS**, **Emerson BD**, **Braith RW**. Exercise training improves endothelial function in young prehypertensives. *Exp Biol Med (Maywood)* 238: 433–441, 2013. doi: 10.1177/1535370213477600.

306. **Hambrecht R**, **Fiehn E**, **Weigl C**, **Gielen S**, **Hamann C**, **Kaiser R**, **Yu J**, **Adams V**, **Niebauer J**, **Schuler G**. Regular Physical Exercise Corrects Endothelial Dysfunction and Improves Exercise Capacity in Patients With Chronic Heart Failure. *Circulation* 98: 2709–2715, 1998. doi: 10.1161/01.CIR.98.24.2709.

307. **Hambrecht R**, **Wolf A**, **Gielen S**, **Linke A**, **Hofer J**, **Erbs S**, **Schoene N**, **Schuler G**. Effect of Exercise on Coronary Endothelial Function in Patients with Coronary Artery Disease. *N Engl J Med* 342: 454–460, 2000. doi: 10.1056/NEJM200002173420702.

308. **Edwards DG**, **Schofield RS**, **Lennon SL**, **Pierce GL**, **Nichols WW**, **Braith RW**. Effect of exercise training on endothelial function in men with coronary artery disease. *The American Journal of Cardiology* 93: 617–620, 2004. doi: 10.1016/j.amjcard.2003.11.032.

309. **Linke Axel**, **Schoene Nina**, **Gielen Stephan**, **Hofer Jürgen**, **Erbs Sandra**, **Schuler Gerhard**, **Hambrecht Rainer**. Endothelial dysfunction in patients with chronic heart failure: systemic effects of lower-limb exercise training. *Journal of the American College of Cardiology* 37: 392–397, 2001. doi: 10.1016/S0735-1097(00)01108-6.

310. **Green DJ**, **Walsh JH**, **Maiorana A**, **Best MJ**, **Taylor RR**, **O'Driscoll JG**. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *American Journal of Physiology-Heart and Circulatory Physiology* 285: H2679–H2687, 2003. doi: 10.1152/ajpheart.00519.2003.

311. **Currie KD**, **Thomas SG**, **Goodman JM**. Effects of short-term endurance exercise training on vascular function in young males. *European Journal of Applied Physiology* 107: 211–218, 2009. doi: 10.1007/s00421-009-1116-4.

312. **Hayashi K**, **Sugawara J**, **Komine H**, **Maeda S**, **Yokoi T**. Effects of Aerobic Exercise Training on the Stiffness of Central and Peripheral Arteries in Middle-Aged Sedentary Men. *The Japanese Journal of Physiology* 55: 235–239, 2005. doi: 10.2170/jjphysiol.S2116.

313. **Kakiyama T**, **Sugawara J**, **Murakami H**, **Maeda S**, **Kuno S**, **Matsuda M**. Effects of short-term endurance training on aortic distensibility in young males. *Medicine and science in sports and exercise* 37: 267–271, 2005.

314. **Moreau KL**, **Donato AJ**, **Seals DR**, **DeSouza CA**, **Tanaka H**. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovascular Research* 57: 861–868, 2003. doi: 10.1016/S0008-6363(02)00777-0.

315. **Jenkins NT**, **Martin JS**, **Laughlin MH**, **Padilla J**. Exercise-induced signals for vascular endothelial adaptations: implications for cardiovascular disease. *Current cardiovascular risk reports* 6: 331–346, 2012.

316. **Padilla J**, **Simmons GH**, **Bender SB**, **Arce-Esquivel AA**, **Whyte JJ**, **Laughlin MH**. Vascular effects of exercise: endothelial adaptations beyond active muscle beds. *Physiology* 26: 132–145, 2011.

317. **Montero D**, **Roche E**, **Martinez-Rodriguez A**. The impact of aerobic exercise training on arterial stiffness in pre- and hypertensive subjects: A systematic review and meta-analysis. *International Journal of Cardiology* 173: 361–368, 2014. doi: 10.1016/j.ijcard.2014.03.072.

318. **Beck DT**, **Martin JS**, **Casey DP**, **Braith RW**. Exercise Training Reduces Peripheral Arterial Stiffness and Myocardial Oxygen Demand in Young Prehypertensive Subjects. *American Journal of Hypertension* 26: 1093–1102, 2013. doi: 10.1093/ajh/hpt080.

319. **Aizawa K**, **Shoemaker JK**, **Overend TJ**, **Petrella RJ**. Effects of lifestyle modification on central artery stiffness in metabolic syndrome subjects with pre-hypertension and/or pre-diabetes. *Diabetes research and clinical practice* 83: 249–256, 2009.

320. **Figueroa A**, **Kalfon R**, **Madzima TA**, **Wong A**. Whole-body vibration exercise training reduces arterial stiffness in postmenopausal women with prehypertension and hypertension. *Menopause* 21: 131–136, 2014.

321. **Collier SR**, **Frechette V**, **Sandberg K**, **Schafer P**, **Ji H**, **Smulyan H**, **Fernhall B**. Sex differences in resting hemodynamics and arterial stiffness following 4 weeks of resistance versus aerobic exercise training in individuals with pre-hypertension to stage 1 hypertension. *Biology of sex differences* 2: 1–7, 2011.

322. **Aizawa K**, **Petrella RJ**. Acute and chronic impact of dynamic exercise on arterial stiffness in older hypertensives. *The open cardiovascular medicine journal* 2: 3, 2008.

323. **Ferrier KE**, **Waddell TK**, **Gatzka CD**, **Cameron JD**, **Dart AM**, **Kingwell BA**. Aerobic exercise training does not modify large-artery compliance in isolated systolic hypertension. *Hypertension* 38: 222–226, 2001.

324. **Madden KM**, **Lockhart C**, **Cuff D**, **Potter TF**, **Meneilly GS**. Aerobic training-induced improvements in arterial stiffness are not sustained in older adults with multiple cardiovascular risk factors. *J Hum Hypertens* 27: 335–339, 2013. doi: 10.1038/jhh.2012.38.

325. **Mitchell GF**, **Parise H**, **Benjamin EJ**, **Larson MG**, **Keyes MJ**, **Vita JA**, **Vasan RS**, **Levy D**. Changes in Arterial Stiffness and Wave Reflection With Advancing Age in Healthy Men and Women. *Hypertension* 43: 1239–1245, 2004. doi: 10.1161/01.HYP.0000128420.01881.aa.

326. **Vaitkevicius PV**, **Fleg JL**, **Engel JH**, **O'Connor FC**, **Wright JG**, **Lakatta LE**, **Yin FC**, **Lakatta EG**. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 88: 1456–1462, 1993. doi: 10.1161/01.CIR.88.4.1456.

327. **Tanaka H**, **Dinenno FA**, **Monahan KD**, **Clevenger CM**, **DeSouza CA**, **Seals DR**. Aging, Habitual Exercise, and Dynamic Arterial Compliance. *Circulation* 102: 1270–1275, 2000. doi: 10.1161/01.CIR.102.11.1270.

328. **Kingwell BA**. Large Artery Stiffness: Implications For Exercise Capacity And Cardiovascular Risk. *Clinical and Experimental Pharmacology and Physiology* 29: 214–217, 2002. doi: 10.1046/j.1440-1681.2002.03622.x.

329. **Seals DR**, **DeSouza CA**, **Donato AJ**, **Tanaka H**. Habitual exercise and arterial aging. *Journal of Applied Physiology* 105: 1323–1332, 2008. doi: 10.1152/japplphysiol.90553.2008.

330. **Shibata S**, **Levine BD**. Biological aortic age derived from the arterial pressure waveform. *Journal of Applied Physiology* 110: 981–987, 2011. doi: 10.1152/japplphysiol.01261.2010.

331. **Gates PE**, **Tanaka H**, **Graves J**, **Seals DR**. Left ventricular structure and diastolic function with human ageing: Relation to habitual exercise and arterial stiffness. *European Heart Journal* 24: 2213–2220, 2003. doi: 10.1016/j.ehj.2003.09.026.

332. **Cameron JD**, **Dart AM**. Exercise training increases total systemic arterial compliance in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 266: H693–H701, 1994. doi: 10.1152/ajpheart.1994.266.2.H693.

333. **Tabara Y**, **Yuasa T**, **Oshiumi A**, **Kobayashi T**, **Miyawaki Y**, **Miki T**, **Kohara K**. Effect of Acute and Long-Term Aerobic Exercise on Arterial Stiffness in the Elderly. *Hypertens Res* 30: 895–902, 2007. doi: 10.1291/hypres.30.895.

334. **Shibata S**, **Levine BD**. Effect of exercise training on biologic vascular age in healthy seniors. *American Journal of Physiology-Heart and Circulatory Physiology* 302: H1340–H1346, 2012. doi: 10.1152/ajpheart.00511.2011.

335. **TIPTON CM**. 13 Exercise, Training and Hypertension: An Update. *Exercise and sport sciences reviews* 19: 447–506, 1991.

336. **Meredith I**, **Friberg P**, **Jennings G**, **Dewar E**, **Fazio V**, **Lambert G**, **Esler M**. Exercise training lowers resting renal but not cardiac sympathetic activity in humans. *Hypertension* 18: 575–582, 1991.

337. **Iwasaki K**, **Zhang R**, **Zuckerman JH**, **Levine BD**. Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? *Journal of Applied Physiology* 95: 1575–1583, 2003.

338. **Roveda F**, **Middlekauff HR**, **Rondon MUP**, **Reis SF**, **Souza M**, **Nastari L**, **Barretto ACP**, **Krieger EM**, **Negrão CE**. The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *Journal of the American College of Cardiology* 42: 854–860, 2003.

339. **Pescatello LS**, **Franklin BA**, **Fagard R**, **Farquhar WB**, **Kelley GA**, **Ray CA**. Exercise and hypertension. *Medicine & Science in Sports & Exercise* 36: 533–553, 2004.

340. **Ray CA**, **Hume KM**. Sympathetic neural adaptations to exercise training in humans: insights from microneurography. *Medicine and science in sports and exercise* 30: 387–391, 1998.

341. **Carter JR**, **Ray CA**. Sympathetic neural adaptations to exercise training in humans. *Autonomic Neuroscience* 188: 36–43, 2015.

342. **Laterza MC**, **de Matos LD**, **Trombetta IC**, **Braga AM**, **Roveda F**, **Alves MJ**, **Krieger EM**, **Negrao CE**, **Rondon MU**. Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension* 49: 1298–1306, 2007.

343. **Straznicky NE**, **Lambert EA**, **Nestel PJ**, **McGrane MT**, **Dawood T**, **Schlaich MP**, **Masuo K**, **Eikelis N**, **De Courten B**, **Mariani JA**. Sympathetic neural adaptation to hypocaloric diet with or without exercise training in obese metabolic syndrome subjects. *Diabetes* 59: 71–79, 2010.

344. **Martinez DG**, **Nicolau JC**, **Lage RL**, **Toschi-Dias E**, **de Matos LDNJ**, **Alves MJNN**, **Trombetta IC**, **Dias da Silva VJ**, **Middlekauff HR**, **Negrão CE**, **Rondon MUPB**. Effects of Long-Term Exercise Training on Autonomic Control in Myocardial Infarction

Patients. *Hypertension* 58: 1049–1056, 2011. doi: 10.1161/HYPERTENSIONAHA.111.176644.

345. **Malik M**, **Hnatkova K**, **Huikuri HV**, **Lombardi F**, **Schmidt G**, **Zabel M**. CrossTalk proposal: Heart rate variability is a valid measure of cardiac autonomic responsiveness. *J Physiol* 597: 2595–2598, 2019. doi: 10.1113/JP277500.

346. **Billman GE**. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physio* 4, 2013. doi: 10.3389/fphys.2013.00026.

347. **Houle MS**, **Billman GE**. Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity. *American Journal of Physiology-Heart and Circulatory Physiology* 276: H215–H223, 1999. doi: 10.1152/ajpheart.1999.276.1.H215.

348. **Collier SR**, **Kanaley JA**, **Carhart Jr R**, **Frechette V**, **Tobin MM**, **Bennett N**, **Luckenbaugh AN**, **Fernhall B**. Cardiac autonomic function and baroreflex changes following 4 weeks of resistance versus aerobic training in individuals with pre-hypertension. *Acta Physiologica* 195: 339–348, 2009. doi: 10.1111/j.1748-1716.2008.01897.x.

349. **Pescatello LS**, **Buchner DM**, **Jakicic JM**, **Powell KE**, **Kraus WE**, **Bloodgood B**, **Campbell WW**, **Dietz S**, **Dipietro L**, **George SM**, **Macko RF**, **Mctiernan A**, **Pate RR**, **Piercy KL**. Physical Activity to Prevent and Treat Hypertension: A Systematic Review. *Medicine & Science in Sports & Exercise* 51: 1314–1323, 2019. doi: 10.1249/MSS.0000000000001943.

350. **Cornelissen VA**, **Fagard RH**, **Coeckelberghs E**, **Vanhees L**. Impact of Resistance Training on Blood Pressure and Other Cardiovascular Risk Factors. *Hypertension* 58: 950–958, 2011. doi: 10.1161/HYPERTENSIONAHA.111.177071.

351. **Murtagh EM**, **Nichols L**, **Mohammed MA**, **Holder R**, **Nevill AM**, **Murphy MH**. The effect of walking on risk factors for cardiovascular disease: An updated systematic review and meta-analysis of randomised control trials. *Preventive Medicine* 72: 34–43, 2015. doi: 10.1016/j.ypmed.2014.12.041.

352. **MacDonald HV**, **Johnson BT**, **Huedo-Medina TB**, **Livingston J**, **Forsyth KC**, **Kraemer WJ**, **Farinatti PTV**, **Pescatello LS**. Dynamic Resistance Training as Stand-Alone Antihypertensive Lifestyle Therapy: A Meta-Analysis. *Journal of the American Heart Association* 5: e003231, [date unknown]. doi: 10.1161/JAHA.116.003231.

353. **Dickinson HO**, **Mason JM**, **Nicolson DJ**, **Campbell F**, **Beyer FR**, **Cook JV**, **Williams B**, **Ford GA**. Lifestyle interventions to reduce raised blood pressure: a systematic review of randomized controlled trials [Online]. *Journal of Hypertension* 24, 2006. https://journals.lww.com/jhypertension/Fulltext/2006/02000/Lifestyle_interventions_to_reduce_raised_blood.2.aspx.

354. **FAGARD RH**. Exercise characteristics and the blood pressure response to dynamic physical training [Online]. *Medicine & Science in Sports & Exercise* 33, 2001. https://journals.lww.com/acsm-msse/Fulltext/2001/06001/Exercise_characteristics_and_the_blood_pressure.18.aspx.

355. **Blumenthal JA**, **Hinderliter AL**, **Smith PJ**, **Mabe S**, **Watkins LL**, **Craighead L**, **Ingle K**, **Tyson C**, **Lin P-H**, **Kraus WE**, **Liao L**, **Sherwood A**. Effects of Lifestyle Modification on Patients With Resistant Hypertension: Results of the TRIUMPH Randomized Clinical Trial. *Circulation* 144: 1212–1226, 2021. doi: 10.1161/CIRCULATIONAHA.121.055329.

356. **Dimeo F**, **Pagonas N**, **Seibert F**, **Arndt R**, **Zidek W**, **Westhoff TH**. Aerobic exercise reduces blood pressure in resistant hypertension. *Hypertension* 60: 653–658, 2012.

357. **Calhoun DA**, **Jones D**, **Textor S**, **Goff DC**, **Murphy TP**, **Toto RD**, **White A**, **Cushman WC**, **White W**, **Sica D**, **Ferdinand K**, **Giles TD**, **Falkner B**, **Carey RM**. Resistant Hypertension: Diagnosis, Evaluation, and Treatment. *Hypertension* 51: 1403–1419, 2008. doi: 10.1161/HYPERTENSIONAHA.108.189141.

358. **Nocon M**, **Hiemann T**, **Müller-Riemenschneider F**, **Thalau F**, **Roll S**, **Willich SN**. Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. *European journal of cardiovascular prevention and rehabilitation* 15: 239–246, 2008. doi: 10.1097/HJR.0b013e3282f55e09.

359. **Sofi F**, **Capalbo A**, **Cesari F**, **Abbate R**, **Gensini GF**. Physical activity during leisure time and primary prevention of coronary heart disease: an updated meta-analysis of cohort studies. *European journal of cardiovascular prevention and rehabilitation* 15: 247–257, 2008. doi: 10.1097/HJR.0b013e3282f232ac.

360. **Ekblom-Bak E**, **Ekblom B**, **Söderling J**, **Börjesson M**, **Blom V**, **Kallings LV**, **Hemmingsson E**, **Andersson G**, **Wallin P**, **Ekblom Ö**. Sex- and age-specific associations between cardiorespiratory fitness, CVD morbidity and all-cause mortality in 266.109 adults. *Preventive Medicine* 127: 105799, 2019. doi: 10.1016/j.ypmed.2019.105799.

361. **Lee D**, **Sui X**, **Artero EG**, **Lee I-M**, **Church TS**, **McAuley PA**, **Stanford FC**, **Kohl HW**, **Blair SN**. Long-Term Effects of Changes in Cardiorespiratory Fitness and Body Mass Index on All-Cause and Cardiovascular Disease Mortality in Men. *Circulation* 124: 2483–2490, 2011. doi: 10.1161/CIRCULATIONAHA.111.038422.

362. **Juraschek SP**, **Blaha MJ**, **Whelton SP**, **Blumenthal R**, **Jones SR**, **Keteyian SJ**, **Schairer J**, **Brawner CA**, **Al-Mallah MH**. Physical Fitness and Hypertension in a Population at Risk for Cardiovascular Disease: The Henry Ford ExercIse Testing (FIT) Project. *Journal of the American Heart Association* 3: e001268, [date unknown]. doi: 10.1161/JAHA.114.001268.

363. **Farrell SW**, **Finley CE**, **Haskell WL**, **Grundy SM**. Is There a Gradient of Mortality Risk among Men with Low Cardiorespiratory Fitness? *Medicine & Science in Sports & Exercise* 47: 1825–1832, 2015. doi: 10.1249/MSS.0000000000000608.

364. **Holmlund T**, **Ekblom B**, **Börjesson M**, **Andersson G**, **Wallin P**, **Ekblom-Bak E**. Association between change in cardiorespiratory fitness and incident hypertension in Swedish adults. .

365. **Barlow CE**, **LaMonte MJ**, **FitzGerald SJ**, **Kampert JB**, **Perrin JL**, **Blair SN**. Cardiorespiratory Fitness Is an Independent Predictor of Hypertension Incidence among Initially Normotensive Healthy Women. *American Journal of Epidemiology* 163: 142–150, 2006. doi: 10.1093/aje/kwj019.

366. **Lazarevic G**, **Antic S**, **Vlahovic P**, **Djordjevic V**, **Zvezdanovic L**, **Stefanovic V**. Effects of aerobic exercise on microalbuminuria and enzymuria in type 2 diabetic patients. *Renal failure* 29: 199–205, 2007.

367. **Calle-Pascual A**, **Martin-Alvarez P**, **Reyes C**, **Calle J**. Regular physical activity and reduced occurrence of microalbuminuria in type 2 diabetic patients. *Diabète & métabolisme* 19: 304–309, 1993.

368. **Finkelstein J**, **Joshi A**, **Hise MK**. Association of physical activity and renal function in subjects with and without metabolic syndrome: a review of the Third National Health and Nutrition Examination Survey (NHANES III). *American journal of kidney diseases* 48: 372–382, 2006.

369. **Metcalf PA**, **Baker J**, **Scragg R**, **Dryson E**, **Scott A**, **Wild C**. Albuminuria in people at least 40 years old: effect of alcohol consumption, regular exercise, and cigarette smoking. *Clinical chemistry* 39: 1793–1797, 1993.

370. **Robinson ES**, **Fisher ND**, **Forman JP**, **Curhan GC**. Physical Activity and Albuminuria. *American Journal of Epidemiology* 171: 515–521, 2010. doi: 10.1093/aje/kwp442.

371. **Brigadski T**, **Leßmann V**. The physiology of regulated BDNF release. *Cell and Tissue Research* 382: 15–45, 2020. doi: 10.1007/s00441-020-03253-2.

372. **Walsh JJ**, **Tschakovsky ME**. Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. *Appl Physiol Nutr Metab* 43: 1095–1104, 2018. doi: 10.1139/apnm-2018-0192.

373. **Prigent-Tessier A**, **Quirié A**, **Maguin-Gaté K**, **Szostak J**, **Mossiat C**, **Nappey M**, **Devaux S**, **Marie C**, **Demougeot C**. Physical training and hypertension have opposite effects on endothelial brain-derived neurotrophic factor expression. *Cardiovascular Research* 100: 374–382, 2013. doi: 10.1093/cvr/cvt219.

374. **Fujimura HA C Anthony; Chen, Ruoyan; Nakamura, Takashi; Nakahashi, Takeshi; Kambayashi, Jun-ichi; Sun, Bing; Tandon, Narendra N**. Brain-derived Neurotrophic

Factor Is Stored in Human Platelets and Released by Agonist Stimulation. *Thromb Haemost* 87: 728–734, 2017. doi: 10.1055/s-0037-1613072.

375. **Cowansage K**, **LeDoux J**, **Monfils M-H**. Brain-Derived Neurotrophic Factor: A Dynamic Gatekeeper of Neural Plasticity. *Current molecular pharmacology* 3: 12–29, 2010. doi: 10.2174/1874467211003010012.

376. **Gottmann K**, **Mittmann T**, **Lessmann V**. BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Experimental Brain Research* 199: 203–234, 2009. doi: 10.1007/s00221-009-1994-z.

377. **LIPSKY RH**, **MARINI AM**. Brain-Derived Neurotrophic Factor in Neuronal Survival and Behavior-Related Plasticity. *Annals of the New York Academy of Sciences* 1122: 130–143, 2007. doi: 10.1196/annals.1403.009.

378. **Rasmussen P**, **Brassard P**, **Adser H**, **Pedersen MV**, **Leick L**, **Hart E**, **Secher NH**, **Pedersen BK**, **Pilegaard H**. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology* 94: 1062–1069, 2009. doi: 10.1113/expphysiol.2009.048512.

379. **Binder DK**, **Scharfman HE**. Mini Review. *Growth Factors* 22: 123–131, 2004. doi: 10.1080/08977190410001723308.

380. **Neeper SA**, **Góauctemez-Pinilla F**, **Choi J**, **Cotman C**. Exercise and brain neurotrophins. *Nature* 373: 109–109, 1995. doi: 10.1038/373109a0.

381. **Aguiar AS**, **Speck AE**, **Prediger RDS**, **Kapczinski F**, **Pinho RA**. Downhill training upregulates mice hippocampal and striatal brain-derived neurotrophic factor levels. *Journal of Neural Transmission* 115: 1251–1255, 2008. doi: 10.1007/s00702-008-0071-2.

382. **Aguiar AS**, **Castro AA**, **Moreira EL**, **Glaser V**, **Santos ARS**, **Tasca CI**, **Latini A**, **Prediger RDS**. Short bouts of mild-intensity physical exercise improve spatial learning and memory in aging rats: Involvement of hippocampal plasticity via AKT, CREB and BDNF signaling. *Mechanisms of Ageing and Development* 132: 560–567, 2011. doi: 10.1016/j.mad.2011.09.005.

383. **Vaynman S**, **Ying Z**, **Gomez-Pinilla F**. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience* 20: 2580–2590, 2004. doi: 10.1111/j.1460-9568.2004.03720.x.

384. **Uysal N**, **Kiray M**, **Sisman A**, **Camsari U**, **Gencoglu C**, **Baykara B**, **Cetinkaya C**, **Aksu I**. Effects of voluntary and involuntary exercise on cognitive functions, and VEGF and BDNF levels in adolescent rats. *Biotechnic & Histochemistry* 90: 55–68, 2015. doi: 10.3109/10520295.2014.946968.

385. **FERRIS LT**, **WILLIAMS JS**, **SHEN C-L**. The Effect of Acute Exercise on Serum Brain-Derived Neurotrophic Factor Levels and Cognitive Function [Online]. *Medicine &*

Science in Sports & Exercise 39, 2007. https://journals.lww.com/acsm-msse/Fulltext/2007/04000/The_Effect_of_Acute_Exercise_on_Serum.20.aspx.

386. **Gold SM**, **Schulz K-H**, **Hartmann S**, **Mladek M**, **Lang UE**, **Hellweg R**, **Reer R**, **Braumann K-M**, **Heesen C**. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *Journal of Neuroimmunology* 138: 99–105, 2003. doi: 10.1016/S0165-5728(03)00121-8.

387. **Schmolesky MT**, **Webb DL**, **Hansen RA**. The effects of aerobic exercise intensity and duration on levels of brain-derived neurotrophic factor in healthy men. *Journal of sports science & medicine* 12: 502–511, 2013.

388. **Berchtold NC**, **Chinn G**, **Chou M**, **Kesslak JP**, **Cotman CW**. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience* 133: 853–861, 2005. doi: 10.1016/j.neuroscience.2005.03.026.

389. **Cotman CW**, **Berchtold NC**. Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences* 25: 295–301, 2002. doi: 10.1016/S0166-2236(02)02143-4.

390. **Erickson KI**, **Miller DL**, **Roecklein KA**. The Aging Hippocampus: Interactions between Exercise, Depression, and BDNF. *The Neuroscientist (Baltimore, Md)* 18: 82–97, 2012. doi: 10.1177/1073858410397054.

391. **Small SA**, **Tsai WY**, **DeLaPaz R**, **Mayeux R**, **Stern Y**. Imaging hippocampal function across the human life span: Is memory decline normal or not? *Annals of Neurology* 51: 290–295, 2002. doi: 10.1002/ana.10105.

392. **L. A. van de Pol**, **A. Hensel**, **F. Barkhof**, **H. J. Gertz**, **P. Scheltens**, **W. M. van der Flier**. Hippocampal atrophy in Alzheimer disease: Age matters. *Neurology* 66: 236, 2006. doi: 10.1212/01.wnl.0000194240.47892.4d.

393. **Raz N**, **Lindenberger U**, **Rodrigue KM**, **Kennedy KM**, **Head D**, **Williamson A**, **Dahle C**, **Gerstorf D**, **Acker JD**. Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers. *Cerebral Cortex* 15: 1676–1689, 2005. doi: 10.1093/cercor/bhi044.

394. **Pajonk F-G**, **Wobrock T**, **Gruber O**, **Scherk H**, **Berner D**, **Kaizl I**, **Kierer A**, **Müller S**, **Oest M**, **Meyer T**, **Backens M**, **Schneider-Axmann T**, **Thornton AE**, **Honer WG**, **Falkai P**. Hippocampal Plasticity in Response to Exercise in Schizophrenia. *Archives of General Psychiatry* 67: 133–143, 2010. doi: 10.1001/archgenpsychiatry.2009.193.

395. **Erickson KI**, **Voss MW**, **Prakash RS**, **Basak C**, **Szabo A**, **Chaddock L**, **Kim JS**, **Heo S**, **Alves H**, **White SM**, **Wojcicki TR**, **Mailey E**, **Vieira VJ**, **Martin SA**, **Pence BD**, **Woods JA**, **McAuley E**, **Kramer AF**. Exercise training increases size of hippocampus and

improves memory. *Proceedings of the National Academy of Sciences* 108: 3017–3022, 2011. doi: 10.1073/pnas.1015950108.

396. **Lin J**, **Chan SK**, **Lee EH**, **Chang WC**, **Tse M**, **Su WW**, **Sham P**, **Hui CL**, **Joe G**, **Chan CL**, **Khong PL**, **So KF**, **Honer WG**, **Chen EY**. Aerobic exercise and yoga improve neurocognitive function in women with early psychosis. *npj Schizophrenia* 1: 15047, 2015. doi: 10.1038/npjschz.2015.47.

397. **Rosano C**, **Guralnik J**, **Pahor M**, **Glynn NW**, **Newman AB**, **Ibrahim TS**, **Erickson K**, **Cohen R**, **Shaaban CE**, **MacCloud RL**, **Aizenstein HJ**. Hippocampal Response to a 24-Month Physical Activity Intervention in Sedentary Older Adults. *The American Journal of Geriatric Psychiatry* 25: 209–217, 2017. doi: 10.1016/j.jagp.2016.11.007.

398. **Jonasson LS**, **Nyberg L**, **Kramer AF**, **Lundquist A**, **Riklund K**, **Boraxbekk C-J**. Aerobic Exercise Intervention, Cognitive Performance, and Brain Structure: Results from the Physical Influences on Brain in Aging (PHIBRA) Study [Online]. *Frontiers in Aging Neuroscience* 8, 2017. https://www.frontiersin.org/articles/10.3389/fnagi.2016.00336.

399. **Thomas AG**, **Dennis A**, **Rawlings NB**, **Stagg CJ**, **Matthews L**, **Morris M**, **Kolind SH**, **Foxley S**, **Jenkinson M**, **Nichols TE**, **Dawes H**, **Bandettini PA**, **Johansen-Berg H**. Multi-modal characterization of rapid anterior hippocampal volume increase associated with aerobic exercise. *NeuroImage* 131: 162–170, 2016. doi: 10.1016/j.neuroimage.2015.10.090.

400. **Henriette van Praag**, **Tiffany Shubert**, **Chunmei Zhao**, **Fred H. Gage**. Exercise Enhances Learning and Hippocampal Neurogenesis in Aged Mice. *J Neurosci* 25: 8680, 2005. doi: 10.1523/JNEUROSCI.1731-05.2005.

401. **van Praag H**, **Kempermann G**, **Gage FH**. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience* 2: 266–270, 1999. doi: 10.1038/6368.

402. **Pereira AC**, **Huddleston DE**, **Brickman AM**, **Sosunov AA**, **Hen R**, **McKhann GM**, **Sloan R**, **Gage FH**, **Brown TR**, **Small SA**. An *in vivo* correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA* 104: 5638–5643, 2007. doi: 10.1073/pnas.0611721104.

403. **Maass A**, **Düzel S**, **Goerke M**, **Becke A**, **Sobieray U**, **Neumann K**, **Lövden M**, **Lindenberger U**, **Bäckman L**, **Braun-Dullaeus R**, **Ahrens D**, **Heinze H-J**, **Müller NG**, **Düzel E**. Vascular hippocampal plasticity after aerobic exercise in older adults. *Molecular Psychiatry* 20: 585–593, 2015. doi: 10.1038/mp.2014.114.

404. **Lisanne F ten Brinke**, **Niousha Bolandzadeh**, **Lindsay S Nagamatsu**, **Chun Liang Hsu**, **Jennifer C Davis**, **Karim Miran-Khan**, **Teresa Liu-Ambrose**. Aerobic exercise increases hippocampal volume in older women with probable mild cognitive impairment: a 6-month randomised controlled trial. *Br J Sports Med* 49: 248, 2015. doi: 10.1136/bjsports-2013-093184.

405. **Morris JK**, **Vidoni ED**, **Johnson DK**, **Van Sciver A**, **Mahnken JD**, **Honea RA**, **Wilkins HM**, **Brooks WM**, **Billinger SA**, **Swerdlow RH**, **Burns JM**. Aerobic exercise for Alzheimer's disease: A randomized controlled pilot trial. *PLOS ONE* 12: e0170547, 2017. doi: 10.1371/journal.pone.0170547.

406. **Malchow B**, **Keeser D**, **Keller K**, **Hasan A**, **Rauchmann B-S**, **Kimura H**, **Schneider-Axmann T**, **Dechent P**, **Gruber O**, **Ertl-Wagner B**, **Honer WG**, **Hillmer-Vogel U**, **Schmitt A**, **Wobrock T**, **Niklas A**, **Falkai P**. Effects of endurance training on brain structures in chronic schizophrenia patients and healthy controls. *Schizophrenia Research* 173: 182–191, 2016. doi: 10.1016/j.schres.2015.01.005.

407. **Morris Z**, **Whiteley WN**, **Longstreth WT**, **Weber F**, **Lee Y-C**, **Tsushima Y**, **Alphs H**, **Ladd SC**, **Warlow C**, **Wardlaw JM**, **Al-Shahi Salman R**. Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ* 339: b3016, 2009. doi: 10.1136/bmj.b3016.

408. **Raoul P. Kloppenborg**, **Paul J. Nederkoorn**, **Mirjam I. Geerlings**, **Esther van den Berg**. Presence and progression of white matter hyperintensities and cognition. *Neurology* 82: 2127, 2014. doi: 10.1212/WNL.0000000000000505.

409. **Brickman AM**, **Zahodne LB**, **Guzman VA**, **Narkhede A**, **Meier IB**, **Griffith EY**, **Provenzano FA**, **Schupf N**, **Manly JJ**, **Stern Y**, **Luchsinger JA**, **Mayeux R**. Reconsidering harbingers of dementia: progression of parietal lobe white matter hyperintensities predicts Alzheimer's disease incidence. *Neurobiology of Aging* 36: 27–32, 2015. doi: 10.1016/j.neurobiolaging.2014.07.019.

410. **Roderick A. Corriveau**, **Walter J. Koroshetz**, **Jordan T. Gladman**, **Sophia Jeon**, **Debra Babcock**, **David A. Bennett**, **S. Thomas Carmichael**, **Susan L.-J. Dickinson**, **Dennis W. Dickson**, **Marian Emr**, **Howard Fillit**, **Steven M. Greenberg**, **Michael L. Hutton**, **David S. Knopman**, **Jennifer J. Manly**, **Karen S. Marder**, **Claudia S. Moy**, **Creighton H. Phelps**, **Paul A. Scott**, **William W. Seeley**, **Beth-Anne Sieber**, **Nina B. Silverberg**, **Margaret L. Sutherland**, **Angela Taylor**, **Christine L. Torborg**, **Salina P. Waddy**, **Amelie K. Gubitz**, **David M. Holtzman**. Alzheimer's Disease–Related Dementias Summit 2016: National research priorities. *Neurology* 89: 2381, 2017. doi: 10.1212/WNL.0000000000004717.

411. **Kapasi A**, **DeCarli C**, **Schneider JA**. Impact of multiple pathologies on the threshold for clinically overt dementia. *Acta Neuropathologica* 134: 171–186, 2017. doi: 10.1007/s00401-017-1717-7.

412. **Jack CR**, **Bennett DA**, **Blennow K**, **Carrillo MC**, **Dunn B**, **Haeberlein SB**, **Holtzman DM**, **Jagust W**, **Jessen F**, **Karlawish J**, **Liu E**, **Molinuevo JL**, **Montine T**, **Phelps C**, **Rankin KP**, **Rowe CC**, **Scheltens P**, **Siemers E**, **Snyder HM**, **Sperling R**, **Elliott C**, **Masliah E**, **Ryan L**, **Silverberg N**. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia* 14: 535–562, 2018. doi: 10.1016/j.jalz.2018.02.018.

413. **Tseng BY**, **Gundapuneedi T**, **Khan MA**, **Diaz-Arrastia R**, **Levine BD**, **Lu H**, **Huang H**, **Zhang R**. White matter integrity in physically fit older adults. *NeuroImage* 82: 510–516, 2013. doi: 10.1016/j.neuroimage.2013.06.011.

414. **Alan J. Gow**, **Mark E. Bastin**, **Susana Muñoz Maniega**, **Maria C. Valdés Hernández**, **Zoe Morris**, **Catherine Murray**, **Natalie A. Royle**, **John M. Starr**, **Ian J. Deary**, **Joanna M. Wardlaw**. Neuroprotective lifestyles and the aging brain. *Neurology* 79: 1802, 2012. doi: 10.1212/WNL.0b013e3182703fd2.

415. **Ho AJ**, **Raji CA**, **Becker JT**, **Lopez OL**, **Kuller LH**, **Hua X**, **Dinov ID**, **Stein JL**, **Rosano C**, **Toga AW**, **Thompson PM**. The effects of physical activity, education, and body mass index on the aging brain. *Human Brain Mapping* 32: 1371–1382, 2011. doi: 10.1002/hbm.21113.

416. **Sexton CE**, **Betts JF**, **Demnitz N**, **Dawes H**, **Ebmeier KP**, **Johansen-Berg H**. A systematic review of MRI studies examining the relationship between physical fitness and activity and the white matter of the ageing brain. *NeuroImage* 131: 81–90, 2016. doi: 10.1016/j.neuroimage.2015.09.071.

417. **Radak Z**, **Hart N**, **Sarga L**, **Koltai E**, **Atalay M**, **Ohno H**, **Boldogh I**. Exercise Plays a Preventive Role Against Alzheimer's Disease. *JAD* 20: 777–783, 2010. doi: 10.3233/JAD-2010-091531.

418. **Larson EB**, **Wang L**, **Bowen JD**, **McCormick WC**, **Teri L**, **Crane P**, **Kukull W**. Exercise Is Associated with Reduced Risk for Incident Dementia among Persons 65 Years of Age and Older. *Ann Intern Med* 144: 73–81, 2006. doi: 10.7326/0003-4819-144-2-200601170-00004.

419. **van Gelder BM**, **Tijhuis MAR**, **Kalmijn S**, **Giampaoli S**, **Nissinen A**, **Kromhout D**. Physical activity in relation to cognitive decline in elderly men. *Neurology* 63: 2316, 2004. doi: 10.1212/01.WNL.0000147474.29994.35.

420. **Ainslie PN**, **Cotter JD**, **George KP**, **Lucas S**, **Murrell C**, **Shave R**, **Thomas KN**, **Williams MJA**, **Atkinson G**. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *The Journal of Physiology* 586: 4005–4010, 2008. doi: 10.1113/jphysiol.2008.158279.

421. **Thomas BP**, **Yezhuvath US**, **Tseng BY**, **Liu P**, **Levine BD**, **Zhang R**, **Lu H**. Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO2. *Journal of Magnetic Resonance Imaging* 38: 1177–1183, 2013. doi: 10.1002/jmri.24090.

422. **Tarumi T**, **Gonzales MM**, **Fallow B**, **Nualnim N**, **Pyron M**, **Tanaka H**, **Haley AP**. Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults [Online]. *Journal of Hypertension* 31, 2013. https://journals.lww.com/jhypertension/Fulltext/2013/12000/Central_artery_stiffness,_neuropsychological.13.aspx.

423. **Murrell CJ**, **Cotter JD**, **Thomas KN**, **Lucas SJE**, **Williams MJA**, **Ainslie PN**. Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: Effect of age and 12-week exercise training. *Age (Dordr)* 35: 905–920, 2013. doi: 10.1007/s11357-012-9414-x.

424. **Kleinloog JPD**, **Mensink RP**, **Ivanov D**, **Adam JJ**, **Uludağ K**, **Joris PJ**. Aerobic Exercise Training Improves Cerebral Blood Flow and Executive Function: A Randomized, Controlled Cross-Over Trial in Sedentary Older Men [Online]. *Frontiers in Aging Neuroscience* 11, 2019. https://www.frontiersin.org/articles/10.3389/fnagi.2019.00333.

425. **Akazawa N**, **Choi Y**, **Miyaki A**, **Sugawara J**, **Ajisaka R**, **Maeda S**. Aerobic exercise training increases cerebral blood flow in postmenopausal women. *Artery Research* 6: 124–129, 2012. doi: 10.1016/j.artres.2012.05.003.

426. **Viboolvorakul S**, **Patumraj S**. Exercise Training Could Improve Age-Related Changes in Cerebral Blood Flow and Capillary Vascularity through the Upregulation of VEGF and eNOS. *BioMed Research International* 2014: 230791, 2014. doi: 10.1155/2014/230791.

427. **Intlekofer KA**, **Cotman CW**. Exercise counteracts declining hippocampal function in aging and Alzheimer's disease. *Neurobiology of Disease* 57: 47–55, 2013. doi: 10.1016/j.nbd.2012.06.011.

428. **Paillard T**, **Rolland Y**, **de Souto Barreto P**. Protective Effects of Physical Exercise in Alzheimer's Disease and Parkinson's Disease: A Narrative Review. *J Clin Neurol* 11: 212, 2015. doi: 10.3988/jcn.2015.11.3.212.

429. **Lange-Asschenfeldt C**, **Kojda G**. Alzheimer's disease, cerebrovascular dysfunction and the benefits of exercise: From vessels to neurons. *Experimental Gerontology* 43: 499–504, 2008. doi: 10.1016/j.exger.2008.04.002.

430. **Guimarães FC**, **Amorim PR dos S**, **dos Reis FF**, **Bonoto RT**, **de Oliveira WC**, **Moura TA da S**, **de Assis CL**, **Palotás A**, **Lima LM**. Physical Activity and Better Medication Compliance Improve Mini-Mental State Examination Scores in the Elderly. *Dementia and Geriatric Cognitive Disorders* 39: 25–31, 2014. doi: 10.1159/000366413.

431. **Nemati Karimooy H**, **Hosseini M**, **Nemati M**, **Esmaily HO**. Lifelong physical activity affects mini mental state exam scores in individuals over 55 years of age. *Journal of Bodywork and Movement Therapies* 16: 230–235, 2012. doi: 10.1016/j.jbmt.2011.08.003.

432. **Ries JD**, **Hutson J**, **Maralit LA**, **Brown MB**. Group Balance Training Specifically Designed for Individuals With Alzheimer Disease: Impact on Berg Balance Scale, Timed Up and Go, Gait Speed, and Mini-Mental Status Examination [Online]. *Journal of Geriatric Physical Therapy* 38, 2015. https://journals.lww.com/jgpt/Fulltext/2015/10000/Group_Balance_Training_Specifically_Designed_for.4.aspx.

433. **Molloy DW**, **Beerschoten DA**, **Borrie MJ**, **Crilly RG**, **Cape RDT**. Acute Effects of Exercise on Neuropsychological Function in Elderly Subjects. *Journal of the American Geriatrics Society* 36: 29–33, 1988. doi: 10.1111/j.1532-5415.1988.tb03430.x.

434. **Brunt VE**, **Minson CT**. Heat therapy: mechanistic underpinnings and applications to cardiovascular health. *Journal of Applied Physiology* 130: 1684–1704, 2021. doi: 10.1152/japplphysiol.00141.2020.

435. **Cheng JL**, **MacDonald MJ**. Effect of heat stress on vascular outcomes in humans. *Journal of Applied Physiology* 126: 771–781, 2019. doi: 10.1152/japplphysiol.00682.2018.

436. **Pizzey FK**, **Smith EC**, **Ruediger SL**, **Keating SE**, **Askew CD**, **Coombes JS**, **Bailey TG**. The effect of heat therapy on blood pressure and peripheral vascular function: A systematic review and meta-analysis. *Experimental Physiology* 106: 1317–1334, 2021. doi: 10.1113/EP089424.

437. **Cullen T**, **Clarke ND**, **Hill M**, **Menzies C**, **Pugh CJA**, **Steward CJ**, **Thake CD**. The health benefits of passive heating and aerobic exercise: To what extent do the mechanisms overlap? *Journal of Applied Physiology* 129: 1304–1309, 2020. doi: 10.1152/japplphysiol.00608.2020.

438. **Laukkanen T**, **Kunutsor SK**, **Khan H**, **Willeit P**, **Zaccardi F**, **Laukkanen JA**. Sauna bathing is associated with reduced cardiovascular mortality and improves risk prediction in men and women: a prospective cohort study. *BMC Medicine* 16: 219, 2018. doi: 10.1186/s12916-018-1198-0.

439. **Zaccardi F**, **Laukkanen T**, **Willeit P**, **Kunutsor SK**, **Kauhanen J**, **Laukkanen JA**. Sauna Bathing and Incident Hypertension: A Prospective Cohort Study. *American Journal of Hypertension* 30: 1120–1125, 2017. doi: 10.1093/ajh/hpx102.

440. **Miyamoto H**, **Kai H**, **Nakaura H**, **Osada K**, **Mizuta Y**, **Matsumoto A**, **Imaizumi T**. Safety and Efficacy of Repeated Sauna Bathing in Patients With Chronic Systolic Heart Failure: A Preliminary Report. *Journal of Cardiac Failure* 11: 432–436, 2005. doi: 10.1016/j.cardfail.2005.03.004.

441. **Lee E**, **Kolunsarka IA**, **Kostensalo J**, **Ahtiainen JP**, **Haapala EA**, **Willeit P**, **Kunutsor SK**, **Laukkanen JA**. The effects of regular sauna bathing in conjunction with exercise on cardiovascular function: A multi-arm randomized controlled trial. .

442. **Laukkanen T**, **Kunutsor SK**, **Zaccardi F**, **Lee E**, **Willeit P**, **Khan H**, **Laukkanen JA**. Acute effects of sauna bathing on cardiovascular function. *Journal of Human Hypertension* 32: 129–138, 2018. doi: 10.1038/s41371-017-0008-z.

443. **Lee E**, **Laukkanen T**, **Kunutsor SK**, **Khan H**, **Willeit P**, **Zaccardi F**, **Laukkanen JA**. Sauna exposure leads to improved arterial compliance: Findings from a non-randomised experimental study. *Eur J Prev Cardiolog* 25: 130–138, 2018. doi: 10.1177/2047487317737629.

444. **Gravel H**, **Coombs GB**, **Behzadi P**, **Marcoux-Clément V**, **Barry H**, **Juneau M**, **Nigam A**, **Gagnon D**. Acute effect of Finnish sauna bathing on brachial artery flow-mediated dilation and reactive hyperemia in healthy middle-aged and older adults. *Physiological Reports* 7: e14166, 2019. doi: 10.14814/phy2.14166.

445. **Gravel H**, **Behzadi P**, **Cardinal S**, **Barry H**, **Neagoe P-E**, **Juneau M**, **Nigam A**, **Sirois MG**, **Gagnon D**. Acute Vascular Benefits of Finnish Sauna Bathing in Patients With Stable Coronary Artery Disease. *Canadian Journal of Cardiology* 37: 493–499, 2021. doi: 10.1016/j.cjca.2020.06.017.

446. **Gayda M**, **Bosquet L**, **Paillard F**, **Garzon M**, **Sosner P**, **Juneau M**, **Bélanger M**, **Nigam A**. Effects of Sauna Alone Versus Postexercise Sauna Baths on Short-term Heart Rate Variability in Patients With Untreated Hypertension [Online]. *Journal of Cardiopulmonary Rehabilitation and Prevention* 32, 2012. https://journals.lww.com/jcrjournal/Fulltext/2012/05000/Effects_of_Sauna_Alone_Versus_Postexercise_Sauna.5.aspx.

447. **Miyata M**, **Tei C**. Waon Therapy for Cardiovascular Disease: *Circulation Journal* advpub: 1002100612–1002100612, 2010. doi: 10.1253/circj.CJ-09-0939.

448. **Kihara T**, **Miyata M**, **Fukudome T**, **Ikeda Y**, **Shinsato T**, **Kubozono T**, **Fujita S**, **Kuwahata S**, **Hamasaki S**, **Torii H**, **Lee S**, **Toda H**, **Tei C**. Waon therapy improves the prognosis of patients with chronic heart failure. *Journal of Cardiology* 53: 214–218, 2009. doi: 10.1016/j.jjcc.2008.11.005.

449. **Imamura Masakazu**, **Biro Sadatoshi**, **Kihara Takashi**, **Yoshifuku Shiro**, **Takasaki Kunitsugu**, **Otsuji Yutaka**, **Minagoe Shinichi**, **Toyama Yoshifumi**, **Tei Chuwa**. Repeated thermal therapy improves impaired vascular endothelial function in patients with coronary risk factors. *Journal of the American College of Cardiology* 38: 1083–1088, 2001. doi: 10.1016/S0735-1097(01)01467-X.

450. **Kihara Takashi**, **Biro Sadatoshi**, **Imamura Masakazu**, **Yoshifuku Shiro**, **Takasaki Kunitsugu**, **Ikeda Yoshiyuki**, **Otuji Yutaka**, **Minagoe Shinichi**, **Toyama Yoshifumi**, **Tei Chuwa**. Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. *Journal of the American College of Cardiology* 39: 754–759, 2002. doi: 10.1016/S0735-1097(01)01824-1.

451. **Sobajima M**, **Nozawa T**, **Fukui Y**, **Ihori H**, **Ohori T**, **Fujii N**, **Inoue H**. Waon Therapy Improves Quality of Life as Well as Cardiac Function and Exercise Capacity in Patients With Chronic Heart Failure. *International Heart Journal* 56: 203–208, 2015. doi: 10.1536/ihj.14-266.

452. **Ikeda Y**, **Biro S**, **Kamogawa Y**, **Yoshifuku S**, **Eto H**, **Orihara K**, **Kihara T**, **Tei C**. Repeated Thermal Therapy Upregulates Arterial Endothelial Nitric Oxide Synthase Expression in Syrian Golden Hamsters. *JAPANESE CIRCULATION JOURNAL* 65: 434–438, 2001. doi: 10.1253/jcj.65.434.

453. **Ikeda Y**, **Biro S**, **Kamogawa Y**, **Yoshifuku S**, **Eto H**, **Orihara K**, **Yu B**, **Kihara T**, **Miyata M**, **Hamasaki S**, **Otsuji Y**, **Minagoe S**, **Tei C**. Repeated Sauna Therapy Increases Arterial Endothelial Nitric Oxide Synthase Expression and Nitric Oxide Production in Cardiomyopathic Hamsters. *Circulation Journal* 69: 722–729, 2005. doi: 10.1253/circj.69.722.

454. **Akasaki Y**, **Miyata M**, **Eto H**, **Shirasawa T**, **Hamada N**, **Ikeda Y**, **Biro S**, **Otsuji Y**, **Tei C**. Repeated Thermal Therapy Up-Regulates Endothelial Nitric Oxide Synthase and Augments Angiogenesis in a Mouse Model of Hindlimb Ischemia. *Circulation Journal* 70: 463–470, 2006. doi: 10.1253/circj.70.463.

455. **Miyauchi T**, **Miyata M**, **Ikeda Y**, **Akasaki Y**, **Hamada N**, **Shirasawa T**, **Furusho Y**, **Tei C**. Waon Therapy Upregulates Hsp90 and Leads to Angiogenesis Through the Akt-Endothelial Nitric Oxide Synthase Pathway in Mouse Hindlimb Ischemia. *Circulation Journal* 76: 1712–1721, 2012. doi: 10.1253/circj.CJ-11-0915.

456. **Inoue S**, **Takemoto M**, **Chishaki A**, **Ide T**, **Nishizaka M**, **Miyazono M**, **Sawatari H**, **Sunagawa K**. Leg Heating Using Far Infra-red Radiation in Patients with Chronic Heart Failure Acutely Improves the Hemodynamics, Vascular Endothelial Function, and Oxidative Stress. *Internal Medicine* 51: 2263–2270, 2012. doi: 10.2169/internalmedicine.51.7115.

457. **Fujita S**, **Ikeda Y**, **Miyata M**, **Shinsato T**, **Kubozono T**, **Kuwahata S**, **Hamada N**, **Miyauchi T**, **Yamaguchi T**, **Torii H**, **Hamasaki S**, **Tei C**. Effect of Waon Therapy on Oxidative Stress in Chronic Heart Failure. *Circulation Journal* 75: 348–356, 2011. doi: 10.1253/circj.CJ-10-0630.

458. **Sobajima M**, **Nozawa T**, **Ihori H**, **Shida T**, **Ohori T**, **Suzuki T**, **Matsuki A**, **Yasumura S**, **Inoue H**. Repeated sauna therapy improves myocardial perfusion in patients with chronically occluded coronary artery-related ischemia. *International Journal of Cardiology* 167: 237–243, 2013. doi: 10.1016/j.ijcard.2011.12.064.

459. **Lin C-C**, **Liu X-M**, **Peyton K**, **Wang H**, **Yang W-C**, **Lin S-J**, **Durante W**. Far Infrared Therapy Inhibits Vascular Endothelial Inflammation via the Induction of Heme Oxygenase-1. *Arteriosclerosis, Thrombosis, and Vascular Biology* 28: 739–745, 2008. doi: 10.1161/ATVBAHA.107.160085.

460. **Park J-H**, **Lee S**, **Cho D-H**, **Park YM**, **Kang D-H**, **Jo I**. Far-infrared radiation acutely increases nitric oxide production by increasing Ca2+ mobilization and Ca2+/calmodulin-dependent protein kinase II-mediated phosphorylation of endothelial nitric oxide synthase at serine 1179. *Biochemical and Biophysical Research Communications* 436: 601–606, 2013. doi: 10.1016/j.bbrc.2013.06.003.

461. **Shinsato T**, **Miyata M**, **Kubozono T**, **Ikeda Y**, **Fujita S**, **Kuwahata S**, **Akasaki Y**, **Hamasaki S**, **Fujiwara H**, **Tei C**. Waon therapy mobilizes CD34+ cells and improves peripheral arterial disease. *Journal of Cardiology* 56: 361–366, 2010. doi: 10.1016/j.jjcc.2010.08.004.

462. **Ohori T**, **Nozawa T**, **Ihori H**, **Shida T**, **Sobajima M**, **Matsuki A**, **Yasumura S**, **Inoue H**. Effect of Repeated Sauna Treatment on Exercise Tolerance and Endothelial Function in Patients With Chronic Heart Failure. *The American Journal of Cardiology* 109: 100–104, 2012. doi: 10.1016/j.amjcard.2011.08.014.

463. **Kuwahata S**, **Miyata M**, **Fujita S**, **Kubozono T**, **Shinsato T**, **Ikeda Y**, **Hamasaki S**, **Kuwaki T**, **Tei C**. Improvement of autonomic nervous activity by Waon therapy in patients with chronic heart failure. *Journal of Cardiology* 57: 100–106, 2011. doi: 10.1016/j.jjcc.2010.08.005.

464. **Kohara K**, **Tabara Y**, **Ochi M**, **Okada Y**, **Ohara M**, **Nagai T**, **Ohyagi Y**, **Igase M**. Habitual hot water bathing protects cardiovascular function in middle-aged to elderly Japanese subjects. *Scientific Reports* 8: 8687, 2018. doi: 10.1038/s41598-018-26908-1.

465. **Maeda T**, **Mimori K**, **Suzuki S**, **Horiuchi T**, **Makino N**. Preventive and promotive effects of habitual hot spa-bathing on the elderly in Japan. *Scientific Reports* 8: 133, 2018. doi: 10.1038/s41598-017-18488-3.

466. **Choudhury B**. *Bikram yoga*. Collins, 2007.

467. **Abel AN**, **Lloyd LK**, **Williams JS**, **Miller BK**. Physiological characteristics of long-term Bikram yoga practitioners. *Journal of Exercise Physiology Online* 15: 32–39, 2012.

468. **Miranda Hurtado M**, **Meza Valladares C**, **Eblen-Zajjur A**, **Rodriguez-Fernandez M**. Acute Cardiovascular Responses to a Session of Bikram Yoga: A Pilot Uncontrolled Trial. *The Journal of Alternative and Complementary Medicine* 25: 398–405, 2019. doi: 10.1089/acm.2018.0261.

469. **Marger CF**, **Hicklin LK**, **Garner DP**. Effects of Bikram yoga on body composition, blood pressure, and sleep patterns in adult practitioners. *Journal of Basic & Applied Sciences* 12, 2016.

470. **Hewett ZL**, **Pumpa KL**, **Smith CA**, **Fahey PP**, **Cheema BS**. Effect of a 16-week Bikram yoga program on heart rate variability and associated cardiovascular disease risk factors in stressed and sedentary adults: A randomized controlled trial. *BMC Complementary and Alternative Medicine* 17: 226, 2017. doi: 10.1186/s12906-017-1740-1.

471. **Tracy BL**, **Hart CEF**. Bikram Yoga Training and Physical Fitness in Healthy Young Adults [Online]. *The Journal of Strength & Conditioning Research* 27, 2013. https://journals.lww.com/nsca-jscr/fulltext/2013/03000/bikram_yoga_training_and_physical_fitness_in.35.aspx.

472. **Hunter SD**, **Dhindsa MS**, **Cunningham E**, **Tarumi T**, **Alkatan M**, **Nualnim N**, **Elmenshawy A**, **Tanaka H**. The effect of Bikram yoga on endothelial function in young and middle-aged and older adults. *Journal of Bodywork and Movement Therapies* 21: 30–34, 2017. doi: 10.1016/j.jbmt.2016.06.004.

473. **Hunter SD**, **Laosiripisan J**, **Elmenshawy A**, **Tanaka H**. Effects of yoga interventions practised in heated and thermoneutral conditions on endothelium-dependent vasodilatation: The Bikram yoga heart study. *Experimental Physiology* 103: 391–396, 2018. doi: 10.1113/EP086725.

474. **Hunter SD**, **Dhindsa MS**, **Cunningham E**, **Tarumi T**, **Alkatan M**, **Nualnim N**, **Tanaka H**. The Effect of Bikram Yoga on Arterial Stiffness in Young and Older Adults. *The Journal of Alternative and Complementary Medicine* 19: 930–934, 2013. doi: 10.1089/acm.2012.0709.

475. **Hunter SD**, **Dhindsa MS**, **Cunningham E**, **Tarumi T**, **Alkatan M**, **Nualnim N**, **Tanaka H**. Impact of Hot Yoga on Arterial Stiffness and Quality of Life in Overweight/Obese Adults. *Journal of Physical Activity and Health* 13: 1360–1363, 2016. doi: 10.1123/jpah.2016-0170.

476. **Hunter SD**, **Dhindsa M**, **Cunningham E**, **Tarumi T**, **Alkatan M**, **Tanaka H**. Improvements in glucose tolerance with Bikram yoga in older obese adults: A pilot study. *Journal of bodywork and movement therapies* 17: 404–407, 2013.

477. **Fox RH**, **Goldsmith R**, **Kidd DJ**, **Lewis HE**. Blood flow and other thermoregulatory changes with acclimatization to heat. *The Journal of Physiology* 166: 548–562, 1963. doi: 10.1113/jphysiol.1963.sp007122.

478. **Buono MJ**, **Numan TR**, **Claros RM**, **Brodine SK**, **Kolkhorst FW**. Is active sweating during heat acclimation required for improvements in peripheral sweat gland function? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 297: R1082–R1085, 2009. doi: 10.1152/ajpregu.00253.2009.

479. **Taylor NAS**. Human Heat Adaptation. In: *Comprehensive Physiology*, p. 325–365.

480. **Fox RH**, **Goldsmith R**, **Kidd DJ**, **Lewis HE**. Acclimatization to heat in man by controlled elevation of body temperature. *The Journal of Physiology* 166: 530–547, 1963. doi: 10.1113/jphysiol.1963.sp007121.

481. **Brunt VE**, **Jeckell AT**, **Ely BR**, **Howard MJ**, **Thijssen DHJ**, **Minson CT**. Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 311: R1060–R1067, 2016. doi: 10.1152/ajpregu.00301.2016.

482. **Hemingway HW**, **Richey RE**, **Moore AM**, **Olivencia-Yurvati AH**, **Kline GP**, **Romero SA**. Acute heat exposure protects against endothelial ischemia-reperfusion injury in aged humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 322: R360–R367, 2022. doi: 10.1152/ajpregu.00336.2021.

483. **Engelland RE**, **Hemingway HW**, **Tomasco OG**, **Olivencia-Yurvati AH**, **Romero SA**. Acute lower leg hot water immersion protects macrovascular dilator function following

ischaemia–reperfusion injury in humans. *Experimental Physiology* 105: 302–311, 2020. doi: 10.1113/EP088154.

484. **Leicht CA**, **Kouda K**, **Umemoto Y**, **Banno M**, **Kinoshita T**, **Moriki T**, **Nakamura T**, **Bishop NC**, **Goosey-Tolfrey VL**, **Tajima F**. Hot water immersion induces an acute cytokine response in cervical spinal cord injury. *European Journal of Applied Physiology* 115: 2243–2252, 2015. doi: 10.1007/s00421-015-3206-9.

485. **Hoekstra SP**, **Bishop NC**, **Faulkner SH**, **Bailey SJ**, **Leicht CA**. Acute and chronic effects of hot water immersion on inflammation and metabolism in sedentary, overweight adults. .

486. **Roxburgh BH**, **Campbell HA**, **Cotter JD**, **Reymann U**, **Williams MJA**, **Gwynne-Jones D**, **Thomas KN**. Acute and adaptive cardiovascular and metabolic effects of passive heat therapy or high-intensity interval training in patients with severe lower-limb osteoarthritis. *Physiological Reports* 11: e15699, 2023. doi: 10.14814/phy2.15699.

487. **Francisco MA**, **Colbert C**, **Larson EA**, **Sieck DC**, **Halliwill JR**, **Minson CT**. Hemodynamics of postexercise versus post-hot water immersion recovery. *Journal of Applied Physiology* 130: 1362–1372, 2021. doi: 10.1152/japplphysiol.00260.2020.

488. **Rissanen JA**, **Häkkinen K**, **Laukkanen JA**, **Häkkinen A**. Acute Hemodynamic Responses to Combined Exercise and Sauna. *INT J SPORTS MED* 41: 824–831, 2020. doi: 10.1055/a-1186-1716.

489. **Brunt VE**, **Eymann TM**, **Francisco MA**, **Howard MJ**, **Minson CT**. Passive heat therapy improves cutaneous microvascular function in sedentary humans via improved nitric oxide-dependent dilation. *Journal of Applied Physiology* 121: 716–723, 2016. doi: 10.1152/japplphysiol.00424.2016.

490. **Bonaca MP**, **Creager MA**. Pharmacological Treatment and Current Management of Peripheral Artery Disease. *Circ Res* 116: 1579–1598, 2015. doi: 10.1161/CIRCRESAHA.114.303505.

491. **Ritossa F**. A new puffing pattern induced by temperature shock and DNP in drosophila. *Experientia* 18: 571–573, 1962. doi: 10.1007/BF02172188.

492. **Tissiéres A**, **Mitchell HK**, **Tracy UM**. Protein synthesis in salivary glands of Drosophila melanogaster: Relation to chromosome puffs. *Journal of Molecular Biology* 84: 389–398, 1974. doi: 10.1016/0022-2836(74)90447-1.

493. **Feder ME**, **Hofmann GE**. HEAT-SHOCK PROTEINS, MOLECULAR CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological Physiology. *Annu Rev Physiol* 61: 243–282, 1999. doi: 10.1146/annurev.physiol.61.1.243.

494. **Ely BR**, **Clayton ZS**, **McCurdy CE**, **Pfeiffer J**, **Needham KW**, **Comrada LN**, **Minson CT**. Heat therapy improves glucose tolerance and adipose tissue insulin signaling in

polycystic ovary syndrome. *American Journal of Physiology-Endocrinology and Metabolism* 317: E172–E182, 2019. doi: 10.1152/ajpendo.00549.2018.

495. **Lee BJ**, **Mackenzie RWA**, **Cox V**, **James RS**, **Thake CD**. Human Monocyte Heat Shock Protein 72 Responses to Acute Hypoxic Exercise after 3 Days of Exercise Heat Acclimation. *BioMed Research International* 2015: 849809, 2015. doi: 10.1155/2015/849809.

496. **Magalhães F de C**, **Amorim FT**, **Passos RLF**, **Fonseca MA**, **Oliveira KPM**, **Lima MRM**, **Guimarães JB**, **Ferreira-Júnior JB**, **Martini ARP**, **Lima NRV**, **Soares DD**, **Oliveira EM**, **Rodrigues LOC**. Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. *Cell Stress and Chaperones* 15: 885–895, 2010. doi: 10.1007/s12192-010-0197-7.

497. **McClung JP**, **Hasday JD**, **He J**, **Montain SJ**, **Cheuvront SN**, **Sawka MN**, **Singh IS**. Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 294: R185–R191, 2008. doi: 10.1152/ajpregu.00532.2007.

498. **Yamada PM**, **Amorim FT**, **Moseley P**, **Robergs R**, **Schneider SM**. Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. *Journal of Applied Physiology* 103: 1196–1204, 2007. doi: 10.1152/japplphysiol.00242.2007.

499. **Brunt VE**, **Wiedenfeld-Needham K**, **Comrada LN**, **Minson CT**. Passive heat therapy protects against endothelial cell hypoxia-reoxygenation via effects of elevations in temperature and circulating factors. *The Journal of Physiology* 596: 4831–4845, 2018. doi: 10.1113/JP276559.

500. **Hafen PS**, **Preece CN**, **Sorensen JR**, **Hancock CR**, **Hyldahl RD**. Repeated exposure to heat stress induces mitochondrial adaptation in human skeletal muscle. *Journal of Applied Physiology* 125: 1447–1455, 2018. doi: 10.1152/japplphysiol.00383.2018.

501. **Hafen PS**, **Abbott K**, **Bowden J**, **Lopiano R**, **Hancock CR**, **Hyldahl RD**. Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization. *Journal of Applied Physiology* 127: 47–57, 2019. doi: 10.1152/japplphysiol.01098.2018.

502. **Pritchard KA Jr**, **Ackerman AW**, **Gross ER**, **Stepp DW**, **Shi Y**, **Fontana JT**, **Baker JE**, **Sessa WC**. Heat Shock Protein 90 Mediates the Balance of Nitric Oxide and Superoxide Anion from Endothelial Nitric-oxide Synthase *. *Journal of Biological Chemistry* 276: 17621–17624, 2001. doi: 10.1074/jbc.C100084200.

503. **García-Cardeña G**, **Fan R**, **Shah V**, **Sorrentino R**, **Cirino G**, **Papapetropoulos A**, **Sessa WC**. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* 392: 821–824, 1998. doi: 10.1038/33934.

504. **Brouet A**, **Sonveaux P**, **Dessy C**, **Balligand J-L**, **Feron O**. Hsp90 Ensures the Transition from the Early Ca2+-dependent to the Late Phosphorylation-dependent Activation of the Endothelial Nitric-oxide Synthase in Vascular Endothelial Growth Factor-exposed Endothelial Cells *. *Journal of Biological Chemistry* 276: 32663–32669, 2001. doi: 10.1074/jbc.M101371200.

505. **Gratton J-P**, **Fontana J**, **O'Connor DS**, **García-Cardeña G**, **McCabe TJ**, **Sessa WC**. Reconstitution of an Endothelial Nitric-oxide Synthase (eNOS), hsp90, and Caveolin-1 Complex in Vitro: EVIDENCE THAT hsp90 FACILITATES CALMODULIN STIMULATED DISPLACEMENT OF eNOS FROM CAVEOLIN-1 *. *Journal of Biological Chemistry* 275: 22268–22272, 2000. doi: 10.1074/jbc.M001644200.

506. **SONG Y**, **ZWEIER JL**, **XIA Y**. Heat-shock protein 90 augments neuronal nitric oxide synthase activity by enhancing Ca2+/calmodulin binding. *Biochemical Journal* 355: 357–360, 2001. doi: 10.1042/bj3550357.

507. **Khurana VG**, **Feterik K**, **Springett MJ**, **Eguchi D**, **Shah V**, **Katusic ZS**. Functional Interdependence and Colocalization of Endothelial Nitric Oxide Synthase and Heat Shock Protein 90 in Cerebral Arteries. *J Cereb Blood Flow Metab* 20: 1563–1570, 2000. doi: 10.1097/00004647-200011000-00006.

508. **Shah V**, **Wiest R**, **Garcia-Cardena G**, **Cadelina G**, **Groszmann RJ**, **Sessa WC**. Hsp90 regulation of endothelial nitric oxide synthase contributes to vascular control in portal hypertension. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 277: G463–G468, 1999. doi: 10.1152/ajpgi.1999.277.2.G463.

509. **Su Y**, **Block ER**. Role of calpain in hypoxic inhibition of nitric oxide synthase activity in pulmonary endothelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 278: L1204–L1212, 2000. doi: 10.1152/ajplung.2000.278.6.L1204.

510. **Billecke SS**, **Bender AT**, **Kanelakis KC**, **Murphy PJM**, **Lowe ER**, **Kamada Y**, **Pratt WB**, **Osawa Y**. hsp90 Is Required for Heme Binding and Activation of Apo-Neuronal Nitric-oxide Synthase: GELDANAMYCIN-MEDIATED OXIDANT GENERATION IS UNRELATED TO ANY ACTION OF hsp90 *. *Journal of Biological Chemistry* 277: 20504–20509, 2002. doi: 10.1074/jbc.M201940200.

511. **Averna M**, **Stifanese R**, **De Tullio R**, **Passalacqua M**, **Salamino F**, **Pontremoli S**, **Melloni E**. Functional Role of HSP90 Complexes with Endothelial Nitric-oxide Synthase (eNOS) and Calpain on Nitric Oxide Generation in Endothelial Cells∗. *Journal of Biological Chemistry* 283: 29069–29076, 2008. doi: 10.1074/jbc.M803638200.

512. **Brunt VE**, **Weidenfeld-Needham KM**, **Comrada LN**, **Francisco MA**, **Eymann TM**, **Minson CT**. Serum from young, sedentary adults who underwent passive heat therapy improves endothelial cell angiogenesis via improved nitric oxide bioavailability. *null* 6: 169–178, 2019. doi: 10.1080/23328940.2019.1614851.

513. **Currie RW**, **Karmazyn M**, **Kloc M**, **Mailer K**. Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circulation Research* 63: 543–549, 1988. doi: 10.1161/01.RES.63.3.543.

514. **Currie RW**, **Tanguay RM**. Analysis of RNA for transcripts for catalase and SP71 in rat hearts after in vivo hyperthermia. *Biochem Cell Biol* 69: 375–382, 1991. doi: 10.1139/o91-057.

515. **Gowda A**, **Yang C**, **Asimakis GK**, **Ruef J**, **Rastegar S**, **Runge MS**, **Motamedi M**. Cardioprotection by Local Heating: Improved Myocardial Salvage After Ischemia and Reperfusion. *The Annals of Thoracic Surgery* 65: 1241–1247, 1998. doi: 10.1016/S0003-4975(98)00117-9.

516. **Yellon DM**, **Pasini E**, **Cargnoni A**, **Marber MS**, **Latchman DS**, **Ferrari R**. The protective role of heat stress in the ischaemic and reperfused rabbit myocardium. *Journal of Molecular and Cellular Cardiology* 24: 895–907, 1992. doi: 10.1016/0022-2828(92)91102-B.

517. **Donnelly TJ**, **Sievers RE**, **Vissern FL**, **Welch WJ**, **Wolfe CL**. Heat shock protein induction in rat hearts. A role for improved myocardial salvage after ischemia and reperfusion? *Circulation* 85: 769–778, 1992. doi: 10.1161/01.CIR.85.2.769.

518. **Saganek LJ**, **Ignasiak DP**, **Batley BL**, **Potoczak RE**, **Dodd G**, **Gallagher KP**. Heat stress increases cardiac HSP72i but fails to reduce myocardial infarct size in rabbits 24 hours later. *Basic Research in Cardiology* 92: 331–338, 1997. doi: 10.1007/BF00788945.

519. **Xi L**, **Chelliah J**, **Nayeem MA**, **Levasseur JE**, **Hess ML**, **Kukreja RC**. Whole Body Heat Shock Fails To Protect Mouse Heart Against Ischemia/Reperfusion Injury: Role of 72 kDa Heat Shock Protein and Antioxidant Enzymes. *Journal of Molecular and Cellular Cardiology* 30: 2213–2227, 1998. doi: 10.1006/jmcc.1998.0781.

520. **Lille S**, **Su C-Y**, **Schoeller T**, **Suchy H**, **Lyons S**, **Russell RC**, **Neumeister M**, **Lai C-C**. Induction of heat-shock protein 72 in rat skeletal muscle does not increase tolerance to ischemia-reperfusion injury. *Muscle & Nerve* 22: 390–393, 1999. doi: 10.1002/(SICI)1097-4598(199903)22:3<390::AID-MUS12>3.0.CO;2-1.

521. **Karmazyn M**, **Mailer K**, **Currie RW**. Acquisition and decay of heat-shock-enhanced postischemic ventricular recovery. *American Journal of Physiology-Heart and Circulatory Physiology* 259: H424–H431, 1990. doi: 10.1152/ajpheart.1990.259.2.H424.

522. **Yamashita N**, **Hoshida S**, **Nishida M**, **Igarashi J**, **Taniguchi N**, **Tada M**, **Kuzuya T**, **Hori M**. Heat Shock-induced Manganese Superoxide Dismutase Enhances the Tolerance of Cardiac Myocytes to Hypoxia–Reoxygenation Injury. *Journal of Molecular and Cellular Cardiology* 29: 1805–1813, 1997. doi: 10.1006/jmcc.1997.0415.

523. **Maloyan A**, **Palmon A**, **Horowitz M**. Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. *American Journal of Physiology-*

Regulatory, Integrative and Comparative Physiology 276: R1506–R1515, 1999. doi: 10.1152/ajpregu.1999.276.5.R1506.

524. **Cheng JL**, **Williams JS**, **Hoekstra SP**, **MacDonald MJ**. Improvements in vascular function in response to acute lower limb heating in young healthy males and females. *Journal of Applied Physiology* 131: 277–289, 2021. doi: 10.1152/japplphysiol.00630.2020.

525. **Zheng Y**, **Im C-N**, **Seo J-S**. Inhibitory effect of Hsp70 on angiotensin II-induced vascular smooth muscle cell hypertrophy. *Experimental & Molecular Medicine* 38: 509–518, 2006. doi: 10.1038/emm.2006.60.

526. **Suzuki K**, **Murtuza B**, **Sammut IA**, **Latif N**, **Jayakumar J**, **Smolenski RT**, **Kaneda Y**, **Sawa Y**, **Matsuda H**, **Yacoub MH**. Heat Shock Protein 72 Enhances Manganese Superoxide Dismutase Activity During Myocardial Ischemia-Reperfusion Injury, Associated With Mitochondrial Protection and Apoptosis Reduction. *Circulation* 106: I–270, 2002. doi: 10.1161/01.cir.0000032880.55215.92.

527. **Sreedhar AS**, **Pardhasaradhi BVV**, **Khar A**, **Srinivas UK**. A cross talk between cellular signalling and cellular redox state during heat-induced apoptosis in a rat histiocytoma. *Free Radical Biology and Medicine* 32: 221–227, 2002. doi: 10.1016/S0891-5849(01)00796-1.

528. **Belhadj Slimen I**, **Najar T**, **Ghram A**, **Dabbebi H**, **Ben Mrad M**, **Abdrabbah M**. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *International Journal of Hyperthermia* 30: 513–523, 2014. doi: 10.3109/02656736.2014.971446.

529. **Araujo J**, **Zhang M**, **Yin F**. Heme Oxygenase-1, Oxidation, Inflammation, and Atherosclerosis [Online]. *Frontiers in Pharmacology* 3, 2012. https://www.frontiersin.org/articles/10.3389/fphar.2012.00119.

530. **Steensberg A**, **Fischer CP**, **Keller C**, **Møller K**, **Pedersen BK**. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology-Endocrinology and Metabolism* 285: E433–E437, 2003. doi: 10.1152/ajpendo.00074.2003.

531. **Hooper PL**. Hot-tub therapy for type 2 diabetes mellitus. *New England Journal of Medicine* 341: 924–925, 1999.

532. **Hesketh K**, **Shepherd SO**, **Strauss JA**, **Low DA**, **Cooper RJ**, **Wagenmakers AJM**, **Cocks M**. Passive heat therapy in sedentary humans increases skeletal muscle capillarization and eNOS content but not mitochondrial density or GLUT4 content. *American Journal of Physiology-Heart and Circulatory Physiology* 317: H114–H123, 2019. doi: 10.1152/ajpheart.00816.2018.

533. **Neff D**, **Kuhlenhoelter AM**, **Lin C**, **Wong BJ**, **Motaganahalli RL**, **Roseguini BT**. Thermotherapy reduces blood pressure and circulating endothelin-1 concentration and

enhances leg blood flow in patients with symptomatic peripheral artery disease. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 311: R392–R400, 2016.

534. **Engelland RE**, **Hemingway HW**, **Tomasco OG**, **Olivencia-Yurvati AH**, **Romero SA**. Neural control of blood pressure is altered following isolated leg heating in aged humans. *American Journal of Physiology-Heart and Circulatory Physiology* 318: H976–H984, 2020.

535. **Thorin E**, **Webb DJ**. Endothelium-derived endothelin-1. *Pflügers Archiv-European Journal of Physiology* 459: 951–958, 2010.

536. **Pearson TA**, **Mensah GA**, **Alexander RW**, **Anderson JL**, **Cannon III RO**, **Criqui M**, **Fadl YY**, **Fortmann SP**, **Hong Y**, **Myers GL**. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *circulation* 107: 499–511, 2003.

537. **Wilson PWF**, **Pencina M**, **Jacques P**, **Selhub J**, **D'Agostino R**, **O'Donnell CJ**. C-Reactive Protein and Reclassification of Cardiovascular Risk in the Framingham Heart Study. *Circulation: Cardiovascular Quality and Outcomes* 1: 92–97, 2008. doi: 10.1161/CIRCOUTCOMES.108.831198.

538. **Laukkanen JA**, **Laukkanen T**. Sauna bathing and systemic inflammation. *Eur J Epidemiol* 33: 351–353, 2018. doi: 10.1007/s10654-017-0335-y.

539. **Kunutsor SK**, **Laukkanen T**, **Laukkanen JA**. Longitudinal associations of sauna bathing with inflammation and oxidative stress: the KIHD prospective cohort study. *Annals of Medicine* 50: 437–442, 2018. doi: 10.1080/07853890.2018.1489143.

540. **Tremblay JC**, **Williams JS**, **Pyke KE**. Ramp and step increases in shear stress result in a similar magnitude of brachial artery flow-mediated dilation. *Eur J Appl Physiol* 119: 611–619, 2019. doi: 10.1007/s00421-018-4049-y.

541. **Corson MA**, **James NL**, **Latta SE**, **Nerem RM**, **Berk BC**, **Harrison DG**. Phosphorylation of Endothelial Nitric Oxide Synthase in Response to Fluid Shear Stress. *Circulation Research* 79: 984–991, 1996. doi: 10.1161/01.RES.79.5.984.

542. **Rubanyi GM**, **Romero JC**, **Vanhoutte PM**. Flow-induced release of endothelium-derived relaxing factor. *American Journal of Physiology-Heart and Circulatory Physiology* 250: H1145–H1149, 1986. doi: 10.1152/ajpheart.1986.250.6.H1145.

543. **Hodges GJ**, **Stewart DG**, **Davison PJ**, **Cheung SS**. Episodic bouts of hyperaemia and shear stress improve arterial blood flow and endothelial function. *European Journal of Applied Physiology* 118: 795–803, 2018. doi: 10.1007/s00421-018-3805-3.

544. **Green DJ**, **Carter HH**, **Fitzsimons MG**, **Cable NT**, **Thijssen DHJ**, **Naylor LH**. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. *The Journal of Physiology* 588: 1571–1577, 2010. doi: 10.1113/jphysiol.2010.186965.

545. **Carter HH**, **Spence AL**, **Atkinson CL**, **Pugh CJA**, **Naylor LH**, **Green DJ**. Repeated core temperature elevation induces conduit artery adaptation in humans. *European Journal of Applied Physiology* 114: 859–865, 2014. doi: 10.1007/s00421-013-2817-2.

546. **Naylor LH**, **Carter H**, **FitzSimons MG**, **Cable NT**, **Thijssen DHJ**, **Green DJ**. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 300: H664–H669, 2011. doi: 10.1152/ajpheart.00985.2010.

547. **Chiesa ST**, **Trangmar SJ**, **González-Alonso J**. Temperature and blood flow distribution in the human leg during passive heat stress. *Journal of Applied Physiology* 120: 1047–1058, 2016. doi: 10.1152/japplphysiol.00965.2015.

548. **Thomas KN**, **van Rij AM**, **Lucas SJE**, **Gray AR**, **Cotter JD**. Substantive hemodynamic and thermal strain upon completing lower-limb hot-water immersion; comparisons with treadmill running. *null* 3: 286–297, 2016. doi: 10.1080/23328940.2016.1156215.

549. **Amin SB**, **Hansen AB**, **Mugele H**, **Willmer F**, **Gross F**, **Reimeir B**, **Cornwell WK**, **Simpson LL**, **Moore JP**, **Romero SA**, **Lawley JS**. Whole body passive heating versus dynamic lower body exercise: a comparison of peripheral hemodynamic profiles. *Journal of Applied Physiology* 130: 160–171, 2021. doi: 10.1152/japplphysiol.00291.2020.

550. **Coombs GB**, **Tremblay JC**, **Shkredova DA**, **Carr JMJR**, **Wakeham DJ**, **Patrician A**, **Ainslie PN**. Distinct contributions of skin and core temperatures to flow-mediated dilation of the brachial artery following passive heating. *Journal of Applied Physiology* 130: 149–159, 2021. doi: 10.1152/japplphysiol.00502.2020.

551. **Dominiczak AF**, **Bohr DF**. Nitric Oxide and Its Putative Role in Hypertension. *Hypertension* 25: 1202–1211, 1995. doi: 10.1161/01.HYP.25.6.1202.

552. **Hu Q**, **Zhu W**, **Zhu Y**, **Zheng L**, **Hughson RL**. Acute effects of warm footbath on arterial stiffness in healthy young and older women. *European Journal of Applied Physiology* 112: 1261–1268, 2012. doi: 10.1007/s00421-011-2066-1.

553. **Ganio MS**, **Brothers RM**, **Shibata S**, **Hastings JL**, **Crandall CG**. Effect of passive heat stress on arterial stiffness. *Experimental Physiology* 96: 919–926, 2011. doi: 10.1113/expphysiol.2011.057091.

554. **Moyen NE**, **Ganio MS**, **Burchfield JM**, **Tucker MA**, **Gonzalez MA**, **Dougherty EK**, **Robinson FB**, **Ridings CB**, **Veilleux JC**. Effect of passive heat stress on arterial stiffness in smokers versus non-smokers. *International Journal of Biometeorology* 60: 499–506, 2016. doi: 10.1007/s00484-015-1046-2.

555. **Schlader ZJ**, **Okada Y**, **Best SA**, **Fu Q**, **Crandall CG**. Arterial stiffness during whole-body passive heat stress in healthy older adults. *Physiological Reports* 7: e14094, 2019. doi: 10.14814/phy2.14094.

556. **Caldwell AR**, **Robinson FB**, **Tucker MA**, **Arcement CH**, **Butts CL**, **McDermott BP**, **Ganio MS**. Effect of passive heat stress and exercise in the heat on arterial stiffness. *European Journal of Applied Physiology* 117: 1679–1687, 2017. doi: 10.1007/s00421-017-3658-1.

557. **Pyke KE**, **Dwyer EM**, **Tschakovsky ME**. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *Journal of Applied Physiology* 97: 499–508, 2004. doi: 10.1152/japplphysiol.01245.2003.

558. **Hunter SD**, **Laosiripisan J**, **Elmenshawy A**. Effects of heated and thermoneutral yoga interventions on arterial stiffness in middle-aged adults. *Complementary Therapies in Medicine* 40: 113–115, 2018. doi: 10.1016/j.ctim.2018.08.005.

559. **Joannides R**, **Costentin A**, **Iacob M**, **Bakkali E-H**, **Richard M-O**, **Thuillez C**. Role Of Arterial Smooth Muscle Tone And Geometry In The Regulation Of Peripheral Conduit Artery Mechanics By Shear Stress. *Clinical and Experimental Pharmacology and Physiology* 28: 1025–1031, 2001. doi: 10.1046/j.1440-1681.2001.03594.x.

560. **Bellien J**, **Favre J**, **Iacob M**, **Gao J**, **Thuillez C**, **Richard V**, **Joannidès R**. Arterial Stiffness Is Regulated by Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor During Changes in Blood Flow in Humans. *Hypertension* 55: 674–680, 2010. doi: 10.1161/HYPERTENSIONAHA.109.142190.

561. **Kinlay S**, **Creager MA**, **Fukumoto M**, **Hikita H**, **Fang JC**, **Selwyn AP**, **Ganz P**. Endothelium-Derived Nitric Oxide Regulates Arterial Elasticity in Human Arteries In Vivo. *Hypertension* 38: 1049–1053, 2001. doi: 10.1161/hy1101.095329.

562. **Madero M**, **Peralta C**, **Katz R**, **Canada R**, **Fried L**, **Najjar S**, **Shlipak M**, **Simonsick E**, **Lakatta E**, **Patel K**, **Rifkin D**, **Hawkins M**, **Newman A**, **Sarnak M**. Association of Arterial Rigidity with Incident Kidney Disease and Kidney Function Decline: The Health ABC Study. *CLIN J AM SOC NEPHROL* 8: 424, 2013. doi: 10.2215/CJN.07900812.

563. **Brengelmann GL**, **Savage MV**. Temperature regulation in the neutral zone. *Annals of the New York Academy of Sciences* 813: 39–50, 1997.

564. **Gisolfi CV**, **Wenger CB**. Temperature regulation during exercise: old concepts, new ideas. *Exercise and sport sciences reviews* 12: 339–372, 1984.

565. **Crandall CG**, **Wilson T**, **Marving J**, **Vogelsang T**, **Kjaer A**, **Hesse B**, **Secher N**. Effects of passive heating on central blood volume and ventricular dimensions in humans. *The Journal of physiology* 586: 293–301, 2008.

566. **Low DA**, **Keller DM**, **Wingo JE**, **Brothers RM**, **Crandall CG**. Sympathetic nerve activity and whole body heat stress in humans. *Journal of Applied Physiology* 111: 1329–1334, 2011. doi: 10.1152/japplphysiol.00498.2011.

567. **Minson CT**, **Wladkowski SL**, **Cardell AF**, **Pawelczyk JA**, **Kenney WL**. Age alters the cardiovascular response to direct passive heating. *Journal of applied physiology* 84: 1323–1332, 1998.

568. **Rowell LB**. Cardiovascular adjustments to thermal stress. .

569. **Rowell LB**, **Brengelmann GL**, **Murray JA**. Cardiovascular responses to sustained high skin temperature in resting man. *Journal of Applied Physiology* 27: 673–680, 1969.

570. **Niimi Y**, **Matsukawa T**, **Sugiyama Y**, **Shamsuzzaman A**, **Mano T**. Comparison [correction of Compariso] of sympathetic nerve response to head-up tilt in summer [correction of sumer] and winter. *Journal of Gravitational Physiology: a Journal of the International Society for Gravitational Physiology* 6: P43-4, 1999.

571. **Horowitz M**, **Meiri U**. Central and peripheral contributions to control of heart rate during heat acclimation. *Pflügers Archiv* 422: 386–392, 1993.

572. **Romero SA**, **Gagnon D**, **Adams AN**, **Cramer MN**, **Kouda K**, **Crandall CG**. Acute limb heating improves macro- and microvascular dilator function in the leg of aged humans. *American Journal of Physiology-Heart and Circulatory Physiology* 312: H89–H97, 2017. doi: 10.1152/ajpheart.00519.2016.

573. **Billman GE**, **Huikuri HV**, **Sacha J**, **Trimmel K**. An introduction to heart rate variability: methodological considerations and clinical applications. *Front Physiol* 6, 2015. doi: 10.3389/fphys.2015.00055.

574. **Radtke T**, **Poerschke D**, **Wilhelm M**, **Trachsel LD**, **Tschanz H**, **Matter F**, **Jauslin D**, **Saner H**, **Schmid J-P**. Acute effects of Finnish sauna and cold-water immersion on haemodynamic variables and autonomic nervous system activity in patients with heart failure. *European journal of preventive cardiology* 23: 593–601, 2016.

575. **Laukkanen T**, **Lipponen J**, **Kunutsor SK**, **Zaccardi F**, **Araújo CGS**, **Mäkikallio TH**, **Khan H**, **Willeit P**, **Lee E**, **Poikonen S**, **Tarvainen M**, **Laukkanen JA**. Recovery from sauna bathing favorably modulates cardiac autonomic nervous system. *Complementary Therapies in Medicine* 45: 190–197, 2019. doi: 10.1016/j.ctim.2019.06.011.

576. **Lansdown A**, **Rees DA**. The sympathetic nervous system in polycystic ovary syndrome: a novel therapeutic target? *Clinical endocrinology* 77: 791–801, 2012.

577. **Wild RA**, **Carmina E**, **Diamanti-Kandarakis E**, **Dokras A**, **Escobar-Morreale HF**, **Futterweit W**, **Lobo R**, **Norman RJ**, **Talbott E**, **Dumesic DA**. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary

Syndrome (AE-PCOS) Society. *The Journal of Clinical Endocrinology & Metabolism* 95: 2038–2049, 2010.

578. **Di Domenico K**, **Wiltgen D**, **Nickel FJ**, **Magalhaes JA**, **Moraes RS**, **Spritzer PM**. Cardiac autonomic modulation in polycystic ovary syndrome: does the phenotype matter? *Fertility and Sterility* 99: 286–292, 2013.

579. **Stener-Victorin E**, **Jedel E**, **Janson PO**, **Sverrisdottir YB**. Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. .

580. **Flouris AD**, **Poirier MP**, **Bravi A**, **Wright-Beatty HE**, **Herry C**, **Seely AJ**, **Kenny GP**. Changes in heart rate variability during the induction and decay of heat acclimation. *European journal of applied physiology* 114: 2119–2128, 2014.

581. **Frank A**, **Belokopytov M**, **Moran D**, **Shapiro Y**, **Epstein Y**. Changes in heart rate variability following acclimation to heat. *Journal of basic and clinical physiology and pharmacology* 12: 19–32, 2001.

582. **Gendron P**, **Gravel H**, **Barry H**, **Gagnon D**. Seven days of hot water heat acclimation does not modulate the change in heart rate variability during passive heat exposure. *Applied Physiology, Nutrition, and Metabolism* 46: 257–264, 2021.

583. **Bailey T**, **Cable N**, **Miller G**, **Sprung V**, **Low D**, **Jones H**. Repeated Warm Water Immersion Induces Similar Cerebrovascular Adaptations to 8 Weeks of Moderate-Intensity Exercise Training in Females. *Int J Sports Med* 37: 757–765, 2016. doi: 10.1055/s-0042-106899.

584. **Farrell SW**, **Kampert JB**, **Kohl HW**, **Barlow CE**, **Macera CA**, **Paffenbarger RS**, **Gibbons LW**, **Blair SN**. Influences of cardiorespiratory fitness levels and other predictors on cardiovascular disease mortality in men. *Med Sci Sports Exerc* 30: 899–905, 1998. doi: 10.1097/00005768-199806000-00019.

585. **Blair SN**, **Kampert JB**, **Kohl HW III**, **Barlow CE**, **Macera CA**, **Paffenbarger RS Jr**, **Gibbons LW**. Influences of Cardiorespiratory Fitness and Other Precursors on Cardiovascular Disease and All-Cause Mortality in Men and Women. *JAMA* 276: 205–210, 1996. doi: 10.1001/jama.1996.03540030039029.

586. **Kravitz BA**, **Corrada MM**, **Kawas CH**. Elevated c-reactive protein levels are associated with prevalent dementia in the oldest-old. *Alzheimers Dement* 5: 318–323, 2009. doi: 10.1016/j.jalz.2009.04.1230.

587. **Knekt P**, **Järvinen R**, **Rissanen H**, **Heliövaara M**, **Aromaa A**. Does sauna bathing protect against dementia? *Preventive Medicine Reports* 20: 101221, 2020. doi: 10.1016/j.pmedr.2020.101221.

588. **Ogawa T**, **Hoekstra SP**, **Kamijo Y-I**, **Goosey-Tolfrey VL**, **Walsh JJ**, **Tajima F. F**, **Leicht CA**. Serum and plasma brain-derived neurotrophic factor concentration are elevated by systemic but not local passive heating. *PLOS ONE* 16: e0260775, 2021. doi: 10.1371/journal.pone.0260775.

589. **Koh SXT**, **Lee JKW**. S100B as a Marker for Brain Damage and Blood–Brain Barrier Disruption Following Exercise. *Sports Medicine* 44: 369–385, 2014. doi: 10.1007/s40279-013-0119-9.

590. **Watson P**, **Shirreffs SM**, **Maughan RJ**. Blood-brain barrier integrity may be threatened by exercise in a warm environment. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 288: R1689–R1694, 2005. doi: 10.1152/ajpregu.00676.2004.

591. **Kojima D**, **Nakamura T**, **Banno M**, **Umemoto Y**, **Kinoshita T**, **Ishida Y**, **Tajima F**. Head-out immersion in hot water increases serum BDNF in healthy males. *null* 34: 834–839, 2018. doi: 10.1080/02656736.2017.1394502.

592. **Shepley BR**, **Ainslie PN**, **Hoiland RL**, **Donnelly J**, **Sekhon MS**, **Zetterberg H**, **Blennow K**, **Bain AR**. Negligible influence of moderate to severe hyperthermia on blood-brain barrier permeability and neuronal parenchymal integrity in healthy men. *Journal of Applied Physiology* 130: 792–800, 2021. doi: 10.1152/japplphysiol.00645.2020.

593. **Glazachev OS**, **Zapara MA**, **Dudnik EN**, **Samartseva VG**, **Susta D**. Repeated hyperthermia exposure increases circulating Brain Derived Neurotrophic Factor levels which is associated with improved quality of life, and reduced anxiety: A randomized controlled trial. *Journal of Thermal Biology* 89: 102482, 2020. doi: 10.1016/j.jtherbio.2019.102482.

594. **Glazachev OS**, **Zapara MA**, **Kryzhanovskaya SY**, **Dudnik EN**, **Yumatov EA**, **Susta D**. Whole-body repeated hyperthermia increases irisin and brain-derived neurotrophic factor: A randomized controlled trial. *Journal of Thermal Biology* 101: 103067, 2021. doi: 10.1016/j.jtherbio.2021.103067.

595. **Goekint M**, **Roelands B**, **Heyman E**, **Njemini R**, **Meeusen R**. Influence of citalopram and environmental temperature on exercise-induced changes in BDNF. *Neuroscience Letters* 494: 150–154, 2011. doi: 10.1016/j.neulet.2011.03.001.

596. **Carter HH**, **Spence AL**, **Pugh CJA**, **Ainslie P**, **Naylor LH**, **Green DJ**. Cardiovascular responses to water immersion in humans: impact on cerebral perfusion. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 306: R636–R640, 2014. doi: 10.1152/ajpregu.00516.2013.

597. **Inaba Y**, **Chen JA**, **Bergmann SR**. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The International Journal of Cardiovascular Imaging* 26: 631–640, 2010. doi: 10.1007/s10554-010-9616-1.

598. **Whelton PK**. The Elusiveness of Population-Wide High Blood Pressure Control. *Annu Rev Public Health* 36: 109–130, 2015. doi: 10.1146/annurev-publhealth-031914-122949.

599. **Crim MT**, **Yoon SS**, **Ortiz E**, **Wall HK**, **Schober S**, **Gillespie C**, **Sorlie P**, **Keenan N**, **Labarthe D**, **Hong Y**. National surveillance definitions for hypertension prevalence and control among adults. *Circulation: Cardiovascular Quality and Outcomes* 5: 343–351, 2012.

600. **John KA**, **Cogswell ME**, **Campbell NR**, **Nowson CA**, **Legetic B**, **Hennis AJM**, **Patel SM**. Accuracy and Usefulness of Select Methods for Assessing Complete Collection of 24-Hour Urine: A Systematic Review. *The Journal of Clinical Hypertension* 18: 456–467, 2016. doi: 10.1111/jch.12763.

601. **Lambers Heerspink HJ**, **Brantsma AH**, **de Zeeuw D**, **Bakker SJL**, **de Jong PE**, **Gansevoort RT**, **for the PREVEND Study Group**. Albuminuria Assessed From First-Morning-Void Urine Samples Versus 24-Hour Urine Collections as a Predictor of Cardiovascular Morbidity and Mortality. *American Journal of Epidemiology* 168: 897–905, 2008. doi: 10.1093/aje/kwn209.

602. **Perloff D**, **Sokolow M**, **Cowan R**. The Prognostic Value of Ambulatory Blood Pressures. *JAMA* 249: 2792–2798, 1983. doi: 10.1001/jama.1983.03330440030027.

603. **Perloff D**, **Sokolow M**, **Cowan R**, **Juster R**. Prognostic value of ambulatory blood pressure measurements: further analyses. *J Hypertens Suppl* 7: S3-10, 1989.

604. **Verdecchia P**, **Porcellati C**, **Schillaci G**, **Borgioni C**, **Ciucci A**, **Battistelli M**, **Guerrieri M**, **Gatteschi C**, **Zampi I**, **Santucci A**, **Santucci C**, **Reboldi G**. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension* 24: 793–801, 1994. doi: 10.1161/01.HYP.24.6.793.

605. **Verdecchia P**, **Schillaci G**, **Borgioni C**, **Ciucci A**, **Pede S**, **Porcellati C**. Ambulatory Pulse Pressure. *Hypertension* 32: 983–988, 1998. doi: 10.1161/01.HYP.32.6.983.

606. **Verdecchia P**, **Schillaci G**, **Borgioni C**, **Ciucci A**, **Porcellati C**. Prognostic Significance of the White Coat Effect. *Hypertension* 29: 1218–1224, 1997. doi: 10.1161/01.HYP.29.6.1218.

607. **Khattar RS**, **Senior R**, **Lahiri A**. Cardiovascular Outcome in White-Coat Versus Sustained Mild Hypertension. *Circulation* 98: 1892–1897, 1998. doi: 10.1161/01.CIR.98.18.1892.

608. **Zweiker R**, **Eber B**, **Schumacher M**, **Toplak H**, **Klein W**. "Non-dipping" related to cardiovascular events in essential hypertensive patients. *Acta Med Austriaca* 21: 86–89, 1994.

609. **Verdecchia P**, **Schillaci G**, **Gatteschi C**, **Zampi I**, **Battistelli M**, **Bartoccini C**, **Porcellati C**. Blunted nocturnal fall in blood pressure in hypertensive women with future

cardiovascular morbid events. *Circulation* 88: 986–992, 1993. doi: 10.1161/01.CIR.88.3.986.

610. **Verdecchia P**, **Schillaci G**, **Porcellati C**. Dippers versus non-dippers. *J Hypertens Suppl* 9: S42-4, 1991.

611. **Higashi Yukihito**, **Nakagawa Keigo**, **Kimura Masashi**, **Noma Kensuke**, **Hara Keiko**, **Sasaki Satoshi**, **Goto Chikara**, **Oshima Tetsuya**, **Chayama Kazuaki**, **Yoshizumi Masao**. Circadian variation of blood pressure and endothelial function in patients with essential hypertension. *Journal of the American College of Cardiology* 40: 2039–2043, 2002. doi: 10.1016/S0735-1097(02)02535-4.

612. **Farquhar William B.**, **Edwards David G.**, **Jurkovitz Claudine T.**, **Weintraub William S.** Dietary Sodium and Health. *Journal of the American College of Cardiology* 65: 1042–1050, 2015. doi: 10.1016/j.jacc.2014.12.039.

613. **Jones DW**. Dietary Sodium and Blood Pressure. *Hypertension* 43: 932–935, 2004. doi: 10.1161/01.HYP.0000126610.89002.c6.

614. **Verhave JC**, **Hillege HL**, **Burgerhof JGM**, **Janssen WMT**, **Gansevoort RT**, **Navis GJ**, **de Zeeuw D**, **de Jong PE**, **The PREVEND study group**. Sodium intake affects urinary albumin excretion especially in overweight subjects. *Journal of Internal Medicine* 256: 324–330, 2004. doi: 10.1111/j.1365-2796.2004.01390.x.

615. **du Cailar G**, **Ribstein J**, **Mimran A**. Dietary sodium and target organ damage in essential hypertension. *American Journal of Hypertension* 15: 222–229, 2002. doi: 10.1016/S0895-7061(01)02287-7.

616. **Shykoff BE**, **Naqvi SS**, **Menon AS**, **Slutsky AS**. Respiratory sinus arrhythmia in dogs. Effects of phasic afferents and chemostimulation. *J Clin Invest* 87: 1621–1627, 1991. doi: 10.1172/JCI115176.

617. **Brown TE**, **Beightol LA**, **Koh J**, **Eckberg DL**. Important influence of respiration on human R-R interval power spectra is largely ignored. *Journal of Applied Physiology* 75: 2310–2317, 1993. doi: 10.1152/jappl.1993.75.5.2310.

618. **Cooke WH**, **Cox JF**, **Diedrich AM**, **Taylor JA**, **Beightol LA**, **Ames JE**, **Hoag JB**, **Seidel H**, **Eckberg DL**. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *American Journal of Physiology-Heart and Circulatory Physiology* 274: H709–H718, 1998. doi: 10.1152/ajpheart.1998.274.2.H709.

619. **Pitzalis MV**, **Mastropasqua F**, **Massari F**, **Forleo C**, **Di Maggio M**, **Passantino A**, **Colombo R**, **Di Biase M**, **Rizzon P**. Short- and long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. *Cardiovascular Research* 32: 226–233, 1996. doi: 10.1016/0008-6363(96)00086-7.

620. **Ziemssen T**, **Gasch J**, **Ruediger H**. Influence of ECG Sampling Frequency on Spectral Analysis of RR Intervals and Baroreflex Sensitivity Using the EUROBAVAR Data set. *Journal of Clinical Monitoring and Computing* 22: 159–168, 2008. doi: 10.1007/s10877-008-9117-0.

621. **Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology**. Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use [Online]. [date unknown]. https://www.ahajournals.org/doi/full/10.1161/01.CIR.93.5.1043 [15 Mar. 2022].

622. **Lorenzo S**, **Halliwill JR**, **Sawka MN**, **Minson CT**. Heat acclimation improves exercise performance. *Journal of Applied Physiology* 109: 1140–1147, 2010. doi: 10.1152/japplphysiol.00495.2010.

623. **Elliott JE**, **Duke JW**, **Hawn JA**, **Halliwill JR**, **Lovering AT**. Increased cardiac output, not pulmonary artery systolic pressure, increases intrapulmonary shunt in healthy humans breathing room air and 40% O2. *The Journal of Physiology* 592: 4537–4553, 2014. doi: 10.1113/jphysiol.2014.274829.

624. **Stout RL**, **Wessel HU**, **Paul MH**. Pulmonary blood flow determined by continuous analysis of pulmonary N2O exchange. *Journal of Applied Physiology* 38: 913–918, 1975. doi: 10.1152/jappl.1975.38.5.913.

625. **Gan K**, **Nishi I**, **Chin I**, **Slutsky A**. On-line determination of pulmonary blood flow using respiratory inert gas analysis. *IEEE Transactions on Biomedical Engineering* 40: 1250–1259, 1993.

626. **Johnson BD**, **Beck KC**, **Proctor DN**, **Miller J**, **Dietz NM**, **Joyner MJ**. Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. *Journal of Applied Physiology* 88: 1650–1658, 2000. doi: 10.1152/jappl.2000.88.5.1650.

627. **Triebwasser J**, **Johnson R**, **Burpo R**, **Campbell J**, **Reardon W**, **Blomqvist C**. Noninvasive determination of cardiac output by a modified acetylene rebreathing procedure utilizing mass spectrometer measurements. *Aviat Space Environ Med* 48: 203–209, 1977.

628. **Yeh MP**, **Gardner RM**, **Adams TD**, **Yanowitz FG**. Computerized determination of pneumotachometer characteristics using a calibrated syringe. *Journal of Applied Physiology* 53: 280–285, 1982. doi: 10.1152/jappl.1982.53.1.280.

629. **Laurent S**, **Cockcroft J**, **Van Bortel L**, **Boutouyrie P**, **Giannattasio C**, **Hayoz D**, **Pannier B**, **Vlachopoulos C**, **Wilkinson I**, **Struijker-Boudier H**, **on behalf of the European Network for Non-invasive Investigation of Large Arteries**. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European Heart Journal* 27: 2588–2605, 2006. doi: 10.1093/eurheartj/ehl254.

630. **O'Rourke MF**, **Staessen JA**, **Vlachopoulos C**, **Duprez D**, **Plante G érard E**. Clinical applications of arterial stiffness; definitions and reference values. *American Journal of Hypertension* 15: 426–444, 2002. doi: 10.1016/S0895-7061(01)02319-6.

631. **Adji A**, **O'Rourke MF**, **Namasivayam M**. Arterial Stiffness, Its Assessment, Prognostic Value, and Implications for Treatment. *American Journal of Hypertension* 24: 5–17, 2011. doi: 10.1038/ajh.2010.192.

632. **Van Bortel LM**, **Laurent S**, **Boutouyrie P**, **Chowienczyk P**, **Cruickshank JK**, **De Backer T**, **Filipovsky J**, **Huybrechts S**, **Mattace-Raso FUS**, **Protogerou AD**, **Schillaci G**, **Segers P**, **Vermeersch S**, **Weber T**, **on behalf of the Artery Society the ES of HWG on VS and F and the EN for N**. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity [Online]. *Journal of Hypertension* 30, 2012. https://journals.lww.com/jhypertension/Fulltext/2012/03000/Expert_consensus_document_on_the_measurement_of.1.aspx.

633. **Avolio AP**, **Deng FQ**, **Li WQ**, **Luo YF**, **Huang ZD**, **Xing LF**, **O'Rourke MF**. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 71: 202–210, 1985. doi: 10.1161/01.CIR.71.2.202.

634. **Butlin M**, **Qasem A**, **Battista F**, **Bozec E**, **McEniery CM**, **Millet-Amaury E**, **Pucci G**, **Wilkinson IB**, **Schillaci G**, **Boutouyrie P**, **Avolio AP**. Carotid-femoral pulse wave velocity assessment using novel cuff-based techniques: comparison with tonometric measurement [Online]. *Journal of Hypertension* 31, 2013. https://journals.lww.com/jhypertension/Fulltext/2013/11000/Carotid_femoral_pulse_wave_velocity_assessment.21.aspx.

635. **Gedikli O**, **Ozturk S**, **Yilmaz H**, **Baykan M**, **Kiris A**, **Durmus I**, **Baltaci D**, **Karahan C**, **Celik S**. Relationship Between Arterial Stiffness and Myocardial Damage in Patients With Newly Diagnosed Essential Hypertension. *American Journal of Hypertension* 21: 989–993, 2008. doi: 10.1038/ajh.2008.235.

636. **Rossi SH**, **McQuarrie EP**, **Miller WH**, **Mackenzie RM**, **Dymott JA**, **Moreno MU**, **Taurino C**, **Miller AM**, **Neisius U**, **Berg GA**, **Valuckiene Z**, **Hannay JA**, **Dominiczak AF**, **Delles C**. Impaired renal function impacts negatively on vascular stiffness in patients with coronary artery disease. *BMC Nephrology* 14: 173, 2013. doi: 10.1186/1471-2369-14-173.

637. **Cruickshank K**, **Riste L**, **Anderson SG**, **Wright JS**, **Dunn G**, **Gosling RG**. Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance. *Circulation* 106: 2085–2090, 2002. doi: 10.1161/01.CIR.0000033824.02722.F7.

638. **Chirinos JA**, **Zambrano JP**, **Chakko S**, **Veerani A**, **Schob A**, **Willens HJ**, **Perez G**, **Mendez AJ**. Aortic Pressure Augmentation Predicts Adverse Cardiovascular Events in

Patients With Established Coronary Artery Disease. *Hypertension* 45: 980–985, 2005. doi: 10.1161/01.HYP.0000165025.16381.44.

639. **Nichols WW**, **Denardo SJ**, **Davidson JB**, **Huo T**, **Bairey Merz CN**, **Pepine CJ**. Association of aortic stiffness and wave reflections with coronary flow reserve in women without obstructive coronary artery disease: An ancillary study from the National Heart, Lung, and Blood Institute–sponsored Women's Ischemia Syndrome Evaluation (WISE). *American Heart Journal* 170: 1243–1254, 2015. doi: 10.1016/j.ahj.2015.08.019.

640. **Borg G**. Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine* 2: 92–98, 1970.

641. **Taylor HL**, **Buskirk E**, **Henschel A**. Maximal Oxygen Intake as an Objective Measure of Cardio-Respiratory Performance. *Journal of Applied Physiology* 8: 73–80, 1955. doi: 10.1152/jappl.1955.8.1.73.

642. **Poole DC**, **Jones AM**. Measurement of the maximum oxygen uptake V̇o2max: V̇o2peak is no longer acceptable. *Journal of Applied Physiology* 122: 997–1002, 2017. doi: 10.1152/japplphysiol.01063.2016.

643. **Astorino TA**. Alterations in VO2max and the VO2 plateau with manipulation of sampling interval. *Clinical Physiology and Functional Imaging* 29: 60–67, 2009. doi: 10.1111/j.1475-097X.2008.00835.x.

644. **Hill DW**, **Stephens LP**, **Blumoff-Ross SA**, **Poole DC**, **Smith JC**. Effect of sampling strategy on measures of V̇O2peak obtained using commercial breath-by-breath systems. *European Journal of Applied Physiology* 89: 564–569, 2003. doi: 10.1007/s00421-003-0843-1.

645. **Kenny GP**, **Giesbrecht GG**, **Thoden JS**. A comparison of human thermoregulatory response following dynamic exercise and warm-water immersion. *European Journal of Applied Physiology and Occupational Physiology* 74: 336–341, 1996. doi: 10.1007/BF02226930.

646. **KARVONEN M**, **KENTALA E**, **MUSTALA O**. The effects of training on heart rate; a longitudinal study. *Ann Med Exp Biol Fenn* 35: 307–315, 1957.

647. **Wegmann M**, **Hecksteden A**, **Poppendieck W**, **Steffen A**, **Kraushaar J**, **Morsch A**, **Meyer T**. Postexercise Hypotension as a Predictor for Long-Term Training-Induced Blood Pressure Reduction: A Large-Scale Randomized Controlled Trial. *Clinical Journal of Sport Medicine* 28: 509–515, 2018. doi: 10.1097/JSM.0000000000000475.

648. **Hecksteden A**, **Grütters T**, **Meyer T**. Association Between Postexercise Hypotension and Long-term Training-Induced Blood Pressure Reduction: A Pilot Study [Online]. *Clinical Journal of Sport Medicine* 23, 2013. https://journals.lww.com/cjsportsmed/Fulltext/2013/01000/Association_Between_Postexercise_Hypotension_and.8.aspx.

649. **Liu S**, **Goodman J**, **Nolan R**, **Lacombe S**, **Thomas SG**. Blood pressure responses to acute and chronic exercise are related in prehypertension. *Medicine & Science in Sports & Exercise* 44: 1644–1652, 2012.

650. **Azevêdo LM**, **Brito LC de**, **Peçanha T**, **Fecchio RY**, **Rezende RA**, **da Silva GV**, **Pio-Abreu A**, **Mion Junior D**, **Halliwill JR**, **Forjaz CL de M**. Can blood pressure decrease after maximal exercise test predict the blood pressure lowering effect of aerobic training in treated hypertensive men? .

651. **Sung K**, **Ryu S**, **Lee J**, **Lee SH**, **Cheong E**, **Hyun Y**, **Lee K**, **Kim H**, **Byrne CD**. Urine Albumin/Creatinine Ratio Below 30 mg/g is a Predictor of Incident Hypertension and Cardiovascular Mortality. *Journal of the American Heart Association* 5: e003245, [date unknown]. doi: 10.1161/JAHA.116.003245.

652. **Parving H-H**, **Persson F**, **Rossing P**. Microalbuminuria: a parameter that has changed diabetes care. *Diabetes research and clinical practice* 107: 1–8, 2015.

653. **Duggal V**, **Thomas I**, **Montez-Rath ME**, **Chertow GM**, **Kurella Tamura M**. National Estimates of CKD Prevalence and Potential Impact of Estimating Glomerular Filtration Rate Without Race [Online]. *Journal of the American Society of Nephrology* 32, 2021. https://journals.lww.com/jasn/Fulltext/2021/06000/National_Estimates_of_CKD_Prevalence_and_Potential.19.aspx.

654. **Kirk I. Erickson**, **Ruchika Shaurya Prakash**, **Michelle W. Voss**, **Laura Chaddock**, **Susie Heo**, **Molly McLaren**, **Brandt D. Pence**, **Stephen A. Martin**, **Victoria J. Vieira**, **Jeffrey A. Woods**, **Edward McAuley**, **Arthur F. Kramer**. Brain-Derived Neurotrophic Factor Is Associated with Age-Related Decline in Hippocampal Volume. *J Neurosci* 30: 5368, 2010. doi: 10.1523/JNEUROSCI.6251-09.2010.

655. **Grundman M**, **Sencakova D**, **Jack CR**, **Petersen RC**, **Kim HT**, **Schultz A**, **Weiner MF**, **DeCarli C**, **DeKosky ST**, **Van Dyck C**. Brain MRI hippocampal volume and prediction of clinical status in a mild cognitive impairment trial. *Journal of Molecular Neuroscience* 19: 23–27, 2002.

656. **Erickson KI**, **Prakash RS**, **Voss MW**, **Chaddock L**, **Hu L**, **Morris KS**, **White SM**, **Wójcicki TR**, **McAuley E**, **Kramer AF**. Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus* 19: 1030–1039, 2009. doi: 10.1002/hipo.20547.

657. **Lakoski SG**, **Cushman M**, **Siscovick DS**, **Blumenthal RS**, **Palmas W**, **Burke G**, **Herrington DM**. The relationship between inflammation, obesity and risk for hypertension in the Multi-Ethnic Study of Atherosclerosis (MESA). *Journal of Human Hypertension* 25: 73–79, 2011. doi: 10.1038/jhh.2010.91.

658. **Sandra Weintraub**, **Sureyya S. Dikmen**, **Robert K. Heaton**, **David S. Tulsky**, **Philip D. Zelazo**, **Patricia J. Bauer**, **Noelle E. Carlozzi**, **Jerry Slotkin**, **David Blitz**, **Kathleen Wallner-Allen**, **Nathan A. Fox**, **Jennifer L. Beaumont**, **Dan Mungas**, **Cindy J. Nowinski**, **Jennifer Richler**, **Joanne A. Deocampo**, **Jacob E. Anderson**, **Jennifer J.**

Manly, **Beth Borosh**, **Richard Havlik**, **Kevin Conway**, **Emmeline Edwards**, **Lisa Freund**, **Jonathan W. King**, **Claudia Moy**, **Ellen Witt**, **Richard C. Gershon**. Cognition assessment using the NIH Toolbox. *Neurology* 80: S54, 2013. doi: 10.1212/WNL.0b013e3182872ded.

659. **Heaton RK**, **Akshoomoff N**, **Tulsky D**, **Mungas D**, **Weintraub S**, **Dikmen S**, **Beaumont J**, **Casaletto KB**, **Conway K**, **Slotkin J**, **Gershon R**. Reliability and Validity of Composite Scores from the NIH Toolbox Cognition Battery in Adults. *Journal of the International Neuropsychological Society* 20: 588–598, 2014. doi: 10.1017/S1355617714000241.

660. **Tulsky DS**, **Carlozzi N**, **Chiaravalloti ND**, **Beaumont JL**, **Kisala PA**, **Mungas D**, **Conway K**, **Gershon R**. NIH Toolbox Cognition Battery (NIHTB-CB): List Sorting Test to Measure Working Memory. *Journal of the International Neuropsychological Society* 20: 599–610, 2014. doi: 10.1017/S135561771400040X.

661. **Tulsky DS**, **Carlozzi NE**, **Chevalier N**, **Espy KA**, **Beaumont JL**, **Mungas D**. V. NIH TOOLBOX COGNITION BATTERY (CB): MEASURING WORKING MEMORY. *Monographs of the Society for Research in Child Development* 78: 70–87, 2013. doi: 10.1111/mono.12035.

662. **Bauer PJ**, **Dikmen SS**, **Heaton RK**, **Mungas D**, **Slotkin J**, **Beaumont JL**. III. NIH TOOLBOX COGNITION BATTERY (CB): MEASURING EPISODIC MEMORY. *Monographs of the Society for Research in Child Development* 78: 34–48, 2013. doi: 10.1111/mono.12033.

663. **Mungas D**, **Heaton R**, **Tulsky D**, **Zelazo PD**, **Slotkin J**, **Blitz D**, **Lai J-S**, **Gershon R**. Factor Structure, Convergent Validity, and Discriminant Validity of the NIH Toolbox Cognitive Health Battery (NIHTB-CHB) in Adults. *Journal of the International Neuropsychological Society* 20: 579–587, 2014. doi: 10.1017/S1355617714000307.

664. **Dikmen SS**, **Bauer PJ**, **Weintraub S**, **Mungas D**, **Slotkin J**, **Beaumont JL**, **Gershon R**, **Temkin NR**, **Heaton RK**. Measuring Episodic Memory Across the Lifespan: NIH Toolbox Picture Sequence Memory Test. *Journal of the International Neuropsychological Society* 20: 611–619, 2014. doi: 10.1017/S1355617714000460.

665. **Tombaugh TN**, **McIntyre NJ**. The Mini-Mental State Examination: A Comprehensive Review. *Journal of the American Geriatrics Society* 40: 922–935, 1992. doi: 10.1111/j.1532-5415.1992.tb01992.x.

666. **Margit L. Bleecker**, **Karen Bolla-Wilson**, **Claudia Kawas**, **Jacqueline Agnew**. Age-specific norms for the Mini-Mental State Exam. *Neurology* 38: 1565, 1988. doi: 10.1212/WNL.38.10.1565.

667. **ESCOBAR JI**, **BURNAM A**, **KARNO M**, **FORSYTHE A**, **LANDSVERK J**, **GOLDING JM**. Use of the Mini-Mental State Examination (MMSE) in a Community Population of Mixed Ethnicity: Cultural and Linguistic Artifacts [Online]. *The Journal of*

Nervous and Mental Disease 174, 1986. https://journals.lww.com/jonmd/Fulltext/1986/10000/Use_of_the_Mini_Mental_State_Examination__MMSE__in.5.aspx.

668. **Roselli F**, **Tartaglione B**, **Federico F**, **Lepore V**, **Defazio G**, **Livrea P**. Rate of MMSE score change in Alzheimer's disease: Influence of education and vascular risk factors. *Clinical Neurology and Neurosurgery* 111: 327–330, 2009. doi: 10.1016/j.clineuro.2008.10.006.

669. **O'connor DW**, **Pollitt PA**, **Treasure FP**, **Brook CPB**, **Reiss BB**. The influence of education, social class and sex on Mini-Mental State scores. *Psychological Medicine* 19: 771–776, 1989. doi: 10.1017/S0033291700024375.

670. **Dong Y**, **Sharma VK**, **Chan BP-L**, **Venketasubramanian N**, **Teoh HL**, **Seet RCS**, **Tanicala S**, **Chan YH**, **Chen C**. The Montreal Cognitive Assessment (MoCA) is superior to the Mini-Mental State Examination (MMSE) for the detection of vascular cognitive impairment after acute stroke. *Journal of the Neurological Sciences* 299: 15–18, 2010. doi: 10.1016/j.jns.2010.08.051.

671. **Ercoli LM**, **Siddarth P**, **Dunkin JJ**, **Bramen J**, **Small GW**. MMSE Items Predict Cognitive Decline in Persons with Genetic Risk for Alzheimer's Disease. *J Geriatr Psychiatry Neurol* 16: 67–73, 2003. doi: 10.1177/0891988703016002001.

672. **Guerrero-Berroa E**, **Luo X**, **Schmeidler J**, **Rapp MA**, **Dahlman K**, **Grossman HT**, **Haroutunian V**, **Beeri MS**. The MMSE orientation for time domain is a strong predictor of subsequent cognitive decline in the elderly. *International Journal of Geriatric Psychiatry* 24: 1429–1437, 2009. doi: 10.1002/gps.2282.

673. **Cardoso S**, **Barros R**, **Marôco J**, **de Mendonça A**, **Guerreiro M**. Different MMSE domains are associated to cognitive decline and education. .

674. **Bruno RM**, **Palagini L**, **Gemignani A**, **Virdis A**, **Di Giulio A**, **Ghiadoni L**, **Riemann D**, **Taddei S**. Poor sleep quality and resistant hypertension. *Sleep Medicine* 14: 1157–1163, 2013. doi: 10.1016/j.sleep.2013.04.020.

675. **Fiorentini A**, **Valente R**, **Perciaccante A**, **Tubani L**. Sleep's quality disorders in patients with hypertension and type 2 diabetes mellitus. *International Journal of Cardiology* 114: E50–E52, 2007. doi: 10.1016/j.ijcard.2006.07.213.

676. **Zhang H**, **Li Y**, **Zhao X**, **Mao Z**, **Abdulai T**, **Liu X**, **Tu R**, **Wang Y**, **Qian X**, **Jiang J**, **Tian Z**, **Luo Z**, **Dong X**, **Wang C**, **Bie R**. The association between PSQI score and hypertension in a Chinese rural population: the Henan Rural Cohort Study. *Sleep Medicine* 58: 27–34, 2019. doi: 10.1016/j.sleep.2019.03.001.

677. **Rana BK**, **Panizzon MS**, **Franz CE**, **Spoon KM**, **Jacobson KC**, **Xian H**, **Ancoli-Israel S**, **Lyons M**, **Kremen WS**. Association of Sleep Quality on Memory-Related Executive

Functions in Middle Age. *Journal of the International Neuropsychological Society* 24: 67–76, 2018. doi: 10.1017/S1355617717000637.

678. **Spira AP**, **Chen-Edinboro LP**, **Wu MN**, **Yaffe K**. Impact of sleep on the risk of cognitive decline and dementia [Online]. *Current Opinion in Psychiatry* 27, 2014. https://journals.lww.com/co-psychiatry/Fulltext/2014/11000/Impact_of_sleep_on_the_risk_of_cognitive_decline.17.aspx.

679. **Brant-Zawadzki M**, **Gillan GD**, **Nitz WR**. MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence--initial experience in the brain. *Radiology* 182: 769–775, 1992. doi: 10.1148/radiology.182.3.1535892.

680. **Tournier J-D**, **Calamante F**, **Gadian DG**, **Connelly A**. Direct estimation of the fiber orientation density function from diffusion-weighted MRI data using spherical deconvolution. *NeuroImage* 23: 1176–1185, 2004. doi: 10.1016/j.neuroimage.2004.07.037.

681. **Harms MP**, **Somerville LH**, **Ances BM**, **Andersson J**, **Barch DM**, **Bastiani M**, **Bookheimer SY**, **Brown TB**, **Buckner RL**, **Burgess GC**, **Coalson TS**, **Chappell MA**, **Dapretto M**, **Douaud G**, **Fischl B**, **Glasser MF**, **Greve DN**, **Hodge C**, **Jamison KW**, **Jbabdi S**, **Kandala S**, **Li X**, **Mair RW**, **Mangia S**, **Marcus D**, **Mascali D**, **Moeller S**, **Nichols TE**, **Robinson EC**, **Salat DH**, **Smith SM**, **Sotiropoulos SN**, **Terpstra M**, **Thomas KM**, **Tisdall MD**, **Ugurbil K**, **van der Kouwe A**, **Woods RP**, **Zöllei L**, **Van Essen DC**, **Yacoub E**. Extending the Human Connectome Project across ages: Imaging protocols for the Lifespan Development and Aging projects. *NeuroImage* 183: 972–984, 2018. doi: 10.1016/j.neuroimage.2018.09.060.

682. **Wahlund LO**, **Barkhof F**, **Fazekas F**, **Bronge L**, **Augustin M**, **Sjögren M**, **Wallin A**, **Ader H**, **Leys D**, **Pantoni L**, **Pasquier F**, **Erkinjuntti T**, **Scheltens P**. A New Rating Scale for Age-Related White Matter Changes Applicable to MRI and CT. *Stroke* 32: 1318–1322, 2001. doi: 10.1161/01.STR.32.6.1318.

683. **Scheltens P**, **Barkhof F**, **Leys D**, **Pruvo JP**, **Nauta JJP**, **Vermersch P**, **Steinling M**, **Valk J**. A semiquantative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. *Journal of the Neurological Sciences* 114: 7–12, 1993. doi: 10.1016/0022-510X(93)90041-V.

684. **Hutchins PM**, **Lynch CD**, **Cooney PT**, **Curseen KA**. The microcirculation in experimental hypertension and aging. *Cardiovascular Research* 32: 772–780, 1996. doi: 10.1016/S0008-6363(96)00136-8.

685. **Middleton JP**, **Pun PH**. Hypertension, chronic kidney disease, and the development of cardiovascular risk: a joint primacy. *Kidney International* 77: 753–755, 2010. doi: 10.1038/ki.2010.19.

686. **Bianchi S**, **Bigazzi R**, **Campese VM**. Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. *American journal of kidney diseases* 34: 973–995, 1999.

687. **Pedrinelli R**, **Dell'omo G**, **Catapano G**, **Giampietro O**, **Carmassi F**, **Matteucci E**, **Talarico L**, **Morale M**, **de Negri F**, **di Bello V**, **Melillo E**. Microalbuminuria and endothelial dysfunction in essential hypertension. *The Lancet* 344: 14–18, 1994. doi: 10.1016/S0140-6736(94)91047-2.

688. **Parving H-H**, **Mogensen C**, **Evrin P-E**. Increased urinary albumin-excretion rate in benign essential hypertension. *The Lancet* 303: 1190–1192, 1974.

689. **Gerber LM**, **Shmukler C**, **Alderman MH**. Differences in urinary albumin excretion rate between normotensive and hypertensive, white and nonwhite subjects. *Archives of internal medicine* 152: 373–377, 1992.

690. **Høegholm A**, **Bang LE**, **Kristensen KS**, **Nielsen JW**, **Holm J**. Microalbuminuria in 411 untreated individuals with established hypertension, white coat hypertension, and normotension. *Hypertension* 24: 101–105, 1994.

691. **Bigazzi R**, **Bianchi S**, **Campese VM**, **Baldari G**. Prevalence of microalbuminuria in a large population of patients with mild to moderate essential hypertension. *Nephron* 61: 94–97, 1992.

692. **James PA**, **Oparil S**, **Carter BL**, **Cushman WC**, **Dennison-Himmelfarb C**, **Handler J**, **Lackland DT**, **LeFevre ML**, **MacKenzie TD**, **Ogedegbe O**. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *Jama* 311: 507–520, 2014.

693. **Erley CM**, **Haefele U**, **Heyne N**, **Braun N**, **Risler T**. Microalbuminuria in essential hypertension. Reduction by different antihypertensive drugs. *Hypertension* 21: 810–815, 1993.

694. **Pedersen E**, **Mogensen C**. Effect of antihypertensive treatment on urinary albumin excretion, glomerular filtration rate, and renal plasma flow in patients with essential hypertension. *Scandinavian Journal of Clinical and Laboratory Investigation* 36: 231–237, 1976.

695. **Pasternak M**, **Liu P**, **Quinn R**, **Elliott M**, **Harrison TG**, **Hemmelgarn B**, **Lam N**, **Ronksley P**, **Tonelli M**, **Ravani P**. Association of Albuminuria and Regression of Chronic Kidney Disease in Adults With Newly Diagnosed Moderate to Severe Chronic Kidney Disease. *JAMA Network Open* 5: e2225821–e2225821, 2022. doi: 10.1001/jamanetworkopen.2022.25821.

696. **Walther C**, **Gielen S**, **Hambrecht R**. The Effect of Exercise Training on Endothelial Function in Cardiovascular Disease in Humans [Online]. *Exercise and Sport Sciences*

Reviews 32, 2004. https://journals.lww.com/acsm-essr/Fulltext/2004/10000/The_Effect_of_Exercise_Training_on_Endothelial.2.aspx.

697. **Mancia G**, **Di Rienzo M**, **Parati G**. Ambulatory blood pressure monitoring use in hypertension research and clinical practice. *Hypertension* 21: 510–524, 1993. doi: 10.1161/01.HYP.21.4.510.

698. **Banegas JR**, **Ruilope LM**, **de la Sierra A**, **Vinyoles E**, **Gorostidi M**, **de la Cruz JJ**, **Ruiz-Hurtado G**, **Segura J**, **Rodríguez-Artalejo F**, **Williams B**. Relationship between Clinic and Ambulatory Blood-Pressure Measurements and Mortality. *N Engl J Med* 378: 1509–1520, 2018. doi: 10.1056/NEJMoa1712231.

699. **Minutolo R**, **Agarwal R**, **Borrelli S**, **Chiodini P**, **Bellizzi V**, **Nappi F**, **Cianciaruso B**, **Zamboli P**, **Conte G**, **Gabbai FB**, **De Nicola L**. Prognostic Role of Ambulatory Blood Pressure Measurement in Patients With Nondialysis Chronic Kidney Disease. *Archives of Internal Medicine* 171: 1090–1098, 2011. doi: 10.1001/archinternmed.2011.230.

700. **Park SK**, **Moon SY**, **Oh C-M**, **Ryoo J-H**, **Park MS**. High Normal Urine Albumin-to-Creatinine Ratio Predicts Development of Hypertension in Korean Men. *Circulation Journal* 78: 656–661, 2014. doi: 10.1253/circj.CJ-13-0745.

701. **Ferguson TW**, **Komenda P**, **Tangri N**. Cystatin C as a biomarker for estimating glomerular filtration rate [Online]. *Current Opinion in Nephrology and Hypertension* 24, 2015. https://journals.lww.com/co-nephrolhypertens/Fulltext/2015/05000/Cystatin_C_as_a_biomarker_for_estimating.15.aspx.

702. **Cardoso Jr CG**, **Gomides RS**, **Queiroz ACC**, **Pinto LG**, **da Silveira Lobo F**, **Tinucci T**, **Mion Jr D**, **de Moraes Forjaz CL**. Acute and chronic effects of aerobic and resistance exercise on ambulatory blood pressure. *Clinics* 65: 317–325, 2010.

703. **Cornelissen VA**, **Buys R**, **Smart NA**. Endurance exercise beneficially affects ambulatory blood pressure: a systematic review and meta-analysis. *Journal of hypertension* 31: 639–648, 2013.

704. **Saco-Ledo G**, **Valenzuela PL**, **Ruiz-Hurtado G**, **Ruilope LM**, **Lucia A**. Exercise reduces ambulatory blood pressure in patients with hypertension: a systematic review and meta-analysis of randomized controlled trials. *Journal of the American Heart Association* 9: e018487, 2020.

705. **Lopes S**, **Mesquita-Bastos J**, **Garcia C**, **Bertoquini S**, **Ribau V**, **Teixeira M**, **Ribeiro IP**, **Melo JB**, **Oliveira J**, **Figueiredo D**. Effect of exercise training on ambulatory blood pressure among patients with resistant hypertension: a randomized clinical trial. *JAMA cardiology* 6: 1317–1323, 2021.

706. **Cooper A**, **Moore L**, **McKenna J**, **Riddoch C**. What is the magnitude of blood pressure response to a programme of moderate intensity exercise? Randomised controlled trial

among sedentary adults with unmedicated hypertension. *British Journal of General Practice* 50: 958–962, 2000.

707. **Wijnen J**, **Kool M**, **Van Baak M**, **Kuipers H**, **De Haan C**, **Verstappen F**, **Boudier HS**, **Van Bortel L**. Effect of exercise training on ambulatory blood pressure. *International journal of sports medicine* 15: 10–15, 1994.

708. **Gilders RM**, **Voner C**, **Dudley GA**. Endurance training and blood pressure in normotensive and hypertensive adults. *Medicine and Science in Sports and Exercise* 21: 629–636, 1989.

709. **Marceau M**, **Kouame N**, **Lacourciere Y**, **Cleroux J**. Effects of different training intensities on 24-hour blood pressure in hypertensive subjects. *Circulation* 88: 2803–2811, 1993.

710. **Fagard RH**, **Cornelissen VA**. Effect of exercise on blood pressure control in hypertensive patients. *European journal of cardiovascular prevention and rehabilitation* 14: 12–17, 2007. doi: 10.1097/HJR.0b013e3280128bbb.

711. **Pescatello LS**, **Fargo AE**, **Leach Jr CN**, **Scherzer HH**. Short-term effect of dynamic exercise on arterial blood pressure. *Circulation* 83: 1557–1561, 1991.

712. **Pescatello LS**, **Guidry MA**, **Blanchard BE**, **Kerr A**, **Taylor AL**, **Johnson AN**, **Maresh CM**, **Rodriguez N**, **Thompson PD**. Exercise intensity alters postexercise hypotension. *Journal of hypertension* 22: 1881–1888, 2004.

713. **Quinn T**. Twenty-four hour, ambulatory blood pressure responses following acute exercise: impact of exercise intensity. *Journal of human hypertension* 14: 547–553, 2000.

714. **Swift PA**, **Markandu ND**, **Sagnella GA**, **He FJ**, **MacGregor GA**. Modest salt reduction reduces blood pressure and urine protein excretion in black hypertensives: a randomized control trial. *Hypertension* 46: 308–312, 2005.

715. **Suckling RJ**, **He FJ**, **Markandu ND**, **MacGregor GA**. Modest salt reduction lowers blood pressure and albumin excretion in impaired glucose tolerance and type 2 diabetes mellitus: a randomized double-blind trial. *Hypertension* 67: 1189–1195, 2016.

716. **He FJ**, **Marciniak M**, **Visagie E**, **Markandu ND**, **Anand V**, **Dalton RN**, **MacGregor GA**. Effect of Modest Salt Reduction on Blood Pressure, Urinary Albumin, and Pulse Wave Velocity in White, Black, and Asian Mild Hypertensives. *Hypertension* 54: 482–488, 2009. doi: 10.1161/HYPERTENSIONAHA.109.133223.

717. **Du Cailar G**, **Ribstein J**, **Mimran A**. Dietary sodium and target organ damage in essential hypertension. *American journal of hypertension* 15: 222–229, 2002.

718. **Seals DR**, **Tanaka H**, **Clevenger CM**, **Monahan KD**, **Reiling MJ**, **Hiatt WR**, **Davy KP**, **DeSouza CA**. Blood pressure reductions with exercise and sodium restriction in

postmenopausal women with elevated systolic pressure: role of arterial stiffness. *Journal of the American College of Cardiology* 38: 506–513, 2001.

719. **Bagrov AY**, **Lakatta EG**. The dietary sodium-blood pressure plot "stiffens." *Hypertension* 44: 22–24, 2004.

720. **D'Elia L**, **Galletti F**, **La Fata E**, **Sabino P**, **Strazzullo P**. Effect of dietary sodium restriction on arterial stiffness: systematic review and meta-analysis of the randomized controlled trials. *Journal of hypertension* 36: 734–743, 2018.

721. **Griffin KA**, **Polichnowski A**, **Litbarg N**, **Picken M**, **Venkatachalam MA**, **Bidani AK**. Critical Blood Pressure Threshold Dependence of Hypertensive Injury and Repair in a Malignant Nephrosclerosis Model. *Hypertension* 64: 801–807, 2014. doi: 10.1161/HYPERTENSIONAHA.114.03609.

722. **Karvetti R-L**, **Knuts L-R**. Validity of the 24-hour dietary recall. *Journal of the American Dietetic Association* 85: 1437–1442, 1985. doi: 10.1016/S0002-8223(21)03818-9.

723. **Wang TJ**, **Gona P**, **Larson MG**, **Levy D**, **Benjamin EJ**, **Tofler GH**, **Jacques PF**, **Meigs JB**, **Rifai N**, **Selhub J**, **Robins SJ**, **Newton-Cheh C**, **Vasan RS**. Multiple Biomarkers and the Risk of Incident Hypertension. *Hypertension* 49: 432–438, 2007. doi: 10.1161/01.HYP.0000256956.61872.aa.

724. **Debray A**, **Gravel H**, **Garceau L**, **Bartlett A-A**, **Chaseling GK**, **Barry H**, **Behzadi P**, **Ravanelli N**, **Iglesies-Grau J**, **Nigam A**. Finnish sauna bathing and vascular health of adults with coronary artery disease: a randomized controlled trial. *Journal of Applied Physiology* 135: 795–804, 2023.

725. **Eriksen BO**, **Stefansson VTN**, **Jenssen TG**, **Mathisen UD**, **Schei J**, **Solbu MD**, **Wilsgaard T**, **Melsom T**. Blood pressure and age-related GFR decline in the general population. *BMC Nephrology* 18: 77, 2017. doi: 10.1186/s12882-017-0496-7.

726. **Chen DC**, **McCallum W**, **Sarnak MJ**, **Ku E**. Intensive BP Control and eGFR Declines: Are These Events Due to Hemodynamic Effects and Are Changes Reversible? *Current Cardiology Reports* 22: 117, 2020. doi: 10.1007/s11886-020-01365-3.

727. **Zhang L**, **Wang Y**, **Xiong L**, **Luo Y**, **Huang Z**, **Yi B**. Exercise therapy improves eGFR, and reduces blood pressure and BMI in non-dialysis CKD patients: evidence from a meta-analysis. *BMC Nephrology* 20: 398, 2019. doi: 10.1186/s12882-019-1586-5.

728. **Palatini P**, **Dorigatti F**, **Saladini F**, **Benetti E**, **Mos L**, **Mazzer A**, **Zanata G**, **Garavelli G**, **Casiglia E**. Factors Associated With Glomerular Hyperfiltration in the Early Stage of Hypertension. *American Journal of Hypertension* 25: 1011–1016, 2012. doi: 10.1038/ajh.2012.73.

729. **Reboldi G**, **Verdecchia P**, **Fiorucci G**, **Beilin LJ**, **Eguchi K**, **Imai Y**, **Kario K**, **Ohkubo T**, **Pierdomenico SD**, **Schwartz JE**, **Wing L**, **Saladini F**, **Palatini P**. Glomerular

hyperfiltration is a predictor of adverse cardiovascular outcomes. *Kidney International* 93: 195–203, 2018. doi: 10.1016/j.kint.2017.07.013.

730. **Carter HH**, **Spence AL**, **Atkinson CL**, **Pugh CJA**, **Naylor LH**, **Green DJ**. Repeated core temperature elevation induces conduit artery adaptation in humans. *European Journal of Applied Physiology* 114: 859–865, 2014. doi: 10.1007/s00421-013-2817-2.

731. **Lee E**, **Kolunsarka I**, **Kostensalo J**, **Ahtiainen JP**, **Haapala EA**, **Willeit P**, **Kunutsor SK**, **Laukkanen JA**. Effects of regular sauna bathing in conjunction with exercise on cardiovascular function: a multi-arm, randomized controlled trial. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 323: R289–R299, 2022. doi: 10.1152/ajpregu.00076.2022.

732. **Stabouli S**, **Printza N**, **Zervas C**, **Dotis J**, **Chrysaidou K**, **Maliahova O**, **Antza C**, **Papachristou F**, **Kotsis V**. Comparison of the SphygmoCor XCEL device with applanation tonometry for pulse wave velocity and central blood pressure assessment in youth. *Journal of Hypertension* 37: 1, 2018. doi: 10.1097/HJH.0000000000001819.

733. **McGarity-Shipley EC**, **Schmitter SM**, **Williams JS**, **King TJ**, **McPhee IAC**, **Pyke KE**. The impact of repeated, local heating-induced increases in blood flow on lower limb endothelial function in young, healthy females. *European Journal of Applied Physiology* 121: 3017–3030, 2021. doi: 10.1007/s00421-021-04749-7.

734. **Oyama J**, **Kudo Y**, **Maeda T**, **Node K**, **Makino N**. Hyperthermia by bathing in a hot spring improves cardiovascular functions and reduces the production of inflammatory cytokines in patients with chronic heart failure. *Heart and Vessels* 28: 173–178, 2013. doi: 10.1007/s00380-011-0220-7.

735. **Julius S**. Transition from high cardiac output to elevated vascular resistance in hypertension. *American Heart Journal* 116: 600–606, 1988. doi: 10.1016/0002-8703(88)90557-1.

736. **Véras-Silva AS**, **Mattos KC**, **Gava NS**, **Brum PC**, **Negrão CE**, **Krieger EM**. Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats. *American Journal of Physiology-Heart and Circulatory Physiology* 273: H2627–H2631, 1997. doi: 10.1152/ajpheart.1997.273.6.H2627.

737. **Jennings G**, **Nelson L**, **Dewar E**, **Korner P**, **Esler M**, **Laufer E**. ANTIHYPERTENSIVE AND HEMODYNAMIC-EFFECTS OF ONE YEARS REGULAR EXERCISE. *Journal of Hypertension* 4: S659–S661, 1986.

738. **Jennings G**, **Nelson L**, **Nestel P**, **Esler M**, **Korner P**, **Burton D**, **Bazelmans J**. The effects of changes in physical activity on major cardiovascular risk factors, hemodynamics, sympathetic function, and glucose utilization in man: a controlled study of four levels of activity. *Circulation* 73: 30–40, 1986.

739. **Nelson L**, **Esler M**, **Jennings G**, **Korner P**. EFFECT OF CHANGING LEVELS OF PHYSICAL ACTIVITY ON BLOOD-PRESSURE AND HAEMODYNAMICS IN ESSENTIAL HYPERTENSION. *The Lancet* 328: 473–476, 1986. doi: 10.1016/S0140-6736(86)90354-5.

740. **O'Rourke MF**, **Mancia G**. Arterial stiffness [Online]. *Journal of Hypertension* 17, 1999. https://journals.lww.com/jhypertension/fulltext/1999/17010/arterial_stiffness.1.aspx.

741. **Franklin SS**. Arterial stiffness and hypertension: a two-way street? *Hypertension* 45: 349–351, 2005.

742. **McEniery CM**, **Wilkinson IB**, **Avolio AP**. AGE, HYPERTENSION AND ARTERIAL FUNCTION. *Clinical and Experimental Pharmacology and Physiology* 34: 665–671, 2007. doi: 10.1111/j.1440-1681.2007.04657.x.

743. **Guimarães GV**, **Ciolac EG**, **Carvalho VO**, **D'Avila VM**, **Bortolotto LA**, **Bocchi EA**. Effects of continuous vs. interval exercise training on blood pressure and arterial stiffness in treated hypertension. *Hypertension Research* 33: 627–632, 2010. doi: 10.1038/hr.2010.42.

744. **Scheltens P**, **De Strooper B**, **Kivipelto M**, **Holstege H**, **Chételat G**, **Teunissen CE**, **Cummings J**, **van der Flier WM**. Alzheimer's disease. *The Lancet* 397: 1577–1590, 2021. doi: 10.1016/S0140-6736(20)32205-4.

745. **Nandi A**, **Counts N**, **Chen S**, **Seligman B**, **Tortorice D**, **Vigo D**, **Bloom DE**. Global and regional projections of the economic burden of Alzheimer's disease and related dementias from 2019 to 2050: A value of statistical life approach. *eClinicalMedicine* 51, 2022. doi: 10.1016/j.eclinm.2022.101580.

746. **Patterson C**. World alzheimer report 2018. .

747. **Prince MJ**, **Wu F**, **Guo Y**, **Robledo LMG**, **O'Donnell M**, **Sullivan R**, **Yusuf S**. The burden of disease in older people and implications for health policy and practice. *The Lancet* 385: 549–562, 2015.

748. **Triantafyllou A**, **Ferreira JP**, **Kobayashi M**, **Micard E**, **Xie Y**, **Kearney-Schwartz A**, **Hossu G**, **Rossignol P**, **Bracard S**, **Benetos A**. Longer Duration of Hypertension and MRI Microvascular Brain Alterations Are Associated with Lower Hippocampal Volumes in Older Individuals with Hypertension. *Journal of Alzheimer's Disease* 74: 227–235, 2020. doi: 10.3233/JAD-190842.

749. **Kynast J**, **Lampe L**, **Luck T**, **Frisch S**, **Arelin K**, **Hoffmann K-T**, **Loeffler M**, **Riedel-Heller SG**, **Villringer A**, **Schroeter ML**. White matter hyperintensities associated with small vessel disease impair social cognition beside attention and memory. *J Cereb Blood Flow Metab* 38: 996–1009, 2018. doi: 10.1177/0271678X17719380.

750. **Alosco ML**, **Brickman AM**, **Spitznagel MB**, **Griffith EY**, **Narkhede A**, **Raz N**, **Cohen R**, **Sweet LH**, **Hughes J**, **Rosneck J**, **Gunstad J**. Independent and interactive effects of blood pressure and cardiac function on brain volume and white matter hyperintensities in heart failure. *Journal of the American Society of Hypertension* 7: 336–343, 2013. doi: 10.1016/j.jash.2013.04.011.

751. **Farooq Kamal**, **Cassandra Morrison**, **Josefina Maranzano**, **Yashar Zeighami**, **Mahsa Dadar**. White Matter Hyperintensity Trajectories in Patients With Progressive and Stable Mild Cognitive Impairment. *Neurology* 101: e815, 2023. doi: 10.1212/WNL.0000000000207514.

752. **L. C. Silbert**, **C. Nelson**, **D. B. Howieson**, **M. M. Moore**, **J. A. Kaye**. Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline. *Neurology* 71: 108, 2008. doi: 10.1212/01.wnl.0000316799.86917.37.

753. **Bracko O**, **Cruz Hernández JC**, **Park L**, **Nishimura N**, **Schaffer CB**. Causes and consequences of baseline cerebral blood flow reductions in Alzheimer's disease. *Journal of Cerebral Blood Flow & Metabolism* 41: 1501–1516, 2021.

754. **Korte N**, **Nortley R**, **Attwell D**. Cerebral blood flow decrease as an early pathological mechanism in Alzheimer's disease. *Acta Neuropathologica* 140: 793–810, 2020. doi: 10.1007/s00401-020-02215-w.

755. **Colcombe SJ**, **Erickson KI**, **Scalf PE**, **Kim JS**, **Prakash R**, **McAuley E**, **Elavsky S**, **Marquez DX**, **Hu L**, **Kramer AF**. Aerobic Exercise Training Increases Brain Volume in Aging Humans. *The Journals of Gerontology: Series A* 61: 1166–1170, 2006. doi: 10.1093/gerona/61.11.1166.

756. **Dao E**, **Barha CK**, **Best JR**, **Hsiung G-Y**, **Tam R**, **Liu-Ambrose T**. The Effect of Aerobic Exercise on White Matter Hyperintensity Progression May Vary by Sex. *Canadian Journal on Aging / La Revue canadienne du vieillissement* 38: 236–244, 2019. doi: 10.1017/S0714980818000582.

757. **Folstein MF**, **Robins LN**, **Helzer JE**. The Mini-Mental State Examination. *Archives of General Psychiatry* 40: 812–812, 1983. doi: 10.1001/archpsyc.1983.01790060110016.

758. **Cockrell JR**, **Folstein MF**. Mini-Mental State Examination. In: *Principles and Practice of Geriatric Psychiatry*, p. 140–141.

759. **Buysse DJ**, **Reynolds CF**, **Monk TH**, **Berman SR**, **Kupfer DJ**. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research* 28: 193–213, 1989. doi: 10.1016/0165-1781(89)90047-4.

760. **Buysse DJ**, **Reynolds CF III**, **Monk TH**, **Hoch Carolyn C**, **Yeager AL**, **Kupfer DJ**. Quantification of Subjective Sleep Quality in Healthy Elderly Men and Women Using the Pittsburgh Sleep Quality Index (PSQI). *Sleep* 14: 331–338, 1991. doi: 10.1093/sleep/14.4.331.

761. **Liu R-Q**, **Qian Z**, **Trevathan E**, **Chang J-J**, **Zelicoff A**, **Hao Y-T**, **Lin S**, **Dong G-H**. Poor sleep quality associated with high risk of hypertension and elevated blood pressure in China: results from a large population-based study. *Hypertension Research* 39: 54–59, 2016. doi: 10.1038/hr.2015.98.

762. **Dale AM**, **Fischl B**, **Sereno MI**. Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* 9: 179–194, 1999.

763. **Fischl B**. FreeSurfer. *Neuroimage* 62: 774–781, 2012.

764. **Iglesias JE**, **Augustinack JC**, **Nguyen K**, **Player CM**, **Player A**, **Wright M**, **Roy N**, **Frosch MP**, **McKee AC**, **Wald LL**. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: application to adaptive segmentation of in vivo MRI. *Neuroimage* 115: 117–137, 2015.

765. **Fischl B**, **Salat DH**, **Busa E**, **Albert M**, **Dieterich M**, **Haselgrove C**, **Van Der Kouwe A**, **Killiany R**, **Kennedy D**, **Klaveness S**. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33: 341–355, 2002.

766. **Fischl B**, **Sereno MI**, **Tootell RB**, **Dale AM**. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Human brain mapping* 8: 272–284, 1999.

767. **Fischl B**, **Sereno MI**, **Dale AM**. Cortical surface-based analysis: II: inflation, flattening, and a surface-based coordinate system. *Neuroimage* 9: 195–207, 1999.

768. **Cantero JL**, **Iglesias JE**, **Van Leemput K**, **Atienza M**. Regional hippocampal atrophy and higher levels of plasma amyloid-beta are associated with subjective memory complaints in nondemented elderly subjects. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences* 71: 1210–1215, 2016.

769. **Cotman CW**, **Berchtold NC**, **Christie L-A**. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends in Neurosciences* 30: 464–472, 2007. doi: 10.1016/j.tins.2007.06.011.

770. **Hemingway H**, **Bazzoni A**, **Craighead D**, **Rosenberg H**, **Nguyen K**, **Freeberg K**, **Adam E**, **Longtine A**, **Chonchol M**, **Minson C**. Passive heat therapy improves cognitive and cerebrovascular function in healthy midlife and older adults. *Physiology* 38: 5731414, 2023.

771. **Tapia-Arancibia L**, **Aliaga E**, **Silhol M**, **Arancibia S**. New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Research Reviews* 59: 201–220, 2008. doi: 10.1016/j.brainresrev.2008.07.007.

772. **Söderlund H**, **Nyberg L**, **Adolfsson R**, **Nilsson L-G**, **Launer LJ**. High Prevalence of White Matter Hyperintensities in Normal Aging: Relation to Blood Pressure and Cognition. *Cortex* 39: 1093–1105, 2003. doi: 10.1016/S0010-9452(08)70879-7.

773. **Aribisala BS**, **Morris Z**, **Eadie E**, **Thomas A**, **Gow A**, **Valdés Hernández MC**, **Royle NA**, **Bastin ME**, **Starr J**, **Deary IJ**, **Wardlaw JM**. Blood Pressure, Internal Carotid Artery Flow Parameters, and Age-Related White Matter Hyperintensities. *Hypertension* 63: 1011–1018, 2014. doi: 10.1161/HYPERTENSIONAHA.113.02735.

774. **Baumbach GL**, **Heistad DD**. Cerebral circulation in chronic arterial hypertension. *Hypertension* 12: 89–95, 1988. doi: 10.1161/01.HYP.12.2.89.

775. **McEwen BS**, **Sapolsky RM**. Stress and cognitive function. *Current Opinion in Neurobiology* 5: 205–216, 1995. doi: 10.1016/0959-4388(95)80028-X.

776. **Cabral DF**, **Rice J**, **Morris TP**, **Rundek T**, **Pascual-Leone A**, **Gomes-Osman J**. Exercise for Brain Health: An Investigation into the Underlying Mechanisms Guided by Dose. *Neurotherapeutics* 16: 580–599, 2019. doi: 10.1007/s13311-019-00749-w.

777. **Atkinson G**, **Williamson P**, **Batterham AM**. Issues in the determination of 'responders' and 'non-responders' in physiological research. *Experimental Physiology* 104: 1215–1225, 2019. doi: 10.1113/EP087712.

778. **Montero D**, **Lundby C**. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. *The Journal of Physiology* 595: 3377–3387, 2017. doi: 10.1113/JP273480.

779. **Padilla J**, **Leary E**, **Limberg JK**. Identifying responders vs. non-responders: Incorporation of controls are required for sound statistical inference. *Experimental physiology* 106: 375, 2021.