THE EFFECT OF MILD HYPOHYDRATION ON RESTING AND REFLEX BLOOD PRESSURE REGULATION IN HEALTHY YOUNG AND OLD ADULTS

by

Joseph C. Watso

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Physiology

Spring 2019

THE EFFECT OF MILD HYPOHYDRATION ON RESTING AND REFLEX

BLOOD PRESSURE REGULATION IN HEALTHY YOUNG AND OLD

ADULTS

by

Joseph C. Watso

Approved: _____
John Jeka, Ph.D.
Chair of the Department of Kinesiology & Applied Physiology

Approved: _____
Kathleen S. Matt, Ph.D.
Dean of the College of Health Sciences

Approved: _____
Douglas J. Doren, Ph.D.
Interim Vice Provost for Graduate and Professional Education

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

William B. Farquhar, Ph.D.
Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Megan M. Wenner, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Melissa Witman, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Sean D. Stocker, Ph.D.
Member of dissertation committee

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

xiv

LIST OF ABBREVIATIONS

BP, arterial blood pressure

cBRS, cardiac vagal arterial baroreflex sensitivity

CON, normal hydration control condition

CPT, cold pressor test

ECG, electrocardiogram

HG, handgrip exercise

MSNA, muscle sympathetic nerve activity

NTS, nucleus tractus solitarius

OVLT, organum vasculosum of the lamina terminalis

PEI, post-exercise ischemia

RVLM, rostral ventrolateral medulla

sBRS, sympathetic arterial baroreflex sensitivity

WD, water deprivation

ABSTRACT

Arterial blood pressure (BP) dysregulation is associated with increased future risk of cardiovascular disease, the leading cause of death among adults in the United States. Indices of BP dysregulation include 1) high resting BP (i.e., hypertension), 2) high BP variability, and 3) exaggerated BP responses during exercise. Hypohydration is common among adults in daily life, is associated with future cardiovascular disease risk, and has greater incidence during aging. However, it is unknown whether hypohydration is a causative factor for BP dysregulation. Therefore, we sought to determine if acute hypohydration causes BP dysregulation. In randomized crossover fashion, 45 non-obese and non-hypertensive adults (35 young & 10 old adults with similar body mass index values and habitual physical activity levels) completed: 1) a normally hydrated control condition (CON) via a three-day protocol with prescribed water intake, and 2) a water deprivation condition (WD) via a stepwise reduction in water intake over three-days concluded with a 16-hour water abstention period. All experimental visits were separated by at least one week. No participants were currently taking any anti-hypertensive medications (inclusive of diuretics). Participants collected their urine and underwent ambulatory BP measurements throughout the 24-hour period preceding each experimental visit. On the day of the experimental visit we measured hydration biomarkers and brachial BP (automated oscillometric device). While participants lie quietly in the supine position, we continuously assessed heart rate (single-lead ECG), beat-to-beat BP (photoplethysmography), muscle sympathetic nerve activity (peroneal microneurography; 23 paired recordings), and common femoral

artery blood flow (sonography) at rest and two minutes of isometric handgrip exercise. WD elicited mild hypohydration as evidenced by elevated plasma osmolality, urine osmolality and specific gravity, and thirst rating similarly among young and old adults. Despite mild hypohydration, WD did not increase: 1) resting or ambulatory daytime BP values, 2) resting or ambulatory BP variability, or 3) sympathetic or BP responses during handgrip exercise or the cold pressor test in either young or old adults. Together, our findings suggest that this model of acute mild hypohydration does not alter resting or reflex BP regulation in healthy young and old adults.

Chapter 1

LITERATURE REVIEW

1.1 Blood Pressure Dysregulation is a Primary Risk Factor for Cardiovascular Disease

1.1.1 The predictive value of blood pressure dysregulation at rest and during daily living

Cardiovascular disease is the leading cause of death among adults in the United States (316). According to the World Health Organization, high resting arterial blood pressure (BP) is a primary risk factor for cardiovascular disease development. Although there is conflicting evidence that suggests BP variability does not substantially increase predictive value of cardiovascular risk prediction (29) or target organ damage (300) better than resting systolic BP alone, high BP variability is also independently linked to increased risk for atrial fibrillation (233), and increased mortality in non-hypertensive (120, 166, 194) and hypertensive populations (184, 226, 285). BP variability also predicts risk for cerebral small vessel disease in asymptomatic hypertensives (94).

1.1.2 The predictive value of blood pressure dysregulation during sympathoexcitatory stimuli

High arterial blood pressure (BP) responses to mental stress (95, 190), the cold pressor test (293, 321), static exercise (190), and dynamic exercise (89, 138, 143, 161, 196, 202, 219, 255, 296, 320) have been demonstrated be associated with increased future risk for hypertension, a major risk factor for cardiovascular disease. The prediction power from dynamic exercise testing is improved when considering the

1

absolute workload (138). High BP during exercise is also associated with greater arterial stiffness and endothelial dysfunction (288). With every 10 mmHg increase in exercise systolic BP, there is an 18 cm/s increase in brachial artery pulse wave velocity, highlighting a mechanism that may contribute to exaggerated BP responses during exercise in non-hypertensive individuals (282). Interestingly, even BP responses from seated-rest to resting pre-exercise position have utility in predicting future risk of hypertension (144). Finally, during exercise systolic BP variability is associated with future risk of developing hypertension (24).

In summary, high resting BP, high BP variability, and high BP responses during sympathoexcitatory stimuli are high clinically relevant predictors for cardiovascular disease development. Thus, there is a critical need to determine the factors that contribute to BP dysregulation.

1.2 Blood Pressure Dysregulation in Cardiovascular Disease States

1.2.1 Blood pressure dysregulation at rest and during daily living in cardiovascular disease states

Rats and humans have similar interindividual variability, so studying the pathogenesis of hypertension in rats provides great insight into prevention and treatment strategies for humans (54). Arginine vasopressin has been shown to modulate baroreflex function in spontaneously hypertensive but not Wistar-Kyoto rats (246). Hypertension is associated with chronic sympathoexcitation as a result of alterations in central nervous system signalling in the paraventricular nucleus and rostral ventrolateral medulla (RVLM) (105). Additional support comes from experiments using spinal cord transection to demonstrate that elevated sympathetic outflow contributes to the pathogenesis of high blood pressure development in spontaneously-hypertensive rats

(253). Studies also suggest that high-NaCl causes synaptic plasticity in the brainstem and increases the risk for salt-sensitive hypertension (40). In humans, greater resting muscle sympathetic nerve activity (MSNA) and impaired sympathetic baroreflex function is associated with higher resting BP (260). Hypertension is associated with marked increases in sympathetic activity and may be related to hyperactive renin-angiotensin-aldosterone system and oxidative stress in cardiovascular control centers (i.e., rostral ventrolateral medulla (129). Individuals with resistant hypertension are in a state of marked sympathetic overdrive and have impaired baroreflex mechanisms (111). In addition, non-hypertensive adults with a family history of hypertension have reduced resting cardiac vagal baroreflex sensitivity (284). This is a potential mechanism that predisposes them to altered BP control. Heart failure is characterized by increased resting sympathetic outflow, altered arterial baroreflex regulation of MSNA, and attenuated cardiopulmonary reflex modulation of MSNA (96).

1.2.2 Blood pressure dysregulation during sympathoexcitatory stimuli in cardiovascular disease states

Exaggerated sympathetic and pressor responses in hypertensive individuals can be observed just 10 seconds into static handgrip exercise (112). Hypertensive individuals demonstrate delayed heart rate recovery following metaboreflex activation (231). Augmented BP responses to the cold pressor test have also been demonstrated in hypertensive individuals (88, 134), and those with a family history of hypertension (88). It has been demonstrated that those with more mild forms of hypertension have exaggerated BP responses during mental stress (arithmetic task) and head-up tilt (80), as well as during the cold pressor test and isometric handgrip exercise (51). Related, augmented BP responses during dynamic exercise is associated with masked

hypertension (160). β-blockers attenuate BP responses during dynamic exercise in hypertensive individuals (214). Interestingly, antagonizing mineralocorticoid receptors in spontaneously hypertensive rats may attenuate exercise pressor reflex overactivity (78) and provides new potential treatment target for overactivity exhibited in humans affected by hypertension. Along these lines, heart failure patients also have augmented sympathetic responses during sympathoexcitatory stimuli (96, 191, 256). In summary several cardiovascular disease states are characterized by BP dysregulation, and better understanding this area is of critical importance.

1.3   The Incidence and Clinical Relevance of Human Hypohydration

Hypohydration is defined as a body water deficit caused by acute or chronic dehydration (193). Adults in the United States are not meeting recommendations for proper water consumption (224). Underhydration (159) and blood hypertonicity increases with age (278) and is associated with increased morbidity (151, 243), mortality (309), and health care expenditures (228, 309). In fact, studies have reported that nearly half of emergency room visits in older adults are related to chronic hypohydration (23) and can be avoided (261). Even mild hypohydration is a proposed pathogenic factor for many diseases (186). Finally, acute hypohydration impairs endothelial function in healthy young adults (15) and chronic hypohydration is predictive of future incidence of cardiovascular disease (52). Thus, there is a critical need to determine if hypohydration directly influences risk factors for cardiovascular disease, such as BP regulation.

1.3.1 Mechanisms that maintain body water homeostasis

Salt and water appetite, salt and water excretion, and arginine vasopressin release are the primary regulatory mechanisms that maintain body fluid and electrolyte balance. These mechanisms and their actions are depicted below in Figure 1.1.
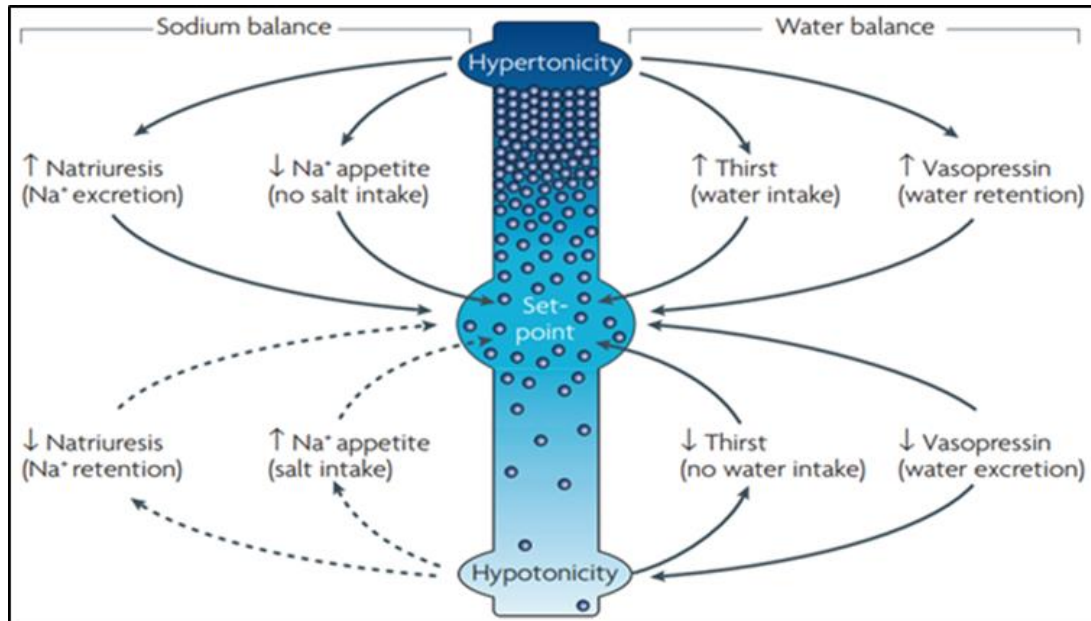
Figure 1.1   Basic Mechanisms of Osmoregulation. Changes in extracellular fluid osmolality modulate homeostatic responses that affect the Na+ balance (left) and the water balance (right) to promote homeostasis according to the principle of negative feedback. Hypertonic and hypotonic conditions lead to proportional changes in the intake or excretion of water and sodium to maintain extracellular fluid osmolality near a constant set point. Dashed lines represent potential homeostatic responses for which experimental data is presently unavailable (32).

Healthy young adults have active thirst mechanisms, tightly controlled hormonal regulation, and ideal organ (e.g. kidney) function to maintain proper body water balance. Small increases in blood sodium concentrations (e.g., ~2-3%) and larger decreases in plasma volume (e.g., ~10%) equally stimulate thirst sensations in healthy young adults.

Hyperosmolality is sensed via mechanical-stretch sensitive transient receptor potential vanilloid channels and subsequently initiate thirst mechanisms to higher brain centers and cause arginine vasopressin release from the posterior pituitary gland to reduce solute (particularly Na^+) concentrations (Figure 1.2). Recently, there is evidence that Na_x channels expressed in specific glial cells in the organum vasculosum of the lamina terminalis (OVLT) act as sensors of increased blood sodium concentrations in body fluids (217). Interestingly, water ingestion restores plasma volume before interstitial and intracellular fluid stores (269).
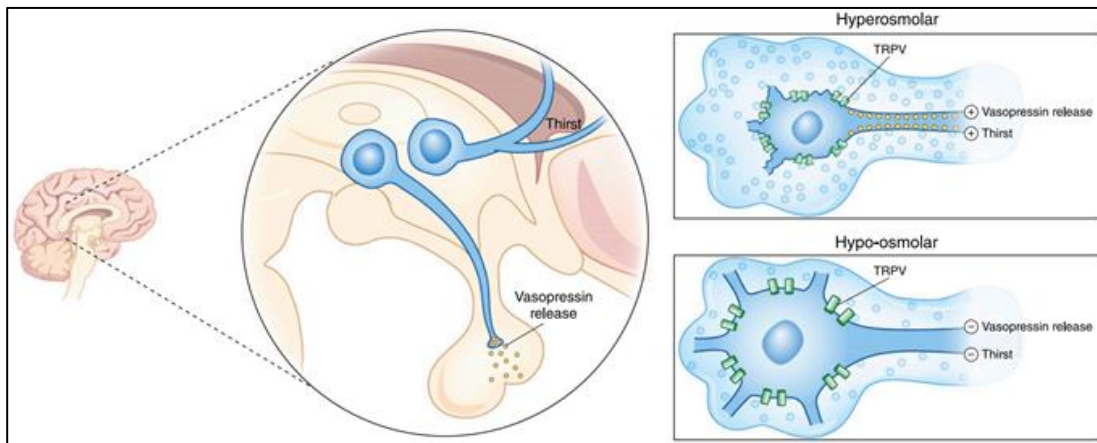
Figure 1.2   Osmoreceptor functions of the organum vasculosum laminae terminalis nuclei and supraoptic nuclei control thirst and vasopressin release. In response to hyperosmolar-induced cell shrinkage, specialized mechanical-stretch transient receptor potential vanilloid cation channels are activated, allowing the influx of positive charges and consequent cell depolarization, provoking action potentials that stimulate thirst and vasopressin release. Conversely, hypo-osmolar cell swelling deactivates these channels, leading to cell hyperpolarization, extinguishing thirst and vasopressin release. Although the exact role of the TRPV channel remains under investigation, its presence is critical in this mechanism. (70). See reference (217) above for recent findings related to these mechanisms.

1.3.2 The effect of age on body water homeostasis

Results from rodent studies demonstrate an altered relation between plasma osmolality and arginine vasopressin release with increasing age (287). Hypohydration increases arginine vasopressin release more in aged rats and impairs renal urine-concentrating ability, potentially a result of increased Aquaporin 2 messenger-ribonucleic acids expression observed in the old rats (286). These data in animals suggest altered arginine vasopressin release and renal function.

In humans, healthy aging is associated with hypohydration because of attenuated plasma arginine vasopressin release, reduced kidney function, and importantly, declines in thirst sensations (46, 92, 164, 179, 252, 262). Old adults have lower total body water than their young counterparts because of a lower set-point and because of water loss related to medication usage (16, 175, 266), and this is independent of body composition (71). The osmotic threshold for thirst is also shifted towards higher plasma osmolality during water deprivation (WD) in older adults, and the relation between plasma osmolality and thirst is attenuated (181).

The National Resident Assessment Instrument policy (102) is one example of a policy aimed to increase awareness for hypohydration and to improve prevention and treatment strategies for hypohydration (45, 195). Strategies include frequent encouragement for drinking (as opposed to infrequent consumption of large amounts of fluid), adaptation of the environment to accommodate proper hydration, and modification of medications to limit potential hypohydration-inducing side effects (93). While there is clear evidence of altered control of body water in aging, it is unclear what the implications are for BP regulation.

1.3.3 The effect of body fluid and electrolyte manipulations on orthostatic tolerance

Moderate hypohydration augments changes in MSNA and total peripheral resistance during orthostasis to compensate for decrements in stroke volume and cardiac output (168), without a change in heart rate responses (57). Specifically, burst amplitude but not burst frequency has been shown to mediate increases in MSNA during progressive central hypovolemia (167). However, tolerance to central hypovolemia does not appear to be related to resting MSNA or sympathetic baroreflex sensitivity (135). Rehydration attenuates arginine vasopressin release, plasma renin activity, and symptoms of overt hypotension during 45 minutes of 70° head-up tilt, suggesting a role for neurohormones and the renin-angiotensin-aldosterone axis in BP maintenance during head-up tilt when hypohydrated (122). Arginine vasopressin release was similar between cardiac-innervated and cardiac-denervated humans during head-down tilt, with plasma volume expansion but no change in plasma osmolality, suggesting that cardiac volume receptors are not the only mechanism for arginine vasopressin release during acute volume shifts (65). Following gastric emptying (via metoclopramide), old adults demonstrated reduced arginine vasopressin responsiveness to standing, despite greater arginine vasopressin release than young adults. This is thought to occur because of increased arginine vasopressin sensitivity to counteract reduced cardiac vagal baroreflex sensitivity (cBRS) in aging (26). This highlights the clinically-relevant role of hydration status in mediating BP regulation during orthostasis.

1.3.4 The effect of hypohydration on mental and physical performance

Mild hypohydration has been demonstrated to deteriorate mental performance in humans (315). Water intake following light exercise has been demonstrated to improve judgement and decision-making performance in healthy adults (229). A study

in healthy young female adults found hypohydration to impair visual and working memory, as well as executive function. Further, drinking water corrected these impairments, suggesting a role for hypohydration *per se* impairing cognitive function in a young healthy cohort (272). Also, during mild hypohydration, young female adults were found to have disrupted mood and concentration (11). While some work has been done on the effect of hypohydration on mental performance, there is a much larger body of research on how hypohydration affects physical performance.

For exercise performance and safety concerns, the American College of Sports Medicine suggests drinking adequate amounts of water before, during, and after physical activity in order to prevent 2% loss in body mass (9). There is evidence that hypohydration impairs exercise performance (248), which is mostly attributed to reductions in stroke volume (110), increased heart rate (6, 106), and impaired thermoregulation (108, 251). These decrements in performance occur independent of thirst (2) and even modest levels of hypohydration can impair performance (212). Further, exercise performance is impaired during hypohydration, even when blinded to hydration condition (intragastric rehydration or no rehydration) (146). Hypovolemic-hyperosmolality increases heat storage because of reduced sweat rate (evaporative heat transfer) and reduced cutaneous blood flow (dry heat transfer) (250). Hyperosmolality also elevates the threshold for both vasodilation and sweating (1, 12), independent of changes in plasma volume (99). Further, hypohydration increases the internal temperature threshold for cutaneous vasodilation during dynamic exercise in a thermoneutral environment, but once vasodilation occurs the slope of forearm blood flow is not different than euhydration (213). Accordingly, hypohydration-induced hyperosmolality attenuates vasodilation in the cutaneous circulation despite high core

temperature (109, 141), likely from increased net filtration in the capillaries (162). These studies provide a link between hypohydration and impaired exercise performance, and increased peripheral (cutaneous) vascular resistance.

1.3.5   Interactions between age, hypohydration, and blood pressure regulation

Results from early experiments administrating intravenous Angiotensin II in baroreceptor intact and denervated dogs suggest that a rise in BP suppresses thirst (171). Additional support for this observation comes from studies conducted in rodents, that observed acute hypertension to inhibit thirst sensations (stimulated by Angiotensin II, hyperosmolality, or hypovolemia) (275) and observed that the arterial baroreceptors mediate the inhibitory effects of acute hypertension on thirst sensations (276). This is problematic as higher resting BP contributes to thirst detriments in older adults (319). Older adults have presented to the hospital with clinical symptoms of severe hypohydration without reporting feelings of thirst (116). Indeed, when young and old adults were matched for body mass loss, old adults had greater increases in plasma osmolality and serum sodium concentrations, but had lower thirst sensations and drank less water (238). During hypertonic saline infusion, old adults have larger increases in plasma osmolality and drink half as much water as young adults, suggesting impaired osmoregulation and thirst sensations (81). In contrast, one study using hypertonic saline infusion, demonstrated that old adults had similar thirst and renal osmoregulatory responses to that of young adults (271). Thus, the responses to acute salt loading remain under debate, however, the influence on age in these responses to acute hyperosmolality are of great importance because of their potential implications for BP regulation.

In humans, when young and old adults were dehydrated and matched for body mass loss, old adults had greater increases in plasma osmolality, serum sodium

10

concentrations, and plasma arginine vasopressin concentrations, but had lower urine osmolality and thirst sensations. There is also evidence of older adults having reduced sensing of solutes in the extracellular fluid (237). Additionally, old adults had increased sensitivity of osmoreceptors during hypohydration (238). A study using a water loading protocol demonstrated reduced glomerular filtration rate in older adults because of changes in water excretion after normalizing to creatinine clearance (68). Gastric emptying (via metoclopramide) increased arginine vasopressin release in both younger and older adults, but older adults had reduced arginine vasopressin responsiveness during standing (26). In contrast to this suggestion, hypertonic saline infusion elicited similar renal osmoregulatory responses in older compared to younger adults in another investigation (271). More studies are needed to better understand the role of hyperosmolality, arginine vasopressin, and water balance during aging given their influences on BP regulation.

1.4 Resting Blood Pressure Regulation and Potential Effects of Hypernatremia and Hypohydration

Body fluid regulation is an important contributor to BP regulation. The key regulators of blood volume include the renin-angiotensin-aldosterone axis and arginine vasopressin. Both the baroreceptors and the sympathetic nervous system are responsible for BP regulation. Below Figure 1.3 depicts the organum vasculosum lamina terminalis and key forebrain areas involved in the control of the endocrine, autonomic, and behavior systems that participate in body fluid regulation (150).

Forebrain Area Innervated	Higher Order Regions	Response System Controlled
Parvocellular PVN	Ventrolateral Medulla and Spinal Cord	Sympathetic Outflow
	Median Eminence/ Anterior Pituitary	CRF/ACTH
Magnocellular PVN/Supraoptic Nucleus	Posterior Pituitary	Vasopressin/ Oxytocin
Perifornical/Lateral Hypothalamus	Basal Ganglia	Thirst
Amygdala	Basal Ganglia	Salt Appetite

Figure 1.3   Key Brain Areas for Endocrine, Autonomic, and Behavioral Function. Organization of structures of the lamina terminalis region (midsagittal representation) and projections from the lamina terminalis to key forebrain area involved in the control of the endocrine, autonomic, and behavioral systems that participate in body fluid regulation. The subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), the median preoptic nucleus, and anterior commissure are adjacent to the lamina terminalis and can be referred to as the structures of the lamina terminalis. CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone; PVN, paraventricular nucleus (150). Independent of the SFO, the area postrema is thought to be responsible for the hypertensive effect during prolonged water deprivation (WD) (60).

The following sections will describe the specific roles of the renin-angiotensin-aldosterone system, arginine vasopressin, the neural and peripheral arcs of the arterial baroreflex, and the cardiopulmonary baroreflex on resting BP regulation.

1.4.1   The renin angiotensin aldosterone system

Blood or plasma volume contraction and hyponatremia stimulate the renin-angiotensin-aldosterone axis to increase sodium and water retention. During volume depletion via 48-hour WD in male rabbits, BP maintenance was predominated by humoral pressor systems (e.g. arginine vasopressin, angiotensin II), rather than the sympathetic nervous system (292), suggesting the renin-angiotensin aldosterone system might play an active role during human hypohydration. In rats, hypohydration has been demonstrated to increase plasma renin activity, even during renal denervation and adrenal demedullation, suggesting sympathoadrenomedullary independent plasma renin activity release to support BP (27). When plasma renin activity is released during hypohydration, angiotensin type-1 receptors in the paraventricular nucleus and rostral ventrolateral medulla become more sensitive (101), suggesting altered renin-angiotensin-aldosterone-mediated BP control. Also in humans, endogenous Angiotensin II is thought to be responsible for greater sympathoexcitation during the modified oxford (242). Thus, there is a body of evidence suggesting that blood volume status and hypohydration affects BP regulation via renin-angiotensin-aldosterone axis related mechanisms.

1.4.2   Arginine vasopressin

Blood or plasma volume contraction and hyperosmolality stimulate arginine vasopressin release from the posterior pituitary to target water retention in the kidneys. Excessive arginine vasopressin release has been suggested to play a role in the development of human hypertension (152). Arginine vasopressin is released via actions of the forebrain and midbrain (97), and supports BP by increasing lumbar sympathetic outflow and heart rate (257), independent of involvement of the subfornical organ (60)

during hypohydration. Rodent models using intravenous arginine vasopressin antagonism demonstrate attenuated pressor and bradycardic effects of α1-adrenergic receptor agonists (e.g., methoxamine, phenylephrine) (136). Arginine vasopressin antagonism in dogs attenuates the depressor and tachycardic effects of system nitric oxide mediated vasodilation (via sodium nitroprusside), with no additive effect of Angiotensin II antagonism, suggesting arginine vasopressin plays a primary role in BP control during potentially hypotensive periods (38). Further, arginine vasopressin blockade during WD causes a significant drop in BP, suggesting its' actions are necessary for BP support during WD (10, 295). Another study in rats built on this notion by demonstrating that administration of intravenous synthetic arginine vasopressin increases BP following WD. Further, they demonstrated that arginine vasopressin blockade lowers BP via reductions in peripheral vascular resistance (7), and this occurs independent of cardiac vagal or arterial baroreceptor input (254). In contrast, another study found that after 24 or 48 hours of WD, arginine vasopressin did not significantly contribute to BP maintenance (91). Though there are some conflicting results, when taken together these studies suggest that arginine vasopressin has an active, if not primary, role in BP regulation and support during WD. However, much of this work has been conducted in animal models, thus more work in humans is warranted.

1.4.3 The neural arc of the arterial baroreflex

The arterial baroreceptors, located in the carotid sinuses and aortic arch, are high pressure stretch receptors responsible for providing input to the hindbrain to modulate sympathetic outflow to maintain BP (97). The sympathetic nervous system is an important regulator of both short-term and long-term BP control (153). Sympathetic nervous system activity in humans can be assessed using microneurography, a term

14

from leading Swedish neurophysiologist Yngve Zotterman. This technique to record efferent sympathetic outflow *in vivo* was developed in Uppsala, Sweden in 1965 by Drs. Hagbarth and Vallbo (298). The peroneal nerve is a common site for assessing sympathetic outflow, with efferent sympathetic fibers being grouped in bundles ranging from 1-44 axons in each of the ~30 nerve fascicles in the common peroneal nerve bundle (290). Healthy adults have a wide range of resting burst frequencies and taller individuals have a longer burst latency (electrocardiogram R-wave time to MSNA peak time) (281). While resting burst frequency varies between healthy individuals, it is reproducible within individuals (69). Interestingly, those with greater resting MSNA have reduced cardiac output, suggesting a compensation response (56). It was demonstrated as early as 1978 that changes in burst frequency and burst amplitude are greater following decreases in BP compared to increases in BP (281).

The earliest work to report an inverse relation between BP and efferent sympathetic outflow was in 1974 (307). In this study, they identified a reverse sigmoid relation between diastolic BP and MSNA in two patients with atrial fibrillation. Later, results from 16 participants demonstrated no correlation between systolic BP and MSNA, a strong correlation between pulse pressure and MSNA, and strong inverse correlations between mean BP/diastolic BP and MSNA (281). Their explanation for these findings were informed by early observations that baroreceptor firing is greatest during systole and silent during diastole (37). Thus, because afferent baroreceptor input completely inhibits MSNA during systole and the duration of baroreceptor silence (i.e., when MSNA is unconstrained) is longer when diastolic BP is lower, the strong inverse relation between diastolic BP and MSNA is physiologically plausible. Indeed, their own past work (47) demonstrated that falls in diastolic BP produce a greater likelihood of

15

burst occurrence. As a result of these experiments and others summarized by Drs. Eckberg and Sleight (84), we now understand the physiology underlying baroreflex control of MSNA. The following section will discuss the role of hypovolemia and hyperosmolality in affecting the arterial baroreflex.

During water deprivation in rats, blood hyperosmolality (i.e., elevated blood osmolality values) was found to influence sympathetic outflow and BP, independent of changes in plasma arginine vasopressin concentrations and blood volume (257). This is likely due to greater sensitivity of the paraventricular nucleus during times of blood hyperosmolality, demonstrated through studies using injections of γ-Aminobutyric acid agonists and glutamate antagonists (101) and studies investigating changes to the intrinsic properties of RVLM neurons (3). In support of these past reports, hypohydrated rats were demonstrated to maintain BP via paraventricular nucleus-driven increases in splanchnic sympathetic outflow that is not synchronized to changes in respiration or heart rate (137). This is thought to occur from central hyperosmolality exciting discrete populations of neurons in the RVLM that increase sympathetic outflow and BP through increased sensitivity of glutamate neurotransmission (274). Importantly, alterations in sympathetic outflow and BP during central hyperosmolality are related to NaCl concentrations *per se*, as eqiu-osmotic sorbitol/mannitol does not produce same OVLT neuronal discharge frequency (169). Additionally, these OVLT neurons sense and respond to increases in extracellular NaCl concentrations (i.e., a body fluid that can be more easily assessed in humans) by increasing sympathetic outflow and BP (35). Other animal studies suggest that activator protein-1 transcription factors are responsible for switching thoracic sympathetic outflow control from the hypothalamus to the commissural nucleus tractus solitarius (NTS) following WD (61). In rats, high salt

feeding to increase peripheral blood sodium concentrations increased BP variability, but not absolute mean BP (267). There is conflicting evidence in the literature suggesting that plasma osmolality does not play a role in altering renal sympathetic outflow during WD and rehydration (258) or in altering cBRS or sympathetic baroreflex sensitivity (sBRS) (292) in animals. While there is a large body of work regarding high elevations in plasma osmolality and blood sodium concentrations may affect BP regulation in animals, there is much less known about the effects of such interventions, such as WD, in humans.

In humans, acute hyperosmolality (via 2,400mg sodium meal) has been demonstrated to increase systolic BP 2 mmHg for every 1 mmol/L increase in blood sodium concentrations (280). Additionally, acute blood hyperosmolality has been shown to increase MSNA at rest (90, 113, 242). Hypertonic saline infusion in humans increases sympathetic baroreflex sensitivity (53, 310), independent of estimated changes in plasma volume (i.e., matched for hematocrit) (310), suggesting hyperosmolality *per se* appears to have a role in altering BP regulation. Additional insight comes from Saitoh et al, who described a bell curve-like relationship between blood volume and baroreflex sensitivity, suggesting similar but opposite changes in baroreflex sensitivity for a given change in blood volume (sensitivity increases with hypervolemia and decreases with hypovolemia) (244). CBRS has been shown to be reduced following exercise-induced hypohydration (55) in young adults. However, the effects of a WD-induced hypohydration on resting BP regulation in humans has not yet been explored. Given both hyperosmolality and hypovolemia appear to increase baroreflex sensitivity, we could speculate that WD, characterized by plasma volume contraction and blood hyperosmolality, would increase baroreflex sensitivity.

1.4.4 Sympathetic vascular transduction

The functional effects of MSNA bursts on the vasculature to change blood flow and BP is termed sympathetic vascular transduction. Briefly, a spike-triggered averaging methodology developed and described by Dr. Fadel's group has recently been used to assess beat-to-beat sympathetic vascular transduction (86). Changes in absolute mean BP and limb vascular conductance is determined at the time of each MSNA burst, referred to as cardiac cycle 0, and followed for 10 subsequent cardiac cycles. This time frame was chosen based on the original work of Wallin and Nerhed (308) demonstrating that the peak increase in mean BP occurs ~5.5 seconds following a burst of MSNA, thus 10 cardiac cycles at a normal basal heart (60 beats/minute) would provide ample time to observe the peak vascular respond to a spontaneous MSNA burst. In addition, work from Dr. Fadel's laboratory using phentolamine to block α-adrenergic receptors has demonstrated the sympathetic origin of the responses and that this window appropriately captures the time course and nadir changes in mean BP and vascular resistance following spontaneous MSNA bursts (87).

1.4.5 The cardiopulmonary baroreflex

Iso-osmotic hypovolemia via furosemide has been demonstrated to increase the gain of the cardiopulmonary baroreflex (i.e., larger increase in vascular resistance for a given decrease in central venous pressure) (289). During graded central hypovolemia (5, 10, 15, 20 Torr of lower-body negative pressure for 5 minutes each) the heart rate response range (the difference between the minimal and maximal responses) tended to be less in the old versus young adults, suggesting blunted responsiveness to graded central hypovolemia with aging. Further, 15 Torr lower-body negative pressure has been shown to increase the gain of carotid-heart rate and carotid-mean BP baroreflexes in

18

young adults but did not affect the gain of the arterial baroreflex in old adults, suggesting unloading the cardiopulmonary baroreceptors is not related to arterial baroreflex function in healthy older adults (263). Old adults were also found to have blunted increases in forearm vascular resistance during 10 to 40 Torr lower-body negative pressure, as well as blunted heart rate increases, but augmented systolic and mean BP increases during 30 and 40 Torr lower-body negative pressure. Additionally, old adults had a greater relation between changes in MSNA and changes in peripheral venous pressure/forearm vascular resistance during 5 to 40 Torr lower-body negative pressure (72). Together, these studies provide evidence that aging is associated with alterations in cardiovascular responses during mild to severe hypovolemia. Given that hypohydration can impose a mild level of hypovolemia, it is important to keep the role of the cardiopulmonary baroreflex in mind when interpreting data from perturbations that cause reductions in plasma volume.

Regarding BP regulation during exercise pressor reflex activation, there is evidence that the cardiopulmonary baroreflex does not modulate (inhibit) sympathetic outflow during handgrip exercise at 30% maximal voluntary contraction (259) or during post-exercise ischemia (247). However, recently, post-exercise ischemia following handgrip exercise at 20% and 30% maximal voluntary contraction was demonstrated not to inhibit MSNA during cardiopulmonary baroreceptor loading, whereas post-exercise ischemia following handgrip exercise at 40% maximal voluntary contraction was found to attenuate cardiopulmonary baroreflex-mediated inhibition of MSNA (156). Thus, there remains conflicting evidence for the role of central hypovolemia in modulating cardiovascular adjustments to exercise pressor reflex activation.

1.4.6 The effects of sex and age on resting blood pressure regulation

Young female adults maintain optimal (<120/80) basal BP via β-receptor mediated vasodilation that offsets α-receptor vasoconstriction (125), which is altered following menopause (124, 125, 127, 128). Young male adults have slightly higher BP than age-matched female adults due to slightly greater sympathetic outflow and total peripheral resistance, as well as the absence of β-receptor mediated vasodilation (125). Throughout the menstrual cycle in female adults, the high estrogen and progesterone (mid-luteal) phase stimulates renin-angiotensin-aldosterone axis and increases sodium retention and expands plasma volume (270). Also, the high-estrogen (late follicular) phase reduces renal arginine vasopressin sensitivity, leading to reduced water retention (270). In young healthy female adults, menstrual cycle status did not influence cBRS or sympathetic vascular transduction, but resting MSNA, plasma norepinephrine concentrations, and sBRS were higher during the midluteal compared to the early follicular phase (201). While oral contraceptive use is associated with increased BP, it does not appear to affect resting MSNA, total peripheral resistance, or cardiac output (127). Although young healthy females have estrogen and progesterone expanding plasma and shifting the osmotic operating point to the left at different points throughout the menstrual cycle, it has only a minor effect on body water balance (311). Mixed findings in the literature suggest that sex hormone fluctuations throughout the menstrual cycle do (49, 50, 103, 148, 176) and do not (197) alter resting and reflex BP regulation.

During aging, resting sympathetic outflow increases and at least partially contributes to increases in resting BP (123). Also, aging has been shown to be associated with impaired arginine vasopressin release during a protocol that included acute dehydration then rehydration (236). Old adults also have a larger increase in BP for a given change in sympathetic nerve activity (i.e., greater sensitivity) (123). This likely

contributes to higher BP variability observed in older adults (59). Further, old adults have delayed heart rate responses to changes in BP, with longer delays observed in old female adults compared to old male adults (198). Old adults have blunted transduction and require greater resting sympathetic outflow to maintain BP (302), partially due to reduced total body water (215). More specifically, old males have a larger drop in BP for each heart beat without a MSNA burst (302), necessitating greater burst frequency to maintain vascular resistance and BP compared to their female counterparts (302). Increased sympathetic vascular transduction in old female adults is likely a result of β-adrenergic receptor involvement during menopause (124, 125, 127, 128). Finally, resting sBRS is thought to be similar between healthy young and old adults (73, 82, 187, 232), but resting cBRS is reduced in healthy aging (82, 147, 173, 187). One study reported that healthy old adults have reductions in the mechanical component of the arterial baroreflex, but because of compensatory mechanisms in the neural component, overall sBRS function is maintained in healthy aging (279). When taken together, these past studies suggest preserved baroreflex control of MSNA but not heart rate. It is currently unclear if mild hypohydration will affect resting baroreflex function in healthy young or old adults.

1.5   Reflex Blood Pressure Regulation

In order to meet the metabolic demands of perturbations, such as exercise, increases in blood flow and BP are needed, however the effect of WD on these responses in humans is not well understood. Studies in rodents demonstrate that the hindbrain is responsible for mediating autonomic cardiovascular reflexes during hypovolemia to maintain BP (97). Hyperosmolality has been demonstrated to enhance sympathoexcitatory and sympathoinhibitory reflexes evoked from the RVLM (5, 267,

21

277). Brooks et al, demonstrated that WD increases excitatory amino acids in the RVLM to support BP (39) via elevations in lumbar sympathetic outflow (41). Arginine vasopressin, a hormone released in response to hypovolemia, works through α2 adrenoceptor dependent mechanisms in the NTS to modulate cardiopulmonary baroreflex function and shift the arterial baroreflex operating point to lower pressures, thus enhancing sympathoinhibitory responses and blunting sympathoexcitatory responses (130). Further, arginine vasopressin-V_1 antagonism has been shown to blunt the pressor effects of central osmolality in dogs (222). These studies suggest arginine vasopressin has a role in BP regulation during WD-induced hypovolemia. The section below describes the integrative cardiovascular responses that occur during acute exercise, the cold pressor test, and voluntary end-expiratory apnea.

1.5.1 Cardiovascular responses during acute exercise

One of the first observations of a BP increasing reflex arising from voluntary contracting muscles in humans was reported in 1937 (8). After many years of research, we now know the sympathetic nervous system to play a role in increasing BP during exercise, and that the increase in BP and MSNA are tightly coupled to the onset of muscular fatigue (142, 256). Specifically, burst amplitude (i.e., efferent sympathetic activity signal strength) increases in an intensity dependent manner throughout increasing levels of exercise (31). The three primary systems responsible for maintaining BP and providing blood flow to active skeletal muscle tissue during exercise are: central command, the arterial baroreflex, and the exercise pressor reflex (142, 256). Below, Figure 1.4 depicts these three primary mechanisms (256).
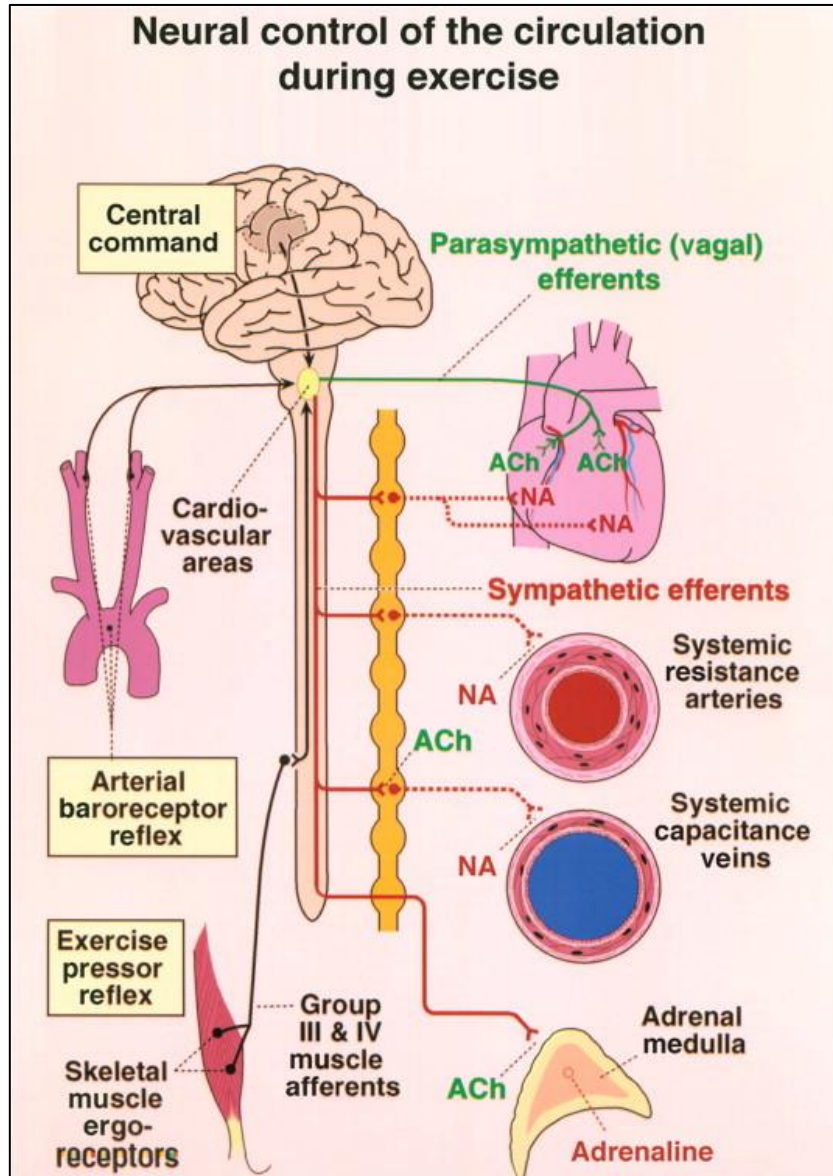
Figure 1.4   Neural Control of the Circulation During Exercise. Neural signals originating from the brain (central command), the aorta and carotid arteries (arterial baroreflex), and skeletal muscle (exercise pressor reflex) are known to modulate sympathetic and parasympathetic nerve activity during exercise. The alterations in autonomic outflow induce changes in heart rate and contractility, changes in the diameter of resistance and capacitance vessels within peripheral tissue beds and release of adrenaline from the medulla of the adrenal gland. As a result, changes in heart rate, stroke volume and systemic vascular resistance mediate alterations in mean arterial pressure appropriate for the intensity and modality of exercise. ACh, acetylcholine; NA, noradrenaline (256).

MSNA during exercise is thought to be uniformly distributed throughout skeletal muscle nerves and is well correlated to plasma norepinephrine concentrations and BP levels, while inversely correlated to limb vascular conductance (i.e., increasing MSNA reduces limb vascular conductance) (69). Heat and exercise-induced hypohydration has been shown to attenuate BP responses (mainly resulting from reductions in cardiac output) and accentuate increases in vascular resistance and plasma norepinephrine concentrations, suggesting greater activation of the sympathetic nervous system (109). Another study demonstrated hypohydration to increase heart rate and plasma arginine vasopressin concentrations in response to cycling exercise (192). Regarding age differences, young and old adults have been demonstrated to have similar sympathetic nervous system responses during static exercise (215), and these responses appear to be independent of relative workload (174). In contrast, however, there is evidence that old compared to young adults have greater BP responses during static exercise (199), dynamic exercise (34), and even dynamic exercise when matched for relative workloads (28). Further, there is one report of old adults have delayed BP recovery from exercise (34). More recent data suggests potentially sex differences with aging, with reports that old female, but not male, adults, have augmented BP responses during handgrip exercise compared to young adults (294). Given the conflicting results about the effects of sex and age on affecting these responses, it is important to determine what populations may have augmented exercise pressor exercise given the prognostic information gained from such results.

1.5.1.1 Central command

In 1972, central command was first described as a feed forward mechanism to control cardiovascular responses derived from the motor cortex (104), specifically in

the anterior cingulate and insular cortices (314). Direct evidence of the involvement of the anterior cingulate region in central command-induced alterations in cardiovascular responses was published in 2018 (107). During neuromuscular blockade to inhibit muscular force production, central command activation increases in MSNA and heart rate (304). It is thought that central command only has a weak influence during isometric exercise in smaller muscle tissues (69). There is evidence that hypohydration reduces resistance exercise performance by, at least in part, attenuating central drive (155). There is a paucity of knowledge of the central command's role in BP regulation during hypohydration, thus work in this area is warranted.

1.5.1.2   The arterial baroreflex

The arterial baroreceptors, located in the carotid sinuses and aortic arch, provide short-term control of BP during exercise by providing input to the brainstem on a beat-to-beat basis. In turn, they assist in modulating sympathetic outflow and heart rate to maintain BP during exercise. During exercise, the baroreceptors actively reset upward to defend higher BP's as exercise intensity increases (62, 142). It is thought that Group III/IV afferent nerve signals are required for resetting of the carotid baroreflex operating points independent of central command (139). Fatiguing handgrip exercise reduces cBRS and post-exercise ischemia (PEI) increases cBRS, but the baroreflex rhythms are not abolished (83). It is unclear if hypohydration would alter arterial baroreflex function during static exercise in humans.

1.5.1.3   The exercise pressor reflex

Activation of afferent Group III and Group IV nerve fibers provide feedback to the brainstem to increase blood flow to active muscle beds. Group III afferent fiber

activation occurs via stretch and pressure, and has been shown to increase sympathetic outflow and BP (131, 157, 158, 273, 313). Group IV afferent nerve fiber activation occurs via local metabolite build up, and also increases sympathetic outflow and BP (131, 158). Early work by Kaufman et al., demonstrated group III afferent fibers to be more sensitive to the mechanical effects of muscle contraction (i.e., physical distortion) whereas group IV afferent fibers are more sensitive to the metabolic byproducts of muscular contraction (i.e., hydrogen ion concentrations, potassium ion concentrations, lactate concentrations, bradykinin concentrations, arachidonic acid concentrations, and adenosine concentrations) in a cat model (157, 158), with later reports suggesting a role for gluconeogenesis to activate IV afferent fibers (69). The exercise pressor reflex can work to increase blood flow and BP independent of central command, as afferent nerve signals can be transmitted to the RVLM and NTS to increase sympathetic outflow and heart rate in decerebrate (i.e., without an intact motor cortex) animals (235, 256). Isometric exercise causes heart rate to increase after a few respiratory cycles, but BP does not increase until ~30 seconds into exercise (297). Because ascorbic acid does not alter sympathetic responses during rhythmic handgrip exercise, reactive oxygen species are not thought to be involved in mechanoreflex feedback mechanisms (211). MSNA and BP responses during metaboreflex isolation are blunted when blood flow is increased via forearm suction (154). In contrast to earlier findings suggesting systemic increases in MSNA during metaboreflex activation (33, 69), recent work suggests MSNA distribution is non-uniform between active and inactive muscle tissues, with reports of no observed increases in MSNA to the contracting ipsilateral muscle (30).

Regarding the potential role of WD-induced hyperosmolality, blood hyperosmolality via high-NaCl feeding has been shown to augment lumbar sympathetic

outflow and BP responses during exercise pressor reflex activation in rodents, as well as to increases vascular resistance (318). Regarding the potential effects of age, old adults have greater increases in cardiac output during moderate intensity plantar flexion exercise. Old adults have been found to have similar cardiovascular responses and similar sympathetic baroreflex function during post-exercise ischemia as young adults, suggesting preserved neural regulation of metaboreflex isolation in healthy aging (114). Further, when another acute sympathoexcitatory stimulus (whole body cooling via skin temperature ~34°C) was superimposed on isometric handgrip exercise, old male and female adults have augmented MSNA at rest but similar responses to isometric handgrip exercise as their young counterparts (115). However, recently it has been reported that old female adults have augmented BP responses during isometric handgrip exercise, potentially due to an inability to properly lower systemic vascular resistance (294). More work is needed to determine the role of WD and age in altering cardiovascular responses during exercise pressor reflex activation.

1.5.2 The cold pressor test

In 1936, Hines et al., demonstrated that placing one's hand into ice-cold water had a BP raising effect through increases in both heart rate and cardiac output (134). MSNA begins to increase 30 seconds into a cold pressor test, around the same time as heart rate responses peak (303). The researchers also observed increases in MSNA to be well correlated to increases in BP (and to a smaller degree venous blood norepinephrine concentrations as well) (303). Later work demonstrated a plateau in MSNA 90 seconds into the cold pressor test, though total peripheral resistance and BP continued to rise throughout the full two minute duration (317). Importantly, this test was found to be reproducible within participants (98). Young and old adults were found

to have similar sympathetic responses during the cold pressor test (215), but more work in this area is needed regarding the effects of hydration status and aging given the divergent results that exist from other sympathoexcitatory stimuli such as exercise.

1.5.3   Voluntary end-expiratory apnea

Hypercapnia and hypoxia increase MSNA (121, 177, 206, 268) by acting on medullary surface central chemoreceptors (43) and carotid body peripheral receptors (180, 306), respectively. Isocapnic hypoxia-induced peripheral chemoreceptor activation resets the baroreflex operating point to a higher BP without changing baroreflex sensitivity (149). Hyperosmolality, via hypertonic saline infusion, increases absolute MSNA during apnea but does not appear to increase responsiveness (i.e., change in MSNA from baseline (113). More work is needed to examine the effects of age and hydration status on these responses.

1.6   The Potential Role of Exercise Training Status and Habitual Physical Activity for Modulating Hypohydration-Induced Changes in Blood Pressure Regulation

Evidence suggests that hyperosmolality is a driving factor for neurally-mediated increases in resting BP variability and cardiovascular reactivity to sympathoexcitatory stimuli (209). This section will describe the role of habitual exercise and physical activity induced increases in plasma volume and decreases in cardiovascular reactivity, as a potential protective mechanism of hypohydration-induced impairments in cardiovascular homeostasis.

1.6.1 The potential for attenuated plasma volume loss during hypohydration in highly physically active individuals

Following exercise training, individuals attain a higher basal plasma volume (21, 64, 264) and should have a smaller relative reduction in plasma volume if given a similar reduction in water intake as an untrained/inactive individual. It is thought that plasma volume expansion accounts for most hypervolemia during the two to four-week training adaptation period before equal increases occur in red blood cell volume (66). Part of the mechanism thought to contribute to plasma volume expansion is reduced urine output via increased aldosterone production and sensitivity (63, 264). Plasma renin activity and serum aldosterone concentrations increase following exercise training and are correlated to sodium retention and to leg volume (edema) (200). Increases in plasma albumin concentrations following exercise training improve the H_2O binding capacity, leading to greater red cell volume (64). Accordingly, exercise-induced increases in plasma volume has been shown to improve orthostatic tolerance and to prevent syncopal symptoms and episodes (208). Additionally, a group of individuals with high BP responses to exercise completed 12 weeks of aerobic exercise training and later were found to have attenuated increases in BP and blood norepinephrine concentrations during a submaximal exercise test (203). Blood volume correlates to VO_{2peak} in post-menopausal female adults, with runners demonstrating the highest blood volumes, followed by swimmers, with both of these groups having greater blood volume values then their inactive counterparts (227). Six months of endurance training in old adults has been shown to increase plasma and blood volume values without any changes in hormones involved in volume regulation, suggesting a resetting of volume receptor thresholds (48). In summary, there is evidence that high levels of exercise training and physical activity may increase plasma volume and protect against WD-induced

reductions in plasma volume contraction. Along the lines of our hypothesis, we would expect this to potentially partially compensate for the hypohydration-induced alterations in BP regulation.

1.6.2 The potential for attenuated cardiovascular responses in highly physically active individuals

There is evidence that wheel-running versus sedentary rats have blunted centrally-mediated increases in sympathetic outflow and BP during rostral ventrolateral medulla neuronal activation, baroreceptor unloading, and vascular α-1 activation (216). A follow up study found GABAergic inhibition in the RVLM enhances BP, but not lumbar sympathetic outflow, responses to glutamate-mediated pressor effects (210). A later study confirmed that active versus sedentary rats have adaptations that attenuate sympathoinhibitory and sympathoexcitatory cardiovascular response mechanisms in the RVLM (77). Additionally, chronic training of anaerobic energy systems in humans may blunt cardiovascular responses during metaboreflex isolation (145). In summary, there is evidence that high habitual physical activity may attenuate cardiovascular responses to sympathoexcitatory stimuli. Whether or not physical activity prevents hypohydration-induced changes in BP regulation in humans remains to be determined.

1.7 Conclusions

Underhydration remains widespread in the United States (100, 132, 163), and is a public health concern, particularly in older adults (252, 278). This is due to reduced thirst sensations (238) and diminished fluid regulation (175, 237) experienced during aging. Hypohydration causes plasma volume contraction and increases plasma osmolality, which may increase resting BP variability and augment cardiovascular reflexes (90, 113, 274). Increased resting BP variability and augmented cardiovascular

30

reactivity are independent predictors for future risk of adverse cardiovascular events (172, 255, 296). While many studies in humans have added to our knowledge of the thermoregulatory and cardiovascular effects of moderate to severe hypohydration (155, 162, 234), many have confounding effects due to the use of exercise and/or heat chambers. We do know that mild hypohydration causes decrements in cognitive function, physical function, and thermoregulation (19, 58, 251), but there are less appreciated changes in resting and reflex BP regulation that may occur. Thus, there is a critical need to determine how mild hypohydration and age impact resting and reflex BP regulation.

Chapter 2

THE EFFECT OF MILD HYPOHYDRATION ON BLOOD PRESSURE VARIABILITY IN YOUNG ADULTS

2.1   Introduction

High arterial blood pressure variability (BPV) is associated with cardiovascular morbidities such as cerebral small vessel disease (94), increased carotid artery intima-media thickness (183), target organ damage (182, 285), hypertensive status (223), and is associated with cardiovascular mortality (185, 241). Water deprivation (WD) causes plasma volume contraction and concentrates electrolytes, causing plasma hypernatremia. High dietary sodium-induced blood hypernatremia increases BPV in rodents (267), however, it remains unknown if hypernatremia affects BPV in humans. This is a clinically relevant issue given that underhydration (defined as chronically low water intake (159)) and is highly prevalent and predictive of future incidence of cardiovascular disease (52). Importantly, underhydration is associated with low water intake but normal plasma osmolality values (not above 290mOsm) (159) but acute reductions in water intake *can* elicit increases in plasma osmolality (283). Therefore, we sought to determine if acute WD-induced blood hypernatremia alters BPV in humans, which could contribute to future development of cardiovascular disease.

Blood hypernatremia is detected by neurons in forebrain circumventricular organs such as the organum vasculosum of the lamina terminalis (OVLT) and subfornical organ (SFO) in the hypothalamus (35). These structures polysynaptically

project to bulbospinal neurons in the rostral ventrolateral medulla (RVLM) to regulate sympathetic outflow and arterial blood pressure (BP) (118). In rodents, WD-induced hyperosmolality has been demonstrated to increase excitatory amino acid neurotransmission in the RVLM to elevate sympathetic outflow and support BP (39, 41). Additionally, high salt feeding-induced hypernatremia in rodents has been demonstrated to increase BPV (267). Based on these published results in animal models, we hypothesized that WD-induced hypernatremia would augment BPV in humans. To test this hypothesis, healthy young adults completed a normal hydration control condition (CON) and a WD condition in randomized crossover fashion. In the laboratory, we assessed resting beat-to-beat BP and a subset of participants underwent ambulatory BP monitoring for the final 24 hours of each hydration condition.

Hypernatremia is proposed to affect central sympathetic circuits involved in BP regulation, thus, we assessed muscle sympathetic nerve activity (MSNA) for determination of spontaneous sympathetic baroreflex function, in addition to cardiac vagal baroreflex function. Further, baroreflex function has been reported to be altered after 11 days of hypertonic cerebroventricular NaCl infusion in rodents, suggesting central actions of NaCl (44) and after five to seven days of high NaCl feeding in humans (17, 67), we speculated that WD-induced hyperosmolality/hypernatremia would also alter baroreflex function. Apart from the central mechanisms, the peripheral arc of BP regulation (i.e., sympathetic vascular transduction defined as vasoconstrictor responses following spontaneous MSNA bursts) also mediates BPV (18, 305). It has been shown that augmented sympathetic vascular transduction is associated with augmented BPV (18). We speculated that WD would augment sympathetic vascular transduction due to previous findings that hypohydration impairs endothelial-dependent dilation (15) and

33

endothelial-dependent vasodilatory signaling blunts α_1-adrenergic vasoconstriction (133), the primary mediator of sympathetic vascular transduction (87). To accomplish this, we employed a signal-averaging method previously described by our laboratory (18). and others (86). In summary, we sought to determine if WD augments BPV, which may contribute to the link between underhydration and cardiovascular disease incidence. We also assessed sympathetic and cardiovagal baroreflex sensitivity and sympathetic vascular transduction as these physiological measures contribute to BPV.

2.2   Methods

2.2.1   Participants.

The Institutional Review Board at the University of Delaware approved this protocol (IRB #1097747) and the study conformed with the most recent version of the *Declaration of Helsinki*. The data reported here are part of an ongoing registered protocol (ClinicalTrials.gov Identifier: NCT03560869). All participants provided verbal and written consent prior to enrollment in the study. During the initial screening visit, participants completed a physical activity readiness questionnaire, medical history questionnaire, and underwent measurements of height and weight. Resting brachial BP measurements were performed in triplicate with participants in the seated position following five minutes of quiet rest (Dash 2000; GE Medical Systems, Milwaukee, WI). Inclusion criteria for this study included: age between 20-40 yrs, resting systolic BP <140 mmHg, resting diastolic BP <90 mmHg, and body mass index <30 kg/m^2 at screening. Study participants were also non-smokers free of any known cardiovascular disease, and had no evidence of metabolic, neurological, renal, or pulmonary disease.

2.2.2 Hydration conditions.

Participants completed two three-day-long hydration conditions separated by at least one week in random order. Female participants were tested in the early follicular phase of the menstrual cycle or placebo phase of oral contraceptives (self-reported). Pilot studies allowed for determination of water intake requirements necessary to observe modest increases in serum sodium concentrations and plasma osmolality. During CON, participants were asked to consume 23mL H_2O/kg body weight/day for days one through three and to consume 250mL H_2O before arriving to the laboratory for testing on day four. The WD condition required participants to consume 23mL H_2O/kg body weight on day one, 17mL H_2O/kg body weight on day two, and 10mL H_2O/kg body weight on day three, followed by a 16-hour water abstention period prior to testing on day four. Participants were asked to refrain from caffeine, alcohol, vigorous exercise, or moderate exercise in the heat on days two and three during both three-day hydration protocols. Participants were given guidance on estimating food portion sizes and keeping a diet log to maintain recommended daily sodium intake (2300mg/day). They were also instructed to consume the same foods during the second three-day hydration condition as reported in their diet log during the first three-day hydration condition. Participants collected their urine in a sterile collection container for 24 hours preceding in-lab testing and reported to the laboratory for testing on the fourth day of each condition after fasting for at least four hours.

2.2.3 Experimental visit.

Upon arrival to the laboratory, participants provided a spot urine sample, were weighed (Tanita Body Composition Analyzer, Model TBF-300A; Arlington Heights, IL), and provided a subjective rating of their thirst and dryness of their mouth using a

Likert scale. Participants laid in the supine position for ≥20 minutes before an intravenous catheter was placed in the antecubital space of the dominant arm for blood sampling. Participants were then outfitted with the required equipment for measurement of MSNA, beat-to-beat BP, brachial BP, heart rate (HR), leg blood flow (described below), and respiratory excursions (via a strain-gauge pneumograph). Data were collected during a 10-minute baseline period as participants rested quietly in a dimly lit, temperature-controlled room (22-24°C).

2.2.4 Blood & urine analysis.

Venous blood samples and the 24-hour urine samples were analyzed for serum and urine electrolyte concentrations (EasyElectrolyte Analyzer; Medica, Beford, MA, USA), and plasma and urine osmolality (3D3 Osmometer; Advanced Instruments, Norwood, MA). Venous blood samples were also analyzed for Hb (Hb 201+; Hemocue, Lake Forest, CA, USA) and Hct (Pre-calibrated Clay Adams, Readacrit Centrifuge; Becton Dickinson, Sparks, MD, USA). Change in plasma volume (expressed as a percentage) was calculated using the following equation (76):

$$\text{Plasma volume (\%)} = ((100 \times (Hb_{CON}/Hb_{WD})) \times (1 - (Hct_{WD}/100))/(1 - Hct_{CON}/100)) - 100$$

Additionally, urine specific gravity was determined for the spot and 24-hour urine samples. Female participants spot urine samples were also used to confirm that they were not pregnant (hCG cassettes, Moore Medical).

2.2.5 Muscle sympathetic nerve activity.

MSNA was directly assessed via microneurography (n=18 pairs of recordings) as previously described by our laboratory (36, 112, 188). Briefly, a tungsten recording microelectrode was inserted percutaneously near the fibular head or in the popliteal

fossa and a reference microelectrode was inserted ≤3 cm from the recording electrode. The nerve recording was amplified (80-90,000x), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-4; University of Iowa, Bioengineering, Iowa City, IA, USA). An adequate nerve recording was confirmed prior to the experimental protocols using the following criteria: absence of afferent nerve activity during light skin stroking, increased efferent nerve activity during voluntary end-expiratory apnea, and pulse synchronous efferent nerve bursts with ≥3 to 1 signal-to-noise ratio.

2.2.6 Arterial blood pressure assessment.

Beat-to-beat BP was measured at the finger of participants' non-dominant hand using photoplethysmography (Finometer; Finapres Medical Systems, the Netherlands) and calibrated to brachial BP according to the manufacturer's recommended calibration procedures (117). Brachial BP was measured using an automated osillometric sphygmomanometer (Dash 2000; GE Medical Systems, WI, USA) and used to verify absolute beat-to-beat BP values. Simultaneously, HR was continuously recorded using standard single-lead ECG (Dash 2000; GE Medical Systems, WI, USA). Respiratory excursions were recorded via a strain gauge pneumograph (Pneumotrace; UFI, CA, USA) placed around the abdomen.

2.2.7 Arterial blood pressure variability.

BP variability was calculated using standard deviation of BP values and using the average real variability (ARV) index. The ARV index calculates the average of the absolute differences between consecutive BP measurements and is thought to provide further prognostic value compared to traditional measures such as standard deviation of

BP (29, 194). BP variability was assessed during 10 minutes of quiet rest using the beat-to-beat BP signal derived from the Finometer (described above). Additionally, ambulatory BP was assessed in a subset of participants (n=23) during the 24-hour periods preceding each experimental visit. Oscar 2 with Sphygmacor, SunTech Medical). The monitor measured BP on the non-dominant arm every 20 minutes from 0601-2200 hours and every 30 minutes from 2201-0600 hours. Participants self-reported sleep and wake times in the laboratory to note the start of 'daytime' and 'nighttime' for the BP values. Data were only included for if participants had at least 15 measurements during the daytime and at least 8 measurements during the nighttime (218).

2.2.8 Femoral artery blood flow.

Continuous measures of common femoral artery (CFA) diameter and velocity were obtained via duplex Doppler ultrasound (GE Logiq P5) as previously described (18, 86). Briefly, a 10- or 12-MHz linear array transducer was selected for optimal image quality and positioned at the CFA via a custom-designed clamp, approximately two to three centimeters proximal to the bifurcation of the superficial and deep femoral arteries. Blood velocity was simultaneously obtained with diameter in pulsed-wave mode at an insonation angle of ≤60° and operating at a linear frequency of 5 MHz. The sample volume encompassed the entire vessel lumen without extending beyond the walls.

2.2.9 Hemodynamic data analysis.

Data were collected continuously at a sampling rate of 1000 Hz using LabChart (LabChart 8.0 Pro, ADInstruments, Colorado Springs, CO, USA). CFA diameter and

blood velocity were analyzed and synchronized with beat-to-beat ECG, BP, and MSNA signals using custom LabVIEW programs. Femoral blood flow was determined via the continuous recordings of CFA diameter and blood velocity and calculated as: $\pi \times (\text{diameter}/2)^2 \times \text{mean blood velocity} \times 60$. Limb vascular conductance (LVC) was determined by dividing femoral blood flow by mean BP, which was calculated as the integral of the BP waveform. Due to technical difficulties, paired LVC was obtained in 14 of the participants (i.e., during four of the 36 visits where MSNA was collected, the leg blood flow data was not satisfactory for analysis).

The sympathetic neurogram was analyzed on a beat-to-beat basis to determine the presence/absence of MSNA bursts using custom LabView software and visually inspected by a lab member blinded to condition and trained in MSNA data analysis and processing (JCW). Bursts were identified in accordance with recent guidelines (265, 312) via the following criteria: (1) >3:1 signal-to-noise ratio, (2) burst morphology consistent with MSNA bursts, and (3) a pulse-synchronous signal. MSNA was quantified as burst frequency (bursts $\bullet$ minute^{-1}), burst incidence (bursts $\bullet$ 100 heart beats^{-1}), and total activity ([(burst frequency $\bullet$ burst amplitude) $\bullet$ minute^{-1}], AU $\bullet$ minute^{-1}) were determined as indices of MSNA. To quantify indices of sympathetic vascular transduction (i.e. the functional effect of individual bursts of MSNA on BP and blood flow), a spike-triggered averaging methodology was used that has been previously described (18, 86). Briefly, each cardiac cycle containing a burst of MSNA was identified and set as cardiac cycle 0 whether or not the burst was a singlet (bursts directly bordered by >1 heartbeat lacking MSNA) or part of a cluster (bursts adjacent to other burst(s) of MSNA). Both mean BP and LVC of cardiac cycle 0 were determined and followed for 10 subsequent cardiac cycles. The absolute change in mean BP and percent

change in LVC was determined in each of these 10 cardiac cycles. These methods reliably quantify beat-to-beat changes in BP and blood flow induced spontaneous MSNA bursts.

2.2.10 Sympathetic baroreflex sensitivity analysis.

Sympathetic baroreflex sensitivity was assessed using in the resting state during spontaneous BP oscillations as previously described (114, 189, 281). This method quantifies baroreflex sensitivity around the operating point and is well correlated to the modified Oxford technique (126). Briefly, each cardiac cycle was assigned to a bin (3mmHg) based on diastolic BP on the corresponding cardiac cycle. Burst incidence and total activity were regressed over diastolic BP bins. All data were weighted to account for the number of cardiac cycles within each bin (114, 189). Bins without MSNA bursts were included in the analysis. Slopes of the linear regressions were used as an index of sympathetic baroreflex sensitivity if they had an r≥0.5 (114, 189, 221).

2.2.11 Cardiac vagal baroreflex sensitivity analysis.

Beat-to-beat time series of systolic BP and R-R interval were analyzed using the sequence method for estimating spontaneous cardiac vagal baroreflex sensitivity (HemoLab version 8.9, Harald Stauss Scientific, Iowa City, IA). A detailed description of this method has been published previously (25). As previously done in our laboratory (17), sequences of four or more consecutive cardiac cycles in which systolic BP and R-R interval change in the same direction were identified as baroreflex sequences. Sequences were detected only when the variation in R-R interval was >0.5 ms and SBP changes were >1 mmHg. A linear regression was applied to each individual sequence, and only those sequences in which R^2 was >0.80 were accepted. Values of cardiac vagal

baroreflex sensitivity were accepted when the number of sequences was ≥3 for both up and down sequences. The slopes of those individual linear regressions were then calculated and averaged for a measure of spontaneous cardiac vagal baroreflex sensitivity. Cardiac vagal baroreflex sensitivity was determined for all sequences combined and separately for up (increase in both SBP and R-R interval) and down (decrease in both SBP and R-R interval) sequences.

2.2.12 Statistical analysis.

Biochemical, MSNA, hemodynamic, and BP variability parameters were compared between the hydration conditions using paired, two-tailed, t-tests. The effects of hydration condition on sympathetic vascular transduction were compared using two-way repeated measures ANOVAs (condition x cardiac cycle number following spontaneous MSNA bursts). Tukey's HSD were used to correct for multiple comparisons (post-hoc analyses for repeated measures ANOVAs). Additionally, peak changes in mean BP and LVC were compared between the hydration conditions using paired, two-tailed, t-tests. All data are presented as means ± SD. All data were analyzed using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA) and significance was set *a priori* at p < 0.05.

2.3 Results

All 35 participants were non-hypertensive and non-obese (Table 2.1). Despite significant changes in several blood and urine hydration markers during WD, all resting hemodynamic measures were not different between conditions (Table 2.2). Plasma volume, estimated by changes in Hb (CON: 13.5±1.5 vs. WD: 13.7±1.4 g • dL^{-1}, p=0.11) and Hct (CON: 40.5±4.0 vs. WD: 40.9±4.1 %, p=0.84), declined (-1.1±8.2%) during

WD. Dietary sodium consumption was similar during each three-day condition (CON: 2151±372 vs. WD: 2184±400 mg • day^{-1}, p=0.72). Modelflow-derived estimates of cardiac output (CON: 5.1±1.0 vs. WD: 5.3±0.9 L • min^{-1}, p=0.28) and total peripheral resistance (CON: 1614±389 vs. WD: 1512±373 dynes • s • cm^{-5}, p=0.27) were not different between conditions. We did not observe reductions in body mass during WD compared to CON (CON: 68.5±13.5 vs. WD: 67.8±13.1 kg, p=0.84).

Resting beat-to-beat and ambulatory daytime systolic BPV (assessed via ARV and SD) was not different between conditions (Figure 2.1). Similarly, we did not observe differences in beat-to-beat diastolic BP ARV (CON: 1.5±0.6 vs. WD: 1.5±0.6 mmHg, p=0.76) and SD (CON: 3.9±1.2 vs. WD: 3.7±1.2 mmHg, p=0.58) or ambulatory daytime diastolic BP ARV (CON: 8.9±2.5 vs. WD: 8.1±1.7 mmHg, p=0.19). Ambulatory daytime diastolic BP SD was higher during CON (CON: 10.5±3.4 vs. WD: 9.0±2.3 mmHg, p=0.04). All indices of nighttime BPV were not different between conditions (p>0.12 for all systolic and diastolic BP ARV and SD values). Twenty-four-hour, daytime, and nighttime BP, and heart rate during ambulatory monitoring were not different between conditions. BP dipping patterns were also similar between experimental conditions (Table 2.3).

Resting sympathetic baroreflex sensitivity (assessed as changes in both burst incidence and total activity) were not different between conditions (Figure 2.2). For cardiac vagal baroreflex sensitivity, overall gain (CON: 24±8 vs. WD: 27±12 ms • mmHg^{-1}, p=0.43), gain from up sequences (CON: 26±13 vs. WD: 27±14 ms • mmHg^{-1}, p=0.74), and gain from down sequences (CON: 23±9 vs. WD: 27±11 ms • mmHg^{-1}, p=0.46) were not different between experimental conditions.

Mean BP changes and LVC percent changes over the 10 cardiac cycles following spontaneous MSNA bursts were not different between conditions. Additionally, peak mean BP and peak LVC changes following spontaneous MSNA bursts were not different between conditions (Figure 2.3). We observed significant correlations between peak mean BP changes attained over the 10 cardiac cycles following spontaneous MSNA bursts and beat-to-beat mean BP ARV within the CON condition ($r=0.50$, $p=0.04$) and within the WD condition ($r=0.47$, $p=0.047$). However, there was little to no relation between changes in sympathetic vascular transduction of mean BP following spontaneous MSNA bursts (WD-CON) and changes in beat-to-beat mean BP ARV (WD-CON) ($r=0.03$, $p=0.91$).

2.4 Discussion

The primary novel finding of this study was that short-term WD did not affect resting beat-to-beat or daytime ambulatory systolic BPV. Similarly, no differences were observed between conditions for sympathetic vascular transduction following spontaneous MSNA bursts. Finally, spontaneous sympathetic and cardiac vagal baroreflex sensitivities were unaffected by short-term WD. Together, these findings suggest that short-term WD elicits mild hypohydration with relative hypernatremia/hyperosmolality, but may not adversely affect resting BP regulation in healthy young adults.

Our strategy to achieve mild – not severe – hypohydration in humans involved short-term WD. These data demonstrate that we were successful in experimentally causing modest increases in plasma osmolality, serum sodium concentrations, urine osmolality, thirst, and urine specific gravity. The increases in pOsm (~4 mOsm/kg H_2O) represent physiologically relevant changes and are similar to those observed in previous

studies examining the physiological effects of mild hypohydration (55, 272). Based on published reference values for hydration biomarkers in humans, plasma osmolality and 24-hour urine volume values in the present study suggest participants were "well-hydrated/euhydrated" during CON and "slightly dehydrated" during WD (14). The change in hydration status achieved here increases relevance to the general public, as mild hypohydration is a contributing factor in numerous acute and chronic diseases (186), with underhydration predicting future incidence of diabetes (243) and heart disease (52). Other investigations have demonstrated that mild hypohydration affects cerebrovascular responses during the cold pressor test (234), impairs exercise performance (212), reduces overall cognitive performance (315) and executive function (272). Our focus to examine the effect of mild hypohydration on BPV is highly clinically-relevant given that high BPV is predictive of several disease states and mortality in humans (182, 183, 185, 223, 241, 285).

Our hypothesis was developed, in part, by prior observations from rodent studies suggesting that hypernatremia induced by high salt feeding increases BPV (267) and that 48 hours of WD increases excitatory amino acid neurotransmission in the RVLM to elevate sympathetic outflow and BP (39, 41). Additionally, one study in rodents reported that sympathetic blockade (via $\alpha 1$- and $\beta 1$- adrenergic receptor antagonists) significantly attenuated the increases in BP brought about by 48 hours of WD (299), suggesting changes in BP following WD could be mediated by the sympathetic nervous system. We reasoned that short-term WD in humans would augment BPV, and that this may be partially attributed to alterations in sympathetic vascular transduction and/or arterial baroreflex function. A key difference between the high salt feeding study and the WD protocol employed in the present study is time of exposure to

44

hypernatremia/hyperosmolality. Therefore, the lack of differences in the current experiment may simply reflect too short a time frame for any increases in serum sodium concentrations and plasma osmolality to sensitize central sympathetic networks. While a study design including 48 hours of WD in humans (to match rodent studies) could potentially explain such discrepancies, it would be not be tolerable to complete or ethical to impose in human subjects.

While changes in BPV were not observed in the present study, our findings are consistent with a previous study in humans that reported no change in the power spectral density of mean BP, a measure of BPV in the frequency domain, following a furosemide-induced hypohydration protocol (220). However, a key difference is that in the present study WD increased plasma osmolality (~4 mOsm/kg H_2O) but barely changed plasma volume (-1% reduction during WD), whereas in the previous study there was reports of plasma osmolality or blood electrolyte concentrations but there were reports of moderate plasma volume contraction (-10% following furosemide administration) (220). Thus, because furosemide normally elicits iso-osmotic hypovolemia, the previous study does not necessarily inform our primary hypothesis regarding WD-induced blood hypernatremia/hyperosmolality and BPV.

The Fadel laboratory has described the methodology for quantification of mean BP and LVC changes following spontaneous MSNA bursts and prospectively applied it to gain additional insight into the peripheral arc of the arterial baroreflex (86, 87, 305). Our group recently observed reductions in sympathetic vascular transduction following a low-sodium (1000mg/day) diet compared to a recommended-sodium (2300mg/day) diet (18). Hypohydration has been demonstrated to impair endothelial-dependent dilation (15). Endothelial-dependent vasodilatory signaling has been reported to blunt

α_1-adrenergic vasoconstriction to skeletal muscle beds (133). Therefore, because sympathetic vascular transduction is primarily α_1-adrenergic-mediated (87), we reasoned that mild hypohydration would augment sympathetic vascular transduction as a result of reduced vasodilatory signaling. During CON, we observed a significant relation between sympathetic vascular transduction of mean BP changes following spontaneous MSNA bursts and beat-to-beat mean BP ARV. This is in agreement with previous reports from Dr. Fadel's group (305) and our group (18). Therefore, we would have expected any increase in beat-to-beat mean BP ARV to be associated with an increase in peak mean BP changes following spontaneous MSNA bursts. Consistent with the lack of change in BPV between conditions, we observed no change in sympathetic vascular transduction of spontaneous MSNA bursts between conditions.

While changes in arterial baroreflex function were not observed in the present study, our findings are consistent with a previous study in humans that reported no change in sympathetic baroreflex sensitivity following a water-restriction protocol (242). However, a key difference is that in the present study WD increased plasma osmolality (~4 mOsm/kg H_2O), whereas in the previous study there was no observed change in plasma osmolality (euhydration: 286±1 vs. dehydration: 286±1 mOsm/kg H_2O). This is likely because in the previous study, fluid intake was limited to 10mL fluid/kg body weight for the 24-hour period preceding the dehydrated visit, whereas in the present study participants reduced their water intake over three days and then completed a 16-hour water abstention prior to testing. As discussed above, the relatively shorter timeframe of hypernatremia or the difference in magnitude of changes in blood sodium concentrations may explain why no changes in sympathetic baroreflex sensitivity were observed in the present study. Another study investigating the effects

of dehydration on baroreflex function noted a trend for lower sympathetic baroreflex gain following dehydration induced by 90 minutes of acute aerobic exercise compared to intravenous rehydration ~30 minutes later (55). While insightful, these data could have potentially been influenced by the prior bout of exercise (about one to two hours post exercise). A consideration in the present study design was to impose mild hypohydration without the potential confounding effects of heat stress and/or exercise as performed other previous studies (55, 240).

2.5 Limitations

The primary objective of this study was to determine if mild hypohydration-induced hypernatremia/hyperosmolality affects BP variability in young adults. First, this study provides insight into how mild, but not severe hypohydration affects BP variability on a beat-to-beat basis in a controlled laboratory setting and during activities of daily living. While plasma osmolality and 24-hour urine volume values suggest participants were "slightly dehydrated" during WD, urine osmolality and specific gravity values indicate suggest participants were "well-hydrated/euhydrated" during WD (14). Nevertheless, there were clear differences in hydration status between the two conditions among these participants. Second, while future studies may be warranted to explore potential sex differences in resting BP regulation during mild hypohydration, preliminary analyses within this dataset suggest no sex differences are present in any of the measures reported here. Third, our conclusions are restricted to healthy young adults, and it is possible WD in older adults *would* elicit alterations in BP variability (and overall resting BP regulation). Specifically, postmenopausal females lose the protective effects of estrogen and MSNA increases with age, with resting MSNA becoming more tightly coupled to BP (125). Thus, a future study comparing the effects

of mild hypohydration on BP variability in young and older (postmenopausal) female adults is warranted. Fourth, we did not measure renin-angiotensin-aldosterone system hormones or arginine vasopressin, which are known to play a role in BP regulation during hypohydration. Future studies addressing the limitations of this study discussed above are warranted.

2.6   Summary

These data suggest that acute WD does not augment resting beat-to-beat or ambulatory BP variability in healthy young adults. Further, our findings suggest that mild hypohydration does not impact resting sympathetic vascular transduction or spontaneous sympathetic baroreflex function in healthy young adults.

Table 2.1   Participant screening measures.

Screening characteristics	
Number (F/M)	35 (17/18)
Age, yr	25 ± 4
Body mass, kg	70 ± 14
Body mass index, kg $\cdot$ m^{-2}	23 ± 3
Systolic BP, mmHg	107 ± 11
Diastolic BP, mmHg	60 ± 7

Data are presented as mean $\pm$ SD. BP, arterial blood pressure.

Table 2.2   Biochemical and resting hemodynamic measures

	Control	Water deprivation	p
Plasma osmolality, mOsm • kg H_2O^{-1}	288 ± 4	292 ± 5	**<0.01**
Serum sodium, mmol • L^{-1}	141.0 ± 2.3	142.3 ± 2.1	**<0.01**
Serum chloride, mmol • L^{-1}	103.7 ± 3.0	105.3 ± 2.6	**<0.01**
Serum potassium, mmol • L^{-1}	4.07 ± 0.30	4.14 ± 0.41	0.33
Urine osmolality, mOsm • kg H_2O^{-1}	480 ± 161	711 ± 199	**<0.01**
Spot urine specific gravity	1.016 ± 0.007	1.022 ± 0.004	**<0.01**
24-hour urine specific gravity	1.013 ± 0.005	1.019 ± 0.005	**<0.01**
Urine rate, L • $24h^{-1}$	1.3 ± 0.6	0.8 ± 0.3	**<0.01**
Thirst rating	3 ± 3	7 ± 2	**<0.01**
Systolic BP, mmHg	110 ± 9	112 ± 9	0.18
Mean BP, mmHg	80 ± 6	80 ± 6	0.46
Diastolic BP, mmHg	64 ± 6	64 ± 6	0.88
Heart rate, beats • min^{-1}	58 ± 7	58 ± 8	0.96
Burst frequency, bursts • min^{-1}	15 ± 7	14 ± 7	0.55
Burst incidence, bursts • 100 heart $beats^{-1}$	26 ± 14	25 ± 13	0.54
Total activity, AU • min^{-1}	298 ± 387	210 ± 163	0.26
Mean femoral blood flow, mL • min^{-1}	410 ± 228	456 ± 213	0.28

Data are presented as mean ± SD, paired, two-tailed, t-tests, bold text denotes $P < 0.05$. Arterial blood pressure (BP) was measured at the brachial artery.

Table 2.3   Ambulatory blood pressure measures

	Control	Water deprivation	p
24h systolic BP, mmHg	117 ± 11	116 ± 11	0.75
24h diastolic BP, mmHg	66 ± 6	66 ± 6	0.41
Daytime systolic BP, mmHg	121 ± 12	120 ± 11	0.84
Daytime diastolic BP, mmHg	70 ± 6	69 ± 6	0.38
Nighttime systolic BP, mmHg	105 ± 10	105 ± 12	0.87
Nighttime diastolic BP, mmHg	56 ± 6	56 ± 7	0.84
24h heart rate, beats • min^{-1}	67 ± 8	66 ± 7	0.70
Daytime heart rate, beats • min^{-1}	83 ± 7	83 ± 8	0.92
Nighttime heart rate, beats • min^{-1}	57 ± 7	56 ± 6	0.29
Nighttime systolic BP dipping, %	12 ± 5	12 ± 5	0.93
Nighttime diastolic BP dipping, %	19 ± 7	18 ± 6	0.27

Data are presented as mean ± SD, paired, two-tailed, t-tests, bold text denotes P < 0.05. Arterial blood pressure (BP) was measured at the brachial artery.
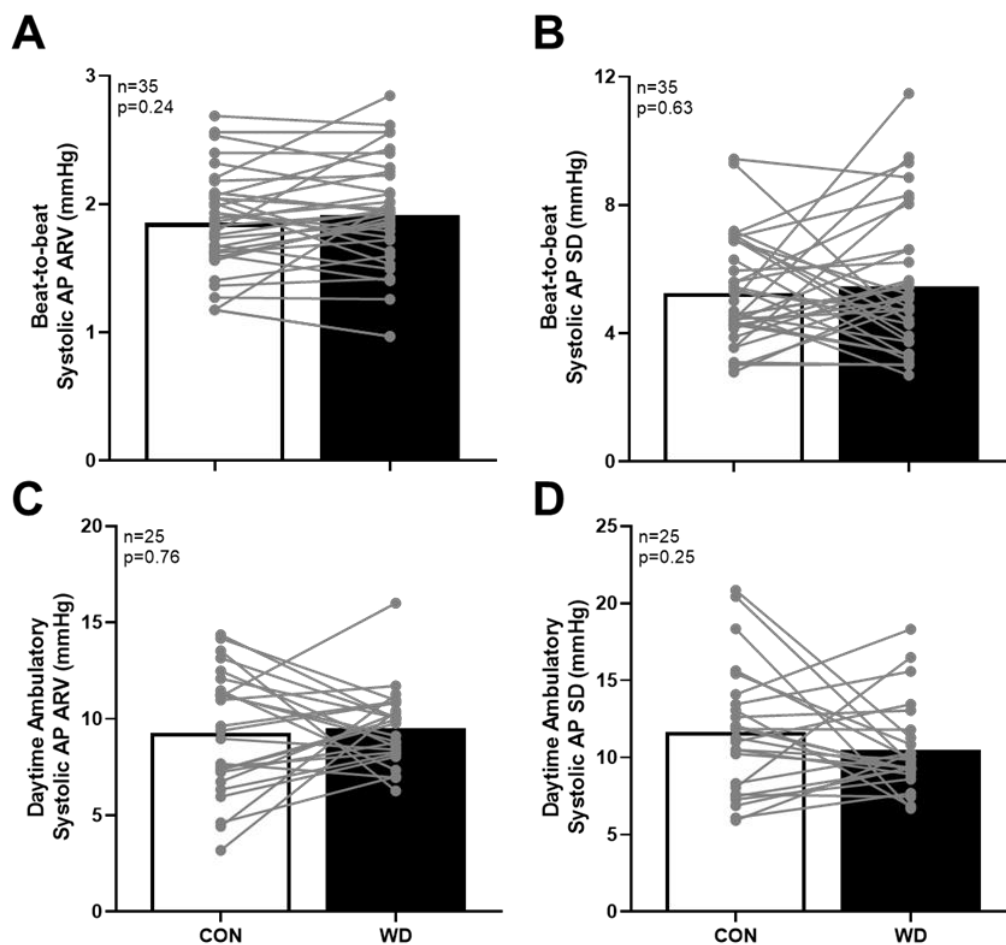
Figure 2.1   Summary data with individual data points are presented above for beat-to-beat systolic arterial blood pressure (BP) (A) average real variability (ARV) and (B) standard deviation (SD) during quiet rest in the supine position. Additionally, summary data with individual data points are presented for daytime ambulatory systolic BP (C) ARV and (D) SD. Open bars indicate the normally hydrated control condition (CON) and closed bars indicate the water deprived condition (WD). Data were compared with paired, two-tailed, t-tests with significance set at $p<0.05$.
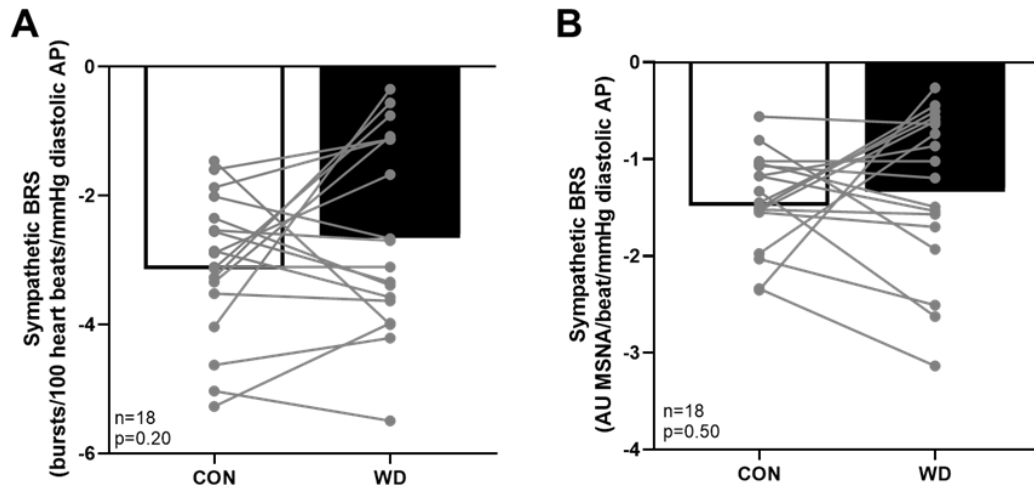
Figure 2.2   Summary data with individual data points are presented above for the slopes of the linear regressions between muscle sympathetic nerve activity (A) burst incidence and diastolic arterial blood pressure (BP) and (B) total activity and diastolic BP during quiet rest in the supine position. Open bars indicate the normally hydrated control condition (CON) and closed bars indicate the water deprived condition (WD). Data were compared with paired, two-tailed, t-tests with significance set at $p < 0.05$.
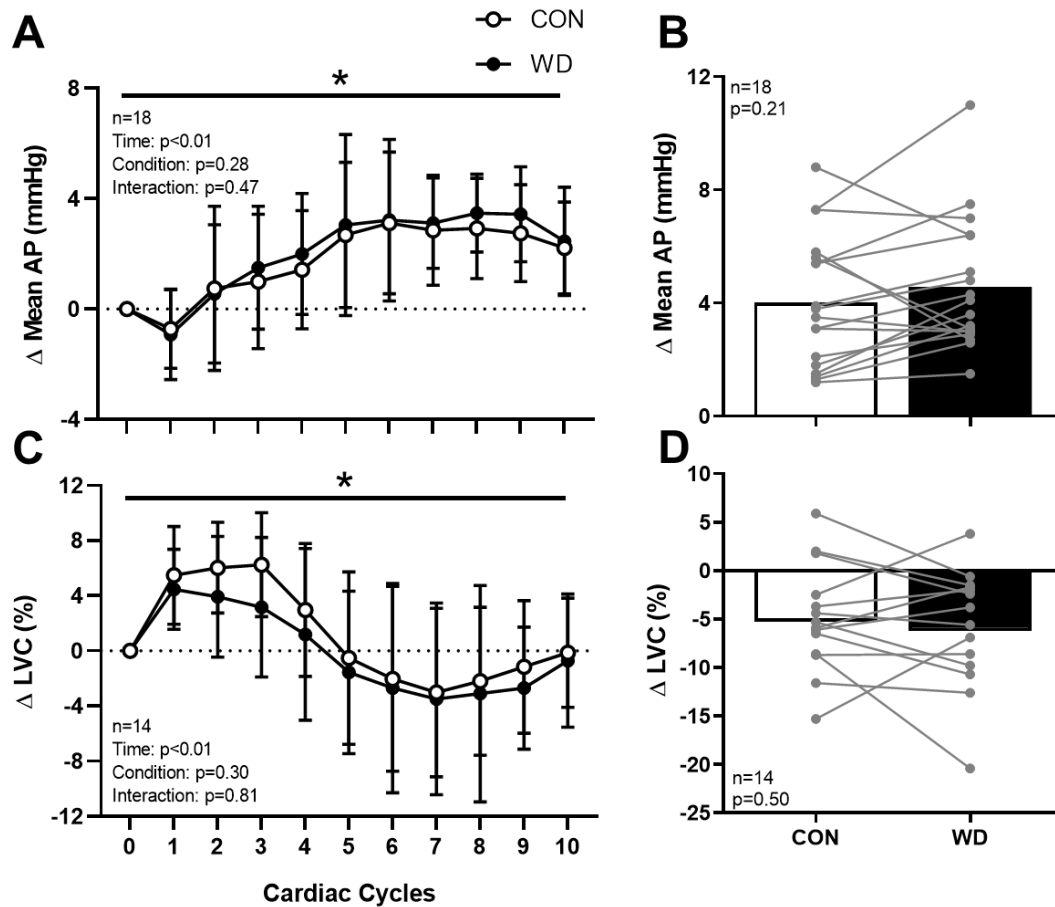
Figure 2.3   Summary data (mean ± standard deviation) are presented above showing the effect of spontaneous muscle sympathetic nerve activity (MSNA) bursts on (A) mean arterial blood pressure (BP) and (C) limb vascular conductance (LVC) over 10 cardiac cycles during quiet rest in the supine position. Additionally, summary data with individual data points are presented for peak (B) absolute increases in mean BP and (D) percent decreases in LVC over the 10 cardiac cycles immediately following spontaneous MSNA bursts. Open circles and bars indicate the normally hydrated control condition (CON) and closed circles and bars indicate the water deprived condition (WD). Data were compared with two-way (time x condition) repeated measures ANOVAs (panels B & D) and paired, two-tailed, t-tests (panels A & C) with significance set at $p < 0.05$. * denotes a significant time effect.

Chapter 3

THE EFFECT OF MILD HYPOHYDRATION ON CARDIOVASCULAR RESPONSES TO STATIC EXERCISE IN YOUNG ADULTS

3.1 Introduction

High salt feeding in salt-resistant rodents augments sympathetic and pressor responses to activation of various reflexes, including sciatic afferent nerve stimulation (267) and the exercise pressor reflex (318). One mechanism hypothesized to underlie these effects is hypernatremia acting centrally to sensitize neurons of the rostral ventrolateral medulla (RVLM). Hypernatremia is detected by neurons in forebrain circumventricular organs such as the organum vasculosum of the lamina terminalis (OVLT) or subfornical organ (SFO). These structures polysynaptically project to bulbospinal neurons in the RVLM to regulate sympathetic nerve activity (SNA) and arterial blood pressure (BP) (118). Chronic increases in dietary salt intake have been reported to exaggerate sympathoexcitatory and sympathoinhibitory responses evoked from the RVLM, such as local injection of various neurotransmitters including L-glutamate and GABA. These chronic increases in dietary salt intake also produce parallel elevations in plasma sodium concentrations ($[Na^+]$) and osmolality (pOsm) (5, 119). Importantly, lesion of the anteroventral 3rd ventricular region that contains OVLT and SFO neurons prevents the salt-induced changes in RVLM neurons and exaggerated reflexes (4, 267).

Water deprivation (WD) is another model characterized by plasma hypernatremia/hyperosmolality as a result of plasma volume contraction. It has been reported that hypernatremia sensed through forebrain osmoreceptors increases excitatory amino acid neurotransmission in the RVLM to elevate SNA and support BP (39, 41). In addition, WD has been shown to augment pressor responses to unilateral RVLM microinjection of L-glutamate, suggesting increased sensitivity of RVLM neurons to excitatory amino acids during severe dehydration in rodents (39). Thus, WD should augment SNA and BP responses to sympathoexcitatory stimuli that depend on glutamatergic neurotransmission in the RVLM.

In humans, hypohydration is known to impair physical performance (58, 212, 251), cognitive function (19, 272, 315), and endothelial function (15). Hypohydration contributes to disease states (186), and low water intake is predictive of future incidence of diabetes (243) and heart disease (52). Despite the widespread deleterious effects of hypohydration on physiological function and health outcomes, the effect of hypohydration on reflex cardiovascular control in humans remains unknown. An augmented BP response during exercise (i.e., exaggerated exercise pressor reflex activation) is an independent predictor of future cardiovascular disease risk in humans (190, 255, 296). However, it is unclear if WD augments sympathetic and pressor responses during exercise pressor reflex activation in humans.

The overall purpose of this study was to determine if WD-induced hypernatremia/hyperosmolality augments SNA and pressor responses. To fill the gaps in knowledge discussed above, we examined the effect of WD on sympathetic and pressor responses during exercise pressor reflex activation. We hypothesized that WD would induce hypernatremia/hyperosmolality and consequently augment sympathetic

and pressor responses in young adults. To test our hypothesis, we employed a step-wise reduction in water intake over three days followed by a 16h WD. In random crossover fashion, we recorded muscle SNA and beat-to-beat BP in healthy young adults during isometric handgrip exercise followed by post-exercise ischemia (metaboreflex isolation) following WD and a normal hydration control condition.

3.2   Materials and Methods

3.2.1   Participants.

The Institutional Review Board at the University of Delaware approved this protocol and the study conformed with the most recent version of the *Declaration of Helsinki*. The data reported here are part of an ongoing registered protocol (ClinicalTrials.gov Identifier: NCT03560869). All participants provided verbal and written consent prior to enrollment in the study. During the initial screening visit, participants completed a physical activity readiness questionnaire, medical history questionnaire, and underwent measurements of height and weight. Resting brachial BP measurements were performed in triplicate with participants in the seated position following five minutes of quiet rest (Dash 2000; GE Medical Systems, Milwaukee, WI). Inclusion criteria for this study included: age between 20-40 yrs, resting systolic BP <140 mmHg, resting diastolic BP <90 mmHg, and body mass index <30 kg/m^2 at screening. Study participants were also non-smokers free of any known cardiovascular disease, and had no evidence of metabolic, neurological, renal, or pulmonary disease.

3.2.2   Hydration conditions.

Participants completed two three-day-long hydration conditions separated by at least one week in random order. Female participants were tested in the early follicular

phase of the menstrual cycle or placebo phase of oral contraceptives (self-reported). Pilot studies allowed for determination of water intake requirements sufficient to observe modest increases in serum $[Na^+]$ and pOsm. The normal hydration control condition (CON) required participants to consume 23mL H_2O/kg body weight/day for days one through three and to consume 250mL H_2O before arriving to the laboratory on day four. The WD condition required participants to consume 23mL H_2O/kg body weight on day one, 17mL H_2O/kg body weight on day two, and 10mL H_2O/kg body weight on day three, followed by a 16-hour water abstention period prior to testing on day four. Participants were asked to refrain from caffeine, alcohol, vigorous exercise, or exercise in the heat on days two and three during both three-day hydration protocols. Participants were given guidance on estimating food portion sizes and keeping a diet log to maintain recommended daily sodium intake (2300mg/day). A research dietitian analyzed participant's three-day diet records for sodium and water content using Nutrient Data System for Research (NDSR). They were also instructed to consume the same foods during the second three-day hydration condition as reported in their diet log during the first three-day hydration condition. Participants collected their urine in a sterile collection container for 24 hours preceding in-lab testing and reported to the laboratory for testing on the fourth day of each condition after fasting for at least four hours.

3.2.3 Experimental visit.

Upon arrival to the laboratory, participants provided a spot urine sample, were weighed (Tanita Body Composition Analyzer, Model TBF-300A; Arlington Heights, IL), and provided a subjective rating of their thirst and dryness of their mouth using a Likert scale. Participants laid in the supine position and an intravenous catheter was

placed in the antecubital space of the dominant arm in retrograde fashion. Maximal voluntary contraction (MVC) was determined using the average handgrip force produced (Grip Force Transducer, ADInstruments, Colorado Springs, CO) during three maximal forearm contractions, separated by a minute each. Participants were then outfitted with the required equipment for measurement of muscle SNA, beat-to-beat BP, brachial BP, heart rate (HR), and respiratory excursions (described below).

Muscle SNA was directly assessed via microneurography at the peroneal nerve. Briefly, a tungsten recording microelectrode was inserted percutaneously near the fibular head or in the popliteal fossa and a reference microelectrode was inserted ≤3 cm from the recording electrode, as previously performed in our laboratory (36). The nerve recording was amplified (x90,000), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-4; University of Iowa, Bioengineering, Iowa City, IA, USA). An adequate nerve recording was confirmed prior to the experimental protocols using the following criteria: absence of afferent nerve activity during light skin stroking, increased efferent nerve activity during voluntary end-expiratory apnea, and pulse synchronous efferent nerve bursts with ≥3 to 1 signal-to-noise ratio.

Beat-to-beat BP was measured at the finger of participants' non-dominant hand using photoplethysmography (Finometer; Finapres Medical Systems, the Netherlands) and calibrated to brachial BP according to the manufacturer's recommended calibration procedures (117). Brachial BP was measured using an automated osillometric sphygmomanometer (Dash 2000; GE Medical Systems, WI, USA) and used to verify absolute beat-to-beat BP values. Simultaneously, HR was continuously recorded using standard single-lead ECG (Dash 2000; GE Medical Systems, WI, USA). Respiratory

excursions were recorded via a strain gauge pneumograph (Pneumotrace; UFI, CA, USA) placed around the abdomen.

3.2.4 Experimental protocol.

Following supine rest for ≥20 minutes a venous blood sample was collected. Data were collected during a 10-minute baseline period as participants rested quietly in a dimly lit, temperature-controlled room (22-24°C). Following baseline data collection, participants performed a two-minute bout of isometric handgrip exercise (HG) with their dominant hand at 30% of MVC, using real-time feedback of force production displayed on a monitor, for assessment of the exercise pressor reflex. Immediately prior to the cessation of HG exercise, post-exercise ischemia (PEI) was performed on the same arm by rapidly inflating an occlusion cuff (Hokanson, Inc, Bellevue, WA, USA) around the upper arm to 250mmHg for three minutes to isolate the metaboreflex component of the exercise pressor reflex. During PEI, a venous blood sample was collected to assess metabolite accumulation during exercise. Following 10-15 of quiet rest, participants performed a two-minute hand-in ice-cold (4°C) H_2O cold pressor test. Muscle SNA, beat-to-beat BP, HR, and respiration were collected continuously during baseline, HG, PEI, and the cold pressor test (n=12 paired recordings during the cold pressor test).

3.2.5 Blood & urine analysis.

Baseline venous blood samples and the 24h urine samples were analyzed for serum/urine electrolyte concentrations (EasyElectrolyte Analyzer; Medica, Beford, MA, USA) and plasma/urine osmolality (3D3 Osmometer; Advanced Instruments, Norwood, MA). Venous blood samples were also analyzed for Hb (Hb 201+; Hemocue,

Lake Forest, CA, USA), Hct (Pre-calibrated Clay Adams, Readacrit Centrifuge; Becton Dickinson, Sparks, MD, USA), pH (Accumet Basic; Fisher Scientific, Hampton, NH, USA), and lactate concentrations (Accutrend; Nova Biomedical, Waltham, MA, USA). Additionally, blood samples collected during PEI were analyzed for lactate concentrations, electrolyte concentrations, and pH. Change in plasma volume (expressed as a percentage) was calculated using the following equation (76):

$$\text{Plasma volume (\%)} = ((100 \times (Hb_1/Hb_2)) \times (1 - (Hct_2/100))/(1 - Hct_1/100)) - 100$$

where subscript "1" represents CON and subscript "2" represents WD. Additionally, the spot urine sample and 24h urine sample were analyzed for urine specific gravity. Spot urine samples from female participants were also used for hormonal pregnancy tests (hCG cassettes, Moore Medical) to confirm that female participants were not pregnant.

3.2.6 Data analysis.

Data were collected continuously at a sampling rate of 1000 Hz using LabChart (LabChart 8.0 Pro, ADInstruments, Colorado Springs, CO, USA). Hemodynamic data were then analyzed offline using custom made LabView programs (National Instruments Corporation, Austin, TX, USA) as described previously (36) by a single investigator (JCW) blinded to participant condition. Briefly the three bursts with the largest height during the overall baseline period were averaged and assigned a value of 100 arbitrary units (AU), and all bursts were scaled accordingly during the perturbations. Burst frequency (BF; bursts • minute^{-1}), burst incidence (BI; bursts • 100 heart beats^{-1}), and total activity (TA; [(BF • burst amplitude) • minute^{-1}], AU • minute^{-1}) were determined as indices of muscle SNA. Absolute muscle SNA (BF, BI, and TA) and BP values were compared at rest between conditions. Absolute change in muscle

SNA and BP measures during the HG, PEI, and the cold pressor test were compared to their respective baseline periods.

3.2.7 Statistical analysis.

Resting anthropometric, muscle SNA, hemodynamic, and biochemical parameters were compared between the hydration conditions using paired, two-tailed, t-tests. Absolute BP values and changes in muscle SNA and BP during HG, PEI, and the cold pressor test were compared to their respective baseline periods using two-way repeated measure ANOVAs (time x condition). To assess metabolites generated during exercise, blood parameters were compared between conditions using two-way repeated measures ANOVAs from the baseline and PEI blood samples. The Wilcoxon matched-pairs signed rank test was employed when the Shapiro-Wilk normality test indicated non-normally distributed data. Tukey's HSD were used to correct for multiple comparisons (post-hoc analyses for repeated measures ANOVAs). All data are presented as means ± SD. All data were analyzed using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA) and significance was set *a priori* at $p < 0.05$.

3.3 Results

Table 3.1 provides anthropometric and hemodynamic data at screening. All 13 participants were non-hypertensive and non-obese.

3.3.1 Biochemical and hemodynamic responses to water deprivation.

Table 3.2 provides 24-hour fluid intake, and blood chemistry from human participants following each hydration protocol. Serum [Na^+], urine osmolality, 24h urine specific gravity, and thirst were elevated during WD ($p<0.05$ for all). Urine rate was significantly lower during WD ($p<0.05$). Also, spot urine specific gravity ($p=0.052$)

and pOsm (p=0.07) had a trend to be elevated during WD (Table 3.2). These biochemical changes were independent of changes in reported sodium consumed (CON: 2813±881 vs. WD: 2834±1304 p=0.96) during each three-day condition. Plasma volume, estimated by changes in Hb (CON: 13.4±1.2 vs. WD: 13.4±1.3 g • dL^{-1}, p=0.29) and Hct (CON: 39.7±2.9 vs. WD: 40.4±3.2 %, p=0.29), declined (-2±7%) during WD. Body mass was not significantly different between conditions (68.6±14.3 vs. 68.3±13.2 kg, p=0.55). No participants reported headaches or light-headedness from the WD protocol. We did not observe differences in resting sympathetic outflow, BP, or HR between hydration conditions (Table 3.3).

3.3.2   Handgrip exercise, post-exercise ischemia, and the cold pressor test.

Sympathetic outflow and BP increased during HG and PEI (Figure 3.1 A-D) to a similar extent in both hydration conditions. Absolute (Table 3.3) and delta (Table 3.4) values for cardiovascular and sympathetic parameters during HG and PEI are displayed below. Rating of perceived exertion (6-20 Borg scale) increased from the first to the second minute of HG exercise but was not different between conditions (CON 12±2 to 15±2, WD: 12±2 vs. 15±3, time: p<0.01, condition: p=0.84, interaction: p=0.72).

We were able to collect venous blood samples during PEI in both experimental visits in nine participants. We were unable to obtain paired blood samples during PEI in four participants due to difficulties placing a retrograde IV catheter, in which cases only a baseline blood sample was collected using a standard butterfly needle. Blood lactate concentrations increased from baseline to PEI in both conditions and were higher during WD PEI compared to CON PEI (CON: 0.8±0.3 to 1.7±0.6 vs. WD: 0.7±0.1 to 2.2±0.8 mmol/L, time: p<0.01, condition: p=0.11, interaction: p=0.04), similarly, blood pH decreased from baseline to PEI in both conditions and was lower during WD PEI

compared to CON PEI (CON: 7.41±0.08 to 7.40±0.07 vs. WD: 7.43±0.06 to 7.37±0.06, time: $p<0.01$, condition: $p=0.66$ interaction: $p=0.05$). Serum potassium concentrations were elevated during PEI compared to baseline in both conditions but were not higher during WD PEI compared to CON PEI (CON: 4.08±0.30 to 5.02±0.39 vs. WD: 4.04±0.36 to 4.98±0.29 mmol/L, time: $p<0.01$, condition: $p=0.53$, interaction: $p=0.71$).

Sympathetic outflow and BP increased during the cold pressor test (Figure 3.2 A-D) to a similar extent in both hydration conditions. Absolute (Table 3.4) and delta (Table 3.5) values for cardiovascular and sympathetic parameters during the cold pressor test are displayed below.

3.4   Discussion

The primary findings were that WD did not augment sympathetic and BP responses during exercise pressor reflex activation or the cold pressor test in healthy young adults. When taken together, these data suggest that WD elicits hypernatremia/hyperosmolality but may not adversely affect reflex cardiovascular control healthy young adults.

Our hypothesis was developed, in part, by prior observations that hypernatremia/hyperosmolality induced by high salt intake exaggerated several sympathetic reflexes and sympathoexcitatory responses evoked from the RVLM (3-5, 140, 230, 267, 318). We reasoned that dehydration-induced hypernatremia would similarly exaggerate sympathetic and pressor responses. Both sympathetic and pressor responses were measured during exercise pressor reflex activation in humans. WD produced a relative hypernatremia but did not exaggerate sympathetic and pressor responses to either stimulus. While absolute burst frequency was higher during the cold pressor test during WD, collectively, these data do not strongly support our hypothesis.

One key difference between the diet studies versus WD from the present study is time of exposure to hypernatremia/hyperosmolality. Studies reporting exaggerated sympathetic responses using dietary salt indicate that such responses are not observed at one week after diet but require two weeks of dietary salt loading (5). Therefore, the lack of differences in the current experiments may simply reflect too short a time frame for any increases in plasma [Na$^+$]/osmolality to sensitize central sympathetic networks.

Previous animal studies have employed a 48h WD model to induce hyperosmolality/hypernatremia and examine changes in BP regulation (39, 41). These studies reported that the resultant hypernatremia works centrally via the forebrain hypothalamus to increase excitatory amino acid neurotransmission to elevate SNA and BP. In addition, these studies also reported RVLM injection of L-glutamate exaggerated pressor responses in WD versus control rats (39). Hence, this latter observation was a key rationale for the current study as these sympathetic reflexes depends on glutamatergic neurotransmission in the RVLM (165). However, in the current study, we did not observe augmented cardiovascular responses during WD in healthy young adults.

In humans, large changes in serum [Na$^+$] and pOsm may be achievable under conditions of more severe dehydration using exercise and/or heat stress. Moderate dehydration in humans has been demonstrated to reduce endurance performance (20), even independent of thirst (2); to increase pain and fatigue during exercise (207); and to reduce thermoregulatory capacity (249) through impaired cutaneous vascular function (109). While studies like these are important to better understand how severe dehydration affects physiological function, we focused on mild hypohydration, in the absence of exercise and/or heat stress, because physiological changes from modest

64

reductions in water intake may be more relevant to the general population. Also, using water intake reduction prevents exercise and/or heat stress induced alterations in BP regulation.

Our strategy to achieve mild – not severe – hypohydration involved short-term WD. Indeed, these data demonstrate that we were successful in experimentally causing modest increases in serum [Na$^+$], urine osmolality, thirst, and urine specific gravity. The increases in serum [Na$^+$] (~2 mmol/L; $p < 0.05$) and represent physiologically relevant changes and are similar to those observed in previous studies examining the physiological effects of mild hypohydration (55, 272). While several variables indicate mild hypohydration was present during WD, pOsm, 24-hour fluid intake, and 24-hour urine volume values indicate participants were "euhydrated/well hydrated" during CON and "very dehydrated" during WD (13).

The change in hydration status achieved here increases relevance to the general public, as mild hypohydration is a contributing factor in numerous acute and chronic diseases (186), with low water intake predicting future incidence of diabetes (243) and heart disease (52). Other investigations have demonstrated that mild hypohydration affects cerebrovascular responses during the cold pressor test (234), impairs exercise performance (212), reduces endothelial function (15), reduces overall cognitive performance (315) and executive function (272). Thus, while mild hypohydration can affect physiological function, we found no evidence of exaggerated sympathetic and pressor responses in healthy young adults.

Findings from previous studies have demonstrated a relation between changes in blood electrolytes/pOsm and sympathetic nervous system control of BP. This was demonstrated in a study partitioning out the individual contributions of serum

[Na$^+$]/pOsm and plasma volume using hypertonic and isotonic hypovolemia (240). In that study, hypovolemia independently modulated HR, and hyperosmolality independently modulated sympathetic nervous system activity to support BP during a head-up tilt challenge. In a study from Rabbitts et al., (242) fluid restriction did not increase pOsm or change resting sympathetic nervous system control of BP. Together, these findings from previous studies suggest that serum [Na$^+$]/pOsm may be related to sympathetic control of BP. However, in the present study, our findings suggest WD-induced increases in serum [Na$^+$] do not augment sympathetic and BP responses to exercise pressor reflex activation in healthy young adults. Finally, intravenous infusion of hypertonic saline has been demonstrated to increase absolute BP and sympathetic outflow during exercise pressor reflex activation. It is important to note that the hypertonic saline infusion from this previous study resulted in 1) plasma volume expansion (+4%) compared to plasma volume contraction (-2%) reported here, and 2) an increase in serum [Na$^+$] of ~4 mmol/L compared to an increase in serum [Na$^+$] of ~2 mmol/L reported here (36). While currently unclear, it is possible that participants in the current study (increase in pOsm of ~1% during WD) did not reach their osmotic threshold (a change in pOsm of 1-2%) (70), which is why resting burst incidence was similar between conditions. This contrasts with our previous study (9) where hypertonic saline infusion increased pOsm by 2% and increased resting burst incidence. Together, the physiological differences resulting from WD versus rapid infusion of hypertonic saline may explain why their effects on sympathetic and pressor responses to static exercise may differ.

3.4.1 Limitations

This study provides insight into how mild, but not severe hypohydration affects sympathetic and pressor responses during static exercise and the cold pressor test. While pOsm, 24-hour total fluid intake, and 24-hour urine volume values indicate participants were "very dehydrated" during WD, urine osmolality and specific gravity values indicate participants were "euhydrated" during WD. Nevertheless, there were clear differences in hydration status between the two conditions among these participants. We did not measure renin-angiotensin-aldosterone system hormones or arginine vasopressin, which are known to play a role in BP regulation during dehydration. This study was not powered to determine sex differences; however, we were able to obtain 13 pairs of human MSNA recordings to test the hypothesis. Future studies are needed to determine the role of age and sex in mediating reflex cardiovascular control during WD. This study does not test whether WD affects other sympathetic and cardiovascular reflexes.

Table 3.1   Participant screening measures

Screening characteristics	
Number (F/M)	13 (6/7)
Age, yr	25 ± 4
Body mass, kg	69 ± 13
Body mass index, kg $\bullet$ m^{-2}	22 ± 3
Systolic BP, mmHg	108 ± 13
Diastolic BP, mmHg	56 ± 7

Data are presented as mean $\pm$ SD. BP,

arterial blood pressure.

Table 3.2   The effect of water deprivation

	Control	Water deprivation	p
24-hour total fluid intake, mL	2456 ± 727	1867 ± 622	**0.01**
Serum sodium, mmol • L^{-1}	140.6 ± 2.1	142.1 ± 1.9	**0.02**
Serum chloride, mmol • L^{-1}	104.4 ± 3.8	106.0 ± 2.6	0.11
Serum potassium, mmol • L^{-1}	4.08 ± 0.30	4.04 ± 0.34	0.73
Plasma osmolality, mOsm • kg H$_2$O^{-1}	290.7 ± 3.0	293.4 ± 4.3	0.07
Urine osmolality, mOsm • kg H$_2$O^{-1}	488 ± 184	699 ± 158	**<0.01**
Spot urine specific gravity	1.016 ± 0.008	1.021 ± 0.006	0.052
24-hour urine specific gravity	1.014 ± 0.006	1.019 ± 0.005	**0.03**
Urine rate, L • 24h^{-1}	1.4 ± 0.7	0.8 ± 0.3	**<0.01**
Thirst rating	3 ± 3	8 ± 2	**<0.01**

Data are presented as mean ± SD, paired, two-tailed, t-tests, bold text denotes p < 0.05. BP, arterial blood pressure; USG, urine specific gravity.

Table 3.3   Absolute sympathetic and cardiovascular responses during exercise pressor reflex activation

	Control	*Water deprivation*
Baseline		
Burst incidence, bursts • 100hb^{-1}	26 ± 14	25 ± 14
Total activity, AU • min^{-1}	321 ± 441	218 ± 176
Systolic BP, mmHg	111 ± 12	113 ± 10
Diastolic BP, mmHg	63 ± 7	62 ± 6
Heart rate, bpm	56 ± 8	57 ± 9
Handgrip exercise		
Burst incidence, bursts • 100hb^{-1}	26 ± 12	25 ± 13
Total activity, AU • min^{-1}	373 ± 283	345 ± 296
Systolic BP, mmHg	121 ± 11*	121 ± 15*
Diastolic BP, mmHg	71 ± 8*	70 ± 11*
Heart rate, bpm	67 ± 10*	69 ± 12*
Post-exercise ischemia		
Burst incidence, bursts • 100hb^{-1}	34 ± 18	35 ± 15*
Total activity, AU • min^{-1}	724 ± 733	631 ± 292
Systolic BP, mmHg	123 ± 14*	122 ± 21
Diastolic BP, mmHg	70 ± 9	69 ± 11
Heart rate, bpm	58 ± 8	59 ± 10

Data are presented as mean ± SD, two-way ANOVA (time x condition). Significant main effect of time for all variables ($p < 0.009$ for all) with no significant main effects of condition or interaction with any variable ($p > 0.41$ for all). Post hoc: * indicates significantly different from respective baseline values, $p < 0.05$. BP, arterial blood pressure.

Table 3.4   Delta sympathetic and cardiovascular responses during exercise pressor reflex activation

	Control	*Water deprivation*
Handgrip exercise		
Δ Burst incidence, bursts • 100hb^{-1}	-1 ± 7	0 ± 7
Δ Total activity, AU • min^{-1}	33 ± 306	127 ± 254
Δ Systolic BP, mmHg	11 ± 7	8 ± 7
Δ Diastolic BP, mmHg	8 ± 6	8 ± 7
Δ Heart rate, bpm	11 ± 6	12 ± 11
Post-exercise ischemia		
Δ Burst incidence, bursts • 100hb^{-1}	7 ± 9	10 ± 9[†]
Δ Total activity, AU • min^{-1}	385 ± 444	414 ± 580
Δ Systolic BP, mmHg	12 ± 7	9 ± 11
Δ Diastolic BP, mmHg	7 ± 6	7 ± 8
Δ Heart rate, bpm	3 ± 3[†]	2 ± 6[†]

Data are presented as mean ± SD, two-way ANOVA (time x condition) for delta (Δ; change from baseline) values. Significant main effect of time for burst incidence, total activity, and heart rate (p < 0.003 for all three variables). There were no significant main effects of condition or interaction with any variable (p > 0.29 for all). Post hoc: [†] indicates significantly different from respective handgrip exercise values, p < 0.05. BP, arterial blood pressure.

Table 3.5   Absolute sympathetic and cardiovascular responses during the cold pressor test

	Control	Water deprivation
Cold pressor baseline		
Burst incidence, bursts • 100hb^{-1}	23 ± 15	23 ± 16
Total activity, AU • min^{-1}	198 ± 222	233 ± 299
Systolic BP, mmHg	111 ± 11	111 ± 17
Diastolic BP, mmHg	63 ± 6	62 ± 6
Heart rate, bpm	56 ± 7	59 ± 8
Cold pressor test		
Burst incidence, bursts • 100hb^{-1}	38 ± 20*	42 ± 16*
Total activity, AU • min^{-1}	1161 ± 1660*	1104 ± 560*
Systolic BP, mmHg	124 ± 16*	122 ± 20*
Diastolic BP, mmHg	73 ± 9*	71 ± 9*
Heart rate, bpm	65 ± 10*	68 ± 11*

Data are presented as mean ± SD, two-way ANOVA (time x condition). Significant main effect of time for all variables ($p < 0.003$ for all) with no significant main effects of condition or interaction for burst incidence, total activity, or systolic and diastolic BP ($p > 0.14$ for all). Heart rate had a trend (main effect of condition p=0.06) to be elevated in the WD condition at both time points. Post hoc: * indicates significantly different from respective baseline values, $p < 0.05$. BP, arterial blood pressure.

Table 3.6　Delta sympathetic and cardiovascular responses during the cold pressor test

	Control	Water deprivation
Cold pressor test		
Δ Burst incidence, bursts • 100hb^{-1}	15 ± 5	19 ± 8
Δ Total activity, AU • min^{-1}	963 ± 374	871 ± 409
Δ Systolic BP, mmHg	13 ± 11	10 ± 8
Δ Diastolic BP, mmHg	10 ± 7	10 ± 6
Δ Heart rate, bpm	8 ± 8	9 ± 7

Data are presented as mean ± SD, paired, two-tailed, t-test for delta (Δ; change from baseline) values. ($p>0.05$ for all). BP, arterial blood pressure.
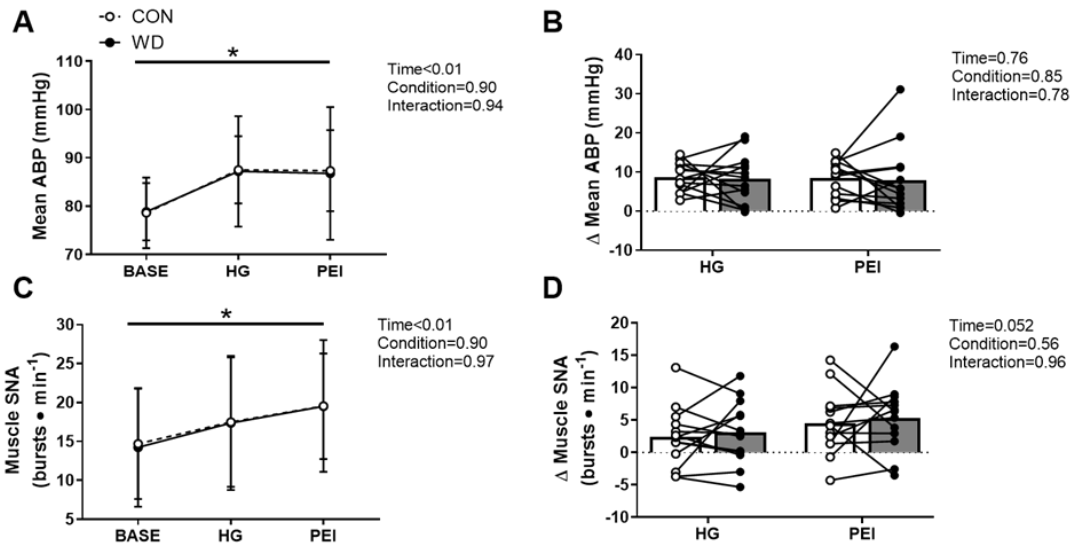
Figure 3.1   Summary data (mean±SD) with individual data points (n=13) of (A) absolute mean BP, (B) mean BP absolute change from baseline, (C) absolute muscle SNA, and (D) muscle SNA absolute change from baseline (expressed as burst frequency for both 5C and 5D) during two minutes of handgrip exercise and three minutes of post-exercise ischemia. Two-way repeated measures ANOVA (time x condition), * denotes $p < 0.05$ for a time effect. BP, arterial blood pressure; CON, control condition; SNA, sympathetic nerve activity; WD, water deprivation condition.
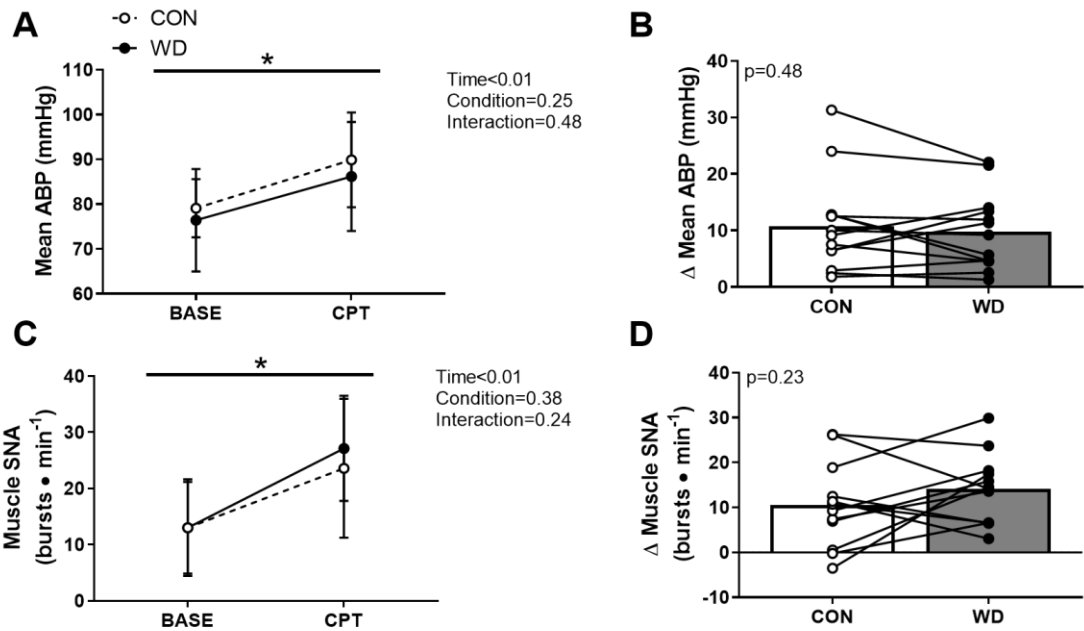
Figure 3.2   Summary data (mean±SD) with individual data points (n=13) of (A) absolute mean BP, (B) mean BP absolute change from baseline, (C) absolute muscle SNA (n=12), and (D) muscle SNA absolute change from baseline (n=12; expressed as burst frequency for both 5C and 5D) during two minutes of a hand-in cold pressor test. Two-way repeated measures ANOVA (time x condition) for panels A & C, paired, two-tailed, t-tests for panels B & D. * denotes $p < 0.05$ for a time effect. BP, arterial blood pressure; CON, control condition; SNA, sympathetic nerve activity; WD, water deprivation condition.

Chapter 4

THE EFFECT OF MILD HYPOHYDRATION ON BLOOD PRESSURE VARIABILITY AND CARDIOVASCULAR REACTIVITY IN HEALTHY AGING

4.1 Introduction

Cardiovascular disease prevalence increases with advancing age and is the leading cause of death among adults in the United States (316). High resting arterial blood pressure (BP) (i.e., hypertension) (22), high BP variability (185, 241), and exaggerated BP responses during exercise (190, 202, 255, 296) are markers of BP dysregulation that are associated with greater future risk for developing cardiovascular disease. Hypertension incidence increases with age (59) and affects nearly eight out of 10 adults over the age of 65 years old (22). Similarly, BP variability (59) and BP responses during exercise (28, 199, 294) are increased during aging. Thus, there is a critical need to determine what physiological factors contribute to these clinically-relevant markers of BP dysregulation that become more pronounced with aging.

Chronic underhydration (low water intake) is associated with greater future incidence of cardiovascular disease (52), although the exact mechanisms underlying this relation are unclear. We do know that old adults are more commonly underhydrated (79) and have a greater incidence of plasma hypertonicity (i.e., high plasma osmolality) (278). This is likely because old adults are not able to regulate body water balance as well as their young counterparts because of reduced thirst sensations (236, 238), lower total body water (71), altered extracellular fluid sensing (237), blunted hormonal (e.g.

antidiuretic hormone) release (26, 236), and impaired kidney function (68). Although the effects of acute mild hypohydration on resting BP, BP variability, and BP responses during exercise in old adults remains unknown.

Previously, we demonstrated that acute mild hypohydration does not affect resting BP (Chapter 2), BP variability (Chapter 2), or BP responses during static exercise (Chapter 3) compared to a normally-hydrated control condition in healthy young adults. The scientific premises for these past studies were based on published data from rodent models of acute water deprivation (WD) affecting sympathetic outflow and BP (39, 41, 42) as well as NaCl-loading studies that suggest high blood sodium concentrations sensitize central sympathetic networks to increase BP variability (267) and BP responses during exercise (318) in rodents. Because old versus young adults have exaggerated increases in blood sodium concentrations (i.e., hypernatremia) following acute WD (238), we hypothesized that more pronounced blood hypernatremia in old versus young adults following acute WD would lead to BP dysregulation.

Therefore, the primary objective of this study was to determine if acute WD causes BP dysregulation in old adults. Thus, we sought to determine if short-term WD affects BP variability and BP responses during isometric exercise in healthy young and old adults. We hypothesized that old versus young adults would have more severe blood hypernatremia following short-term WD and would consequently impair BP regulation. Specifically, we hypothesized that old but not young adults would have elevated BP variability and BP responses during exercise pressor reflex activation.

77

4.2 Methods

4.2.1 Participants.

The Institutional Review Board at the University of Delaware approved this protocol (IRB #1097747) and the study conformed with the most recent version of the *Declaration of Helsinki*. The data reported here are part of a registered protocol (ClinicalTrials.gov Identifier: NCT03560869). All participants provided verbal and written consent prior to enrollment in the study.

During the initial screening visit, young and old participants completed a physical activity readiness questionnaire, medical history questionnaire, and underwent measurements of height and weight. Resting brachial BP measurements were performed in triplicate with participants in the seated position following five minutes of quiet rest (Dash 2000; GE Medical Systems, Milwaukee, WI). Inclusion criteria for this study included: age between 20-35 or 55-75 yrs, resting systolic BP <140 mmHg, resting diastolic BP <90 mmHg, and body mass index <30 kg/m^2 at screening. Study participants were also non-smokers free of any known cardiovascular disease, and had no evidence of metabolic, neurological, renal, or pulmonary disease. Exclusion criteria included previous diagnosis of hypertension and past or current use of antihypertensive medications (e.g., diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and β-blockers). A resting 12-lead electrocardiogram and venous blood sample (assessed for complete blood count, metabolic panel, electrolytes, whole blood analysis, inflammatory markers) was collected at screening in old adults. All old female adults were post-menopausal (self-report).

4.2.2 Hydration conditions.

Participants completed two three-day-long hydration conditions separated by at least one week in random order. Young female participants were tested in the early follicular phase of the menstrual cycle or placebo phase of oral contraceptives (self-reported). Pilot studies allowed for determination of water intake requirements necessary to observe modest increases in serum sodium concentrations and plasma osmolality. During CON, participants were asked to consume 23mL H_2O/kg body weight/day for days one through three and to consume 250mL H_2O before arriving to the laboratory for testing on day four. The WD condition required participants to consume 23mL H_2O/kg body weight on day one, 17mL H_2O/kg body weight on day two, and 10mL H_2O/kg body weight on day three, followed by a 16-hour water abstention period prior to testing on day four. Participants were asked to refrain from caffeine, alcohol, vigorous exercise, or moderate exercise in the heat on days two and three during both three-day hydration protocols. Participants were given guidance on estimating food portion sizes and keeping a diet log to maintain recommended daily sodium intake (2300mg/day). They were also instructed to consume the same foods during the second three-day hydration condition as reported in their diet log during the first three-day hydration condition. Participants collected their urine in a sterile collection container for 24 hours preceding in-lab testing and reported to the laboratory for testing on the fourth day of each condition after fasting for at least four hours.

4.2.3 Experimental visit.

Upon arrival to the laboratory, participants provided a spot urine sample, were weighed (Tanita Body Composition Analyzer, Model TBF-300A; Arlington Heights, IL), and provided a subjective rating of their thirst and dryness of their mouth using a

Likert scale. Participants laid in the supine position for ≥20 minutes before an intravenous catheter was placed in retrograde fashion into the antecubital space of the dominant arm for blood sampling. Participants were then outfitted with the required equipment for measurement of MSNA, beat-to-beat BP, brachial BP, heart rate (HR), leg blood flow (described below), and respiratory excursions (via a strain-gauge pneumograph). Maximal voluntary contraction (MVC) was determined using the average handgrip force produced (Grip Force Transducer, ADInstruments, Colorado Springs, CO) during three maximal forearm contractions, separated by a minute each.

Data were collected during a 10-minute baseline period as participants rested quietly in a dimly lit, temperature-controlled room (22-24°C). Following baseline data collection, participants performed a two-minute bout of isometric handgrip exercise (HG) with their dominant hand at 30% of maximal voluntary contraction, using real-time feedback of force production displayed on a monitor, for assessment of the exercise pressor reflex. Immediately prior to the cessation of HG exercise, post-exercise ischemia (PEI) was performed on the same arm by rapidly inflating an occlusion cuff (Hokanson, Inc, Bellevue, WA, USA) around the upper arm to 250mmHg for three minutes to isolate the metaboreflex component of the exercise pressor reflex. During PEI, a venous blood sample was collected to assess metabolite accumulation during exercise. Following 10-15 of quiet rest, participants performed a two-minute hand-in ice-cold (4ºC) H2O cold pressor test. MSNA, beat-to-beat BP, HR, and respiration were collected continuously during baseline, HG, PEI, and the cold pressor test.

4.2.4 Physical activity monitoring.

Habitual physical activity levels were objectively assessed in a subset of young (n=21) and all old participants during seven consecutive days following the second

randomized experimental visit. Average daily step count and moderate-to-vigorous physical activity duration were determined from validated accelerometers ((239) Actigraph wGT3X-BT, Pensacola, FL, USA) after manual confirmation of wear and non-wear times based on notes from participants' physical activity logs.

4.2.5 Blood & urine analysis.

Venous blood samples and the 24-hour urine samples were analyzed for serum and urine electrolyte concentrations (EasyElectrolyte Analyzer; Medica, Beford, MA, USA), and plasma and urine osmolality (3D3 Osmometer; Advanced Instruments, Norwood, MA). Venous blood samples were also analyzed for Hb (Hb 201+; Hemocue, Lake Forest, CA, USA) and Hct (Pre-calibrated Clay Adams, Readacrit Centrifuge; Becton Dickinson, Sparks, MD, USA). Additionally, blood samples collected during PEI were analyzed for lactate concentrations, electrolyte concentrations, and pH. Change in plasma volume (expressed as a percentage) was calculated using the following equation (76):

$$\text{Plasma volume (\%)} = ((100 \times (Hb_{CON}/Hb_{WD})) \times (1 - (Hct_{WD}/100))/(1 - Hct_{CON}/100)) - 100$$

Additionally, urine specific gravity was determined for the spot and 24-hour urine samples. Female participants spot urine samples were also used to confirm that they were not pregnant (hCG cassettes, Moore Medical).

4.2.6 Muscle sympathetic nerve activity.

MSNA was directly assessed via microneurography as previously described by our laboratory (36, 112, 188). Briefly, a tungsten recording microelectrode was inserted percutaneously near the fibular head or in the popliteal fossa and a reference microelectrode was inserted ≤3 cm from the recording electrode. The nerve recording

was amplified (80-90,000x), bandpass filtered (700-2,000 Hz), rectified, and integrated (time constant 0.1s) using a nerve traffic analyzer (Nerve Traffic Analyzer, model 662c-4; University of Iowa, Bioengineering, Iowa City, IA, USA). An adequate nerve recording was confirmed prior to the experimental protocols using the following criteria: absence of afferent nerve activity during light skin stroking, increased efferent nerve activity during voluntary end-expiratory apnea, and pulse synchronous efferent nerve bursts with ≥3 to 1 signal-to-noise ratio.

4.2.7   Arterial blood pressure assessment.

Beat-to-beat BP was measured at the finger of participants' non-dominant hand using photoplethysmography (Finometer; Finapres Medical Systems, the Netherlands) and calibrated to brachial BP according to the manufacturer's recommended calibration procedures (117). Brachial BP was measured using an automated osillometric sphygmomanometer (Dash 2000; GE Medical Systems, WI, USA) and used to verify absolute beat-to-beat BP values. Simultaneously, HR was continuously recorded using standard single-lead ECG (Dash 2000; GE Medical Systems, WI, USA). Respiratory excursions were recorded via a strain gauge pneumograph (Pneumotrace; UFI, CA, USA) placed around the abdomen.

4.2.8   Arterial blood pressure variability.

BP variability was calculated using standard deviation of BP values and using the average real variability (ARV) index. The ARV index calculates the average of the absolute differences between consecutive BP measurements and is thought to provide further prognostic value compared to traditional measures such as standard deviation of BP (29, 194). BP variability was assessed during 10 minutes of quiet rest using the beat-

to-beat BP signal derived from the Finometer (described above). Additionally, ambulatory BP was assessed in a subset of participants (n=24 young and n=9 old) during the 24-hour periods preceding each experimental visit. Oscar 2 with Sphygmacor, SunTech Medical). The monitor measured BP on the non-dominant arm every 20 minutes from 0601-2200 hours and every 30 minutes from 2201-0600 hours. Participants self-reported sleep and wake times in the laboratory to note the start of 'daytime' and 'nighttime' for the BP values. Data were only included for if participants had at least 15 measurements during the daytime and at least 8 measurements during the nighttime (218).

4.2.9   Hemodynamic data analysis.

Data were collected continuously at a sampling rate of 1000 Hz using LabChart (LabChart 8.0 Pro, ADInstruments, Colorado Springs, CO, USA). The sympathetic neurogram was analyzed on a beat-to-beat basis to determine the presence/absence of MSNA bursts using custom LabView software and visually inspected by a lab member blinded to condition and trained in MSNA data analysis and processing (JCW). Bursts were identified in accordance with recent guidelines (265, 312) via the following criteria: (1) >3:1 signal-to-noise ratio, (2) burst morphology consistent with MSNA bursts, and (3) a pulse-synchronous signal. MSNA was quantified as burst frequency (bursts • minute^{-1}), burst incidence (bursts • 100 heart beats^{-1}), and total activity ([(burst frequency • burst amplitude) • minute^{-1}], AU • minute^{-1}) were determined as indices of MSNA (n=16 paired recordings in young adults and n=5 paired recordings in old adults).

To quantify indices of sympathetic vascular transduction (i.e. the functional effect of individual bursts of MSNA on BP), a spike-triggered averaging methodology

was used that has been previously described (18, 86). Briefly, each cardiac cycle containing a burst of MSNA was identified and set as cardiac cycle 0 whether or not the burst was a singlet (bursts directly bordered by >1 heartbeat lacking MSNA) or part of a cluster (bursts adjacent to other burst(s) of MSNA). The peak change in mean BP over the subsequent 10 cardiac cycles was determined. These methods reliably quantify beat-to-beat changes in BP induced spontaneous MSNA bursts. Limb vascular conductance and common femoral artery hemodynamics were not determined in the present analysis due to the very low sample size of sufficient common femoral artery video recordings collected with old adult cohort (one paired video recording).

Absolute BP and heart rate values during HG, PEI, and the cold pressor test were compared to their respective baseline periods. MSNA measures during perturbations were not determined in the present analysis due to the very low sample size of sufficient paired nerve recordings collected with old adult cohort (four paired recordings for burst frequency incidence and three paired recordings for MSNA total activity).

4.2.10 Sympathetic baroreflex sensitivity analysis.

Sympathetic baroreflex sensitivity was assessed using in the resting state during spontaneous BP oscillations as previously described (114, 189, 281). This method quantifies baroreflex sensitivity around the operating point and is well correlated to the modified Oxford technique (126). Briefly, each cardiac cycle was assigned to a bin (3mmHg) based on diastolic BP on the corresponding cardiac cycle. Burst incidence and total activity (n=16 in young adults and n=5 in old adults) were regressed over diastolic BP bins. All data were weighted to account for the number of cardiac cycles within each bin (114, 189). Bins without MSNA bursts were included in the analysis.

Slopes of the linear regressions were used as an index of sympathetic baroreflex sensitivity if they had an r≥0.5 (114, 189, 221).

4.2.11 Cardiac vagal baroreflex sensitivity analysis

Beat-to-beat time series of systolic BP and R-R interval were analyzed using the sequence method for estimating spontaneous cardiac vagal baroreflex sensitivity (HemoLab version 8.9, Harald Stauss Scientific, Iowa City, IA). A detailed description of this method has been published previously (25). As previously done in our laboratory (17), sequences of four or more consecutive cardiac cycles in which systolic BP and R-R interval change in the same direction were identified as baroreflex sequences. Sequences were detected only when the variation in R-R interval was >0.5 ms and SBP changes were >1 mmHg. A linear regression was applied to each individual sequence, and only those sequences in which R^2 was >0.80 were accepted. Values of cardiac vagal baroreflex sensitivity were accepted when the number of sequences was ≥3 for both up and down sequences. The slopes of those individual linear regressions were then calculated and averaged for a measure of spontaneous cardiac vagal baroreflex sensitivity. Cardiac vagal baroreflex sensitivity was determined for all sequences combined and separately for up (increase in both SBP and R-R interval) and down (decrease in both SBP and R-R interval) sequences.

4.2.12 Statistical analysis.

Screening measures were compared using unpaired, two-tailed, t-tests. Biochemical data, resting BP values, indices of BP variability and baroreflex function were compared using two-way independent group ANOVAs (condition x age group). Absolute BP responses during exercise pressor reflex activation and the cold pressor

test were compared using three-way independent groups ANOVAs (time x condition x age group). Delta BP responses during exercise pressor reflex activation were compared using three-way independent groups ANOVAs (time x condition x age group). Delta BP responses during the cold pressor test were compared using two-way independent groups ANOVAs (condition x age group). Tukey multiple comparison testing was employed in all post-hoc analyses. All data are presented as means ± SD. All data were analyzed using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA) and significance was set *a priori* at $p < 0.05$.

4.3 Results

4.3.1 Resting biochemical and cardiovascular measures.

Participant screening information and habitual physical activity measures are included in Table 4.1. Both age groups had significantly higher spot urine specific gravity, 24-hour urine osmolality, and thirst ratings during WD (Table 4.2). Additionally, spot urine specific gravity values were lower in old compared to young adults in both experimental conditions (Table 4.2). Serum sodium concentrations, plasma osmolality, and 24-hour urine specific gravity were higher and urine rate lower during WD in both groups (Figure 4.1). Plasma osmolality values were higher and 24-hour urine specific gravity values were lower in old compared to young adults during both experimental conditions (Figure 4.1). Body mass, hematocrit values, hemoglobin concentrations, and reported sodium consumed per day was similar between conditions and age groups (Table 4.2). Estimated plasma volume changes from CON to WD were similar between age groups (Young: 0.2±8.2 vs. Old: 0.0±7.4%, p=0.97).

Old adults had higher 24-hour and nighttime diastolic BP values than young adults regardless of condition. Interestingly, within old adults, there were lower resting systolic BP values during WD. Finally, during the CON condition, old adults had lower diastolic BP dipping (Table 4.3).

4.3.2 Blood pressure variability

Resting beat-to-beat and ambulatory daytime systolic BP variability (assessed via ARV and standard deviation) was not different between conditions. Old compared to young adults had higher beat-to-beat systolic ARV and standard deviation, as well as high ambulatory daytime ARV (Figure 4.2).

Resting burst frequency (Young CON: 15±7 vs. WD: 14±7; Old CON: 34±7 vs. WD: 37±8 bursts/minute, interaction: $p=0.19$, condition: $p=0.53$, age: $p<0.01$) and burst incidence values (Young CON: 26±14 vs. WD: 24±13; Old CON: 58±11 vs. WD: 62±15 bursts/minute, interaction: $p=0.28$, condition: $p=0.74$, age: $p<0.01$) were significantly higher in old compared to young adults but were not different between conditions. Peak mean BP changes following spontaneous MSNA bursts were not different between conditions but were lower in the old compared to young adults during both experimental conditions (Young CON: 4.1±2.3 vs. WD: 5.2±3.1; Old CON: 1.4±0.6 vs. WD: 1.1±0.3 mmHg, interaction: $p=0.32$, condition: $p=0.56$, age: $p=0.02$). Resting sBRS values were not different between conditions or age groups (Figure 4.3). Resting cBRS values were lower in old compared to young adults but similar between experimental conditions (Figure 4.3). Resting cBRS values for up (Young CON: 23±10 vs. WD: 28±16; Old CON: 7±2 vs. WD: 8±5 ms/mmHg, interaction: $p=0.45$, condition: $p=0.31$, age: $p=0.03$) and down (Young CON: 26±9 vs. WD: 27±12; Old CON: 9±5 vs. WD: 10±7 ms/mmHg, interaction: $p=0.79$, condition: $p=0.71$, age: $p<0.01$) sequences

were not different between conditions but were significantly lower in old versus young adults.

4.3.3 Cardiovascular measures during exercise pressor reflex activation and the cold pressor test

There were significant time and age effects for absolute systolic, mean, and diastolic BP values, with these measures increasing from baseline to HG and PEI in both groups, and greater BP values in the old compared to young adults, however, responses were not different between conditions (Figure 4.4). Additionally, absolute heart rate values significantly increased from baseline to HG then decreased during PEI in both age groups, however these responses were not different between conditions (Figure 4.4). Changes in systolic and mean BP values from baseline were higher, and heart rate values lower, during PEI compared to HG, however, these responses were not different between age groups or experimental conditions (Figure 4.5). Rating of perceived exertion values increased from minute one of HG to minute two of HG but were not different between conditions of age groups (time: $p<0.01$, condition: $p=0.31$, age: $p=0.36$).

There were significant time and age effects for absolute systolic, mean, and diastolic BP values, with these measures increasing from baseline to the cold pressor test in both groups, and greater BP values in the old compared to young adults, however, responses were not different between conditions (Figure 4.6). Additionally, absolute heart rate values significantly increased from baseline to the cold pressor test in both age groups, however these responses were not different between conditions (Figure 4.6). Changes in systolic BP during the cold pressor test were higher in the old versus young adults, but were not different between experimental conditions (Figure 4.7). Changes in

mean BP, diastolic BP, and heart rate during the cold pressor test were not different between experimental conditions or age groups (Figure 4.7).

4.4 Discussion

The purpose of this investigation was to determine if healthy old compared to young adults had more pronounced blood hypernatremia following short-term WD, and if more pronounced blood hypernatremia elicited BP dysregulation in healthy old adults. The primary preliminary findings from this study suggest that, 1) old and young adults with similar body mass index values and habitual physical activity habits have similar changes in urine and blood hydration markers, including similar increases in serum sodium concentrations following WD, and 2) despite relative blood hypernatremia in both groups did not have changes in BP variability or BP responses during exercise pressor reflex activation or the cold pressor test. Together, these preliminary findings suggest that healthy old adults have similar biochemical responses following short-term WD as young adults.

This work is clinically-relevant because BP dysregulation, including augmented BP variability (185, 241) and BP responses during exercise (190, 202, 255, 296), are predictive of future risk of developing hypertension, a primary risk factor for the leading cause of death in the United States, cardiovascular disease (22). The rationale for this hypothesis that old adults would have more pronounced blood hypernatremia was developed in part by past reports that demonstrated old compared to young adults having larger increases in serum sodium concentrations and plasma osmolality following 24-hour WD (238). However, a limitation of that previous report was the inclusion of only young and old male adults in their investigation, thus, given that our cohort is comprised of male and female young adults and primarily female old adults, it is possible there is

sex-specificity in the biochemical responses to WD during healthy aging. Additionally, the participants in the present study had similar objectively measured physical activity measures and no participants were current smokers whereas the previous study included several current smokers and it is unclear how that may have affected their results (238). Therefore, the present investigation extends the findings of previous work and provides new information regarding potential sex differences in aging related to biochemical responses to WD that should be prospectively investigated.

In chapters 2 and 3, we reported that a stepwise reduction in water intake over three days concluded with a 16-hour water abstention period is a successful model for inducing mild hypohydration, and importantly, elevating serum sodium concentrations. Here, we report significant increases in urine osmolality values, rating of thirst values, serum sodium concentrations, and plasma osmolality values, and decreases in urine flow rate in the WD versus CON condition in both age groups. Thus, when taken together these previous data suggest that this model of WD would be sufficient to answer our hypotheses. We anticipated that old adults would not tolerate the WD as well as young adults because of lower basal total body water (71), altered extracellular fluid sensing (237), blunted hormonal (e.g. antidiuretic hormone) release (26, 236), and impaired kidney function (68). However, we observed similar increases in serum sodium concentrations and plasma osmolality values in our old compared to young adults from CON to WD. These preliminary findings suggest that healthy old adults can buffer changes in water intake in a manner similar to that of their young adult counterparts.

Our findings that healthy old compared to young adults have lower spot and 24-hour urine specific gravity values, regardless of experimental condition, are consistent with previous reports that old adults have a reduced ability to concentrate urine (68, 170,

178, 236). Similarly, our findings that healthy old compared to young adults have higher plasma osmolality values, regardless of experimental condition, are consistent with one previous report that old adults have higher basal plasma osmolality (278). Importantly, these observations were in the context of prescribed water intake amounts and reported sodium consumed for three-days prior to testing being near the recommended daily allowance similar between conditions.

Our findings that healthy old adults have higher resting BP values than their young counterparts is consistent with previous literature (22). Related, a strength of the current study design was the inclusion of old adults who had not been diagnosed with or were currently being treated for hypertension, a population that is known to have increased BP variability (225, 285, 300) and increased BP responses during static handgrip exercise (74, 112), which prevents one potential confounding effects of hypertensive status on our primary outcome measures of BP variability and BP responses to exercise pressor reflex activation. Studies investigating age-related changes in BP regulation independent of hypertensive status can be challenging given that nearly eight out of 10 adults over the age of 65 years have hypertension present.

WD did not affect ambulatory BP values or BP dipping patterns in either age group. Interestingly, we observed attenuated diastolic BP dipping during the nighttime in old compared to young adults in the CON condition. This attenuated diastolic BP dipping in old adults was primarily driven by higher nighttime diastolic BP values as daytime diastolic BP values were similar among the two age groups. This is consistent with one previous study reporting the incidence of irregular BP dipping patterns, including a lack of nighttime BP dipping (i.e., non-dippers), increases with age (75). Inappropriate BP dipping is an independent risk factor for future cardiovascular and

cerebrovascular events in old adults (245, 301) and all-cause mortality in old hypertensive adults (85). Along these lines, it was previously reported that nighttime BP dipping is attenuated in hypertensive compared to non-hypertensive adults (205). In summary, our current findings suggest that healthy non-hypertensive old adults have attenuated diastolic BP dipping compared to young adults, however, this effect is abolished when comparing age groups in the WD condition. This observation warrants future prospective studies to investigate the role of hydration status on BP dipping patterns in aging because of the high clinical relevance irregular BP dipping patterns have on future cardiovascular disease risk.

Our findings that healthy old adults compared to young adults have increased ambulatory BP ARV and beat-to-beat BP variability during CON are consistent with previous reports (59). Similarly, our findings that healthy old compared to young adults have smaller peak increases in mean BP over the 10 cardiac cycles following spontaneous MSNA bursts during CON are also consistent with previous reports (302). Further, our findings that resting sBRS is similar between healthy young and old adults during CON is consistent with previous reports (73, 82, 187, 232). Similarly, our findings that resting cBRS is reduced in healthy aging in the CON condition is also consistent with published findings (82, 147, 173, 187). Contrary to our hypotheses, short-term WD did not significantly affect BP variability, sympathetic vascular transduction, or baroreflex control of MSNA or heart rate at rest in either age group.

Previous animal studies have employed a 48h WD model to induce blood hypernatremia and examine changes in BP regulation (39, 41). These studies reported that WD-induced blood hypernatremia acts centrally in the forebrain hypothalamus to increase excitatory amino acid neurotransmission to elevate SNA and BP. In addition,

92

one of these studies also reported that RVLM injection of L-glutamate exaggerated pressor responses in WD versus control rats (39). Hence, this latter observation was a key rationale for the current study as these sympathetic reflexes depends on glutamatergic neurotransmission in the RVLM (165). In contrast to our hypothesis, WD in the current study did not affect absolute BP values or the change in BP from baseline during exercise pressor reflex activation in healthy young and old adults.

While there were no significant changes in BP responses during exercise between conditions, we did observe differences in absolute BP values between age groups. Our finding that old compared to young adults have higher absolute BP values during handgrip exercise is consistent with previous studies (28, 34). However, in contrast to previous findings (34, 199), we found that young and old adults had similar increases in BP from baseline during exercise pressor reflex activation. Specifically, given that data from a recent investigation suggested that old female adults have higher BP responses than young adults during HG (294), we expected that within our old adults, comprised primarily of female adults, that BP responses would be higher in the old compared to the young group but this was not observed in the present study. Additionally, we observed old versus young adults to have higher systolic BP responses during the cold pressor test regardless of experimental condition. While a past study (215) did not report systolic BP changes during the cold pressor test, they reported that young and old adults had similar increases in mean BP during the cold pressor test, which is consistent with our findings and one other study in male and female adults (291). However, a more recent study (204) reported old versus young male adults to have larger systolic and diastolic BP responses during the cold pressor test. While it is unclear why this was not observed in the present study, the high proportion of female

93

adults in the old participant group may explain differences in findings. The current findings contribute to a growing body of literature highlighting age-related alterations in resting and reflex BP regulation.

4.5   Summary

Our preliminary findings suggest that following short-term WD healthy old adults have similar increases in serum sodium concentrations as young adults. Additionally, despite relative blood hypernatremia following WD in both young and old adults, neither group had increases in resting BP, ambulatory BP, BP variability, or BP responses during exercise pressor reflex activation and the cold pressor test.

Table 4.1   Participant screening measures

Characteristic	Young	Old
Number (F/M)	29 (14/15)	10 (9/1)
Age, yrs	25 ± 4	$67 \pm 7^{\#}$
Body mass, kg	69 ± 14	63 ± 12
Body mass index, kg • m^{-2}	23 ± 3	24 ± 3
Systolic BP, mmHg	107 ± 11	$119 \pm 11^{\#}$
Diastolic BP, mmHg	59 ± 7	$75 \pm 7^{\#}$
Step count, steps • day^{-1}	7124 ± 3902	8072 ± 2682
MVPA, min • day^{-1}	67 ± 33	74 ± 34

Data are presented as mean $\pm$ SD. $^{\#}$ indicates significant age effect. BP, arterial blood pressure; MVPA, moderate-to-vigorous physical activity.

Table 4.2   Biochemical measures and hydration indices

	Young		Old	
	CON	**WD**	**CON**	**WD**
Hematocrit, %	41.1 ± 4.1	40.9 ± 4.4	40.4 ± 2.2	40.5 ± 2.0
Hemoglobin, mg • dL^{-1}	13.7 ± 1.5	13.7 ± 1.5	13.1 ± 0.9	13.1 ± 0.9
Spot urine specific gravity	1.016 ± 0.007	1.022 ± 0.004*	1.011 ± 0.006[#]	1.020 ± 0.004[#]*
24h urine osmolality, mOsm • kg H_2O^{-1}	501 ± 157	738 ± 193*	443 ± 85	644 ± 163*
Body mass, kg	68 ± 14	67 ± 14	61 ± 11	61 ± 12
Thirst rating	3 ± 3	7 ± 2*	3 ± 2	6 ± 3*
Reported sodium consumed, mg • day^{-1}	2120 ± 391	2188 ± 324	2092 ± 341	2064 ± 359

Data are presented as mean ± SD. * indicates significantly different from CON, [#] indicates significantly different from Young group.

Table 4.3   Ambulatory blood pressure measures

	Young		Old	
	CON	**WD**	**CON**	**WD**
24-hour brachial systolic BP, mmHg	117 ± 11	116 ± 11	123 ± 13	125 ± 18
24-hour brachial diastolic BP, mmHg	66 ± 6	66 ± 6	74 ± 9[#]	73 ± 10[#]
Daytime brachial systolic BP, mmHg	121 ± 12	121 ± 11	126 ± 14	128 ± 19
Daytime brachial diastolic BP, mmHg	70 ± 6	69 ± 6	74 ± 11	74 ± 12
Nighttime brachial systolic BP, mmHg	104 ± 10	105 ± 12	113 ± 11	111 ± 13
Nighttime brachial diastolic BP, mmHg	56 ± 6	56 ± 7	67 ± 6[#]	65 ± 7[#]
Brachial systolic BP dipping, %	12 ± 5	12 ± 5	10 ± 7	14 ± 8
Brachial diastolic BP dipping, %	19 ± 7	18 ± 6	12 ± 8[#]	14 ± 8

Data are presented as mean ± SD. * indicates significantly different from CON, [#] indicates significantly different from Young group under respective experimental condition. BP; arterial blood pressure.
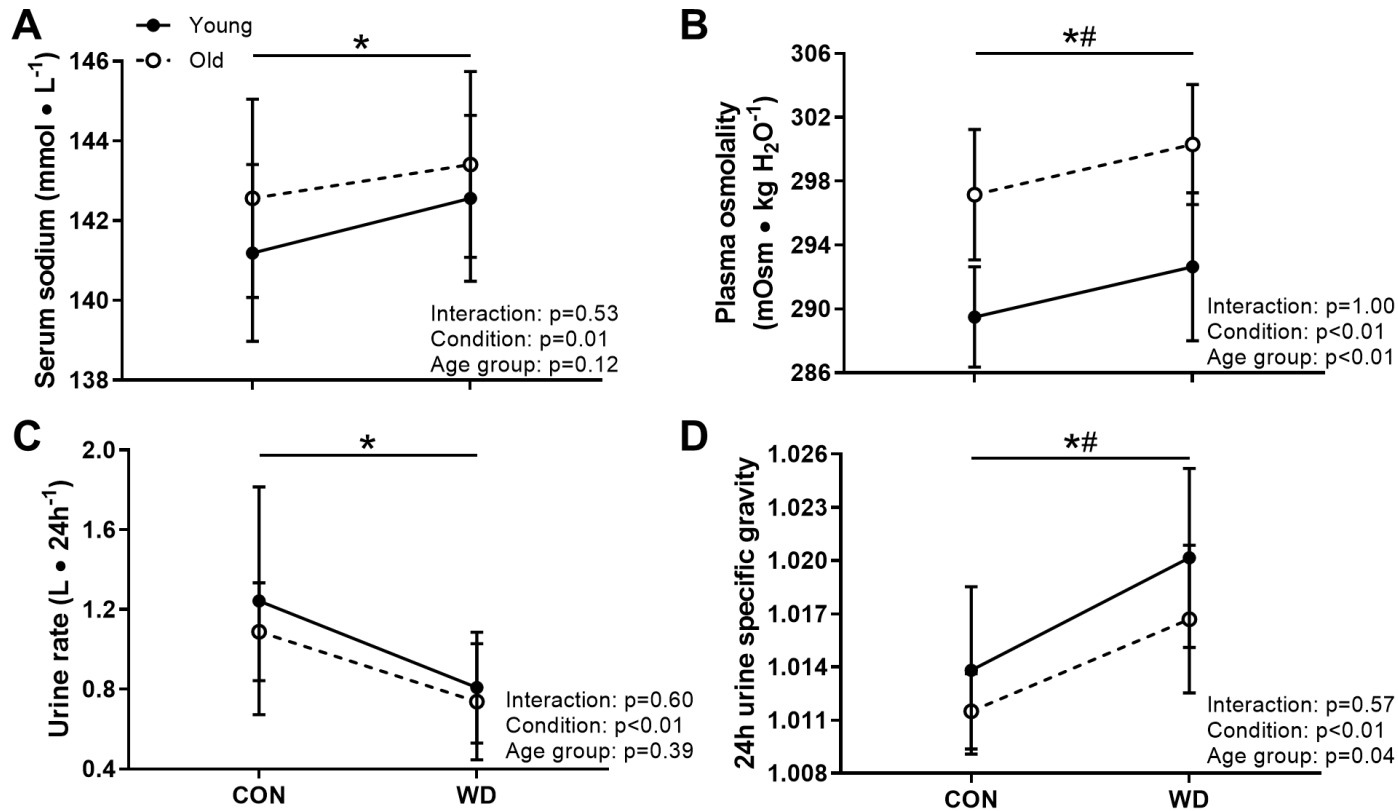
Figure 4.1   Biochemical responses following each hydration protocol. A) Serum sodium concentrations were increased following water deprivation (WD) in both groups. B) Plasma osmolality values were significantly higher in the old compared to young adults, and were higher following WD in both groups, but there was no significant interaction present. C) Urine rates were decreased following WD in both groups. D) Twenty-four-hour urine specific gravity values were significantly lower in the old compared to young adults, and were higher following WD in both groups, but there were no significant interactions present. * indicates a significant condition effect, # indicates a significant age effect
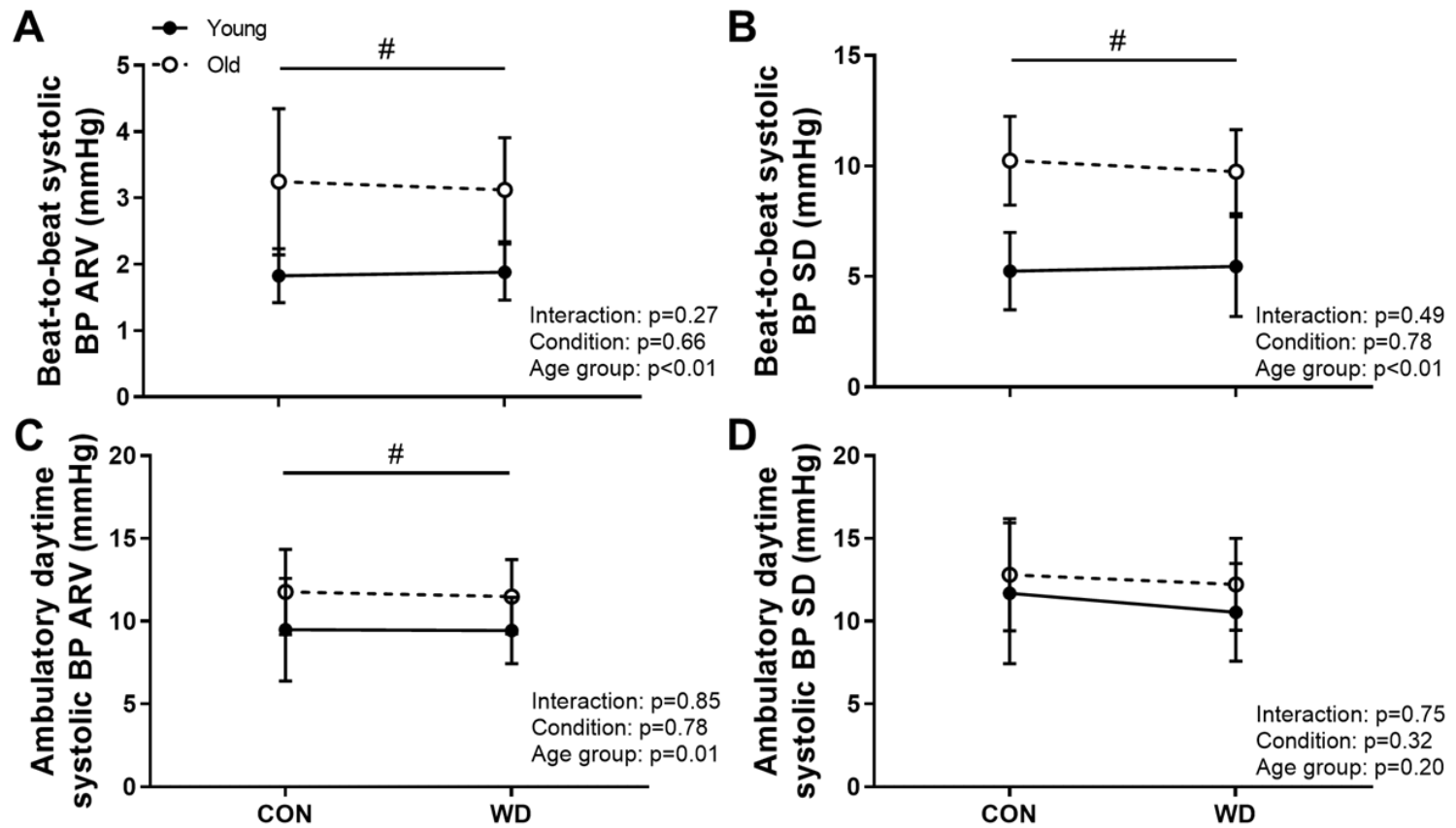
Figure 4.2   Blood pressure variability measures. A) Beat-to-beat systolic blood pressure (BP) average real variability (ARV), B) beat-to-beat systolic BP standard deviation (SD), and C) ambulatory daytime systolic BP ARV were higher in old compared to young adults, however there was no significant effect of condition. D) Ambulatory daytime systolic BP SD was similar between conditions and age groups. # indicates a significant age effect.
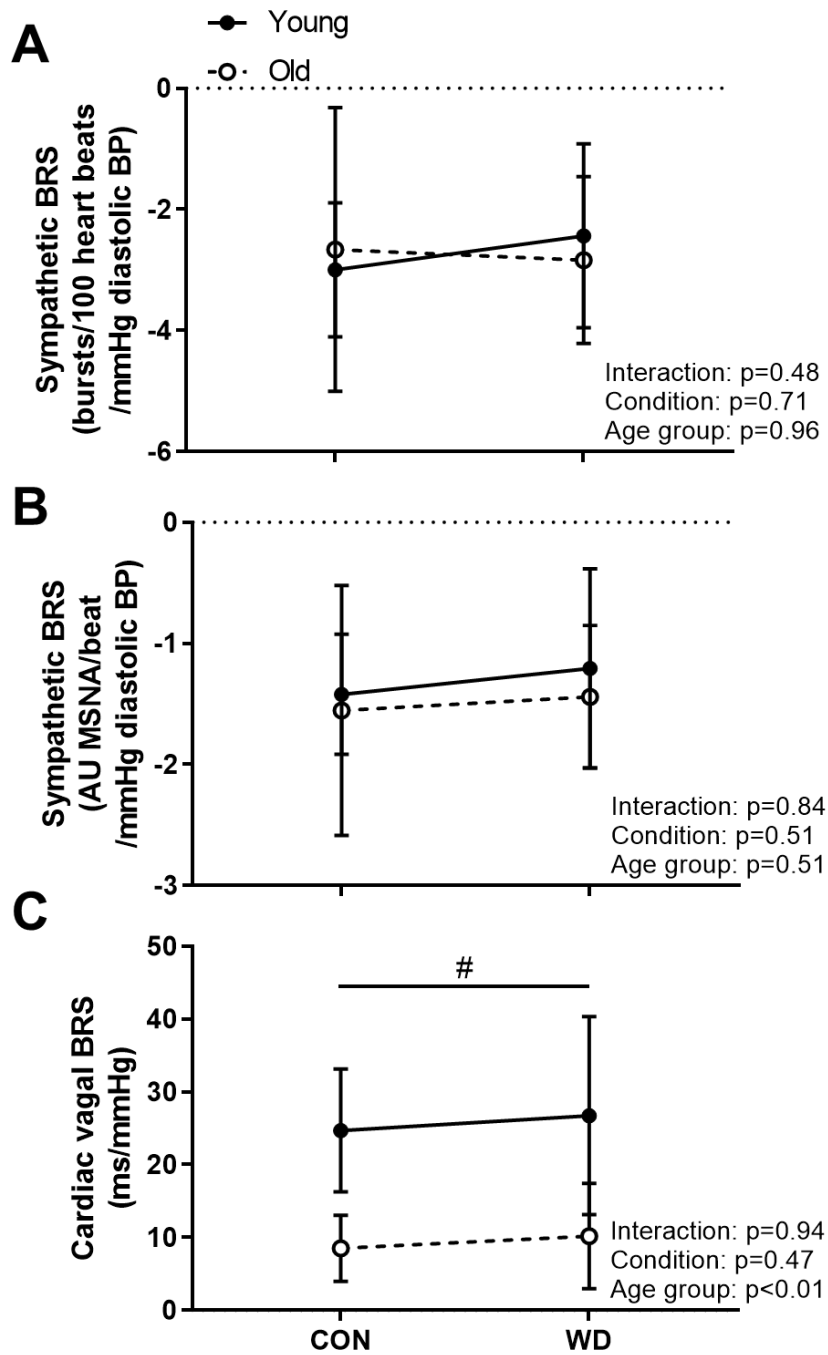
Figure 4.3   Arterial baroreflex sensitivity. A & B) Resting sympathetic (sBRS) and C) cardiac vagal (cBRS) baroreflex sensitivity values were similar between experimental conditions. cBRS values were lower in old compared to young adults. [#] indicates a significant age effect. MSNA, muscle sympathetic nerve activity.
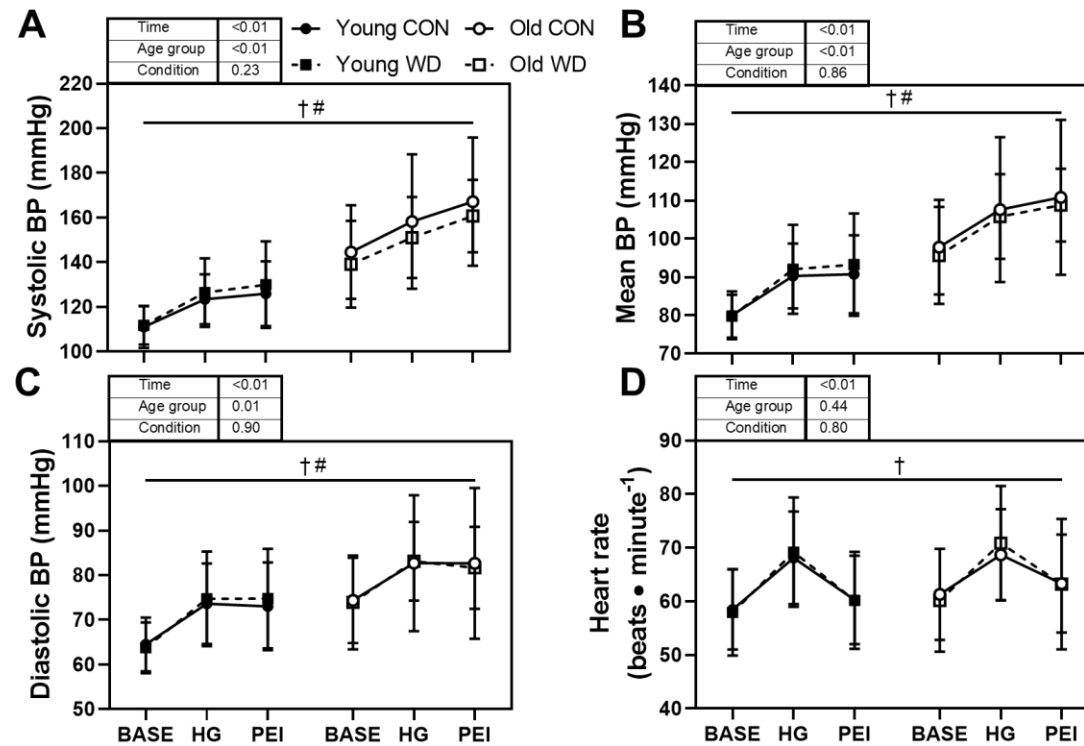
Figure 4.4   Absolute arterial blood pressure and heart rate values during exercise pressor reflex activation. A) Absolute systolic blood pressure (BP) values increased during handgrip exercise (HG) and post-exercise ischemia (PEI) similarly between experimental conditions, but were higher in the old versus young adults at all time points. B) Absolute mean BP values increased during HG and PEI similarly between experimental conditions, but were higher in the old versus young adults at all time points. C) Absolute diastolic BP values increased during HG and PEI similarly between experimental conditions, but were higher in the old versus young adults at all time points. D) Absolute heart rate values increased during HG similarly between experimental conditions and age groups. [†] indicates a significant time effect. [#] indicates a significant age effect.
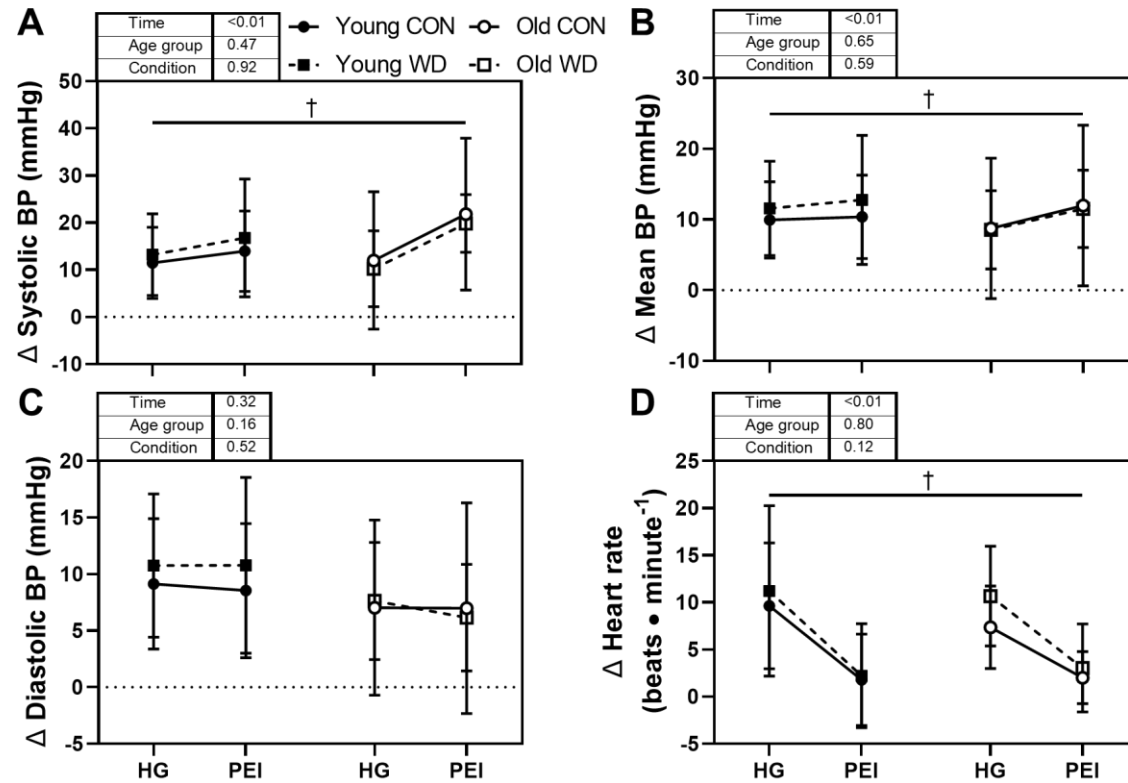
Figure 4.5   Arterial blood pressure and heart rate changes from baseline during exercise pressor reflex activation. A) Changes in systolic blood pressure (BP) values from baseline were higher during post-exercise ischemia (PEI) compared to handgrip exercise (HG), but were not different between age groups or conditions. B) Changes in mean BP values from baseline were higher during PEI compared to HG, but were not different between age groups or conditions. C) Changes in diastolic BP values from baseline were not different between time points, age groups, or experimental conditions. D) Changes in heart rate from baseline were lower during PEI compared to HG, but were not different between age groups or conditions. [†] indicates a significant time effect.
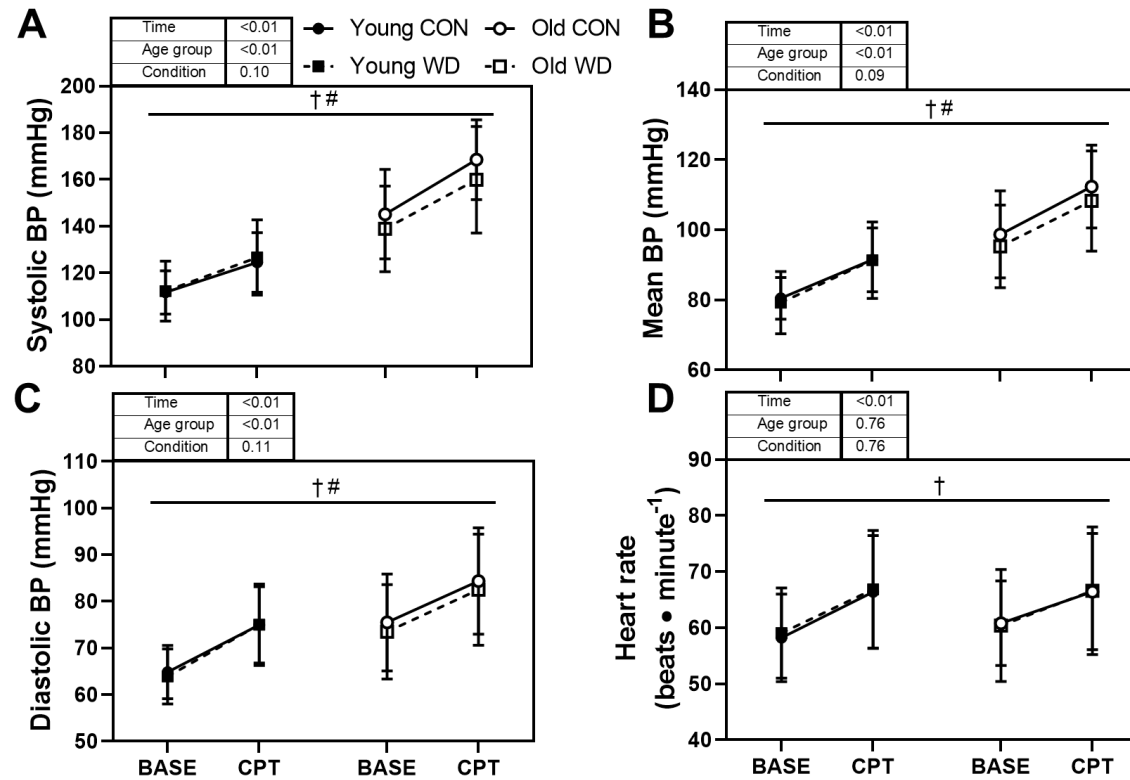
Figure 4.6   Absolute arterial blood pressure and heart rate values during the cold pressor test. A) Absolute systolic blood pressure (BP) values increased during the cold pressor test (CPT) similarly between experimental conditions, but were higher in the old versus young adults at all time points. B) Absolute mean BP values increased during CPT similarly between experimental conditions, but were higher in the old versus young adults at all time points. C) Absolute diastolic BP values increased during CPT similarly between experimental conditions, but were higher in the old versus young adults at all time points. D) Absolute heart rate values increased during HG similarly between experimental conditions and age groups. [†] indicates a significant time effect. [#] indicates a significant age effect.
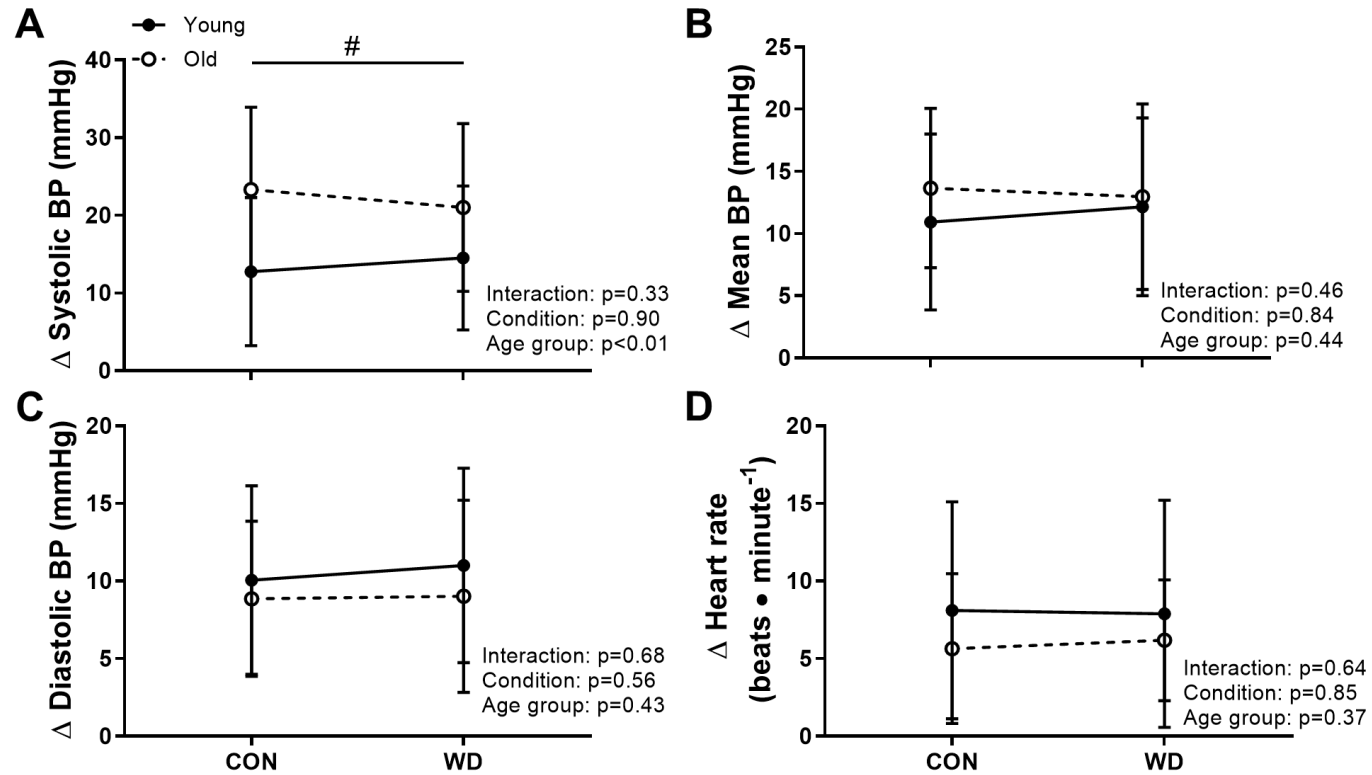
Figure 4.7   Delta arterial blood pressure and heart rate values during the cold pressor test. A) Absolute changes in systolic blood pressure (BP) during the cold pressor test (CPT) were similar between experimental conditions, but were higher in the old versus young adults. B) Absolute changes in mean BP during the CPT were similar between experimental conditions and age groups. C) Absolute changes in diastolic BP during the CPT were similar between experimental conditions and age groups. D) Absolute changes in heart rate during the CPT were similar between experimental conditions and age groups. # indicates a significant age effect.

Chapter 5

DISSERTATION CONCLUSIONS

5.1   Summary

The overall intent of this study was to determine if mild hypohydration affects arterial blood pressure (BP) regulation in healthy adults. Our model of hypohydration, consisting of three-day stepwise reductions in water intake concluded with a 16-hour water abstention period, was effective in eliciting relative blood hypernatremia in healthy young male and female adults. Despite the relative hypernatremia, we did not observe any effect of hypohydration on resting BP, ambulatory BP, or BP responses during static exercise in healthy young adults. While young adults may have compensatory mechanisms sufficient to counteract mild hypohydration, we reasoned that old adults may not be able to regulate blood electrolyte concentrations and volume, and consequently BP regulation based on previously published evidence.

In contrast to our hypothesis, our data in a cohort of healthy non-hypertensive and non-obese old adults who had similar body mass index values and objectively measured physical activity levels compared to our cohort of healthy young adults, suggest that healthy old adults have similar increases in serum sodium concentrations following hypohydration as healthy young adults. Consistent with the modest blood hypernatremia imposed within old adults relative to young adults, we did not observe any effect of hypohydration on resting BP, ambulatory BP, or BP responses during static exercise or the cold pressor test. However, consistent with previously published evidence, we did observe old adults compared to young adults had lower urine specific

gravity values, higher resting BP, greater resting and ambulatory BP variability, reduced cardiac vagal baroreflex sensitivity, higher absolute BP values during static exercise and the cold pressor test, and higher delta systolic BP values during the cold pressor test.

Collectively, these data demonstrate that during healthy aging, there may be a preserved ability to prevent short-term water restriction-induced blood hypernatremia and any potentially related hypohydration-induced BP dysregulation. Our current findings also provide additional evidence for the age-related alterations in blood pressure regulation in the absence of the potential confounding effects of differences in body mass index or objectively assessed physical activity that sometimes confound experiments investigating the effects of age independently. Namely, under control conditions, several measures of resting and reflex blood pressure regulation were altered in healthy old compared to young adults.

5.2 Perspectives

BP dysregulation is a primary risk factor for developing cardiovascular disease, the leading cause of death in the United States (22, 316). These findings inform us about how hypohydration, a public health problem primarily in old adults, impacts BP regulation in healthy aging. When taken together, our findings in healthy young and old adults suggest that healthy aging is associated with a maintained ability to prevent hypohydration-induced severe blood hypernatremia and prevent hypohydration-induced BP dysregulation at rest, during daily living, and during static exercise. Finally, given our study design, we had the advantage to provide additional insight into the age-related changes in BP regulation that occur independent of age group differences in body mass index and habitual physical activity levels.

Future research investigating the effects of chronic and/or more severe hypohydration is warranted. Further, because hypertension affects nearly five out of 10 adults over the age of 20 years old (22), future studies should be conducted to determine the interactions between mild hypohydration and BP regulation in hypertensive adults.

REFERENCES

1. **Aaron G. Lynn, Daniel Gagnon, Konrad Binder, Robert C. Boushel and Glen P. Kenny.** Divergent roles of plasma osmolality and the baroreflex on sweating and skin blood flow. *Am J Physiol Regul Integr Comp Physiol* 302: 5: 634-642, 2012.

2. **Adams JD, Sekiguchi Y, Suh H, Seal AD, Sprong CA, Kirkland TW and Kavouras SA.** Dehydration Impairs Cycling Performance, Independently of Thirst: A Blinded Study. *Med Sci Sports Exerc* 2018.

3. **Adams JM, McCarthy JJ and Stocker SD.** Excess dietary salt alters angiotensinergic regulation of neurons in the rostral ventrolateral medulla. *Hypertension* 52: 5: 932-937, 2008.

4. **Adams JM, Bardgett ME and Stocker SD.** Ventral lamina terminalis mediates enhanced cardiovascular responses of rostral ventrolateral medulla neurons during increased dietary salt. *Hypertension* 54: 2: 308-314, 2009.

5. **Adams JM, Madden CJ, Sved AF and Stocker SD.** Increased Dietary Salt Enhances Sympathoexcitatory and Sympathoinhibitory Responses From the Rostral Ventrolateral Medulla. *Hypertension* 50: 2: 354-359, 2007.

6. **Adams WM, Ferraro EM, Huggins RA and Casa DJ.** Influence of body mass loss on changes in heart rate during exercise in the heat: a systematic review. *J Strength Cond Res* 28: 8: 2380-2389, 2014.

7. **Aisenbrey GA, Handelman WA, Arnold P, Manning M and Schrier RW.** Vascular effects of arginine vasopressin during fluid deprivation in the rat. *J Clin Invest* 67: 4: 961-968, 1981.

8. **Alam M and Smirk FH.** Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol* 89: 4: 372-383, 1937.

9. **American College of Sports Medicine, Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ and Stachenfeld NS.** American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 2: 377-390, 2007.

10. **Andrews CE,Jr and Brenner BM.** Relative contributions of arginine vasopressin and angiotensin II to maintenance of systemic arterial pressure in the anesthetized water-deprived rat. *Circ Res* 48: 2: 254-258, 1981.

11. **Armstrong LE, Ganio MS, Casa DJ, Lee EC, McDermott BP, Klau JF, Jimenez L, Le Bellego L, Chevillotte E and Lieberman HR.** Mild dehydration affects mood in healthy young women. *J Nutr* 142: 2: 382-388, 2012.

12. **Armstrong LE, Maresh CM, Gabaree CV, Hoffman JR, Kavouras SA, Kenefick RW, Castellani JW and Ahlquist LE.** Thermal and circulatory responses during exercise: effects of hypohydration, dehydration, and water intake. *J Appl Physiol* 82: 6: 2028-2035, 1997.

13. **Armstrong LE.** Hydration Biomarkers During Daily Life: Recent Advances and Future Potential. *Nutrition Today* 47: 4: S3, 2012.

14. **Armstrong LE, Pumerantz AC, Fiala KA, Roti MW, Kavouras SA, Casa DJ and Maresh CM.** Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab* 20: 2: 145, 2010.

15. **Arnaoutis G, Kavouras SA, Stratakis N, Likka M, Mitrakou A, Papamichael C, Sidossis LS and Stamatelopoulos K.** The effect of hypohydration on endothelial function in young healthy adults. *Eur J Nutr* 56: 3: 1211-1217, 2017.

16. **Ayus JC and Arieff AI.** Abnormalities of water metabolism in the elderly. *Semin Nephrol* 16: 4: 277-288, 1996.

17. **Babcock MC, Brian MS, Watso JC, Edwards DG, Stocker SD, Wenner MM and Farquhar WB.** Alterations in dietary sodium intake affect cardiovagal baroreflex sensitivity. *Am J Physiol Regul Integr Comp Physiol* 315: 4: R695, 2018.

18. **Babcock MC, Robinson AT, Migdal KU, Watso JC, Wenner MM, Stocker SD and Farquhar WB.** Reducing Dietary Sodium to 1000 mg per Day Reduces

Neurovascular Transduction Without Stimulating Sympathetic Outflow. *Hypertension* 2018.

19. **Backes TP and Fitzgerald K.** Fluid consumption, exercise, and cognitive performance. *Biol Sport* 33: 3: 291-296, 2016.

20. **Bardis CN, Kavouras SA, Kosti L, Markousi M and Sidossis LS.** Mild hypohydration decreases cycling performance in the heat. *Med Sci Sports Exerc* 45: 9: 1782-1789, 2013.

21. **Ben Abderrahman A, Prioux J, Chamari K, Ben Ounis O, Tabka Z and Zouhal H.** Running interval training and estimated plasma-volume variation. *Int J Sports Physiol Perform* 8: 4: 358-365, 2013.

22. **Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, Djousse L, Elkind MSV, Ferguson JF, Fornage M, Jordan LC, 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, O'Flaherty M, Pandey A, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Spartano NL, Stokes A, Tirschwell DL, Tsao CW, Turakhia MP, VanWagner LB, Wilkins JT, Wong SS and Virani SS.** Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* CIR0000000000000659, 2019.

23. **Bennett JA, Thomas V and Riegel B.** Unrecognized chronic dehydration in older adults: examining prevalence rate and risk factors. *J Geron Nurs* 30: 11: 22, 2004.

24. **Berger A, Grossman E, Katz M, Kivity S, Klempfner R, Segev S, Goldenberg I, Sidi Y and Maor E.** Exercise systolic blood pressure variability is associated with increased risk for new-onset hypertension among normotensive adults. *J Am Soc Hypertens* 10: 6: 535.e2, 2016.

25. **Bertinieri G, di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A and Mancia G.** A new approach to analysis of the arterial baroreflex. *J Hypertens Suppl* 3: 3: 79, 1985.

26. **Bevilacqua M, Norbiato G, Chebat E, Raggi U, Cavaiani P, Guzzetti R and Bertora P.** Osmotic and nonosmotic control of vasopressin release in the elderly: effect of metoclopramide. *J Clin Endocrinol Metab* 65: 6: 1243-1247, 1987.

27. **Blair ML, Woolf PD and Felten SY.** Sympathetic activation cannot fully account for increased plasma renin levels during water deprivation. *Am J Physiol* 272: 4 Pt 2: 1197, 1997.

28. **Bogaard HJ, Woltjer HH, Dekker BM, van Keimpema AR, Postmus PE and de Vries PM.** Haemodynamic response to exercise in healthy young and elderly subjects. *Eur J Appl Physiol Occup Physiol* 75: 5: 435-442, 1997.

29. **Boggia J, Asayama K, Li Y, Hansen TW, Mena L and Schutte R.** Cardiovascular risk stratification and blood pressure variability on ambulatory and home blood pressure measurement. *Curr Hypertens Rep* 16: 9: 470-480, 2014.

30. **Boulton D, Taylor CE, Green S and Macefield VG.** The metaboreflex does not contribute to the increase in muscle sympathetic nerve activity to contracting muscle during static exercise in humans. *J Physiol* 2018.

31. **Boulton D, Taylor CE, Macefield VG and Green S.** Effect of contraction intensity on sympathetic nerve activity to active human skeletal muscle. *Front Physiol* 5: 194, 2014.

32. **Bourque CW.** Central mechanisms of osmosensation and systemic osmoregulation. *Nature Rev Neuro* 9: 7: 519-531, 2008.

33. **Boushel R.** Muscle metaboreflex control of the circulation during exercise. *Acta Physiol* 199: 4: 367-383, 2010.

34. **Boutcher SH and Stocker D.** Cardiovascular responses to light isometric and aerobic exercise in 21- and 59-year-old males. *Eur J Appl Physiol* 80: 3: 220-226, 1999.

35. **Brian J Kinsman, Haley N Nation and Sean D Stocker.** Hypothalamic Signaling in Body Fluid Homeostasis and Hypertension. *Curr Hyper Rep* 19: 1, 2017.

36. **Brian MS, Matthews EL, Watso JC, Babcock MC, Wenner MM, Rose WC, Stocker SD and Farquhar WB.** The influence of acute elevations in plasma osmolality and serum sodium on sympathetic outflow and blood pressure responses to exercise. *J Neurophysiol* 2017.

37. **Bronk DW and Stella G.** Afferent impulses in the carotid sinus nerve I. The relation of the discharge from single end organs to arterial blood pressure. *J Cell Compar Physiol* 1: 1: 113-130, 1932.

38. **Brooks VL.** Vasopressin and ANG II in the control of ACTH secretion and arterial and atrial pressures. *Am J Physiol* 256: 2 Pt 2: 339, 1989.

39. **Brooks VL, Freeman KL and Clow KA.** Excitatory amino acids in rostral ventrolateral medulla support blood pressure during water deprivation in rats. *Am J Physiol Heart Circ Physiol* 286: 5: 1642, 2004.

40. **Brooks VL, Haywood JR and Johnson AK.** Translation of salt retention to central activation of the sympathetic nervous system in hypertension. *Clin Exp Pharmacol Physiol* 32: 5-6: 426-432, 2005.

41. **Brooks VL, Qi Y and O'Donaughy TL.** Increased osmolality of conscious water-deprived rats supports arterial pressure and sympathetic activity via a brain action. *Am J Physiol Regul Integr Comp Physiol* 288: 5: 1248, 2005.

42. **Brooks VL, Freeman KL and O'Donaughy TL.** Acute and chronic increases in osmolality increase excitatory amino acid drive of the rostral ventrolateral medulla in rats. *Am J Physiol Regul Integr Comp Physiol* 287: 6: 1359, 2004.

43. **Bruce EN and Cherniack NS.** Central chemoreceptors. *J Appl Physiol* 62: 2: 389-402, 1987.

44. **Buñag RD and Miyajima E.** Baroreflex impairment precedes hypertension during chronic cerebroventricular infusion of hypertonic sodium chloride in rats. *J Clin Invest* 74: 6: 2065-2073, 1984.

45. **Bunn D, Jimoh F, Wilsher SH and Hooper L.** Increasing fluid intake and reducing dehydration risk in older people living in long-term care: a systematic review. *J Am Med Direct Assoc* 16: 2: 101-113, 2015.

46. **Burger SG, Kayser-Jones J and Bell JP.** Malnutrition and Dehydration in Nursing Homes: Key Issues in Prevention and Treatment. *Commonwealth Fund* 2000.

47. **Burke D, Sundlöf G and Wallin G.** Postural effects on muscle nerve sympathetic activity in man. *J Physiol* 272: 2: 399-414, 1977.

48. **Carroll JF, Convertino VA, Wood CE, Graves JE, Lowenthal DT and Pollock ML.** Effect of training on blood volume and plasma hormone concentrations in the elderly. *Med Sci Sports Exerc* 27: 1: 79-84, 1995.

49. **Carter JR, Fu Q, Minson CT and Joyner MJ.** Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertension* 61: 2: 395-399, 2013.

50. **Carter JR, Lawrence JE and Klein JC.** Menstrual cycle alters sympathetic neural responses to orthostatic stress in young, eumenorrheic women. *Am J Physiol Endocrinol Metab* 297: 1: 85, 2009.

51. **Carthy ER, White L, Russell FD, Holmes M, Leicht AS, Brooks PR, Hitchen-Holmes D and Askew CD.** Cardiovascular responsiveness to sympathoexcitatory stress in subjects with and without mild hypertension. *Clin Physiol Func Imag* 35: 2: 150-158, 2015.

52. **Chan J, Knutsen SF, Blix GG, Lee JW and Fraser GE.** Water, other fluids, and fatal coronary heart disease: the Adventist Health Study. *Am J Epidemiol* 155: 9: 827-833, 2002.

53. **Charkoudian N, Eisenach JH, Joyner MJ, Roberts SK and Wick DE.** Interactions of plasma osmolality with arterial and central venous pressures in control of sympathetic activity and heart rate in humans. *Am J Physiol Heart Circ Physiol* 289: 6: 2456, 2005.

54. **Charkoudian N, Gusman E, Joyner MJ, Wallin BG and Osborn J.** Integrative mechanisms of blood pressure regulation in humans and rats: cross-species similarities. *Am J Physiol Regul Integr Comp Physiol* 298: 3: 755, 2010.

55. **Charkoudian N, Halliwill JR, Morgan BJ, Eisenach JH and Joyner MJ.** Influences of hydration on post-exercise cardiovascular control in humans. *J Physiol* 552: Pt 2: 635-644, 2003.

56. **Charkoudian N, Joyner MJ, Barnes SA, Johnson CP, Eisenach JH, Dietz NM and Wallin BG.** Relationship between muscle sympathetic nerve activity and systemic hemodynamics during nitric oxide synthase inhibition in humans. *Am J Physiol Heart Circ Physiol* 291: 3: 1378, 2006.

57. **Cheuvront SN, Ely BR, Kenefick RW, Buller MJ, Charkoudian N and Sawka MN.** Hydration assessment using the cardiovascular response to standing. *Eur J Appl Physiol* 112: 12: 4081-4089, 2012.

58. **Cheuvront SN and Kenefick RW.** Dehydration: physiology, assessment, and performance effects. *Comp Physiol* 257-285, 2014.

59. **Cicconetti P, Cacciafesta M, Migliori M, Di Gioacchino CF, Vetta F, Chiarotti F and Marigliano V.** Influence of sex and age on blood pressure variability. *Arch Geron Geri* 30: 3: 225-236, 2000.

60. **Collister JP, Nahey DB, Hendel MD and Brooks VL.** Roles of the subfornical organ and area postrema in arterial pressure increases induced by 48-h water deprivation in normal rats. *Physiol Rep* 2: 1: e00191, 2014.

117

61. **Colombari DSA, Colombari E, Freiria-Oliveira AH, Antunes VR, Yao ST, Hindmarch C, Ferguson AV, Fry M, Murphy D and Paton JFR.** Switching control of sympathetic activity from forebrain to hindbrain in chronic dehydration. *J Physiol* 589: 18: 4457-4471, 2011.

62. **Convertino VA.** Effects of exercise and inactivity on intravascular volume and cardiovascular control mechanisms. *Acta Astronaut* 27: 123-129, 1992.

63. **Convertino VA.** Blood volume: its adaptation to endurance training. *Med Sci Sports Exerc* 23: 12: 1338-1348, 1991.

64. **Convertino VA, Brock PJ, Keil LC, Bernauer EM and Greenleaf JE.** Exercise training-induced hypervolemia: role of plasma albumin, renin, and vasopressin. *J Appl Physiol Respir Environ Exerc Physiol* 48: 4: 665-669, 1980.

65. **Convertino VA, Thompson CA, Benjamin BA, Keil LC, Savin WM, Gordon EP, Haskell WL, Schroeder JS and Sandler H.** Haemodynamic and ADH responses to central blood volume shifts in cardiac-denervated humans. *Clin Physiol* 10: 1: 55-67, 1990.

66. **Convertino VA.** Blood volume response to physical activity and inactivity. *Am J Med Sci* 334: 1: 72-79, 2007.

67. **Creager MA, Roddy MA, Holland KM, Hirsch AT and Dzau VJ.** Sodium depresses arterial baroreceptor reflex function in normotensive humans. *Hypertension* 17: 6 Pt 2: 989-996, 1991.

68. **Crowe MJ, Forsling ML, Rolls BJ, Phillips PA, Ledingham JG and Smith RF.** Altered water excretion in healthy elderly men. *Age Ageing* 16: 5: 285-293, 1987.

69. **D R Seals and R G Victor.** Regulation of muscle sympathetic nerve activity during exercise in humans. *Exerc Sports Sci Rev* 19: 313-349, 1991.

70. **Danziger J and Zeidel ML.** Osmotic homeostasis. *Clin J Am Soc Nephrol* 10: 5: 852-862, 2015.

71. **Davy KP and Seals DR.** Total blood volume in healthy young and older men. *J Appl Physiol* 76: 5: 2059-2062, 1994.

72. **Davy KP, Seals DR and Tanaka H.** Augmented cardiopulmonary and integrative sympathetic baroreflexes but attenuated peripheral vasoconstriction with age. *Hypertension* 32: 2: 298-304, 1998.

73. **Davy KP, Tanaka H, Andros EA, Gerber JG and Seals DR.** Influence of age on arterial baroreflex inhibition of sympathetic nerve activity in healthy adult humans. *Am J Physiol* 275: 5 Pt 2: 1768, 1998.

74. **Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ and Farquhar WB.** Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 299: 5: 1318, 2010.

75. **Deng M, Chen D, Dong Y, Lu P, Zhan B, Xu J, Ji X, Li P and Cheng X.** Independent association between age and circadian systolic blood pressure patterns in adults with hypertension. *J Clin Hypertens* 19: 10: 948-955, 2017.

76. **Dill DB and Costill DL.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 2: 247-248, 1974.

77. **Dombrowski MD and Mueller PJ.** Sedentary conditions and enhanced responses to GABA in the RVLM: role of the contralateral RVLM. *Am J Physiol Regul Integr Comp Physiol* 313: 2: R168, 2017.

78. **Downey RM, Vongpatanasin W, Mitchell JH, Smith SA and Mizuno M.** Mineralocorticoid receptor antagonists attenuate exaggerated exercise pressor reflex responses in hypertensive rats.(RESEARCH ARTICLE: Integrative Cardiovascular Physiology and Pathophysiology). *Am J Physiol* 313: 4: 788, 2017.

79. **Drewnowski A, Rehm CD and Constant F.** Water and beverage consumption among adults in the United States: cross-sectional study using data from NHANES 2005-2010. *BMC Public Health* 13: 1068, 2013.

80. **Drummond PD.** Cardiovascular reactivity in mild hypertension. *J Psychosom Res* 27: 4: 291-297, 1983.

81. **Dyke M, Davis K, Clark B, Fish L, Elahi D and Minaker K.** Effects of hypertonicity on water intake in the elderly: An age-related failure. *Geriatr Nephrol Urol* 7: 1: 11-66, 1997.

82. **Ebert TJ, Morgan BJ, Barney JA, Denahan T and Smith JJ.** Effects of aging on baroreflex regulation of sympathetic activity in humans. *Am J Physiol* 263: 3 Pt 2: 798, 1992.

83. **Eckberg DL, Cooke WH, Diedrich A, Levine BD, Pawelczyk JA, Buckey JC, Ertl AC, Biaggioni I, Cox JF, Robertson D, Baisch FJ, Blomqvist CG, Kuusela TA and Tahvanainen KUO.** Human baroreflex rhythms persist during handgrip and muscle ischaemia. *Acta Physiol* 209: 2: 114-123, 2013.

84. **Eckberg DL and Sleight P.** *Human baroreflexes in health and disease.* Oxford: Clarendon Press, 1992.

85. **Fagard RH, Celis H, Thijs L, Staessen JA, Clement DL, De Buyzere ML and De Bacquer DA.** Daytime and nighttime blood pressure as predictors of death and cause-specific cardiovascular events in hypertension. *Hypertension* 51: 1: 55-61, 2008.

86. **Fairfax ST, Padilla J, Vianna LC, Davis MJ and Fadel PJ.** Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. *Am J Physiol Heart Circ Physiol* 304: 5: 759, 2013.

87. **Fairfax ST, Holwerda SW, Credeur DP, Zuidema MY, Medley JH, Dyke PC, Wray DW, Davis MJ and Fadel PJ.** The role of α-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. *J Physiol* 591: 14: 3637-3649, 2013.

88. **Farah K, Keshav G and Pawan S.** Autonomic reactivity to cold pressor test in prehypertensive and hypertensive medical students. *Indian J Physiol Pharm* 55: 3: 246, 2011.

89. **Farah R, Shurtz-Swirski R and Nicola M.** High blood pressure response to stress ergometry could predict future hypertension. *Eur J Intern Med* 20: 4: 366-368, 2009.

90. **Farquhar WB, Wenner MM, Delaney EP, Prettyman AV and Stillabower ME.** Sympathetic neural responses to increased osmolality in humans. *Am J Physiol Heart Circ Physiol* 291: 5: 2181, 2006.

91. **Fejes-Tóth G, Náray-Fejes-Tóth A and Ratge D.** Evidence against role of antidiuretic hormone in support of blood pressure during dehydration. *Am J Physiol* 249: 1 Pt 2: 42, 1985.

92. **Ferreira-Pêgo C, Guelinckx I, Moreno L, Kavouras S, Gandy J, Martinez H, Bardosono S, Abdollahi M, Nasseri E, Jarosz A, Babio N and Salas-Salvadó J.** Total fluid intake and its determinants: cross-sectional surveys among adults in 13 countries worldwide. *Eur J Nutr* 54: S2: 35-43, 2015.

93. **Ferry M.** Strategies for ensuring good hydration in the elderly. *Nutr Rev* 63: 6 Pt 2: 22, 2005.

94. **Filomena J, Riba-Llena I, Vinyoles E, Tovar JL, Mundet X, Castane X, Vilar A, Lopez-Rueda A, Jimenez-Balado J, Cartanya A, Montaner J, Delgado P and ISSYS Investigators.** Short-Term Blood Pressure Variability Relates to the Presence of Subclinical Brain Small Vessel Disease in Primary Hypertension. *Hypertension* 66: 3: 634-640, 2015.

95. **Flaa A, Eide IK, Kjeldsen SE and Rostrup M.** Sympathoadrenal stress reactivity is a predictor of future blood pressure: an 18-year follow-up study. *Hypertension* 52: 2: 336-341, 2008.

96. **Floras JS.** Sympathetic Nervous System Activation in Human Heart Failure: Clinical Implications of an Updated Model. *J Am Coll Cardiol* 54: 5: 375-385, 2009.

97. **Flynn FW and Stricker EM.** Hypovolemia stimulates intraoral intake of water and NaCl solution in intact rats but not in chronic decerebrate rats. *Physiol Behav* 80: 2-3: 281-287, 2003.

98. **Fonkoue IT and Carter JR.** Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol* 309: 11: R1386, 2015.

99. **Fortney SM, Wenger CB, Bove JR and Nadel ER.** Effect of hyperosmolality on control of blood flow and sweating. *J Appl Physiol Respir Environ Exerc Physiol* 57: 6: 1688-1695, 1984.

100. **Frangeskou M, Lopez-Valcarcel B and Serra-Majem L.** Dehydration in the elderly: A review focused on economic burden. *J Nutr Health Aging* 19: 6: 619-627, 2015.

101. **Freeman KL and Brooks VL.** AT(1) and glutamatergic receptors in paraventricular nucleus support blood pressure during water deprivation. *Am J Physiol Regul Integr Comp Physiol* 292: 4: 1675, 2007.

102. **Fries BE, Hawes C, Morris JN, Phillips CD, Mor V and Park PS.** Effect of the National Resident Assessment Instrument on Selected Health Conditions and Problems. *J Am Geriatr Soc* 45: 8: 994-1001, 1997.

103. **Fu Q, Okazaki K, Shibata S, Shook RP, VanGunday TB, Galbreath MM, Reelick MF and Levine BD.** Menstrual cycle effects on sympathetic neural responses to upright tilt. *J Physiol* 587: Pt 9: 2019-2031, 2009.

104. **G. M. Goodwin, D. I. McCloskey and J. H. Mitchell.** Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *J Physiol* 226: 1: 173-190, 1972.

105. **Gabor A and Leenen FHH.** Central neuromodulatory pathways regulating sympathetic activity in hypertension. *J Appl Physiol* 113: 8: 1294-1303, 2012.

106. **Ganio MS, Wingo JE, Carrolll CE, Thomas MK and Cureton KJ.** Fluid ingestion attenuates the decline in VO2peak associated with cardiovascular drift. *Med Sci Sports Exerc* 38: 5: 901-909, 2006.

107. **Gillies MJ, Huang Y, Hyam JA, Aziz TZ and Green AL.** Direct neurophysiological evidence for a role of the human anterior cingulate cortex in central command. *Auton Neurosci* 2018.

108. **González-Alonso J, Mora-Rodríguez R, Below PR and Coyle EF.** Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol* 82: 4: 1229-1236, 1997.

109. **González-Alonso J, Mora-Rodríguez R, Below PR and Coyle EF.** Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol* 79: 5: 1487-1496, 1995.

110. **González-Alonso J, Mora-Rodríguez R and Coyle EF.** Stroke volume during exercise: interaction of environment and hydration. *Am J Physiol Heart Circ Physiol* 278: 2: 321, 2000.

111. **Grassi G, Seravalle G, Brambilla G, Pini C, Alimento M, Facchetti R, Spaziani D, Cuspidi C and Mancia G.** Marked sympathetic activation and baroreflex dysfunction in true resistant hypertension. *Int J Cardiol* 177: 3: 1020-1025, 2014.

112. **Greaney JL, Edwards DG, Fadel PJ and Farquhar WB.** Rapid onset pressor and sympathetic responses to static handgrip in older hypertensive adults. *J Human Hyper* 29: 7: 402, 2015.

113. **Greaney JL, Ray CA, Prettyman AV, Edwards DG and Farquhar WB.** Influence of increased plasma osmolality on sympathetic outflow during apnea. *Am J Physiol Regul Integr Comp Physiol* 299: 4: 1091, 2010.

114. **Greaney JL, Schwartz CE, Edwards DG, Fadel PJ and Farquhar WB.** The neural interaction between the arterial baroreflex and muscle metaboreflex is preserved in older men. *Exp Physiol* 98: 10: 1422-1431, 2013.

115. **Greaney JL, Stanhewicz AE, Kenney WL and Alexander LM.** Muscle sympathetic nerve activity during cold stress and isometric exercise in healthy older adults. *J Appl Physiol* 117: 6: 648, 2014.

116. **Gross CR, Lindquist RD, Woolley AC, Granieri R, Allard K and Webster B.** Clinical indicators of dehydration severity in elderly patients. *J Emerg Med* 10: 3: 267-274, 1992.

117. **Guelen I, Westerhof BE, Van Der Sar, Gertrude L, Van Montfrans GA, Kiemeneij F, Wesseling KH and Bos WJ.** Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Press Monit* 8: 1: 27-30, 2003.

118. **Guyenet PG.** The sympathetic control of blood pressure. *Nat Rev Neurosci* 7: 5: 335-346, 2006.

119. **Habecker BA, Grygielko ET, Huhtala TA, Foote B and Brooks VL.** Ganglionic tyrosine hydroxylase and norepinephrine transporter are decreased by increased sodium chloride in vivo and in vitro. *Auton Neurosci* 107: 2: 85-98, 2003.

120. **Hansen TW, Jeppesen J, Rasmussen S, Ibsen H and Torp-Pedersen C.** Ambulatory blood pressure and mortality: a population-based study. *Hypertension* 45: 4: 499-504, 2005.

121. **Hardy JC, Gray K, Whisler S and Leuenberger U.** Sympathetic and blood pressure responses to voluntary apnea are augmented by hypoxemia. *J Appl Physiol* 77: 5: 2360-2365, 1994.

122. **Harrison MH, Geelen G, Keil LC, Wade CA, Hill LC, Kravik SE and Greenleaf JE.** Effect of hydration on plasma vasopressin, renin, and aldosterone responses to head-up tilt. *Aviat Space Environ Med* 57: 5: 420-425, 1986.

123. **Hart EC and Charkoudian N.** Sympathetic neural regulation of blood pressure: influences of sex and aging. *Physiol* 29: 1: 8-15, 2014.

124. **Hart EC and Charkoudian N.** Sympathetic neural mechanisms in human blood pressure regulation. *Curr Hypertens Rep* 13: 3: 237-243, 2011.

125. **Hart EC, Joyner MJ, Wallin BG and Charkoudian N.** Sex, ageing and resting blood pressure: gaining insights from the integrated balance of neural and haemodynamic factors. *J Physiol* 590: Pt 9: 2069-2079, 2012.

126. **Hart EC, Joyner MJ, Wallin BG, Karlsson T, Curry TB and Charkoudian N.** Baroreflex control of muscle sympathetic nerve activity: a nonpharmacological measure of baroreflex sensitivity. *Am J Physiol Heart Circ Physiol* 298: 3: 816, 2010.

127. **Harvey RE, Hart EC, Charkoudian N, Curry TB, Carter JR, Fu Q, Minson CT, Joyner MJ and Barnes JN.** Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension* 66: 3: 590-597, 2015.

128. **Harvey RE, Barnes JN, Charkoudian N, Curry TB, Eisenach JH, Hart EC and Joyner MJ.** Forearm vasodilator responses to a β-adrenergic receptor agonist in premenopausal and postmenopausal women. *Physiol Rep* 2: 6: 2014.

129. **Haspula D and Clark MA.** Neuroinflammation and sympathetic overactivity: Mechanisms and implications in hypertension. *Auton Neurosci* 2018.

130. **Hasser EM, Bishop VS and Hay M.** Interactions between vasopressin and baroreflex control of the sympathetic nervous system. *Clin Exp Pharm Physiol* 24: 1: 102-108, 1997.

131. **Hayes SG, Kindig AE and Kaufman MP.** Comparison between the effect of static contraction and tendon stretch on the discharge of group III and IV muscle afferents. *J Appl Physiol* 99: 5: 1891-1896, 2005.

132. **Hayley J. Miller.** Dehydration in the Older Adult. *J Geront Nurs* 41: 9: 8, 2015.

133. **Hearon CM, Kirby BS, Luckasen GJ, Larson DG and Dinenno FA.** Endothelium-dependent vasodilatory signalling modulates α1 -adrenergic vasoconstriction in contracting skeletal muscle of humans. *J Physiol* 594: 24: 7435-7453, 2016.

134. **Hines EA and Brown GE.** The cold pressor test for measuring the reactibility of the blood pressure: Data concerning 571 normal and hypertensive subjects. *Am Heart J* 11: 1: 1-9, 1936.

135. **Hinojosa-Laborde C, Ryan KL, Rickards CA and Convertino VA.** Resting sympathetic baroreflex sensitivity in subjects with low and high tolerance to central hypovolemia induced by lower body negative pressure. *Front Physiol* 5: 241, 2014.

136. **Hiwatari M and Johnston CI.** Involvement of vasopressin in the cardiovascular effects of intracerebroventricularly administered alpha 1-adrenoceptor agonists in the conscious rat. *J Hyper* 3: 6: 613, 1985.

137. **Holbein WW, Bardgett ME and Toney GM.** Blood pressure is maintained during dehydration by hypothalamic paraventricular nucleus-driven tonic sympathetic nerve activity. *J Physiol* 592: 17: 3783-3799, 2014.

138. **Holmqvist L, Mortensen L, Kanckos C, Ljungman C, Mehlig K and Manhem K.** Exercise blood pressure and the risk of future hypertension. *J Hum Hypertens* 26: 12: 691-695, 2012.

139. **Hureau TJ, Weavil JC, Thurston TS, Broxterman RM, Nelson AD, Bledsoe AD, Jessop JE, Richardson RS, Wray DW and Amann M.** Identifying the role of group III/IV muscle afferents in the carotid baroreflex control of mean arterial pressure and heart rate during exercise. *J Physiol* 2018.

140. **Ito S, Gordon FJ and Sved AF.** Dietary salt intake alters cardiovascular responses evoked from the rostral ventrolateral medulla. *Am J Physiol* 276: 6 Pt 2: 1600, 1999.

141. **Ito T, Itoh T, Hayano T, Yamauchi K and Takamata A.** Plasma hyperosmolality augments peripheral vascular response to baroreceptor unloading during heat stress. *Am J Physiol Regul Integr Comp Physiol* 289: 2: R440, 2005.

142. **J. W Williamson, P. J Fadel and J. H Mitchell.** New insights into central cardiovascular control during exercise in humans: a central command update. *Exp Physiol* 91: 1: 51-58, 2006.

143. **Jae SY, Franklin BA, Choo J, Choi Y and Fernhall B.** Exaggerated Exercise Blood Pressure Response During Treadmill Testing as a Predictor of Future Hypertension in Men: A Longitudinal Study. *Am J Hypertens* 28: 11: 1362-1367, 2015.

144. **Jae SY, Kurl S, Laukkanen JA, Choi Y, Park WH, Bunsawat K, Heffernan KS, Fernhall B, Kang S and Park JB.** Usefulness of blood pressure rise prior to exercise stress testing to predict the risk of future hypertension in normotensive Korean men. *Am J Cardiol* 114: 8: 1238-1242, 2014.

145. **James P Fisher and Michael J White.** Muscle afferent contributions to the cardiovascular response to isometric exercise. *Exp Physiol* 89: 6: 639-646, 2004.

146. **James LJ, Moss J, Henry J, Papadopoulou C and Mears SA.** Hypohydration impairs endurance performance: a blinded study. *Physiol Rep* 5: 12: 10.14814/phy2.13315, 2017.

147. **James MA, Robinson TG, Panerai RB and Potter JF.** Arterial baroreceptor-cardiac reflex sensitivity in the elderly. *Hypertension* 28: 6: 953-960, 1996.

148. **Jarvis SS, Shibata S, Bivens TB, Okada Y, Casey BM, Levine BD and Fu Q.** Sympathetic activation during early pregnancy in humans. *J Physiol* 590: 15: 3535-3543, 2012.

149. **John R Halliwill, Barbara J Morgan and Nisha Charkoudian.** Peripheral Chemoreflex and Baroreflex Interactions in Cardiovascular Regulation in Humans. *J Physiol* 552: 1: 295-302, 2003.

150. **Johnson AK.** The sensory psychobiology of thirst and salt appetite. *Med Sci Sports Exerc* 39: 8: 1388-1400, 2007.

151. **Johnson EC, Bardis CN, Jansen LT, Adams JD, Kirkland TW and Kavouras SA.** Reduced water intake deteriorates glucose regulation in patients with type 2 diabetes. *Nutr Res* 43: 25-32, 2017.

152. **Johnston C.** Vasopressin in Circulatory Control and Hypertension. *J Hyper* 3: 6: 557-569, 1985.

153. **Joyner MJ, Charkoudian N and Wallin BG.** A sympathetic view of the sympathetic nervous system and human blood pressure regulation. *Exp Physiol* 93: 6: 715-724, 2008.

154. **Joyner MJ and Wieling W.** Increased muscle perfusion reduces muscle sympathetic nerve activity during handgripping. *J Appl Physiol* 75: 6: 2450-2455, 1993.

155. **Judelson DA, Maresh CM, Farrell MJ, Yamamoto LM, Armstrong LE, Kraemer WJ, Volek JS, Spiering BA, Casa DJ and Anderson JM.** Effect of hydration state on strength, power, and resistance exercise performance. *Med Sci Sports Exerc* 39: 10: 1817-1824, 2007.

156. **Katayama K, Kaur J, Young BE, Barbosa TC, Ogoh S and Fadel PJ.** High intensity muscle metaboreflex activation attenuates cardiopulmonary baroreflex-mediated inhibition of muscle sympathetic nerve activity. *J Appl Physiol* 2018.

157. **Kaufman MP, Longhurst JC, Rybicki KJ, Wallach JH and Mitchell JH.** Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *J Appl Physiol Respir Environ Exerc Physiol* 55: 1 Pt 1: 105-112, 1983.

158. **Kaufman MP and Hayes SG.** The Exercise Pressor Reflex. *Clin Auton Res* 12: 6: 429-439, 2002.

159. **Kavouras SA.** Hydration, dehydration, underhydration, optimal hydration: are we barking up the wrong tree? *Eur J Nutr* 2019.

160. **Kayrak M, Bacaksiz A, Vatankulu MA, Ayhan SS, Kaya Z, Ari H, Sonmez O and Gok H.** Exaggerated blood pressure response to exercise--a new portent of masked hypertension. *Clin Exp Hypertens* 32: 8: 560-568, 2010.

161. **Keller K, Stelzer K, Ostad MA and Post F.** Impact of exaggerated blood pressure response in normotensive individuals on future hypertension and prognosis: Systematic review according to PRISMA guideline. *Adv Med Sci* 62: 2: 317-329, 2017.

162. **Kenefick RW, Sollanek KJ, Charkoudian N and Sawka MN.** Impact of skin temperature and hydration on plasma volume responses during exercise. *J Appl Physiol* 117: 4: 413-420, 2014.

163. **Kenney EL, Long MW, Cradock AL and Gortmaker SL.** Prevalence of Inadequate Hydration Among US Children and Disparities by Gender and Race/Ethnicity: National Health and Nutrition Examination Survey, 2009-2012. *Am J Public Health* 105: 8: e118, 2015.

164. **Kenney WL and Chiu P.** Influence of age on thirst and fluid intake. *Med Sci Sports Exerc* 33: 9: 1524-1532, 2001.

165. **Kiely JM and Gordon FJ.** Non-NMDA receptors in the rostral ventrolateral medulla mediate somatosympathetic pressor responses. *J Auton Nerv Syst* 43: 3: 231-239, 1993.

166. **Kikuya M, Hozawa A, Ohokubo T, Tsuji I, Michimata M, Matsubara M, Ota M, Nagai K, Araki T, Satoh H, Ito S, Hisamichi S and Imai Y.** Prognostic significance of blood pressure and heart rate variabilities: the Ohasama study. *Hypertension* 36: 5: 901-906, 2000.

167. **Kimmerly DS and Shoemaker JK.** Hypovolemia and MSNA discharge patterns: assessing and interpreting sympathetic responses. *Am J Physiol Heart Circ Physiol* 284: 4: 1198, 2003.

168. **Kimmerly DS and Shoemaker JK.** Hypovolemia and neurovascular control during orthostatic stress. *Am J Physiol Heart Circ Physiol* 282: 2: 645, 2002.

169. **Kinsman BJ, Browning KN and Stocker SD.** NaCl and osmolarity produce different responses in organum vasculosum of the lamina terminalis neurons, sympathetic nerve activity and blood pressure. *J Physiol* 595: 18: 6187-6201, 2017.

170. **Kirkland JL, Lye M, Levy DW and Banerjee AK.** Patterns of urine flow and electrolyte excretion in healthy elderly people. *Br Med J* 287: 6406: 1665-1667, 1983.

171. **Klingbeil CK, Brooks VL, Quillen EW,Jr and Reid IA.** Effect of baroreceptor denervation on stimulation of drinking by angiotensin II in conscious dogs. *Am J Physiol* 260: 3 Pt 1: 333, 1991.

172. **Krakoff LR.** Blood Pressure Out of the Office: Its Time Has Finally Come. *Am J Hypertens* 29: 3: 289-295, 2016.

173. **Laitinen T, Hartikainen J, Vanninen E, Niskanen L, Geelen G and Länsimies E.** Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol* 84: 2: 576-583, 1998.

174. **Lalande S, Sawicki CP, Baker JR and Shoemaker JK.** Effect of age on the hemodynamic and sympathetic responses at the onset of isometric handgrip exercise. *J Appl Physiol* 116: 2: 222-227, 2014.

175. **Lavizzo-Mourey RJ.** Dehydration in the elderly: a short review. *J Natl Med Assoc* 79: 10: 1033-1038, 1987.

176. **Lawrence JE, Ray CA and Carter JR.** Vestibulosympathetic reflex during the early follicular and midluteal phases of the menstrual cycle. *Am J Physiol Endocrinol Metab* 294: 6: 1046, 2008.

177. **Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C and Sinoway L.** Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. *J Appl Physiol* 79: 2: 581-588, 1995.

178. **Lindeman RD, Tobin J and Shock NW.** Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc* 33: 4: 278-285, 1985.

179. **Luckey AE and Parsa CJ.** Fluid and Electrolytes in the Aged. *Arch Surg* 138: 10: 1055-1060, 2003.

180. **Lugliani R, Whipp BJ, Seard C and Wasserman K.** Effect of bilateral carotid-body resection on ventilatory control at rest and during exercise in man. *N Engl J Med* 285: 20: 1105-1111, 1971.

181. **Mack GW, Weseman CA, Langhans GW, Scherzer H, Gillen CM and Nadel ER.** Body fluid balance in dehydrated healthy older men: thirst and renal osmoregulation. *J Appl Physiol* 76: 4: 1615-1623, 1994.

182. **Madden JM, O'Flynn AM, Dolan E, Fitzgerald AP and Kearney PM.** Short-term blood pressure variability over 24 h and target organ damage in middle-aged men and women. *J Hum Hypertens* 29: 12: 719-725, 2015.

183. **Mancia G, Parati G, Hennig M, Flatau B, Omboni S, Glavina F, Costa B, Scherz R, Bond G and Zanchetti A.** Relation between blood pressure variability and carotid artery damage in hypertension: baseline data from the European Lacidipine Study on Atherosclerosis (ELSA). *J Hypertens* 19: 11: 1981-1989, 2001.

184. **Mancia G and Parati G.** Ambulatory blood pressure monitoring and organ damage. *Hypertension* 36: 5: 894-900, 2000.

185. **Mancia G, Bombelli M, Facchetti R, Madotto F, Corrao G, Trevano FQ, Grassi G and Sega R.** Long-term prognostic value of blood pressure variability in the general population: results of the Pressioni Arteriose Monitorate e Loro Associazioni Study. *Hypertension* 49: 6: 1265-1270, 2007.

186. **Manz F.** Hydration and Disease. *J Am Coll Nutr* 26: Supplement 5: 541S, 2007.

187. **Matsukawa T, Sugiyama Y and Mano T.** Age-related changes in baroreflex control of heart rate and sympathetic nerve activity in healthy humans. *J Auton Nerv Syst* 60: 3: 209-212, 1996.

188. **Matthews EL, Brian MS, Coyle DE, Edwards DG, Stocker SD, Wenner MM and Farquhar WB.** Peripheral venous distension elicits a blood pressure raising reflex in young and middle-aged adults. *Am J Physiol Regul Integr Comp Physiol* 310: 11: 1128, 2016.

189. **Matthews EL, Brian MS, Edwards DG, Stocker SD, Wenner MM and Farquhar WB.** Blood pressure responses to dietary sodium: Association with autonomic cardiovascular function in normotensive adults. *Auton Neurosci* 208: 51-56, 2017.

190. **Matthews KA, Woodall KL and Allen MT.** Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension* 22: 4: 479-485, 1993.

191. **McClain J, Hardy C, Enders B, Smith M and Sinoway L.** Limb congestion and sympathoexcitation during exercise. Implications for congestive heart failure. *J Clin Invest* 92: 5: 2353-2359, 1993.

192. **McConell GK, Burge CM, Skinner SL and Hargreaves M.** Influence of ingested fluid volume on physiological responses during prolonged exercise. *Acta Physiol Scand* 160: 2: 149-156, 1997.

193. **McDermott BP, Anderson SA, Armstrong LE, Casa DJ, Cheuvront SN, Cooper L, Kenney WL, O'Connor FG and Roberts WO.** National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *J Athl Train* 52: 9: 877-895, 2017.

194. **Mena LJ, Felix VG, Melgarejo JD and Maestre GE.** 24-Hour Blood Pressure Variability Assessed by Average Real Variability: A Systematic Review and Meta-Analysis. *J Am Heart Assoc* 6: 10: 1-10, 2017.

195. **Mentes J.** Oral hydration in older adults: greater awareness is needed in preventing, recognizing, and treating dehydration. *Am J Nurs* 106: 6: 40, 2006.

196. **Michaelides AP, Liakos CI, Vyssoulis GP, Chatzistamatiou EI, Markou MI, Tzamou V and Stefanadis CI.** The interplay of exercise heart rate and blood pressure as a predictor of coronary artery disease and arterial hypertension. *J Clin Hypertens* 15: 3: 162-170, 2013.

197. **Middlekauff HR, Park J and Gornbein JA.** Lack of effect of ovarian cycle and oral contraceptives on baroreceptor and nonbaroreceptor control of sympathetic nerve activity in healthy women. *Am J Physiol Heart Circ Physiol* 302: 12: 2560, 2012.

198. **Milan-Mattos JC, Porta A, Perseguini NM, Minatel V, Rehder-Santos P, Takahashi ACM, Mattiello SM and Catai AM.** Influence of age and gender on the phase and strength of the relation between heart period and systolic blood pressure spontaneous fluctuations. *J Appl Physiol* 124: 3: 791-804, 2018.

199. **Milia R, Roberto S, Mulliri G, Loi A, Marcelli M, Sainas G, Milia N, Marongiu E and Crisafulli A.** Effect of aging on hemodynamic response to metaboreflex activation. *Eur J Appl Physiol* 115: 8: 1693-1703, 2015.

200. **Milledge JS, Bryson EI, Catley DM, Hesp R, Luff N, Minty BD, Older MW, Payne NN, Ward MP and Withey WR.** Sodium balance, fluid homeostasis and the renin-aldosterone system during the prolonged exercise of hill walking. *Clin Sci* 62: 6: 595-604, 1982.

201. **Minson CT, Halliwill JR, Young TM and Joyner MJ.** Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* 101: 8: 862-868, 2000.

202. **Miyai N, Arita M, Miyashita K, Morioka I, Shiraishi T and Nishio I.** Blood pressure response to heart rate during exercise test and risk of future hypertension. *Hypertension* 39: 3: 761-766, 2002.

203. **Miyai N, Arita M, Miyashita K, Morioka I, Shiraishi T, Nishio I and Takeda S.** Antihypertensive effects of aerobic exercise in middle-aged normotensive men with exaggerated blood pressure response to exercise. *Hypertens Res* 25: 4: 507-514, 2002.

204. **Monahan KD, Feehan RP, Sinoway LI and Gao Z.** Contribution of sympathetic activation to coronary vasodilatation during the cold pressor test in healthy men: effect of ageing. *J Physiol* 591: 11: 2937-2947, 2013.

205. **Morfis L and Howes LG.** Nocturnal fall in blood pressure in the elderly is related to presence of hypertension and not age. *Blood Pressure Monit* 6: 5: 274-278, 1997.

206. **Morgan BJ, Denahan T and Ebert TJ.** Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea. *J Appl Physiol* 74: 6: 2969-2975, 1993.

207. **Moyen NE, Ganio MS, Wiersma LD, Kavouras SA, Gray M, McDermott BP, Adams JD, Binns AP, Judelson DA, McKenzie AL, Johnson EC, Muñoz CX, Kunces LJ and Armstrong LE.** Hydration status affects mood state and pain sensation during ultra-endurance cycling. *J Sports Sci* 33: 18: 1962-1969, 2015.

208. **Mtinangi BL and Hainsworth R.** Effects of moderate exercise training on plasma volume, baroreceptor sensitivity and orthostatic tolerance in healthy subjects. *Exp Physiol* 84: 1: 121-130, 1999.

209. **Mueller PJ.** Exercise training and sympathetic nervous system activity: evidence for physical activity dependent neural plasticity. *Clin Exp Pharmacol Physiol* 34: 4: 377-384, 2007.

210. **Mueller PJ and Mischel NA.** Selective enhancement of glutamate-mediated pressor responses after GABAA receptor blockade in the RVLM of sedentary versus spontaneous wheel running rats. *Frontiers* 3: 2012.

211. **Muller MD, Drew RC, Cui J, Blaha CA, Mast JL and Sinoway LI.** Effect of oxidative stress on sympathetic and renal vascular responses to ischemic exercise. *Physiol Rep* 1: 3: n/a, 2013.

212. **Murray B.** Hydration and Physical Performance. *Am Coll Nutrition* 26: Supplement 5: 542S, 2007.

213. **Nadel ER, Fortney SM and Wenger CB.** Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol Respir Environ Exerc Physiol* 49: 4: 715-721, 1980.

214. **Neus H, von Eiff AW, Friedrich G, Ruddel H and Schulte W.** The effect of beta receptor blockers and diuretics on blood pressure reactivity. *Z Kardiol* 72: 10: 617-621, 1983.

215. **Ng AV, Callister R, Johnson DG and Seals DR.** Sympathetic neural reactivity to stress does not increase with age in healthy humans. *Am J Physiol* 267: 1 Pt 2: 344, 1994.

216. **Nicholas A. Mischel and Patrick J. Mueller.** (In)activity-dependent alterations in resting and reflex control of splanchnic sympathetic nerve activity. *J Appl Physiol* 111: 6: 1854-1862, 2011.

217. **Nomura K, Hiyama TY, Sakuta H, Matsuda T, Lin C, Kobayashi K, Kobayashi K, Kuwaki T, Takahashi K, Matsui S and Noda M.** [Na+] Increases in Body Fluids Sensed by Central Nax Induce Sympathetically Mediated Blood Pressure Elevations via H+-Dependent Activation of ASIC1a. *Neuron* 101: 1: 75.e6, 2019.

218. **O'Brien E, Coats A, Owens P, Petrie J, Padfield PL, Littler WA, de Swiet M and Mee F.** Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British hypertension society. *BMJ* 320: 7242: 1128-1134, 2000.

219. **Odahara T, Irokawa M, Karasawa H and Matsuda S.** Detection of exaggerated blood pressure response using laboratory of physical science protocol and risk of future hypertension. *J Occup Health* 52: 5: 278-286, 2010.

220. **Ogawa Y, Iwasaki K, Aoki K, Saitoh T, Kato J and Ogawa S.** Dynamic cerebral autoregulation after mild dehydration to simulate microgravity effects. *Aviat Space Environ Med* 80: 5: 443-447, 2009.

221. **Ogoh S, Fisher JP, Young CN, Raven PB and Fadel PJ.** Transfer function characteristics of the neural and peripheral arterial baroreflex arcs at rest and during postexercise muscle ischemia in humans. *Am J Physiol Heart Circ Physiol* 296: 5: 1416, 2009.

222. **Ota K, Kimura T, Matsui K, Iitake K, Shoji M, Inoue M, Sato K, Ohta M, Yamamoto T and Yoshinaga K.** Effects of central osmotic stimulation on vasopressin and enkephalin release into the blood and cerebrospinal fluid and blood pressure. *Acta Endocrinol* 122: 1: 62-70, 1990.

223. **Palatini P, Penzo M, Racioppa A, Zugno E, Guzzardi G, Anaclerio M and Pessina AC.** Clinical relevance of nighttime blood pressure and of daytime blood pressure variability. *Arch Intern Med* 152: 9: 1855-1860, 1992.

224. **Panel on Dietary Reference Intakes for Electrolytes and Water and Standing Committee on the Scientific Evaluation of Dietary Reference Intakes.** *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate.* Washington: National Academies Press, 2004.

225. **Parati G, Pomidossi G, Albini F, Malaspina D and Mancia G.** Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. *J Hypertens* 5: 1: 93-98, 1987.

226. **Parati G, Ochoa JE, Lombardi C and Bilo G.** Assessment and management of blood-pressure variability. *Nature* 10: 3: 143, 2013.

227. **Parker Jones P, Davy KP, Desouza CA and Tanaka H.** Total blood volume in endurance-trained postmenopausal females: relation to exercise mode and maximal aerobic capacity. *Acta Physiol Scand* 166: 4: 327-333, 1999.

228. **Pash E, Parikh N and Hashemi L.** Economic Burden Associated With Hospital Postadmission Dehydration. *J Parenteral Enteral Nutr* 38: 2_suppl: 64S, 2014.

229. **Patsalos OC and Thoma V.** Water supplementation after dehydration improves judgment and decision-making performance. *Psychol Res* 2019.

230. **Pawloski-Dahm CM and Gordon FJ.** Increased dietary salt sensitizes vasomotor neurons of the rostral ventrolateral medulla. *Hypertension* 22: 6: 929-933, 1993.

231. **Peçanha T, Brito LC, Fecchio RY, Sousa PN, da Silva Junior, Natan Daniel, Abreu AP, Silva GV, Mion-Junior D and Forjaz, Cláudia Lúcia de Moraes.** Metaboreflex activation delays heart rate recovery after aerobic exercise in never-treated hypertensive men. *J Physiol* 594: 21: 6211-6223, 2016.

232. **Peinado AB, Harvey RE, Hart EC, Charkoudian N, Curry TB, Nicholson WT, Wallin BG, Joyner MJ and Barnes JN.** Neural control of blood pressure in women: differences according to age. *Clin Auton Res* 27: 3: 157-165, 2017.

233. **Perkiömäki JS, Nortamo S, Ylitalo A, Kesäniemi A, Ukkola O and Huikuri HV.** Ambulatory Blood Pressure Characteristics and Long-Term Risk for Atrial Fibrillation. *Am J Hypertension* hpw149, 2016.

234. **Perry BG, Bear TLK, Lucas SJE and Mündel T.** Mild dehydration modifies the cerebrovascular response to the cold pressor test. *Exp Physiol* 101: 1: 135-142, 2016.

235. **Person RJ.** Somatic and vagal afferent convergence on solitary tract neurons in cat: electrophysiological characteristics. *Neuroscience* 30: 2: 283-295, 1989.

236. **Phillips PA, Bretherton M, Risvanis J, Casley D, Johnston C and Gray L.** Effects of drinking on thirst and vasopressin in dehydrated elderly men. *Am J Physiol* 264: 5 Pt 2: 877, 1993.

237. **Phillips PA, Johnston CI and Gray L.** Disturbed fluid and electrolyte homoeostasis following dehydration in elderly people. *Age Ageing* 22: 1: S33, 1993.

238. **Phillips PA, Rolls BJ, Ledingham JG, Forsling ML, Morton JJ, Crowe MJ and Wollner L.** Reduced thirst after water deprivation in healthy elderly men. *N Engl J Med* 311: 12: 753-759, 1984.

239. **Plasqui G and Westerterp KR.** Physical activity assessment with accelerometers: an evaluation against doubly labeled water. *Obesity* 15: 10: 2371-2379, 2007.

240. **Posch AM, Luippold AJ, Mitchell KM, Bradbury KE, Kenefick RW, Cheuvront SN and Charkoudian N.** Sympathetic neural and hemodynamic responses to head-up tilt during iso-osmotic and hyper-osmotic hypovolemia. *J Neurophysiol* 2232-2237, 2017.

241. **Pringle E, Phillips C, Thijs L, Davidson C, Staessen JA, de Leeuw PW, Jaaskivi M, Nachev C, Parati G, O'Brien ET, Tuomilehto J, Webster J, Bulpitt CJ and Fagard RH.** Systolic blood pressure variability as a risk factor for stroke and cardiovascular mortality in the elderly hypertensive population. *J Hypertens* 21: 12: 2251-2257, 2003.

242. **Rabbitts JA, Strom NA, Sawyer JR, Curry TB, Dietz NM, Roberts SK, Kingsley-Berg SM and Charkoudian N.** Influence of endogenous angiotensin II on control of sympathetic nerve activity in human dehydration. *J Physiol* 587: Pt 22: 5441-5449, 2009.

243. **Roussel R, Fezeu L, Bouby N, Balkau B, Lantieri O, Alhenc-Gelas F, Marre M and Bankir L.** Low water intake and risk for new-onset hyperglycemia. *Diabetes Care* 34: 12: 2551-2554, 2011.

244. **Saitoh T, Ogawa Y, Aoki K, Shibata S, Otsubo A, Kato J and Iwasaki K.** Bell-shaped relationship between central blood volume and spontaneous baroreflex function. *Auton Neurosci* 143: 1-2: 46-52, 2008.

245. **Salles GF, Reboldi G, Fagard RH, Cardoso CRL, Pierdomenico SD, Verdecchia P, Eguchi K, Kario K, Hoshide S, Polonia J, de la Sierra A, Hermida RC, Dolan E, O'Brien E and Roush GC.** Prognostic Effect of the Nocturnal Blood Pressure Fall in Hypertensive Patients: The Ambulatory Blood Pressure Collaboration in Patients With Hypertension (ABC-H) Meta-Analysis. *Hypertension* 67: 4: 693-700, 2016.

246. **Sampey DB, Burrell LM and Widdop RE.** Vasopressin V2 receptor enhances gain of baroreflex in conscious spontaneously hypertensive rats. *Am J Physiol* 276: 3 Pt 2: 872, 1999.

247. **Sanders JS and Ferguson DW.** Cardiopulmonary baroreflexes fail to modulate sympathetic responses during isometric exercise in humans: direct evidence from microneurographic studies. *J Am Coll Cardiol* 12: 5: 1241-1251, 1988.

248. **Savoie F, Kenefick R, Ely B, Cheuvront S and Goulet E.** Effect of Hypohydration on Muscle Endurance, Strength, Anaerobic Power and Capacity and Vertical Jumping Ability: A Meta-Analysis. *Sports Med* 45: 8: 1207-1227, 2015.

249. **Sawka MN.** Physiological consequences of hypohydration: exercise performance and thermoregulation. *Med Sci Sports Exerc* 24: 6: 657-670, 1992.

250. **Sawka MN, Francesconi RP, Young AJ and Pandolf KB.** Influence of hydration level and body fluids on exercise performance in the heat. *JAMA* 252: 9: 1165-1169, 1984.

251. **Sawka MN, Cheuvront SN and Kenefick RW.** High skin temperature and hypohydration impair aerobic performance. *Exp Physiol* 97: 3: 327-332, 2012.

252. **Schols JM, Groot, C P G M de, Cammen, T J M van der and Olde Rikkert, M G M.** Preventing and treating dehydration in the elderly during periods of illness and warm weather. *J Nutr Health Aging* 13: 2: 150-157, 2009.

253. **Schramm LP and Chornoboy ES.** Sympathetic activity in spontaneously hypertensive rats after spinal transection. *Am J Physiol* 243: 5: 506, 1982.

254. **Schreihofer AM, Stricker EM and Sved AF.** Chronic nucleus tractus solitarius lesions do not prevent hypovolemia-induced vasopressin secretion in rats. *Am J Physiol* 267: 4 Pt 2: 965, 1994.

255. **Schultz MG, Otahal P, Picone DS and Sharman JE.** Clinical Relevance of Exaggerated Exercise Blood Pressure. *J Am Coll Cardiol* 66: 16: 1843-1845, 2015.

256. **Scott A Smith, Jere H Mitchell and Mary G Garry.** The mammalian exercise pressor reflex in health and disease. *Exp Physiol* 91: 1: 89-102, 2006.

257. **Scrogin KE, Grygielko ET and Brooks VL.** Osmolality: a physiological long-term regulator of lumbar sympathetic nerve activity and arterial pressure. *Am J Physiol* 276: 6 Pt 2: 1579, 1999.

258. **Scrogin KE, McKeogh DF and Brooks VL.** Is osmolality a long-term regulator of renal sympathetic nerve activity in conscious water-deprived rats? *Am J Physiol Regul Integr Comp Physiol* 282: 2: 560, 2002.

259. **Seals DR.** Cardiopulmonary baroreflexes do not modulate exercise-induced sympathoexcitation. *J Appl Physiol* 64: 5: 2197-2203, 1988.

260. **Seravalle G, Lonati L, Buzzi S, Cairo M, Quarti Trevano F, Dell'Oro R, Facchetti R, Mancia G and Grassi G.** Sympathetic nerve traffic and baroreflex function in optimal, normal, and high-normal blood pressure states. *J Hyper* 33: 7: 1411-1417, 2015.

261. **Shanley L, Mittal V and Flores G.** Preventing dehydration-related hospitalizations: a mixed-methods study of parents, inpatient attendings, and primary care physicians. *Hosp Pediatr* 3: 3: 204-211, 2013.

262. **Sheehy CM, Perry PA and Cromwell SL.** Dehydration: biological considerations, age-related changes, and risk factors in older adults. *Biol Res Nurs* 1: 1: 30-37, 1999.

263. **Shi X, Gallagher KM, Welch-O'Connor RM and Foresman BH.** Arterial and cardiopulmonary baroreflexes in 60- to 69- vs. 18- to 36-yr-old humans. *J Appl Physiol* 80: 6: 1903-1910, 1996.

264. **Shoemaker JK, Green HJ, Ball-Burnett M and Grant S.** Relationships between fluid and electrolyte hormones and plasma volume during exercise with training and detraining. *Med Sci Sports Exerc* 30: 4: 497-505, 1998.

265. **Shoemaker JK, Klassen SA, Badrov MB and Fadel PJ.** Fifty years of microneurography: learning the language of the peripheral sympathetic nervous system in humans. *J Neurophysiol* 119: 5: 1731-1744, 2018.

266. **Silver AJ.** Aging and risks for dehydration. *Cleve Clin J Med* 57: 4: 341-344, 1990.

267. **Simmonds SS, Lay J and Stocker SD.** Dietary salt intake exaggerates sympathetic reflexes and increases blood pressure variability in normotensive rats. *Hypertension* 64: 3: 583-589, 2014.

268. **Somers VK, Mark AL, Zavala DC and Abboud FM.** Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *J Appl Physiol* 67: 5: 2101-2106, 1989.

269. **Stachenfeld NS.** Acute effects of sodium ingestion on thirst and cardiovascular function. *Curr Sports Med Rep* 7: 4 Suppl: 7, 2008.

270. **Stachenfeld NS and Keefe DL.** Estrogen effects on osmotic regulation of AVP and fluid balance. *Am J Physiol Endocrinol Metab* 283: 4: 711, 2002.

271. **Stachenfeld NS, Mack GW, Takamata A, DiPietro L and Nadel ER.** Thirst and fluid regulatory responses to hypertonicity in older adults. *Am J Physiol* 271: 3 Pt 2: 757, 1996.

272. **Stachenfeld NS, Leone CA, Mitchell ES, Freese E and Harkness L.** Water intake reverses dehydration associated impaired executive function in healthy young women. *Physiol Behav* 2017.

273. **Stebbins CL, Brown B, Levin D and Longhurst JC.** Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *J Appl Physiol* 65: 4: 1539-1547, 1988.

274. **Stocker SD, Lang SM, Simmonds SS, Wenner MM and Farquhar WB.** Cerebrospinal Fluid Hypernatremia Elevates Sympathetic Nerve Activity and Blood Pressure via the Rostral Ventrolateral Medulla. *Hypertension* 66: 6: 1184-1190, 2015.

275. **Stocker SD, Stricker EM and Sved AF.** Acute hypertension inhibits thirst stimulated by ANG II, hyperosmolality, or hypovolemia in rats. *Am J Physiol Regul Integr Comp Physiol* 280: 1: 214, 2001.

276. **Stocker SD, Stricker EM and Sved AF.** Arterial baroreceptors mediate the inhibitory effect of acute increases in arterial blood pressure on thirst. *Am J Physiol Regul Integr Comp Physiol* 282: 6: 1718, 2002.

277. **Stocker SD, Madden CJ and Sved AF.** Excess dietary salt intake alters the excitability of central sympathetic networks. *Physiol Behav* 100: 5: 519-524, 2010.

278. **Stookey JD.** High Prevalence of Plasma Hypertonicity among Community-Dwelling Older Adults: Results from NHANES III. *J Am Diet Assoc* 105: 8: 1231-1239, 2005.

279. **Studinger P, Goldstein R and Taylor JA.** Age- and fitness-related alterations in vascular sympathetic control. *J Physiol* 587: Pt 9: 2049-2057, 2009.

280. **Suckling RJ, He FJ, Markandu ND and MacGregor GA.** Dietary salt influences postprandial plasma sodium concentration and systolic blood pressure. *Kidney Int* 81: 4: 407-411, 2012.

281. **Sundlöf G and Wallin BG.** Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol* 274: 621-637, 1978.

282. **Sung J, Choi SH, Choi Y, Kim D and Park WH.** The relationship between arterial stiffness and increase in blood pressure during exercise in normotensive persons. *J Hypertens* 30: 3: 587-591, 2012.

283. **Szinnai G, Schachinger H, Arnaud MJ, Linder L and Keller U.** Effect of water deprivation on cognitive-motor performance in healthy men and women. *Am J Physiol Regul Integr Comp Physiol* 289: 1: 275, 2005.

284. **Takata S, Iwase N, Okuwa H, Ogawa J, Ikeda T and Hattori N.** Baroreflex function and pressor responsiveness in normotensive young subjects with a family history of hypertension. *Jpn Circ J* 49: 9: 990-996, 1985.

285. **Tatasciore A, Renda G, Zimarino M, Soccio M, Bilo G, Parati G, Schillaci G and De Caterina R.** Awake systolic blood pressure variability correlates with target-organ damage in hypertensive subjects. *Hypertension* 50: 2: 325-332, 2007.

286. **Terashima Y, Kondo K, Inagaki A, Yokoi H, Arima H, Murase T, Iwasaki Y and Oiso Y.** Age-associated decrease in response of rat aquaporin-2 gene expression to dehydration. *Life Sci* 62: 10: 873-882, 1998.

287. **Terwel D and Jolles J.** The relationship between plasma osmolality and plasma vasopressin concentration is altered in old male Lewis rats. *Eur J Endocrinol* 131: 1: 86-90, 1994.

288. **Thanassoulis G, Lyass A, Benjamin EJ, Larson MG, Vita JA, Levy D, Hamburg NM, Widlansky ME, O'Donnell CJ, Mitchell GF and Vasan RS.** Relations of exercise blood pressure response to cardiovascular risk factors and vascular function in the Framingham Heart Study. *Circulation* 125: 23: 2836-2843, 2012.

289. **Thompson CA, Tatro DL, Ludwig DA and Convertino VA.** Baroreflex responses to acute changes in blood volume in humans. *Am J Physiol* 259: 4 Pt 2: 792, 1990.

290. **Tompkins RP, Melling CW, Wilson TD, Bates BD and Shoemaker JK.** Arrangement of sympathetic fibers within the human common peroneal nerve: implications for microneurography. *J Appl Physiol* 115: 10: 1553-1561, 2013.

291. **Tonkin AL and Wing LM.** Effects of age and isolated systolic hypertension on cardiovascular reflexes. *J Hypertens* 12: 9: 1083-1088, 1994.

292. **Trapani AJ, Undesser KP, Keeton TK and Bishop VS.** Neurohumoral interactions in conscious dehydrated rabbit. *Am J Physiol* 254: 2 Pt 2: 338, 1988.

293. **Treiber FA, Kamarck T, Schneiderman N, Sheffield D, Kapuku G and Taylor T.** Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosom Med* 65: 1: 46-62, 2003.

294. **Trinity JD, Layec G, Hart CR and Richardson RS.** Sex-specific impact of aging on the blood pressure response to exercise. *Am J Physiol Heart Circ Physiol* 314: 1: H104, 2017.

295. **Tyagi MG and Thomas M.** Enhanced cardiovascular reactivity to desmopressin in water-restricted rats: facilitatory role of immunosuppression. *Methods Find Exp Clin Pharmacol* 21: 9: 619-624, 1999.

296. **Tzemos N, Lim PO, Mackenzie IS and MacDonald TM.** Exaggerated Exercise Blood Pressure Response and Future Cardiovascular Disease. *J Clin Hyper* 17: 11: 837-844, 2015.

297. **V F Gladwell and J H Coote.** Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *J Physiol* 540: 3: 1095-1102, 2002.

298. **Vallbo AB, Hagbarth K and Wallin BG.** Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* 96: 4: 1262-1269, 2004.

299. **Veitenheimer BJ, Engeland WC, Guzman PA, Fink GD and Osborn JW.** Effect of global and regional sympathetic blockade on arterial pressure during water deprivation in conscious rats. *Am J Physiol Heart Circ Physiol* 303: 8: 1022, 2012.

300. **Veloudi P, Blizzard CL, Head GA, Abhayaratna WP, Stowasser M and Sharman JE.** Blood Pressure Variability and Prediction of Target Organ Damage in Patients With Uncomplicated Hypertension. *Am J Hypertens* 1046-1054, 2016.

301. **Verdecchia P.** Prognostic value of ambulatory blood pressure : current evidence and clinical implications. *Hypertension* 35: 3: 844-851, 2000.

302. **Vianna LC, Hart EC, Fairfax ST, Charkoudian N, Joyner MJ and Fadel PJ.** Influence of age and sex on the pressor response following a spontaneous burst of muscle sympathetic nerve activity. *Am J Physiol Heart Circ Physiol* 302: 11: 2419, 2012.

303. **Victor RG, Leimbach WN,Jr, Seals DR, Wallin BG and Mark AL.** Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* 9: 5: 429-436, 1987.

304. **Victor RG, Secher NH, Lyson T and Mitchell JH.** Central command increases muscle sympathetic nerve activity during intense intermittent isometric exercise in humans. *Circ Res* 76: 1: 127-131, 1995.

305. **Vranish JR, Holwerda SW, Young BE, Credeur DP, Patik JC, Barbosa TC, Keller DM and Fadel PJ.** Exaggerated Vasoconstriction to Spontaneous Bursts of Muscle Sympathetic Nerve Activity in Healthy Young Black Men. *Hypertension* 71: 1: 192-198, 2018.

306. **Wade JG, Larson CP, Hickey RF, Ehrenfeld WK and Severinghaus JW.** Effect of carotid endarterectomy on carotid chemoreceptor and baroreceptor function in man. *N Engl J Med* 282: 15: 823-829, 1970.

307. **Wallin BG, Delius W and Sundlöf G.** Human muscle nerve sympathetic activity in cardiac arrhythmias. *Scand J Clin Lab Invest* 34: 4: 293-300, 1974.

308. **Wallin BG and Nerhed C.** Relationship between spontaneous variations of muscle sympathetic activity and succeeding changes of blood pressure in man. *J Auton Nerv Syst* 6: 3: 293-302, 1982.

309. **Warren JL, Bacon WE, Harris T, McBean AM, Foley DJ and Phillips C.** The burden and outcomes associated with dehydration among US elderly, 1991. *Am J Public Health* 84: 8: 1265-1269, 1994.

310. **Wenner MM, Rose WC, Delaney EP, Stillabower ME and Farquhar WB.** Influence of plasma osmolality on baroreflex control of sympathetic activity. *Am J Physiol Heart Circ Physiol* 293: 4: 2313, 2007.

311. **Wenner MM and Stachenfeld NS.** Blood pressure and water regulation: understanding sex hormone effects within and between men and women. *J Physiol* 590: 23: 5949-5961, 2012.

312. **White DW, Shoemaker JK and Raven PB.** Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neuro* 193: 12-21, 2015.

313. **Williamson JW, Crandall CG, Potts JT and Raven PB.** Blood pressure responses to dynamic exercise with lower-body positive pressure. *Med Sci Sports Exerc* 26: 6: 701-708, 1994.

314. **Williamson JW, McColl R, Mathews D, Mitchell JH, Raven PB and Morgan WP.** Brain activation by central command during actual and imagined handgrip under hypnosis. *J Appl Physiol* 92: 3: 1317-1324, 2002.

315. **Wilson MG and Morley JE.** Impaired cognitive function and mental performance in mild dehydration. *Eur J Clin Nutr* 57: S2: S29, 2003.

316. **Xu J, Murphy SL, Kochanek KD, Bastian B and Arias E.** Deaths: Final Data for 2016. *Natl Vital Stat Rep* 67: 5: 1-76, 2018.

317. **YAMAMOTO K, IWASE S and MANO T.** Responses of Muscle Sympathetic Nerve Activity and Cardiac Output to the Cold Pressor Test. *Jap J Physiol* 42: 2: 239-252, 1992.

318. **Yamauchi K, Tsuchimochi H, Stone AJ, Stocker SD and Kaufman MP.** Increased dietary salt intake enhances the exercise pressor reflex. *Am J Physiol Heart Circ Physiol* 306: 3: 450, 2014.

319. **Young CN, Stillabower ME, DiSabatino A and Farquhar WB.** Venous smooth muscle tone and responsiveness in older adults. *J Appl Physiol * 101: 5: 1362-1367, 2006.

320. **Zelinka T, Hradec J and Hampl J.** [Does a hypertensive reaction to dynamic exercise imply an increased risk of developing essential hypertension?]. *Cas Lek Cesk* 132: 2: 53-56, 1993.

321. **Zhao Q, Gu D, Lu F, Mu J, Wang X, Ji X, Hu D, Ma J, Huang J, Li J, Chen J, Cao J, Chen CS, Chen J, Rice TK and He J.** Blood Pressure Reactivity to the Cold Pressor Test Predicts Hypertension Among Chinese Adults: The GenSalt Study. *Am J Hypertens* 28: 11: 1347-1354, 2015.

HUMAN SUBJECTS RESEARCH APPROVAL LETTER

UNIVERSITY OF DELAWARE

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE: August 8, 2017

TO: Joseph Watso, BS
FROM: University of Delaware IRB

STUDY TITLE: [1097747-1] Sympathetic Reactivity to Water Restriction in Young and Old Adults

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: August 8, 2017
EXPIRATION DATE: July 18, 2018
REVIEW TYPE: Full Committee Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB (HUMANS) has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that <u>informed consent</u> is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.