Aging in a Warming Climate: Physiological and Cognitive Health Risks of Extreme Heat Events

by

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DISSERTATION ABSTRACT

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Doctor of Philosophy in Human Physiology

Title: Aging in a Warming Climate: Physiological and Cognitive Health Risks of Extreme Heat Events

As climate change progresses and the frequency and intensity of heat waves continues to increase, the health and safety of older individuals is at risk. Aging is associated with reductions in thermoregulatory function and cardiovascular capacity, putting older individuals at elevated risk for the development of adverse heat-related health outcomes during extreme heat events. Among the top causes of hospitalizations during heat waves is acute kidney injury risk, which is characterized by a sudden decline in kidney function and may result from dehydration, reductions in renal blood flow, and/or renal oxidative stress and inflammation. The in-hospital mortality rate for older patients with acute kidney injury ranges from 15-40%. Older adults are also at an increased risk of developing classic heat stroke, which involves inflammation-induced endothelial dysfunction and a resultant hypercoagulable state. Mortality rates in those affected can reach over 50%, and survivors oftentimes experience long-term consequences including an elevated risk of future morbidity and mortality. While the thermoregulatory adjustments to prolonged, extreme heat exposure in older versus young have been well documented, there is need for a deeper look into the pathophysiology associated with the development of heat-related health risks in older adults. Moreover, cognitive function during prolonged heat stress remains an understudied aspect of age-related susceptibility to heat, as cognition represents a crucial aspect of thermoregulation that involves the ability to practice heat-relief seeking behaviors aimed at reducing thermal strain and heat accumulation. Thus, this dissertation aimed to identify age-related differences in endothelial function and coagulation, cognitive function, and kidney function and acute kidney injury risk in response to 6 hours of extreme heat exposure with light exercise to mimic activities of daily living throughout heat exposure. Our findings demonstrate that despite exhibiting similar thermoregulatory responses to heat stress, older adults displayed reduced thermotolerance, increased cardiovascular strain, and evidence of endothelial injury. Additionally, older adults exhibited impairments in kidney function that persisted during the

recovery period following heat exposure, despite experiencing elevations in biomarkers of acute kidney injury risk comparable to those observed in young adults. However, no changes in cognitive function were observed in either age group during prolonged, extreme heat stress. We anticipate these findings will support efforts to uncover age-related susceptibility to adverse heat-related health outcomes and urge researchers to continue to investigate mechanisms that extend beyond the thermoregulatory adjustments to heat stress. Our results provide evidence that age-related reductions in endothelial and renal function likely contribute to the increased vulnerability to heat-related morbidity and mortality among older adults, even when thermoregulatory responses appear relatively preserved.

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CHAPTER 1
ORIGINAL PROPOSAL

Heatwaves are defined as prolonged periods of extremely hot weather, and climate change is increasing the frequency, duration, and intensity of these extreme heat events[1]. Cardiovascular mortality comprises more than one-half of excess deaths due to heatwaves[2], as prolonged exposure to high ambient temperature elicits cardiovascular strain that contributes negatively to health outcomes. Among the most vulnerable to adverse heat events are elderly persons, and in some instances, over 90% of excess deaths during heatwaves occur in this population[3]. With the population of the world rapidly aging, the number of people aged 65 and over in the United States is projected to increase to nearly 80 million by 2040 [4] and by the year 2050, there will be more people over 60 years old than under 15 years old in the world for the first time in history[5]. Thus, there should be rising concern over the safety of elderly individuals as higher environmental temperatures along with more frequent and intense heatwaves are expected to occur.

Aging is associated with reduced thermoregulatory and cardiovascular efficiency[6–8], chronic inflammation[9], increased blood coagulation potential[10–13], structural and functional alterations in kidney function[14–17], and cognitive decline[18]. Cognitive function is important to consider in the context of heat exposure as behavior, such as seeking relief from heat and proper hydration, is the primary thermoeffector in the prevention of morbidity and mortality during heatwaves[19,20]. These age-related physiological changes contribute to an elevated susceptibility among the elderly to heat-related complications, including an increased risk of classic heat stroke and acute kidney injury (AKI).

Acute kidney injury (AKI) is one of the top two causes of hospitalizations in older adults during heatwaves [21,22]. Additionally, recent studies have shown that heat stress-induced AKI is not temporary and often progresses to chronic kidney disease (CKD)[23,24]. However, heat stress-induced AKI has never been experimentally studied in the context of aging, and research on the mechanisms of AKI is still in its infancy. Furthermore, the consequences of extreme heat exposure are not always immediate, as cardiovascular mortality can occur 0-3 days following extreme heat exposure [25,26]. Up to 60% of patients with classic heat stroke are hospitalized or die within one day of illness onset [27], and the risk of cardiovascular disease is elevated following a

single heat stroke occurrence [28]. In terms of AKI, even rapid recovery (≤2 days) from one episode of AKI is associated with a 43% increase of chronic kidney disease within one year [29].

PURPOSE, SPECIFIC AIMS, AND HYPOTHESES

In the face of a warming planet and aging population, our population encounters growing risk of poor environmental, economic, and worldwide health problems. Therefore, characterizing the acute and chronic effects of prolonged extreme heat exposure in a young and aged population is crucial to understand the effect of aging on adverse heat-related health outcomes. Importantly, understanding the mechanisms by which heat stress may increase the development of biomarkers related to heat stroke and AKI risk as well as the effects of prolonged heat exposure on cognitive function in aged adults would provide critical insight into the interventions that may help mitigate these risks during heatwaves.

Specific Aim 1: Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining biomarkers related to heat stroke risk.

The main mechanisms contributing to the development of heat stroke are impaired cardiovascular function, endothelial dysfunction, excess inflammation, and coagulation[30–32]. Previous work has clearly defined the negative impact of aging on thermoregulatory capacity and cardiovascular strain during heat stress[6–8]. It is also well known that aging is associated with increased baseline levels of inflammation[9], endothelial dysfunction, and increased blood coagulation potential[10–13], all of which contribute to increased risk for heat-related morbidity and mortality[33]. It remains unknown how these physiological changes associated with aging impact the development of biomarkers related to the culmination of heat stroke during prolonged exposure to heat stress. Utilizing a 6-hour extreme heat event model, we will study markers of inflammation, endothelial dysfunction, and blood coagulability (**Table 1**) before, during, and 24-48 hours following prolonged heat stress in young and aged individuals to determine the impact of aging on acute and chronic physiological responses related to the development of heat stroke and cardiovascular risk following extreme heat exposure. **Hypothesis 1a:** Therefore, we hypothesize that inflammatory markers and blood coagulability will increase in both young and aged individuals after 6 hours of heat stress, however only markers of endothelial dysfunction

will increase in aged individuals, and all these biomarkers will be increased to a greater extent in aged individuals 24-48 hours following heat stress. Due in part to the myriad changes that occur with healthy aging and predispose aged individuals to higher risk of heat-related morbidities, it is important to identify specific functions of aging that may increase heat stroke risk. Therefore, we will elucidate the impact of aging on the development of biomarkers of heat stroke risk.

Hypothesis 1b: We hypothesize that baseline platelet count, D-Dimer levels, and endothelial dysfunction will be associated with greater increases in biomarkers of heat stroke risk.

Markers	**Sample**	**Measurement**
IL-6, IL-1β, IL-1ra, TNFa, LPS	Serum	Inflammation
ICAM-1, syndecan-1, von Willebrand factor antigen	Plasma	Endothelial Function
Platelets, aPTT, PT, D-dimer, fibrinogen, Protein C	Whole blood/Plasma	Coagulation

Table 1: Markers for Aim 1. Interleukin (IL), IL-1 receptor antagonist (IL-1ra), tumor necrosis factor alpha (TNFa), lipopolysaccharide (LPS), intercellular adhesion molecule 1 (ICAM-1), von Willebrand factor antigen (vWFa), activated partial thromboplastin time (aPTT), prothrombin time (PT).

Specific Aim 2: Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining biomarkers of kidney injury risk. During heat stress, the kidneys maintain blood pressure, conserve water and electrolytes, and redirect blood flow to the skin in an effort to promote heat loss. When coupled with dehydration, heat stress amplifies processes that may result in kidney-related pathology such as acute kidney injury (AKI)[34,35]. The pathophysiology of AKI is complex, with the most common cause being ischemia resulting in cellular injury and organ dysfunction, and adults aged 65 years and older are likely at risk of AKI associated with heat stress[36]. In an 8-hour model of extreme heat exposure in young, healthy men, the product of IGFBP7 and TIMP-2 ([IGFBP7·TIMP-2]), the only US Food and Drug Administration-approved approved biomarker for assessing kidney injury risk [37], increased in a wet bulb temperature environment of 31°C and was worsened at a wet bulb temperature of 34°C[38]. This outcome is likely to be exacerbated in older individuals who undergo age-dependent changes in kidney structure and function over time, including reduced glomerular filtration rate (GFR), diminished

kidney reserve, reduced renal blood flow, and augmented basal activation of the polyol-fructokinase pathway[14–17,39]. Additionally, older adults may be less likely to maintain hydration due to attenuated thirst sensation caused by dysfunction of the central nervous system mechanisms controlling thirst, prescription medication use, and/or physical/cognitive impairments[40]. Research on the mechanisms of AKI is still in its infancy, and interestingly, heat stress-induced AKI has never been experimentally studied in the context of aging. Additionally, approximately one-third of older adults ≥65 years who survive AKI do not fully recover kidney function[41]. Thus, understanding the impact of aging on the development of biomarkers related to kidney injury risk during prolonged heat stress is crucial. We will examine biomarkers of kidney injury risk and the etiology underlying kidney injury risk (**Table 2**) before, during, and 24-48 hours following 6 hours of extreme heat exposure. **Hypothesis 2a:** We hypothesize that increases in urinary IGFBP-7 and TIMP-2 will be greater in aged individuals exposed to 6 hours of extreme heat when compared to young individuals, with greater sustained elevations 24-48 hours post-heat event. Additionally, it is unknown if the etiology of heat-induced kidney injury risk differs in young and aged individuals. **Hypothesis 2b:** Therefore, we hypothesize that KIM-1, a biomarker of potential kidney injury at the proximal tubule, will be greater in aged individuals than young individuals, and aged individuals will exhibit greater markers of kidney inflammation and oxidative (IL-17a and IL-18) stress due to aging-induced changes in kidney function.

Markers	**Sample**	**Measurement**
IL-17a	Plasma	Etiology
IL-18	Urine	Etiology
L-FABP	Urine	Etiology
IGFBP7	Urine	Injury risk
TIMP-2	Urine	Injury risk
NGAL	Urine	Injury risk
KIM-1	Urine	Injury risk/location
TRX-1	Urine	Etiology
TBARS	Urine	Etiology

Table 2: Markers of kidney injury for Aim 2. Interleukin (IL), liver type fatty acid binding protein (L-FABP), insulin-like growth factor-binding protein 7 (IGFBP7), tissue inhibitor

metalloproteinase 2 (TIMP-2), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), thioredoxin-1 (TRX-1), thiobarbituric acid reactive substances (TBARS).

Specific Aim 3: Effect of Prolonged Heat Stress on Cognitive Function

Heat exchange between the skin and environment can be modified by both autonomic and behavioral thermoeffector responses, with behavior being the most powerful thermoeffector during heat stress[42]. The use of air conditioning, moving to cooler locations, and adequate hydration are all linked to reductions in morbidity and mortality during heatwaves[19,20]. However, aging results in cognitive decline and a reduced ability to sense one's thermal state which may result in an inability to modify behavior and seek relief from heat[19,43]. Additionally, cognitive function declines with prolonged heat stress even beyond vulnerable populations as demonstrated by Laurent et al.[44], whose findings revealed that individuals living in non-AC buildings experienced reductions in cognitive function during a heatwave as assessed by working memory and selective attention/processing speed. Other studies have reported a decline in cognitive function following a bout of passive heat stress (>30 min) which may be attributed to dehydration[45,46], hyperventilation-induced hypocapnia[47,48], and reductions in cerebral blood flow[49,50]. Additionally, cerebral blood flow (CBF) is attenuated in the elderly and aging-induced cognitive deterioration may be affected by this attenuation[51]. During passive heat stress, CBF and arterial P_{CO2} are reduced due to hyperventilatory hypocapnia and reductions in venous return and stroke volume[52]. Thus, aged individuals may be at great risk for decrements in cognitive function during prolonged heat stress. These effects may further exacerbate the risk of developing adverse health outcomes during extreme heat conditions, particularly in an aged population who already face decrements in cognitive health, thermal sensing, and cerebral blood flow. **Hypothesis 3a:** We hypothesize that cognitive function will decline to a greater extent in aged vs young individuals. **Hypothesis 3b:** Further, we hypothesize that decrements in cerebral blood flow in aged individuals will be correlated to the level of cognitive decline reached as a result of prolonged heat stress.

SIGNIFICANCE

This work advances our understanding of the risk normal aging imposes on health during exposure to extreme heat events and will help uncover specific functions of aging related to increased risk for the development of adverse health outcomes during environmental heatwaves. Previous work has clearly demonstrated that aging results in reduced thermoregulatory efficiency and increased cardiovascular strain during prolonged heat exposure which contribute negatively to health outcomes. Results from **aim 1** will extend previous findings to further elucidate the effects of aging on physiological responses to heat stress related to the development of heat stroke. Findings from this work will provide valuable clinical insight into specific age-related factors that lead to increased risk for the development of heat stroke when compared to a younger population. Under specific **aim 2,** we will identify the impact of aging on kidney injury risk and its etiology. Lastly, specific **aim 3** will examine the impact of heat stress and aging on cognitive function.

CHAPTER II
REVIEW OF THE LITERATURE AND NEW INSIGHTS

INTRODUCTION

In a letter written to his future great-granddaughter in the year 2100, United Nations Secretary-General António Guterres wrote:

"Today, our world stands at a crossroads, with two paths before us that will have a direct impact on your future. The first leads to a future of relentless temperature rise, deadly droughts and famines, melting glaciers, and rising seas… The second path leads to the legacy you deserve: [inclusive of] better health."[53]

Earth's temperature once remained relatively constant many centuries ago, but over the past century, rapid technological advancements and industrialization have profoundly altered Earth's climate, resulting in widespread environmental degradation and global warming[54]. Changes to our environment have been taking place since the 19th century through human activities that have increased greenhouse gases in the Earth's atmosphere, thus resulting in an increased average global temperature[54]. Since 1850, Earth's temperature has risen by an average of 0.06°C (0.11°F) per decade, and since 1982, the rate of warming is now more than three times that, at 0.20°C (0.36°F) per decade[55]. The environmental impacts of rising temperatures include soil degradation, desertification, loss of biodiversity, degradation of ecosystems, acidification of the oceans, and more, all of which negatively impact human health[54]. It is projected that 3.3 to 3.6 billion people are already extremely vulnerable to the impacts of climate change[56], and with Earth's temperature projected to continue rising, it can only be assumed that increasingly more individuals will be at risk.

Among the most at-risk for poor health outcomes during periods of extreme environmental temperatures are individuals aged 65 years and older. Heat-related mortality is substantially higher in adults over 50 than adults under 50, with risk increasing exponentially with advancing age[57] as heat-vulnerability is influenced by advanced age more than any other non-modifiable risk factor[58–60]. The World Health Organization has projected that annual heat-

related deaths in individuals aged ≥65 years will increase by as much as 250,000 by mid-century[61]. Impaired thermoregulatory function and hemodynamic instability contribute to the development of heat-vulnerability with aging[60,62], resulting in greater heat storage and higher core body temperatures compared to younger adults[63]. In addition to increasing global temperatures, the population of the world is rapidly aging. The number of people aged 65 years and older in the United States is projected to increase to nearly 80 million by 2040[4] and by the year 2050, there will be more people over 60 years old than under 15 years old in the world for the first time in history[5]. Thus, there should be rising concern over the safety of elderly individuals as higher environmental temperatures along with more frequent and intense heat waves are expected to occur.

PART I:

Thermoregulation in Young vs Older Adults

When ambient temperature exceeds skin temperature, heat gain surpasses heat loss, resulting in increased heat storage. Thus, the thermoregulatory system must adjust a variety of physiological mechanisms to promote heat loss in an effort to regulate core temperature and maintain heat balance. The rise in body temperature is sensed by thermoreceptors in the central nervous system and skin, triggering thermoeffector mechanisms to promote heat loss and prevent continued increases in body temperature[64]. The primary mechanisms to promote heat loss are sympathetically driven cutaneous vasodilation and eccrine sweating.

Cutaneous vasodilation increases skin blood flow to support convective heat loss to the environment, necessitating a redistribution of blood away from the splanchnic and renal beds[65]. To maintain arterial pressure under the resulting drop in peripheral resistance, cardiac output must increase, driven primarily by an elevation in heart rate with stroke volume maintained or slightly increased[66]. Sweating promotes cooling and evaporative heat loss[67], and sweat evaporation provides the greatest means for heat dissipation[64]. These thermoregulatory responses to passive heat stress were described by Rowell in the 1960s. Using water-perfused suits to increase skin temperature to 40.5°C, Rowell observed a nearly doubling of cardiac output from resting values, stroke volume was increased slightly, and splanchnic and renal blood flow was

redistributed to the skin with a resultant decrease in right atrial mean pressure, where mean arterial pressure was maintained by an increase in inotropic function of the heart[66].

Aging is associated with reduced thermoregulatory efficiency and greater cardiovascular strain due to age-related changes in blood flow to the skin, sweating, and cardiovascular function[60]. Seminal work by Kenney[68] first demonstrated blunted elevations in forearm blood flow during progressive heat stress in old compared to young men and women. Impairments in the vascular responses to heat stress with normal aging are primarily due to attenuated nitric oxide (NO)-mediated vasodilation resulting from reduced NO bioavailability and elevated reactive oxygen species (ROS)[69,70]. Blunted autonomic control of cutaneous circulation[71] and reduced cardiac reserve[65] also contribute to compromised skin blood flow in older adults undergoing heat stress. In addition to the reduced efficacy of cutaneous vasodilation, increases in cardiac output are also attenuated due to a lack of ability to maintain stroke volume whereas younger adults can maintain or even slightly increase stroke volume via increased contractility of the heart[65]. Although absolute heart rate responses used to augment cardiac output are similar in young and older heat-stressed adults, older adults operate at a higher percentage of their age-predicted maximum[72]. Consequently, they rely on a greater proportion of their heart rate reserve to support increases in cardiac output, resulting in greater cardiovascular strain[72]. In addition to reduced cutaneous perfusion, aging is associated with a decreased sweat rate[73,74] and decreased sweat output per gland, ultimately lowering whole-body sweat production and diminishing the capacity for evaporative heat loss[76]. When changes in sweating and skin blood flow cannot support a sufficient rate of heat loss (e.g., physical activity in hot or humid conditions, reductions in normal thermoregulatory function due to aging), body core temperature will continue to rise. If the increase in body core temperature is not controlled, it may progress to heat-related illness and ultimately death[60]. In addition to the reduced thermoregulatory efficiency already faced, older adults are more likely to experience chronic health conditions (e.g., cardiovascular disease, hypertension, obesity, diabetes) and take prescription medicines that can impact their ability to thermoregulate[77,78].

Early observations in the thermoregulatory responses to heat stress in young vs older adults involved exercise in the heat, where it was uncovered that older adults exhibited higher core body temperatures and reduced activation of sweat glands than their younger counterparts for matched exercise workloads[75]. Among the first controlled human studies directly comparing

the physiological responses to heat stress in young vs older adults was conducted by Inoue and Shibasaki[79], where they observed attenuated cutaneous vasodilation and reduced local sweat production at several skin sites in older adult men during a 60 min lower extremity hot water bath, resulting in larger rises in core temperature for the same heat load. Additional, more recent investigations have been made using longer duration heat exposures. Kenny et al.[63] examined the impact of 3 hours of continuous heat stress at 44°C and 30% relative humidity on hyperthermia and cardiovascular strain in young and older adults. They concluded that while there were no differences in cardiac output, blood pressure, or heart rate, in healthy, habitually active individuals, older adults experienced greater heat gain and attenuated limb perfusion than younger adults[63].

New Insights

More recently, investigations implementing longer, realistic simulated heat event conditions to quantify the physiological and health impacts of heat waves have also been employed. Meade et al.[80] utilized a 9-hour simulated heat event at 40°C and 9% relative humidity to investigate the effect of a daylong heat exposure in healthy young and older adults, as well as older adults with chronic conditions, on thermoregulatory function. They determined that older adults sustain greater heat storage and core temperatures than their young counterparts during 9 h of resting heat exposure, and age-related increases in core temperature were exacerbated in older adults with chronic conditions including type 2 diabetes and hypertension, common heat-vulnerability-linked health conditions. Additionally, they found that older adults experiencing greater thermal strain compared to their younger counterparts reported similar levels of heat-related symptoms and lower overall mood disturbance[81]. These findings suggest that older adults may underestimate their level of thermal strain, which may further contribute to their increased heat-vulnerability as perceptual state greatly influences behavioral responses to the heat[82,83].

In an effort to increase ecological validity, studies are now implementing activities of daily living into their heat exposures to more accurately represent the potential impacts climate change will continue to have on the safety and health of older adults. McKenna et al.[84] implemented two 3-hour extreme heat exposures with accompanying activities of daily living

throughout the heat exposure via light cycling at ~3 METs every hour for 5 min. Their results corroborate previous data that older adults are at greater risk for hyperthermia than younger individuals, with the extent of thermal strain more accurately reflecting what would occur during realistic heat wave conditions. Thus, future studies should continue to aim to investigate the impact of warmer environmental temperatures on the potential risks older adults face during heat waves using realistic heat wave conditions and activities of daily living in their simulated heat event models, with a focus on the development of biomarkers related to heat illness and injury.

PART II:

Behavioral Thermoregulation and Cognitive Function During Heat Stress

In addition to autonomic thermoeffector responses, behavior is also an important means for body temperature control and is the most powerful thermoregulatory effector mechanism[85,86]. In contrast to involuntary physiological responses, thermoregulatory behaviors are motivated and goal-oriented, emerging through reinforcement learning and driven by the expectation of reward, which in this case, is to reduce thermal discomfort and avoid thermal strain[86,87]. These behaviors include moving to a cooler location or using air conditioning, drinking water, etc., and have been linked to lower morbidity and mortality during heat waves[59,60,88]. However, older adults experience impaired cognition and/or ability to sense their own thermal state, which may interfere with their capacity to modify their behavior[89], and heat can exacerbate these cognitive decrements. Large cohort and national survey studies have linked high ambient temperature exposure with reduced cognitive performance and accelerated cognitive decline in middle-aged and older adults[90], and chronic exposure to extreme heat is associated with faster cognitive decline among socially vulnerable older adults aged 52 years and older[91]. Although the exact mechanisms by which extreme temperatures affect cognitive function are not fully understood, the physiological changes associated with heat stress, including increased core body temperature[92], dehydration[93], and altered cerebral blood flow[94] may impact neural activity and cognitive processes.

Previous research has shown that cognitive function declines with prolonged heat stress even beyond vulnerable populations as demonstrated by Laurent et al.[95], whose findings revealed that young individuals living in non-air conditioned buildings experienced reductions in

cognitive function during a heat wave as assessed by tests examining working memory and selective attention/processing speed. Additionally, to our knowledge, only one study has investigated the effects on environmental heat stress on cognitive function in older adults. Older adults were exposed to temperatures of 24°C and 32°C, but investigators did not find differences in cognitive performance assessed via the Cambridge Neuropsychological Test Automated Battery (CANTAB) between the two experimental temperatures[96]. However, in a subgroup of individuals exposed to greater relative humidity than those in the control session, the subgroup presented worse cognitive performance, and fitness level was associated with reduced cognitive vulnerability to heat stress. Thus, it is likely that higher environmental temperatures have a greater effect on cognition, and this corroborates the suggestion that future research should utilize realistic heat wave conditions to examine the impact of extreme heat on cognition among older individuals, aiming to uncover how these potential decrements in cognitive function can impact health outcomes during heat waves.

New Insights

A recent review by Yin et al.[97] showed that extreme temperatures were associated with reduced cognitive function across hourly, daily, and yearly scales. They found that males and older individuals were among the most vulnerable to extreme temperatures, and that individuals living in warmer areas were less susceptible to the cognitive effects of high environmental temperatures. In a cross-sectional study using data from the 2018 wave of the China Health and Retirement Longitudinal Study[98] and controlling for covariates and meteorological variables, the number of days of high temperature exposure was negatively associated with cognitive function[99].

Despite increasing interest in the effects of heat stress on cognition, several important gaps remain in the literature. Most experimental studies have examined short-duration heat exposures and are typically conducted in young, healthy adults, limiting their relevance to populations most vulnerable to heat-related adverse health outcomes. Additionally, many laboratory studies utilize passive heating protocols that do not reflect the light physical activity commonly performed during activities of daily living. Consequently, relatively little is known about how prolonged heat exposure, similar to conditions experienced during heat waves,

influences cognitive function across different age groups, particularly in older adults. Addressing these gaps is important for understanding whether prolonged heat exposure may contribute to cognitive impairment and increase the risk of adverse health outcomes by limiting the ability of older individuals to practice heat-relief seeking behaviors aimed at reducing the incidence of morbidity and mortality.

PART III:

Vascular Function

In addition to the thermoregulatory impairments that occur with normal aging, vascular function is also altered. As previously described, older adults experience attenuated cutaneous vasodilation because of reduced NO-bioavailability-mediated endothelial dysfunction. This impairment in endothelial function not only increases the chance for greater heat storage during heat exposure but is one of the most clinically important drivers of cardiovascular disease (CVD)[100], and may also contribute to poor health outcomes during heat events.

Older adults exhibit vascular dysfunction not only via endothelium-dependent dilation[69,70], but also through functional (e.g., altered autocrine and paracrine signaling) and structural (e.g., vessel fibrosis) changes that lead to stiffening of the large arteries[100–102]. Together, arterial stiffening with endothelial dysfunction directly contribute to the progressive increases in systolic blood pressure and a subsequent widening of pulse pressure that occur with advancing age[103], and is likely involved in the reduced ability of the systemic circulation to respond to heat exposure. The changes in cardiovascular function that occur with normal aging are driven by two molecular processes: oxidative stress and chronic, low-grade vascular inflammation, which act in a feed-forward manner to amplify each other[103]. These processes result in reduced NO bioavailability[104,105], altered major arterial structural proteins[106,107], and promote the development of cardiovascular disease[108–110], all of which can exacerbate the effects of prolonged, extreme heat exposure on cardiovascular function and lead to increased rates of morbidity and mortality in older adults. In addition to its role in producing and secreting molecules important for vasodilation, the endothelium also plays a crucial role in the suppression of inflammation, coagulation, and the development of atherosclerotic plaques[103].

During heat waves, older adults are at increased risk of classic heat stroke[111,112]. Mortality rates in those affected can reach over 50%[111,113], and survivors oftentimes experience long-term consequences including an elevated risk of future morbidity and mortality[114]. Heat stroke arises from heat-induced cytotoxicity in vulnerable tissues (e.g., the brain, vasculature, and gastrointestinal tract) triggering systemic inflammatory response syndrome (SIRS) and disseminated intravascular coagulopathy (DIC)[111,112]. These processes drive central nervous system dysfunction and multi-organ damage and can be fatal without prompt treatment.

All the aforementioned mechanisms associated with aging may influence the risk of developing of heat stroke. Greater levels of hyperthermia via reduced thermoregulatory efficiency and inadequate cardiovascular responses to support increased circulatory demand, particularly when coupled with dehydration, means that older adults are at greater risk for extreme core temperature elevations and cardiovascular collapse, setting the stage for the development of SIRS, DIC, and resultant organ damage[112]. More specifically, older adults are likely predisposed to SIRS as aging is associated with chronic, low grade inflammation due to elevated basal levels of pro-inflammatory cytokines and reductions in their anti-inflammatory and antioxidant counterparts[115,116]. This presents a reduced ability for older adults to buffer inflammatory and oxidative insults, resulting in greater inflammatory and oxidative tissue damage during heat stress as demonstrated by Wright et al.[117], where older men experienced greater levels of circulating pro-inflammatory cytokines during intermittent work in the heat compared to their younger counterparts, despite achieving similar thermal, cardiovascular, and perceptual strain.

The risk of thrombosis in response to heat was apparently first suggested from a study that measured changes in blood properties in young healthy adults during 6 hours of laboratory-based heat exposure at 41°C and 15-25% humidity[118]. This study aimed to draw parallels from a 1976 London heat wave that was associated with greater risk of coronary and cerebral thrombosis. Researchers found that the 6 hour heat exposure resulted in hemoconcentration and increased blood viscosity, platelet, and neutrophil counts.

As mentioned, heat stroke can result in DIC, which is characterized by the development of diffuse microvascular emboli[112] due to heat-induced damage to the vascular endothelium[119]. Additionally, heat stress elevates core temperature and decreases central blood volume, creating a hypercoagulable milieu[120]. In older adults, this state is exacerbated by endothelial

dysfunction[102], age-related vascular remodeling[121], and hematological changes such as platelet aggregation and higher levels of clotting factors[122,123]. Additionally, aging is associated with increased levels of blood coagulation factors such as factor VII, factor VIII, and fibrinogen[124], all risk factors for thrombotic diseases[125]. Collectively, these increase thrombotic risk and predispose older adults to acquired coagulopathies[126].

It is known that chronic heat exposures (i.e., heat therapy) and even acute passive heating can improve vascular function via increased shear rate and expression of heat shock proteins (HSPs), improvements in NO bioavailability, and reductions in markers of oxidative stress and inflammation[103]. Interestingly, a study in eight healthy, young men examined hemostatic responses to 42-59 min of hot water immersion, during which water temperature was progressively increased (maximum 41°C) until core temperature reached ~39°C[127]. Hot water immersion resulted in hemoconcentration, a shortened activated partial thromboplastin time (aPTT), increased platelet count, decreased plasminogen activator inhibitor-1 (PAI-1) activity, and a transient reduction in fibrinogen concentration followed by increased fibrinogen during recovery[127]. Despite these heat-induced alterations in hemostatic variables, there was no evidence of overt activation of the intrinsic or extrinsic coagulation pathways, nor increases in fibrin degradation products, indicating preserved hemostatic control and no increased thrombotic risk in young, healthy men. However, it remains unknown whether similar compensatory control of coagulation and fibrinolysis is maintained in older adults during prolonged heat exposure.

New Insights

A recent, large time-series study examined associations between short-term apparent temperature exposure and multiple coagulation indices in the general population[128]. Apparent temperature considers environmental temperature, relative humidity, and wind speed on temperature perception, and was associated with significant alterations in coagulation parameters, including shortened prothrombin time (PT), shortened activated partial thromboplastin time (aPTT), and increased fibrinogen concentrations, consistent with a shift toward a more pro-coagulant state during heat exposure. Importantly, these associations were observed at the population level, independent of overt heat illness, suggesting that ambient heat alone can influence hemostatic balance. Additionally, McCormick et al.[129] recently observed

autophagic impairments paired with greater apoptotic signaling and inflammatory responses in older compared to young adults during 9 h of heat exposure at 40°C and 9% relative humidity, highlighting greater vulnerability to heat-induced cellular injury with aging and thus a potentially heightened susceptibility to the development of SIRS. To date, no study has investigated the impact of prolonged heat exposure on vascular endothelial function and coagulation in young versus older adults. Thus, there is no direct evidence to support the observed physiological responses associated with heat stress to be linked with greater risk of thrombosis. Given that mortality rates in those who develop heat stroke can reach over 50%[111,113] and heat-related mortality for people over 65 years of age increased by approximately 85% between 2000–2004 and 2017–2021[130], research on the physiological responses to heat stress associated with the pathophysiology of heat stroke is warranted.

PART IV:

Acute Kidney Injury

The kidneys are essential for many homeostatic functions including blood pressure regulation, acid/base balance, and water and electrolyte conservation. During heat stress, these functions are challenged as the kidneys must simultaneously support the maintenance of blood pressure, limit fluid and electrolyte losses, and aid in the redirection of blood flow to the skin for the promotion of heat loss. Dehydration is a common occurrence during heat stress, as water lost via sweating is often not sufficiently replaced with fluid intake, even when fluids are readily available[131]. These changes elicit a coordinated hormonal response aimed at conserving body water, including activation of the renin–angiotensin–aldosterone system in response to reduced renal perfusion and the release of vasopressin (antidiuretic hormone) in response to hyperosmolality[132]. Collectively, these mechanisms reduce urine output, increase urine concentration[133], and decrease renal blood flow in an effort to preserve blood pressure and circulating volume[134–139].

Autonomic control of kidney function stems from the renal sympathetic nerves which play an important role in modifying renal blood flow, glomerular filtration rate (GFR), tubular sodium and water reabsorption, and coordinating the release of hormones to increase blood pressure by vasoconstricting in response to different stimuli[140]. Kidney function is most

commonly quantified as a function of GFR, which reflects the ability of the kidneys to filter blood. In healthy individuals, the kidneys typically function at ~75% of their maximum filtration capacity, allowing the kidneys to preserve GFR in states where GFR would be reduced, such as with aging where there is a loss of nephron mass or partial loss of individual nephron filtering ability[141]. Thus, the preservation of kidney function via hyperfiltration reflects the kidneys' reserve capacity to augment GFR[140]. One of the most common means to estimate GFR is through creatinine clearance. Creatinine enters the circulation at a relatively constant rate as a result of the nonenzymatic dehydration of creatine in skeletal muscle[142]. Urine output may be quantified as total volume or flow rate, while urine concentration can be evaluated using measures such as osmolality, specific gravity, and urinary creatinine concentration[140]. Reductions in urine output and/or increases in urine concentration represent normal renal adaptations aimed at conserving water and electrolytes, particularly during conditions of physiological stress such as heat exposure.

One of the top two causes of hospitalizations during heat waves is acute kidney injury (AKI)[143–145], secondary to fluid and electrolyte imbalances[143,146] which act as a precursor to the development of AKI[147]. AKI is defined as an acute reduction in kidney function (e.g., GFR)[148–150] with increasing concentrations of products of nitrogen metabolism in the plasma (e.g., creatinine) which may also manifest with a decrease in urine output[151]. Those most at risk of developing AKI are individuals in occupational settings consistently exposed to heat stress and adults aged 65 years and older[140]. The primary causes of AKI include ischemia, hypoxia, or nephrotoxicity[152]. AKI pathophysiology can be divided into three categories: prerenal, intrinsic, and postrenal (obstructive). Prerenal AKI is the most common cause of AKI and results from hypoperfusion of the kidneys secondary to reductions in blood volume[153], leading to reductions in renal blood flow and GFR and a resultant ischemic environment in the renal vasculature[140]. Intrinsic AKI most commonly results from ischemia or sepsis, with epithelial cell injury occurring in the proximal tubule[153]. Lastly, the least common type of AKI is postrenal AKI which results from obstruction of the ureters, bladder outlet, or urethra[153].

Biomarkers of AKI are highly developed and the product of insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) has been approved by the FDA as a tool for assessing the risk of AKI [37], outperforming over 330 biomarkers in predicting the incidence of AKI in clinical settings[154]. In renal epithelial cells,

IGFBP7 and TIMP-2 are proteins that induce G1 cell cycle arrest during kidney injury[155–157]. Interpretation of these AKI biomarkers in non-clinical situations reflects an increased potential for the development of AKI due to underlying pathophysiological processes taking place[158]. Additional biomarkers inform the location and mechanism of kidney injury and stress, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), monocyte chemoattractant protein-1 (MCP-1), thioredoxin-1 (TRX-1), interleukin-17a, and more.

In the context of heat stress, AKI risk rises when dehydration and hyperthermia increase. AKI risk can be further exacerbated by exercising in the heat, where previous research has speculated the proximal tubules to likely be the site of renal pathophysiology as indicated by a larger increase in IGFBP7 compared to TIMP-2, where IGFBP7 is preferentially secreted in the renal proximal tubules and TIMP-2 in the distal tubules[37,140,158]. This tubular injury is likely caused by numerous mechanisms primarily stemming from a reduction in renal blood flow[140]. Animal studies indicate that reduced renal cortical blood flow[159,160] compromises oxygen delivery to this location[161], creating an oxygen supply/demand mismatch as Na+/K+ pump activity increases to conserve fluid[162,163]. Thus, tissue oxygenation may become compromised and ATP production for normal cell functioning is limited[164]. Dehydration further amplifies this demand for ATP due to an increased drive for water and electrolyte conservation[162,163]. The presence of uric acid generated as a product of purine metabolism and the breakdown of ATP[165] is also common during heat stress and dehydration[166,167] and may contribute to increases in AKI risk as uric acid can produce additional reactive oxygen species, damaging cells and triggering inflammation in kidney tissue. These pathophysiological pathways that result in ATP depletion is magnified in the presence of inflammation[168], which may be amplified by exercise-induced inflammation, elevations in core temperature, and aging.

Multiple epidemiological studies have demonstrated that AKI risk is greater in older adults[169–171], and a clear age-dependent relationship between AKI and older age has been confirmed[172,173]. Adults aged 65+ are 10 times more likely to develop AKI compared to adults <40 years[174], and the in-hospital mortality rate for older patients with AKI ranges from 15-40%[175,176]. In addition to a reduction in total renal mass with aging[177], GFR begins to decline in a at a rate of approximately 1 ml/min/year above the age of 45[178–180], renal blood flow is reduced by 10% per decade above age 40[181], glomerulosclerosis may occur[182], there is a thickening of large vessels' walls[183], and there is a reduction in the amount and length of tubules in the

kidneys[177]. Aging also impairs the kidneys' ability to conserve water and respond to dehydration[140]. Additionally, age-related changes in body composition increase susceptibility to volume depletion[147]. A decrease in the fat-free mass observed in older adults is associated with a decrease in muscle mass, total body water, and bone mass. The severity of dehydration is determined by the proportion of total body water lost rather than the absolute volume lost. Thus, even minimal changes in body composition can have substantial effects on overall fluid volume in older adults[184]. In conclusion, the physiological changes associated with aging put older adults at greater risk for developing AKI when exposed to hot environmental temperatures.

Early evidence linking heat stress to AKI emerged from studies of adults under 45 years old participating in military physical training in hot conditions[150,185,186]. These findings proved to be demonstrative of a larger issue among workers in occupational settings who are regularly exposed to heat stress[158,187,188]. As previously mentioned, older adults are also at risk of developing AKI during heat exposure, thus, there is a crucial need to understand the mechanisms underlying these epidemiological observations to develop strategies aimed at mitigating the risk of AKI during heat stress. To date, numerous studies have demonstrated that AKI risk rises during experimental heat exposures. Hess et al.[189] investigated the effect of 8 hours of heat stress with no fluid intake at current and projected extreme heat event conditions (wet bulb temperatures of 31°C and 34°C) on AKI risk in young, healthy men. Researchers found that [IGFBP7·TIMP-2] increased in both trials, with a greater increase in biomarkers of AKI risk during the projected wet bulb temperature trial of 34 °C. This was accompanied by increases in urinary thioredoxin-1 (TRX-1) and plasma IL-17a, markers of renal oxidative stress and inflammation, respectively. These findings indicate that current and projected heat wave conditions increase AKI risk in young, healthy men with both the proximal and distal tubules as the locations of potential renal injury and oxidative stress and inflammation as the likely mechanisms for AKI risk[189]. It is important to note however, that fluid intake was not allowed which does not accurately reflect real-world conditions, where water is likely readily available.

New Insights

More recently, researchers have begun conducting laboratory-based research on kidney function and AKI risk and its etiology in young vs older adults. McKenna et al.[190] found that

older adults were at greater risk of AKI following a 3 h exposure to a hot, dry environment at 47°C and 15% relative humidity with activities of daily living and 3 mL water/kg body mass consumed every hour. Older adults experienced greater increases in creatinine, their primary outcome for assessing kidney function, and cystatin C, their secondary outcome, both of which are kidney function biomarkers that are often used in clinical settings to estimate glomerular filtration rates. However, the older adults became more hyperthermic in this study and no biomarkers of AKI risk or its etiology were measured, thus the role of age in AKI risk during heat stress could not be elucidated. To address these limitations, McKenna et al.[191] went on to use water-perfused suits and activities of daily living to induce the same level of hyperthermia in young and older adults, aiming to better interpret the etiology of AKI risk in older adults compared to younger by examining both renal and gastrointestinal responses to heat stress. The authors uncovered no differences in kidney function between hyperthermia-matched groups but observed higher gastroduodenal permeability in older adults and highlight that heat stress impairs kidney function and elevates markers of kidney injury, but these responses were not modulated by age during controlled hyperthermia. Participants were heated for just 72 ± 4 min in the young cohort and 67 ± 10 in the older cohort to increase core temperature by 2°C. While the increase in core temperature in both groups is significant, the time frame in which they were heated is extremely short and not representative of real-world scenarios in which older adults experience heat waves. Recent work by Vliora et al.[192] also employed a 3 h extreme heat exposure at 44°C and 30% RH in both young and older adults, however water consumption was not permitted, and participants remained seated for the entirety of the heat exposure. They found that older adults experienced inflammation and increased AKI risk as indicated by increases in c-reactive protein (CRP) and neutrophil gelatinase-associated lipocalin (NGAL), an early biomarker of AKI risk, from baseline to end of heat exposure. Additionally, they observed elevations in serum klotho following heat exposure in the young adults, indicating cytoprotection in the young, but not older adults which may contribute to increased susceptibility and heat-related cellular stress faced by older adults during heat waves.

The resulting conclusions from these studies highlight the need for a more integrative look at the pathophysiology of AKI risk in young vs older adults using real-world heat wave conditions while examining defined biomarkers of AKI risk and it's etiology. While hyperthermia-controlled studies provide a more mechanistic look into age-related differences in

heat-induced illness and injury with high internal validity, studies implementing ecologically valid heat wave conditions are also crucial to fully understand heat-induced AKI risk in older adults.

PART V:

Conclusions and Remaining Gaps in Literature

Heat-related illness ranges from mild heat exhaustion to life-threatening heat stroke; however, milder forms of heat-related injury are less clearly defined and underrecognized and may present greater risks in older adults who are already facing elevated susceptibility to heat. A critical step in understanding the risks of a warming climate is the identification and characterization of the physiological mechanisms underlying heat-related injury across multiple organ systems in populations most vulnerable to an increasingly hot climate. Much of the existing literature has focused on short-duration heating protocols without form of activities of daily living, or hyperthermia-matched protocols that, although mechanistically informative, may underestimate the true physiological burden experienced during real-world heat waves in at-risk populations.

Importantly, the kidney and vascular endothelium represent critical, yet understudied targets of heat-related injury, and the effect of prolonged heat exposure using realistic heat wave conditions on cognitive function has never been experimentally studied in the context of aging. Age-related decrements in thermoregulatory and cardiovascular function have been defined and evaluated in the context of laboratory-based heat exposures and have set the precedent for the increased risk of poor health outcomes during heat waves that older adults face. However, few studies have examined renal, vascular, and cognitive function under controlled yet ecologically relevant heat wave conditions, and even fewer studies have investigated these physiological pathways/functions using comprehensive biomarker-based approaches. Studies utilizing high internal validity are crucial to understanding causal relations between increases in core body temperature and physiological outcomes related to heat-induced illness and injury, much of which is still underexplored when it comes to the isolated effect of age on heat-induced injury. However, it can also be argued that studies conducted under ecologically valid, real-world heat wave conditions are essential for accurately characterizing health risk in older adults. Thus,

while hyperthermia-controlled paradigms are critical for isolating age-specific pathophysiological mechanisms, real-world simulations provide crucial insight into the magnitude of risk older adults face relative to younger adults, better informing public health guidance, clinical risk stratification, and heat-mitigation strategies.

Furthermore, while heat exposure has been shown to acutely elevate biomarkers of kidney injury and inflammation, their persistence following heat exposure are not well characterized, particularly in older adults. It is unknown how older adults recover from an extreme heat exposure compared to their younger counterparts, and whether decrements in physiological function persist past the acute response. This gap in knowledge limits our ability to identify early indicators of heat-related injury, assess recovery trajectories, and develop targeted interventions aimed at reducing downstream or delayed physiological effects on health outcomes.

Collectively, these gaps underscore the need for integrative, laboratory-based investigations that combine prolonged, realistic heat exposure with comprehensive evaluations of renal, vascular, and cognitive outcomes across age groups. Addressing these limitations is essential for improving climate resiliency and reducing heat-related health burdens.

CHAPTER III

CHALLENGES AND ALTERNATIVE APPROACHES

OVERVIEW

During the proposal and completion of this dissertation, several considerations and challenges were presented, resulting in changes to the study design and outcomes measured. All post-heat event measurements were made only ~24 hours after heat exposure, as we removed the 48 hour measurement.

SPECIFIC AIM CONSIDERATIONS

Aim 1 Considerations:

Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining biomarkers related to heat stroke risk.

Although Aim 1 was initially proposed to examine biomarkers associated with heat stroke risk, we reframed this aim to characterize the effects of prolonged heat stress on endothelial function and coagulation potential, two physiological mechanisms underlying heat stroke. This approach allows the aim to be positioned within the broader context of heat-related illness and injury, recognizing that vascular dysfunction and prothrombotic alterations may contribute to adverse health outcomes during prolonged, extreme heat exposure. By focusing on these physiological responses, Aim 1 addresses mechanisms through which prolonged heat stress may increase susceptibility to heat-related morbidity in older adults such as heat stroke. Aim 1 remains relatively the same as originally proposed, however we decided to limit the number of biomarkers analyzed due to time and cost constraints.

It was originally proposed that we measure multiple biomarkers of inflammation, including lipopolysaccharide (LPS), a biomarker of gastrointestinal permeability. New research examining the effect of prolonged heat stress on kidney function and gastrointestinal permeability concluded that older adults exhibited higher gastroduodenal permeability than their younger counterparts during a model of controlled hyperthermia[191]. Although studied in the context of kidney function in the study by McKenna et al.[191], gastrointestinal permeability plays

a role in the systemic inflammatory response to heat stress and the pathophysiology of heat stroke[193–195]. Heat stress elicits vasoconstriction in the splanchnic and renal vascular beds to facilitate the redistribution of blood flow from these central compartments towards the skin to promote heat loss[72,196]. While necessary for thermoregulation, the prolonged relative ischemia to these organs reduces oxygen supply and is thus likely to cause hypoxia-related cell damage[196]. The systemic inflammatory response can therefore be exacerbated by accompanied endotoxemia that develops due to gastrointestinal permeability[197], secondary to hypoxia-related cell damage[196]. Thus, with the knowledge that older adults experience greater gastrointestinal permeability during a controlled hyperthermia exposure[191], we decided to solely examine changes in the inflammatory cytokine interleukin (IL)-6 for a broad characterization of the overall inflammatory response to the 6 hour heat exposure.

As previously proposed, we are measuring flow-mediated dilation (FMD) at 24 h pre- and ~24 h post-heat exposure for the characterization of endothelial function following prolonged heat stress. FMD represents the most commonly used non-invasive measurement of peripheral artery endothelium-dependent dilation and has been shown to be highly correlated with cardiovascular morbidity and mortality[198–200] and to parallel endothelial function in the coronary arteries[201]. Importantly, FMD integrates functional nitric oxide-dependent signaling and vascular responsiveness[202], thereby providing a physiologically meaningful assessment of endothelial health that extends beyond circulating biomarkers alone. Therefore, FMD represents a highly validated and robust measure of endothelial function and has not previously been studied in the context of prolonged heat stress in aging adults.

Given the inclusion of FMD as the primary outcome of endothelial function, circulating biomarkers were refined to complement this functional outcome. Von Willebrand factor (vWF) remained as a secondary marker of endothelial activation and injury, as it is predominantly released following damage or stimulation of the endothelium[194,195]. In contrast, intracellular adhesion molecule (ICAM)-1 and syndecan-1 were removed due to their broader biological roles and lower specificity for acute endothelial injury in this context, particularly after we completed FMD and vWF analyses. ICAM-1 reflects systemic inflammatory activation and leukocyte-endothelial interactions rather than direct endothelial dysfunction[203], while syndecan-1 primarily reflects glycocalyx shedding, which is more consistently observed in severe critical illness (e.g., sepsis or trauma) and is less sensitive to subclinical or transient endothelial stress[204]. Thus, these

markers were determined to be unnecessary to the outcomes observed with FMD and vWF for addressing the primary mechanistic aims of the study.

Similarly, biomarkers of coagulation cascade activation were streamlined to focus on markers most likely to reflect clinically meaningful thrombotic risk in response to heat stress. D-dimer reflects fibrin turnover and is widely used as an early marker of coagulation system activation[205], while Protein C is a key endogenous anticoagulant whose reduction typically signals a shift toward a pro-coagulant state[206]. Additional coagulation measures including platelet count, activated partial thromboplastin time (aPTT), prothrombin time (PT), and fibrinogen were removed, as these indices typically change in parallel with, or secondary to, more pronounced coagulation activation.

Finally, Aim 3 (described below) was simplified and combined with Aim 1 for ease of reporting. In conclusion, these changes prioritize validated functional outcomes and endothelial-specific biomarkers while avoiding redundancy and overinterpretation of insignificant coagulation findings, thereby strengthening the mechanistic focus and interpretability of the study.

Aim 2 Considerations:

Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining biomarkers of kidney injury risk.

At the time of proposing this work, no study had experimentally investigated the impact of prolonged heat exposure on acute kidney injury (AKI) risk in older adults. Thus, we initially proposed a broad panel of biomarkers to comprehensively assess AKI risk and its potential mechanisms in young and older adults. As the study progressed, emerging literature began to address AKI risk during heat exposure in both age groups[190,191]. Thus, we refined our analyses with advice from colleagues with expertise in renal physiology and AKI to focus on outcomes most directly linked to physiological function and clinically meaningful kidney stress.

Rather than continuing with a broad panel of exploratory biomarkers, we prioritized functional indicators of hydration status and renal handling, including urine and serum osmolality and creatinine concentrations as well as urine albumin, concentrations to capture integrated renal responses to prolonged heat exposure. Serum and urine creatinine allow for the calculation of creatinine clearance, which is the most commonly used method used to estimate

glomerular filtration rate (GFR). Urine and serum osmolality have a well-described physiological basis for assessing fluid balance and renal concentrating ability, which are essential to understanding heat-related renal strain[207]. Lastly, urinary albumin assesses glomerular barrier function, and albuminuria can increase before measurable declines in GFR, making it a sensitive marker of early or subclinical kidney injury[208]. Together, urinary albumin, serum and urine creatinine, and serum and urine osmolality provide a complementary assessment of kidney function by capturing glomerular barrier integrity, filtration capacity, and renal water-handling and concentrating ability, thereby allowing evaluation of both early kidney injury and integrated physiological responses to stress.

In parallel, we narrowed our AKI biomarker panel to include insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor metalloproteinase (TIMP)-2, the FDA-approved biomarkers for assessing AKI risk; mechanistic markers thioredoxin (TRX)-1, for assessing oxidative stress and monocyte chemoattractant protein (MCP)-1, for assessing inflammation. As part of this modification and with advice from colleagues, we also decided not to pursue measurements related to the polyol-fructokinase pathway. Although this pathway has been implicated in heat- and dehydration-related kidney injury in a recurrent heat stress animal model[209], it is unknown whether an acute bout of heat stress and dehydration activates this pathway and whether polyol-fructokinase activation differs between young and older adults. Additionally, there are currently no validated biomarkers that allow its activation to be quantified in humans. Without established assays, our attempts to measure this pathway would result in uncertainty and limited interpretability. For this reason, we focused instead on biomarkers and functional outcomes with well-characterized physiological meaning and established reliability.

Aim 3 Considerations:

Effect of Prolonged Heat Stress on Cognitive Function.

We initially considered measuring internal carotid artery (ICA) blood flow velocity and diameter and CO_2 during the heat-stress protocol; however, we elected to remove this approach and instead solely focus on the use of the NIH Toolbox Cognition Battery because the time and procedural demands of ICA and CO_2 measurements would likely interfere with other scheduled assessments and introduce complexity to our already lengthy and demanding heat event protocol. The NIH Toolbox offers a brief, standardized, and validated assessment of cognition across

domains relevant to heat-related performance (working memory, attention/processing speed, executive function), while preserving protocol efficiency and minimizing interference with other assessments.

UPDATED AIMS

Aim 1: Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining endothelial function, coagulation potential, and cognitive function.

Hypothesis 1a: We hypothesize that FMD will decrease in older individuals from pre- to ~24 hours post-heat exposure, with no change in FMD in young adults. Aging introduces physiological changes that significantly increase vulnerability to heat-related illnesses and injuries. These risks extend beyond thermoregulation alone, encompassing critical vascular processes such as endothelial function and blood coagulability, which can lead to life-threatening outcomes. Understanding how aging influences these mechanisms is essential for identifying pathways that contribute to heat-related morbidity and mortality. Therefore, we will examine the impact of aging on biomarkers of endothelial function and coagulation potential within the broader context of heat stress. **Hypothesis 1b:** We hypothesize that vWF, D-dimer, and Protein C will increase in older individuals after the 6 hour heat exposure and remain elevated ~24 h post-heat exposure, with no observed changes in these biomarkers in young adults. Additionally, cognitive function highlights a critical aspect of thermoregulation, as impairments in cognition may reduce one's ability to perform behavioral thermoeffectors that promote heat loss, reduce thermal discomfort, and protect against thermal strain, ultimately reducing the risk of morbidity and mortality during heat waves. **Hypothesis 3a:** We hypothesize that cognitive function will decline to a greater extent in old compared to young individuals during the 6 hour heat exposure.

Aim 2: Effect of Aging on Physiological Responses to a 6-Hour Extreme Heat Event: examining kidney function and biomarkers of kidney injury risk.

Hypothesis 2a: We hypothesize that increases in urinary IGFBP-7 and TIMP-2 will be greater in older individuals exposed to 6 hours of extreme heat when compared to young individuals, with greater sustained elevations ~24 hours post-heat event. Additionally, it is unknown how kidney function and biomarkers of inflammation and oxidative stress differ between young and older individuals during prolonged heat stress. **Hypothesis 2b:** Therefore, we hypothesize that older individuals will exhibit greater reductions in kidney function, as evidenced by reduced creatinine clearance, greater serum osmolality, and albuminuria compared to their younger counterparts. Additionally, we hypothesize that older adults will exhibit a greater increase in markers of kidney inflammation (MCP-1) and oxidative stress (TRX-1) that will remain elevated ~24 h post-heat exposure compared to young adults.

CHAPTER IV
VASCULAR AND COGNITIVE FUNCTION FOLLOWING PROLONGED HEAT EXPOSURE IN YOUNG VS OLDER ADULTS

INTRODUCTION

Climate change poses a significant and escalating threat to human health by driving a global increase in the frequency, intensity, and duration of extreme heat events, or heatwaves[58]. Aging influences heat vulnerability more than any other non-modifiable risk factor[58,59]. Older adults face elevated risk of adverse health outcomes during heatwaves due to the physiological changes associated with aging, including reduced thermoregulatory efficiency[72,210], wherein the two primary autonomic responses for heat dissipation, cutaneous vasodilation and sweating, are impaired[73,210]. In addition to the consequences of climate change, the population of the world is rapidly aging. The number of individuals aged 65 and older in the United States is projected to increase to nearly 80 million by 2040[4], and by 2050, the global population aged ≥60 years is expected to exceed the population under 15 years of age for the first time in history[5]. Consequently, concern over the safety of older adults is warranted as environmental temperatures continue to rise and heat waves become increasingly frequent and intense.

Age-related reductions in thermoregulatory efficiency and hemodynamic stability are thought to be central contributors to heightened heat-vulnerability among aging adults by increasing thermal load and cardiovascular strain during extreme heat exposure. In addition to these thermoregulatory impairments, older adults also exhibit chronic low-grade inflammation[115,116], endothelial dysfunction[121,211], and elevated circulating levels of coagulation factors such as factor VII, factor VIII, and fibrinogen[124], all of which increase the risk for thrombotic disease[125]. Prolonged elevations in core temperature and the resultant cardiovascular strain during extreme heat exposure may directly injure or activate the vascular endothelium[119,212], promoting a pro-thrombotic milieu through increased endothelial permeability[120], release of pro-coagulant factors, and impaired fibrinolysis[213], mechanisms that may be exacerbated in aging adults.

Among thrombotic conditions associated with extreme heat is heat stroke, the incidence of which has increased substantially in recent decades and is expected to continue rising due to climate change, urbanization, and increased life expectancy[214,215]. Up to 60% of individuals with classic heat stroke are hospitalized or die within 24 hours of symptom onset, underscoring the severe and life-threatening nature of this condition[112]. Heat stroke results from heat-induced cytotoxicity that precipitates a systemic inflammatory response syndrome and disseminated intravascular coagulation, ultimately leading to multi-system organ dysfunction and death if left untreated[67].

In addition to autonomic thermoeffector responses, behavior is also an important means for body temperature control and is the most powerful thermoregulatory effector mechanism[85,86]. These behaviors include moving to a cooler location or using air conditioning, drinking water, etc., and have been linked to lower morbidity and mortality during heat waves[59,60,88]. However, older adults may experience impairments in cognition and/or ability to accurately sense their own thermal state, which may interfere with their capacity to modify their behavior[89], and heat can exacerbate these cognitive decrements.

Despite the growing health burden of heat-related morbidity and mortality, most experimental human studies have focused on short-duration or exercise-induced heat stress, which may not accurately reflect the prolonged, sustained heat exposure experienced during heatwaves, particularly in older adults who are disproportionately affected. Moreover, no study to date has directly examined the effects of prolonged, extreme heat exposure on vascular endothelial function, coagulation potential, and cognitive function in young versus older adults. Given that mortality rates in those who develop heatstroke can reach over 50%[111,113] and that heat-related mortality for people aged ≥65 years increased by approximately 85% between 2000–2004 and 2017–2021[216], investigating the vascular and coagulation responses and to prolonged heat stress and its effect on cognitive function across age groups is critically needed. Understanding these responses to prolonged heat exposure is crucial for identifying physiological mechanisms underlying heat-related morbidity and informing strategies to protect vulnerable aging populations during heatwaves.

Therefore, the purpose of the present study was to compare thermoregulatory and cardiovascular responses to 6 h of heat exposure with light exercise designed to mimic activities

of daily living in young and older adults, and to examine age-related differences in heat-induced vascular endothelial dysfunction and coagulation potential. We hypothesized that older adults would experience reduced thermoregulatory function and increased cardiovascular strain, and that prolonged heat exposure would impair endothelial function and promote a pro-coagulant state in older adults as assessed by flow-mediated dilation and biomarkers of endothelial activation/injury and coagulation. A secondary objective of this study was to determine if prolonged heat exposure results in reduced cognitive function and whether there are age-related differences.

MATERIALS AND METHODS

This paper is the first in a pair of research articles examining the effect on prolonged heat exposure on biomarkers of heat-illness and injury risk in young and older adults. Age-related differences in vascular and hemostatic function are discussed herein. The second article reports findings related to kidney function and risk of acute kidney injury (Chapter V).

Ethical Approval

This study was approved by the Institutional Review Board at the University of Oregon. Written informed consent was obtained from all participants in accordance with the principles set forth by the Declaration of Helsinki.

Participants

Thirteen young (18-30) and 14 older (60-80) adults participated in the study. All participants were non-smokers and were excluded if they met any of the following criteria: 1) chronic/diagnosed cardiovascular or cardiorespiratory diseases except stage 1 hypertension; 2) history of hospitalization for cardiovascular events; 3) history of stroke, clotting disorders, or venous thrombosis; 4) prior diagnosis of diabetes, sleep apnea, or obesity; 5) history of heat illness, heat injury, orthostatic intolerance, or hypotension; 6) undergoing routine heat therapy

(hot tubs, saunas, hot bath, hot yoga, etc.); 7) mobility restrictions that interfere with physical activity; 8) BMI <18 kg/m^2 or >35 kg/m^2); 9) systolic BP≥140 mmHg or diastolic BP≥90 mmHg; 10) mini mental state exam (MMSE) score ≤ 27; 11) ongoing use of anticholinergics, aspirin, diuretics, non-steroidal anti-inflammatory drugs (NSAIDs), or more than one blood pressure medication; 12) abnormalities detected suggestive of provocable ischemia or undetected cardiac disease on screening electrocardiogram (see *Screening Visit*); 13) currently pregnant, breast-feeding, or trying to conceive, or 14) undergoing treatment to increase sperm count. We did not account for menstrual cycle phase in the present study. Of the 7 young females who participated in the study, one had an intrauterine device (IUD), one was using oral contraceptives, one was using an etonogestrel implant (Nexplanon), and four were not using any form of contraceptives. Older female participants were post-menopausal, with one participant reporting use of hormone replacement therapy. Participants self-reported as being recreationally active for an average of ~5.5 days per week (young adults) and ~6.9 days per week (older adults) of vigorous and/or moderate physical activity according to the International Physical Activity Questionnaire (IPAQ)[217]. All participants volunteered for a screening visit and 3 experimental visits.

Table 4.1 Participant Characteristics

	Young (n=13)	**Older (n=14)**	**P value**
Males/Females	6/7	6/8	
Age (yr)	24±4	68±6	<0.001
Height (cm)	170±13	167±17	0.578
Body mass (kg)	67.7±10.1	77.1±13.1	0.047
Body mass index (kg/m^2)	23.5±3.5	28.4±8.0	0.049
Body surface area[a] (m^2)	1.79±0.18	1.86±0.23	0.382
Systolic blood pressure (mmHg)	115±12.4	122.5±12.8	0.136
Diastolic blood pressure (mmHg)	75.5±8.5	77±9.6	0.677

Values are presented as means ± standard deviation. Data were analyzed using unpaired t tests.
[a]Body surface area was calculated according to the equation by Du Bois[218].

Screening Visit

During the screening visit, participants were familiarized with all experimental procedures and measurements and completed a medical history form and physical activity questionnaire (IPAQ)[217]. In addition, their height, body mass, and brachial artery blood pressure were measured. A kidney function via urine test was implemented to determine their urine albumin creatinine ratio (uACR). The urine sample was also used to test for pregnancy in individuals with childbearing potential. A mini-mental state exam (MMSE) was administered to ensure participants did not show significant signs of cognitive impairment (MMSE score $\leq$ 27) for the cognitive function aim of the study.

Participants then underwent a metabolic gas production assessment to estimate the workload to be used during the experimental heat event visit. The metabolic test included 10-15 min of cycling with collection and analysis of metabolic gases using indirect calorimetry (PARVO Medics True-One Metabolic Measurement System, ParvoMedics, Salt Lake City, UT) to identify the workload that would elicit a metabolic rate of ~3 METs, VO_2 = 10.5 ml/kg/min[219]. Following the metabolic assessment, participants underwent a functional capacity exercise test while monitored with a with a 12-lead electrocardiogram (ECG) (CardioCardTM, Nasiff Associates, Inc.) to screen for cardiac abnormalities. The functional capacity test began with light cycling on a cycle ergometer at 10 W for 3 min. Immediately following warm-up, the workload increased by 10 W every minute and blood pressure was measured every 3 min until 85% of age-predicted heart rate max was reached. Exercise was terminated and participants were excluded from the study if any of the American College of Sports Medicine (ACSM) absolute indications for exercise cessation were met, or if three or more of the relative indications for exercise cessation were met[220].

Experimental Procedures

Participants completed three consecutive experimental visits including a pre-heat event vascular function testing visit (Visit 1), the heat event visit (Visit 2) and the post-heat event vascular function testing visit (Visit 3). All experimental trials began between 6:00 and 10:00 AM to avoid diurnal variations and started at the same time of day for each participant across their experimental visits. Participants were tested during the months of February to August.

Although participants were tested during the summer months, screening during June-August included the question "Do you spend a lot of time outside in the heat (i.e., multiple times a week for >2 hours at a time)" and participants were excluded if they answered yes.

Participants were instructed to abstain from eating food and consuming caffeine for at least 2 hours, consuming alcohol or using nicotine, tobacco, recreational drugs, supplements and over-the-counter medications for 24 hours, and moderate or vigorous exercise for 24 hours before the start of each session, with the exception of the heat event visit, where participants were given a standardized breakfast to consume at least 1 hour prior to arrival and were allowed to consume caffeine. Participants were asked to consume at least 500 mL the evening before and morning of the heat event visit to ensure adequate hydration. Euhydration was defined as a urine specific gravity (USG) < 1.020[221]. If participants were not euhydrated (USG ≥ 1.020), they were provided 500 mL of water and USG was re-tested before undergoing heat exposure. Urine human chorionic gonadotropin (hCG) was measured to provide a negative pregnancy test before all visits in participants of childbearing potential. Participants were allowed to wear clothing of their choice for the heat event, which included shoes or sandals, shorts or capri pants, and t-shirts or light tops. For the vascular function visits, participants were asked to wear a short-sleeved shirt for the measurement of brachial artery flow-mediated dilation.

Vascular function testing

Participants arrived at the laboratory 24 h before and 24 h after the start of their heat event for experimental Visits 1 and 3. Endothelium-dependent dilation was assessed via flow-mediated dilation (FMD) of the brachial artery in accordance with expert consensus guidelines[202]. Participants were fitted with three ECG electrodes, a blood pressure cuff on their left arm for blood pressure measurements, and an inflatable occlusion cuff on their right lower arm placed 0-3 cm distal to the antecubital fossa and inflated to 250 mmHg for 5 min (E20 Rapid Cuff Inflater, D. E. Hokanson, Bellevue, WA, USA). Participants lay supine with their right arm extended to 80-90° at heart level. Brachial artery diameter and blood velocity were measured via Doppler ultrasonography (L9-3 Probe, Philips iE33, Andover, MA) at baseline for 1 min prior to cuff inflation and again for 3 min following release of the occlusion. Flow-mediated dilation was calculated as the percent change in brachial artery diameter from baseline to peak dilation

throughout the 3 min period following cuff release. Image recordings were then analyzed via custom wall-tracking software (Vascular Research Tools 5; Medical Imaging Applications LLC, Coralville, IA) to determine brachial mean diameter (mm) at baseline and peak brachial diameter following 5 min of cuff occlusion. FMD was calculated as the percent change in diameter from baseline to peak dilation post-occlusion. Following release of the arterial occlusion, blood flow rapidly increases due to the downstream metabolic vasodilation, increasing shear stress on the brachial artery and resulting in vasodilation via shear-induced nitric oxide release[222]. If the first FMD was considered non-physiological (i.e. the participant moved their arm during occlusion and the same position could not be replicated following release), the participants remained supine for a 20 min rest period before the test was repeated.

Shear rate was calculated frame-by-frame from diameter and velocity measurements. The shear stimulus responsible for eliciting dilation was calculated as area under the curve above baseline shear rate from the time of release to peak dilation (SR_{AUC}). FMD was then normalized for shear stimulus by dividing FMD by SR_{AUC}, as previously described and validated[223,224]. Upon completion of the vascular function test for Visit 1, participants were given a core temperature pill to ingest before going to bed and a standardized breakfast (Oats Overnight ®) to consume at least 1 h prior to arriving for the heat event visit.

Experimental heat event

Participants arrived at the laboratory the following morning after Visit 1 for the heat event visit (Visit 2) after eating their provided breakfast and following pre-visit guidelines. Upon arrival, participants were instructed to first void their bladder to provide a urine sample for measurement of hydration status and pregnancy status (if applicable). Before instrumentation, participants' nude body weight was measured and recorded, and a baseline venous blood draw was collected. Participants were then seated for 20 min of baseline rest, followed by baseline measurements of blood pressure, heart rate, core temperature, and perceptual data. After baseline measurements were recorded, participants entered the environmental chamber set at 42°C and 20% relative humidity. These environmental conditions were selected to align with the U.S. National Weather Service criteria for issuing an Excessive Heat Warning, which occurs when the daytime heat index exceeds 40.5°C for at least a 48 hour period[225]. The selected temperature and

humidity for the present study equates to a heat index of 42.4°C, which represents a "danger" level for heat-related illnesses[226,227].

Participants then completed their first set of National Institutes of Health (NIH) toolbox tests for cognitive function administered on an iPad by the same trained researcher for all participants. Following completion, participants were asked to remain seated for the duration of the 6 hour Heat Event. Every hour, participants cycled at a pre-determined workload (3 METs) for 15 min to simulate activities of daily living and were given water (3 mL/kg body weight). At hour 3 of the heat event, participants completed their second set of cognitive function tests and were given a snack (CLIF BAR®, CLIF Bar & Company, Emeryville, CA, USA) after completing the test. At the end of the 6 hour Heat Event, participants completed their final cognitive function test, and a final venous blood sample was collected. Following a short recovery period, participants provided a post-heat exposure nude body weight and were provided with a Gatorade and snack. The morning following the heat event visit, participants arrived at the laboratory for their post-vascular function test, and a final blood draw was performed.

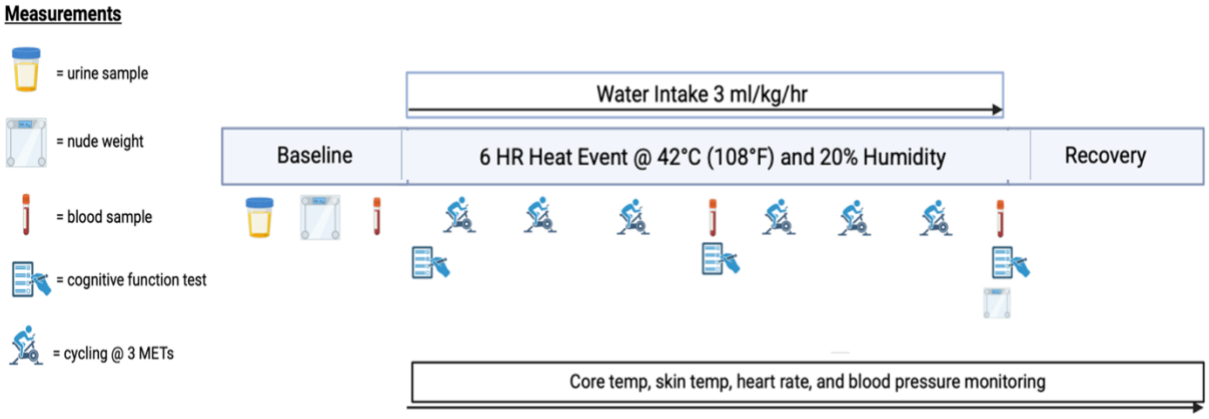


Figure 4.1. Overview of the experimental heat event. Young (n=13) and older (n=14) participants underwent a 6 hour heat exposure at 42°C and 20% relative humidity. To mimic activities of daily living, participants performed six 15 min bouts of light physical activity (~3 METs) every hour of heat exposure. Participants consumed 3 mL/kg body mass of water every hour during the heat exposure. Measurements and assessments included core and skin temperatures, heart rate, blood pressure, sweat loss, blood collections, and cognitive function tests. Created with BioRender.com.

Measurements and Instrumentation

Core temperature was measured every 5 min via ingestible telemetry pill (eCelsius performance pill, BodyCap, Caen, France) swallowed the evening before the start of the heat event visit. Total thermal load expressed as the area under the curve (AUC) was calculated using the trapezoidal method over the duration of heat exposure completed by each participant. Skin temperature was measured continuously as the average of eight thermocron sensors (iButtons, Maxim Integrated, San Jose, CA) located on the forehead, chest, back, forearm, upper arm, abdomen, thigh, and calf for the measurement of skin temperature using the following equation: (0.21× forehead) + (0.06 × forearm) + (0.12 × upper arm) + (0.17 × abdomen) + [0.21 × (chest + back)/2] + (0.15 × thigh) + (0.08 × calf) ([228]. Mean body temperature was calculated with the following equation: Mean body temperature (°C) (0.8 × T_{core}) + (0.2 × T_{skin}), where T_{core} and T_{skin} are core temperature and mean skin temperature. Skin temperature data were converted to 5-min averages. Total sweat loss was calculated as [(pre-heat exposure body weight – post-heat exposure body weight) + (fluid consumed – urine output)] and used to calculate sweat rate: (total sweat loss/total heat exposure time). Percentage changes in body mass were calculated as [(post-heat exposure – pre-heat exposure body weight)/pre-heat exposure body weight] × 100.

Heart rate was measured continuously using a commercially available wireless heart rate monitor (Polar Electro Inc. Lake Success, NY, USA) and is presented at baseline and every 30 min throughout heating. Blood pressure was measured every 20 min using a blood pressure cuff and 3-lead ECG (Tango M2, SunTech Medical, Morrisville, NC) and is presented at baseline and every hour throughout heating. Mean arterial pressure was calculated as one-third systolic pressure plus two-third diastolic pressure, and rate pressure product was calculated as heart rate × systolic blood pressure and are presented at baseline and every hour throughout heating.

Participants were asked to rate their perceived exertion (6–20 scale)[229], thermal sensation (10-point scale; 1 = not hot or cold, 10 = hottest I've ever felt), thermal comfort (TC) (4-point scale; 1 = comfortable, 4 = very uncomfortable)[230], and skin wettedness (-3 to +3 scale; -3 = very dry, 0 = neutral, +3 = very wet) every 20 min during heat exposure and data are presented at baseline and every hour throughout heat exposure.

Cognition

Cognitive function was measured using the NIH Toolbox (NIH Toolbox ® V3 App) tests for cognitive function with the following subdomains of the NIH Toolbox cognition battery: executive function, attention, and processing speed. Attentional control and response inhibition were evaluated with the NIH Toolbox Flanker Inhibitory Control and Attention Test, processing speed was assessed with the NIH Toolbox Pattern Comparison Processing Speed Test, and cognitive flexibility and attention were measured using the NIH Dimensional Change Card Sort Test. All three tests were performed in a randomized order at baseline before heating, halfway through heating, and at the end of the heat event with test instructions given via audio and text on an iPad screen while the same trained researcher pointed out the relevant aspects of the stimuli on the screen during instruction. NIH Toolbox age-adjusted normative scores were used to assess within-group and between-group changes in cognitive performance in response to heat exposure and contextualize performance relative to expected population norms.

Blood Sampling

Venous whole blood samples were collected into four vacutainer tubes (BD, Franklin Lakes, NJ). One sample was collected into a vacutainer treated with potassium ethylenediaminetetraacetic acid (K_2EDTA) and three were collected into serum-separating vacutainers. K_2EDTA samples of whole blood were used to measure hematocrit and hemoglobin, while serum samples rested for 30 minutes to allow for clotting at room temperature before centrifugation. Serum samples were centrifuged at 3000 RPM for 10 minutes, processed and aliquoted into separate microtubes and frozen at -80°C for subsequent analysis. Serum samples were used to determine concentrations of biomarkers via enzyme-linked immunosorbent assays (ELISAs).

Biochemical Analyses

Serum concentrations of interleukin-6 (IL-6), von Willebrand factor (vWF), Protein C, and D-dimer were measured via a commercially available ELISA (ELH-vWF-1, ELH-CoagFXIV-1, ELH-DDIMER-1; RayBiotech Inc.). Although serum was used for vWF, Protein C, and D-dimer assays, all samples were processed under identical conditions to allow reliable

within-subject comparisons despite potential ex vivo effects. To eliminate inter-assay variance, all samples were analyzed in duplicate within the same assay and run by a single technician.

Data and Statistical Analysis

Statistical analyses were performed using Prism 10.4.0 (GraphPad Software, Inc., San Diego, CA). Data were analyzed with a repeated-measures mixed-effects model (Age × Time) to accommodate missing data points, or unpaired, two-tailed t-tests as appropriate. When significant main effects were observed, pairwise comparisons were performed using Šidák's post hoc test . Kaplan–Meier curves were used to depict the proportion of participants completing the 6 h heat exposure. One older participant terminated the heat exposure after ~1 h and was therefore excluded from all analyses requiring prolonged heat exposure (thermoregulatory, cardiovascular, perceptual, and cognitive outcomes). However, because endothelial function and circulating biomarkers were assessed at 24 h pre- and ~24 h post-heat exposure, this participant was retained for analyses of flow-mediated dilation and blood biomarkers. Data are presented as means with 95% confidence intervals. Statistical significance was set a priori at $P < 0.05$, and actual P values are reported where possible.

RESULTS

Thermoregulatory Responses

Tolerance to prolonged heat exposure was assessed using time-to-withdrawal analysis during the 6 h heat exposure. Kaplan–Meier curves demonstrated no difference in early protocol termination in older adults compared with young adults (P=0.074; Fig. 4.2). However, by the end of the 6 h heat exposure, ~85% of young adults (n=11) remained in the protocol compared with ~50% of older adults (n=7). Representative tracings for the core temperature response to heat exposure are depicted in one young and one older adult in Fig. 4.3 to highlight why data were extracted at baseline, end of heating, and final exercise before termination described below.

The thermoregulatory responses to the 6 hour heat exposure are presented in Figs. 4.3-4.9. Core temperature increased from baseline to end of heating in young (P<0.001) and older adults (P<0.001) with no difference between groups (P=0.678; Fig. 4.4A). End core temperature during the last exercise bout completed before protocol termination did not differ between young [37.9°C (37.7, 38.2)] and older adults [38.2°C (37.9, 38.5), P=0.212] (Fig. 4.4B) and peak core temperature did not differ between young [38.0°C (37.8, 38.2)] and older adults [38.3°C (38.0, 38.6), P=0.160] (Fig. 4.4C). The overall change in core temperature from baseline to end of heating did not differ between young [+0.87°C (0.60, 1.13)] and older adults [+1.10°C (0.76, 1.44), P=0.257] (Fig. 4.5A). However, it is important to note that only 11 young and 7 older adults completed the full 6 hour heat exposure. To account for the incomplete data and to better represent the overall thermal load, changes in core temperature are also expressed as the area under the curve (AUC), representing the total thermal load over the duration of heat exposure (°C·h). There was no difference in total thermal load (AUC) between young [2.61°C·h (1.41, 3.80)] and older [2.73°C·h (1.60, 3.86), P=0.875] adults (Fig. 4.5B).

Representative tracings for mean skin and mean body temperature responses to heat exposure are depicted in one young and one older adult in Figs. 4.6A and 4.6B. Mean skin temperature increased from baseline to end of heating in young (P<0.001) and older adults (P<0.001) with no difference between groups (P=0.225; Fig. 4.7A). End mean skin temperature during the last exercise bout completed before protocol termination was greater in young [38.2°C (37.9, 38.5)] compared to older adults [37.7°C (37.3, 38.1), P<0.050] (Fig. 4.7B) and peak mean skin temperature did not differ between young [38.2°C (37.9, 38.5)] and older adults [38.0°C (37.6, 38.4), P=0.305] (Fig. 4.7C). Mean body temperature increased from baseline to end of heating in young (P<0.001) and older adults (P<0.001) with no difference between groups (P=0.734; Fig. 4.8A). End mean body temperature during the last exercise bout completed before protocol termination did not differ between young [37.9°C (37.7, 38.2)] and older adults [38.1°C (37.7, 38.4), P=0.544] (Fig. 4.8B) and peak mean body temperature did not differ between young [38.0°C (37.8, 38.2)] and older adults [38.1°C (37.8, 38.4), P=0.468] (Fig. 4.8C).

Sweat rate did not differ between young [0.38 $L \cdot kg^{-1} \cdot h^{-1}$ (0.32, 0.43)] and older adults [0.36 $L \cdot kg^{-1} \cdot h^{-1}$ (0.31, 0.42), P=0.706] (Fig. 4.9A). However, percent change (Δ) in body mass

was greater in young [-3.16% (-3.64, -2.68)] vs older adults [-2.50% (-2.87, -2.13), P=0.027] (Fig. 4.9B).

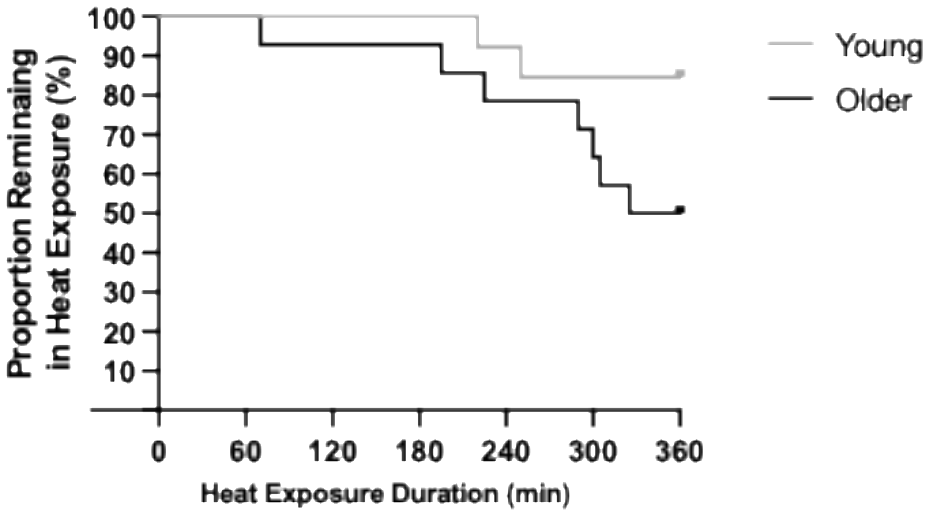


Figure 4.2. Kaplan–Meier curves for the proportion of participants remaining in the 6 h heat exposure protocol as a function of exposure duration. Withdrawal from the protocol for any physiological or safety reason was treated as an event. Curves are shown separately for young and older adults, and group differences were assessed using a log-rank (Mantel–Cox) test.

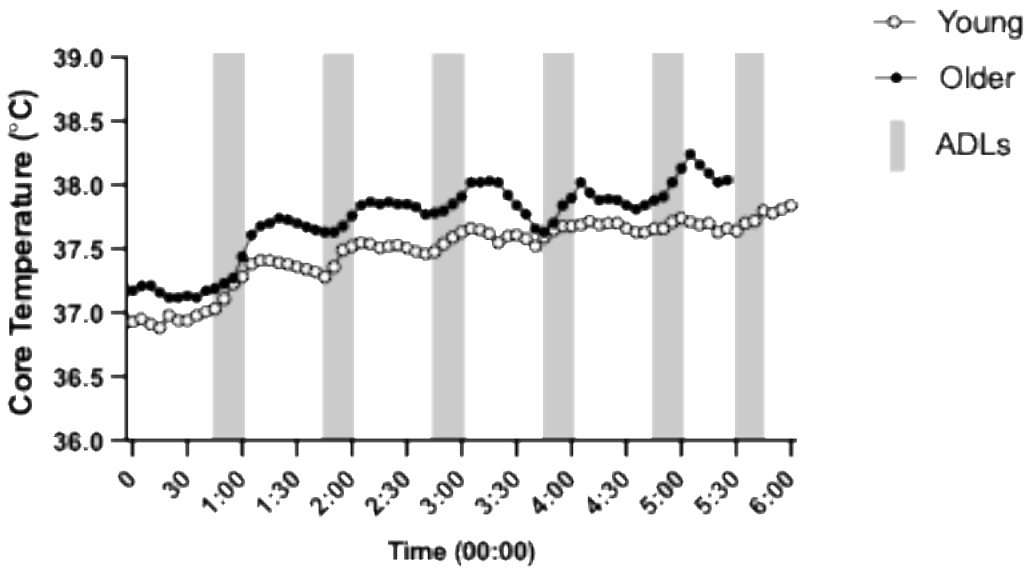


Figure 4.3. Representative tracings for core temperature responses during the 6 h heat exposure in one young and one older adult. Exercise was performed for 15 min every 45 min (ending on the hour), except during the final hour (5:30–5:45). Exercise bouts to mimic activities of daily

living (ADLs) are represented by the gray shaded regions. The tracings demonstrate changes in core temperature with exercise as well as progressive heat accumulation and variability in heat tolerance, including early termination in some older adults.

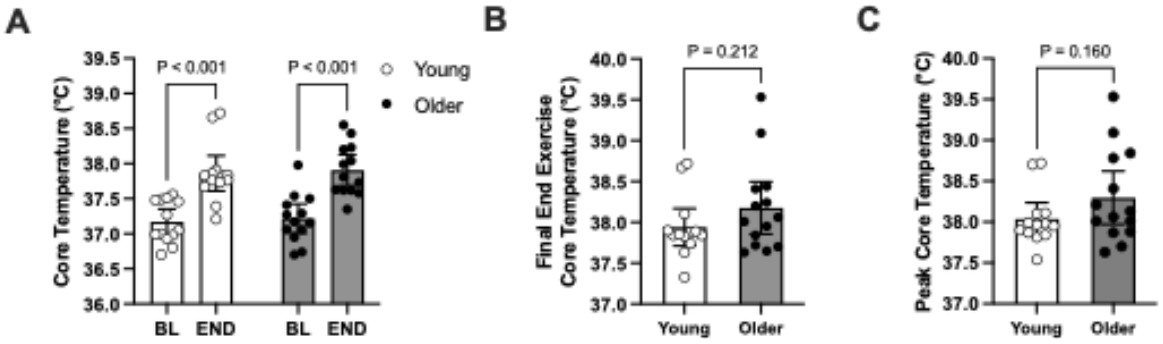


Figure 4.4. (A) Core temperature at baseline (BL) and end of heating (END), (B) core temperature measured at the conclusion of the final 15-min exercise bout (completed at 5:45 or preceding protocol termination), and (C) peak core temperature throughout heat exposure in young and older adults. Data were analyzed with unpaired t-tests or a repeated measures mixed-effects model and pairwise comparisons were made using Šidák's post hoc tests. Data are presented as means with 95% confidence intervals.

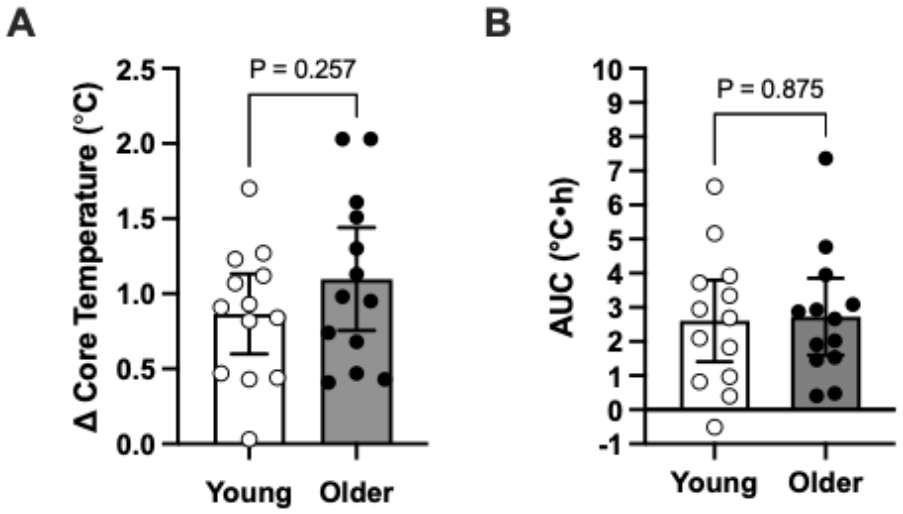


Figure 4.5. (A) Overall change (Δ) in core temperature from baseline to end of heating and (B) cumulative core temperature responses (AUC) during heat exposure in young and older adults. Thermal load is expressed as the area under the curve (AUC; °C·h), calculated over the duration of heat exposure completed by each participant. Participants who terminated the protocol early

contributed partial AUC values reflecting their individual cumulative thermal load, whereas participants who completed the full protocol contributed 6-h AUC values. AUC values therefore represent cumulative exposure rather than standardized 6-h thermal load. Data were analyzed with unpaired t-tests and are presented as means with 95% confidence intervals.

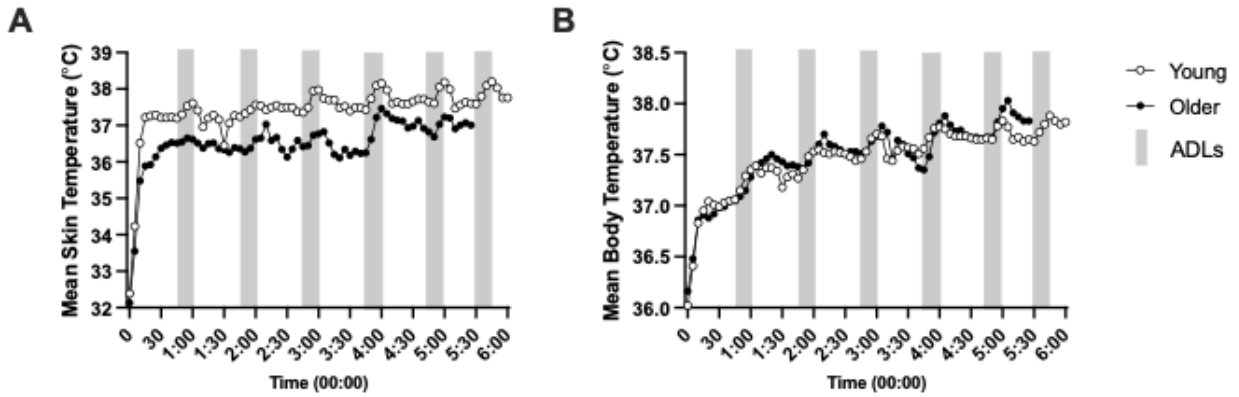


Figure 4.6. Representative tracings for (A) mean skin temperature and (B) mean body temperature responses during the 6 h heat exposure in one young and one older adult. Exercise was performed for 15 min every 45 min (ending on the hour), except during the final hour (5:30–5:45). Exercise bouts to mimic activities of daily living (ADLs) are represented by the gray shaded regions. The tracings demonstrate changes in mean skin and body temperatures with exercise as well as progressive heat accumulation and variability in heat tolerance, including early termination in some older adults.

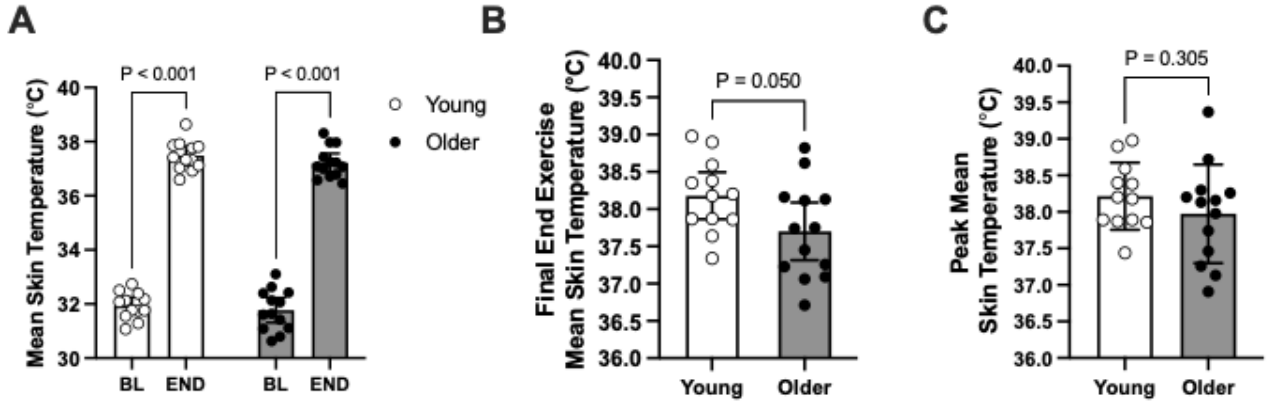


Figure 4.7. (A) Mean skin temperature at baseline (BL) and end of heating (END), (B) mean skin temperature measured at the conclusion of the final 15-min exercise bout (completed at 5:45 or preceding protocol termination), and (C) peak mean skin temperature throughout heat exposure in young and older adults. Data were analyzed with unpaired t-tests or a repeated measures mixed-effects model and pairwise comparisons were made using Šidák's post hoc tests. Data are presented as means with 95% confidence intervals.

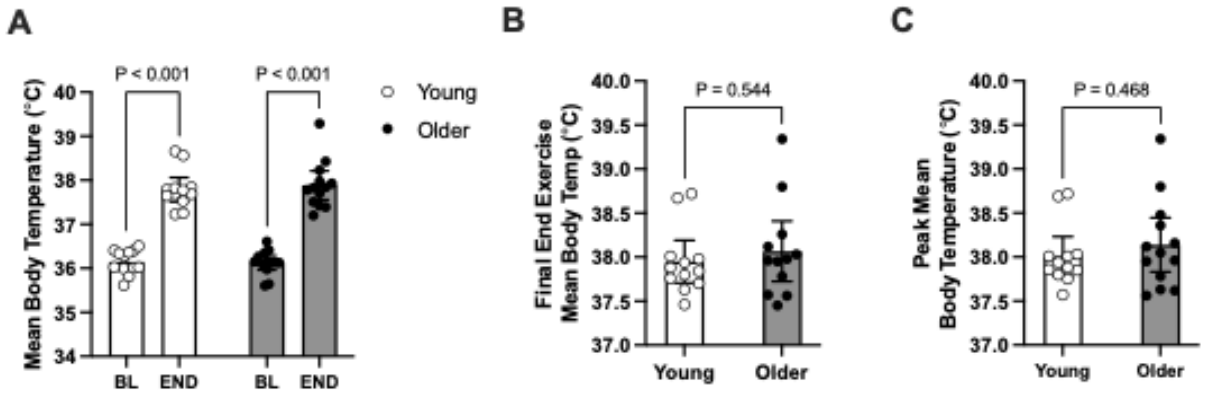


Figure 4.8. (A) Mean body temperature at baseline (BL) and end of heating (END), (B) mean body temperature measured at the conclusion of the final 15-min exercise bout (completed at 5:45 or before protocol termination) and (C) peak mean body temperature throughout heat exposure in young and older adults. Data were analyzed with unpaired t-tests or a repeated measures mixed-effects model and pairwise comparisons were made using Šidák's post hoc tests. Data are presented as means with 95% confidence intervals.

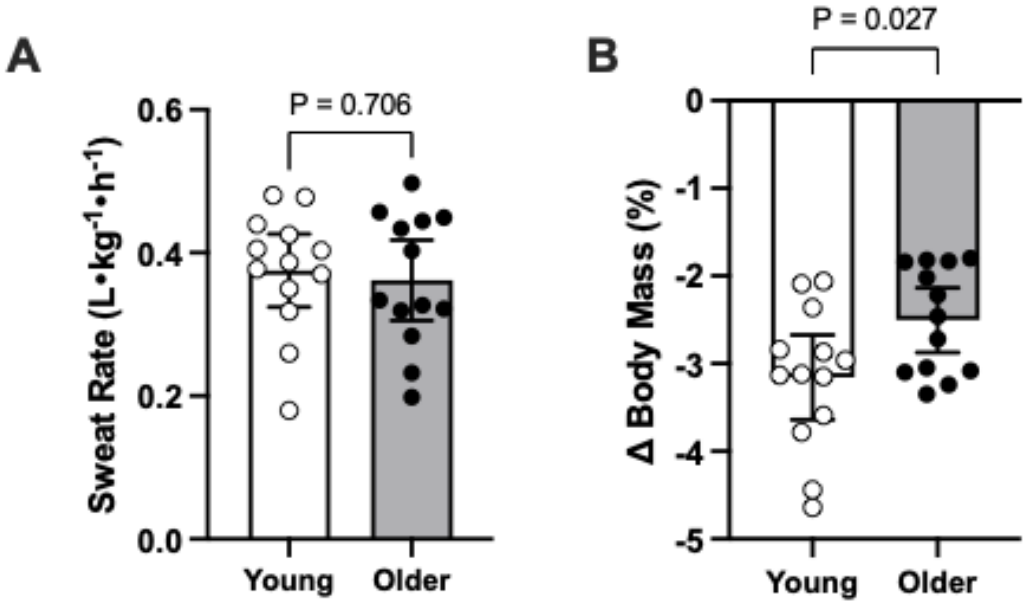


Figure 4.9. (A) Sweat rate and (B) percent change (Δ) in body mass in young and older adults during heat exposure. Sweat rate was calculated as total sweat loss divided by individual heat exposure duration and normalized to body mass. Percent change in body mass reflects the relative degree of dehydration. Data were analyzed using unpaired t-tests and are presented as means with 95% confidence intervals.

Cardiovascular Responses

The cardiovascular responses to heat exposure in young and older adults are presented every 30 min throughout heat exposure in Table 4.2. Heart rate increased over time in both groups ($P<0.001$) and there was a main effect of age ($P=0.028$), with no age × time interaction ($P=0.110$). Systolic blood pressure increased over time in both groups ($P<0.001$) with no effect of age ($P=0.488$), however there was an age × time interaction ($P=0.002$). Diastolic blood pressure increased over time in both groups ($P<0.001$) with a main effect of age ($P=0.029$), and no age × time interaction ($P=0.938$). Mean arterial pressure calculated from systolic and diastolic pressures increased over time in both groups ($P=0.017$) with no effect of age ($P=0.077$) or age × time interaction ($P=0.640$). Rate pressure product calculated as the product of heart rate and

systolic pressure increased over time in both groups ($P<0.001$) with no effect of age ($P=0.217$), however there was an age × time interaction ($P=0.008$).

Table 4.2. Cardiovascular responses at baseline and every 30 min throughout heating in young and older adults.

Time Point	Group	Heart Rate (bpm)	Systolic (mmHg)	Diastolic (mmHg)	Mean (mmHg)	Rate Pressure Product (bpm • mmHg)
Baseline	Young (n=13)	74 (66, 82)	112 (104, 119)	72 (65, 79)	85 (78, 92)	8317 (7261, 9373)
	Older (n=13)	67 (61, 72)	124 (115, 134)	79 (73, 84)	94 (88, 100)*	8277 (7411, 9143)
00:30	Young (n=13)	89 (80, 98)				
	Older (n=13)	74 (69, 79)*				
1:00 (end exercise)	Young (n=13)	110 (103, 117)	125 (118, 132)	63 (57, 70)	84 (79, 89)	13665 (9071, 10511)
	Older (n=13)	105 (95, 115)	141 (129, 154)	71 (64, 79)	93 (86, 100)	14406 (12748, 16063)
1:30	Young (n=13)	91 (86, 95)				
	Older (n=13)	81 (72, 90)				
2:00 (end exercise)	Young (n=13)	113 (103, 123)	126 (117, 135)	63 (56, 70)	84 (79, 89)	14186 (12275, 16096)
	Older (n=13)	107 (98, 116)	133 (121, 145)	72 (64, 81)	92 (84, 100)	14122 (12715, 15529)
2:30	Young (n=13)	92 (87, 97)				
	Older (n=13)	82 (74, 90)				
3:00 (end exercise)	Young (n=13)	119 (109, 130)	124 (114, 134)	60 (54, 65)	80 (75, 84)	15086 (12747, 17424)
	Older (n=13)	112 (103, 120)	129 (118, 140)	69 (62, 76)	89 (81, 96)	14284 (12967, 15601)
3:30	Young (n=13)	98 (90, 106)				
	Older (n=12)	91 (81, 102)				
4:00 (end exercise)	Young (n=11)	132 (119, 145)	132 (120, 145)	60 (54, 67)	84 (81, 88)	17587 (14508, 20665)
	Older (n=11)	118 (106, 129)*	126 (115, 138)	70 (55, 85)*	89 (76, 101)	14799 (12964, 16635)*
4:30	Young (n=11)	104 (96, 113)				
	Older (n=12)	93 (82, 104)				
5:00 (end exercise)	Young (n=11)	133 (118, 149)	130 (120, 140)	58 (51, 65)	82 (78, 86)	17390 (14771, 20009)
	Older (n=10)	118 (106, 130)*	127 (114, 141)	70 (58, 83)*	88 (75, 101)	14668 (12614, 16722)*
5:30	Young (n=11)	116 (102, 130)				

	Older (n=7)	89 (83, 95)				
6:00	Young (n=11)	107 (98, 116)	118 (110, 127)	70 (64, 75)	86 (82, 89)	12683 (11392, 13973)
	Older (n=7)	90 (83, 98)*	110 (98, 123)	77 (65, 88)	88 (77, 99)	9934 (8685, 11184)

Data presented as mean with 95% Cis. * P < 0.05 young vs older.

Perceptual Responses

Perceptual responses during heat exposure are presented in Table 4.3. At baseline, ratings of perceived exertion (RPE), thermal sensation (TS), thermal comfort (TC), and skin wettedness were similar between young and older adults. RPE increased over the course of heating in both groups; however, older adults reported significantly higher RPE compared with young adults at 1, 2, 3, and 5 hours (P<0.05). In contrast, TS, TC, and skin wettedness increased similarly over time in both groups, with no between-group differences observed at any time point. By the end of the protocol, perceptual ratings in both young and older adults reflected marked thermal strain and discomfort, despite comparable subjective thermal and skin wettedness responses between groups.

Table 4.3. Perceptual responses at baseline and every hour throughout heating in young and older adults.

Time Point	Group	RPE (6-20)	TS (0-10)	TC (1-4)	Skin Wettedness (-3-3)
Baseline	Young (n=13)	6 (6, 6)	0 (0, 1)	1 (1, 1)	-1 (-2, 0)
	Older (n=13)	6 (6, 6)	1 (0, 1)	1 (1, 2)	-2 (-3, -1)
1:00 (end exercise)	Young (n=13)	9 (8, 9)	5 (4, 6)	2 (2, 2)	1 (1, 1)
	Older (n=13)	11 (10, 12)*	5 (4, 7)	2 (2, 2)	1 (1, 2)
2:00 (end exercise)	Young (n=13)	9 (8, 10)	6 (5, 7)	2 (2, 2)	2 (2, 2)
	Older (n=13)	12 (10, 13)*	6 (4, 7)	2 (2, 3)	2 (1, 2)
3:00 (end exercise)	Young (n=13)	10 (8, 11)	7 (6, 8)	3 (2, 3)	2 (2, 2)
	Older (n=13)	12 (10, 14)*	5 (4, 7)	3 (2, 3)	2 (1, 2)
4:00 (end exercise)	Young (n=11)	11 (9, 12)	7 (6, 8)	3 (2, 3)	2 (2, 2)
	Older (n=11)	13 (10, 15)	6 (4, 8)	3 (2, 3)	2 (2, 2)
5:00 (end exercise)	Young (n=11)	11 (9, 13)	7 (6, 8)	3 (2, 3)	2 (2, 3)
	Older (n=10)	13 (10, 16)*	6 (4, 8)	3 (2, 3)	2 (2, 2)
6:00	Young (n=11)	9 (7, 11)	7 (5, 8)	3 (2, 3)	2 (2, 2)
	Older (n=10)	10 (7, 14)	5 (3, 7)	2 (1, 3)	2 (1, 2)

RPE, rating of perceived exertion; TS, thermal sensation; TC, thermal comfort. Data presented as mean with 95% CIs. * $P < 0.05$ young vs older.

Vascular Endothelial Function

Brachial artery flow-mediated dilation (FMD) differed by age ($P < 0.001$), with older adults exhibiting lower FMD [4.4% (3.0, 5.8)] compared to young [8.1% (6.3, 9.9), P=0.001] at baseline 24 h before heat exposure (Fig. 4.10A). There was no main effect of time (P=0.067) and no age × time interaction (P=0.403), however there was a trend toward decreasing FMD from 24 h pre- [4.4% (3.0, 5.8)] to ~24 h post heat exposure [3.1% (2.0, 4.2), P=0.062] heat exposure in the older adults. FMD remained lower in older [3.1% (2.0, 4.2)] compared with young adults [7.6% (5.5, 9.7), P<0.001] ~24 h post heat exposure (Fig. 4.10A). After normalization to shear

rate area under the curve, shear-adjusted FMD also differed by age (P=0.004), with lower shear-adjusted FMD in older [2.3% (1.4, 3.2)] compared with young [3.6% (2.5, 4.6), P=0.027] adults at baseline 24 h before heat exposure and at ~24 h post heat exposure [older: 1.8% (1.2, 2.4); young: 3.4% (2.6, 4.2), P=0.004]. There was no main effect of time (P=0.269) and no age × time interaction (P=0.543) for shear-adjusted FMD (Fig. 4.10B). All other FMD determinants are presented in Table 4.4.

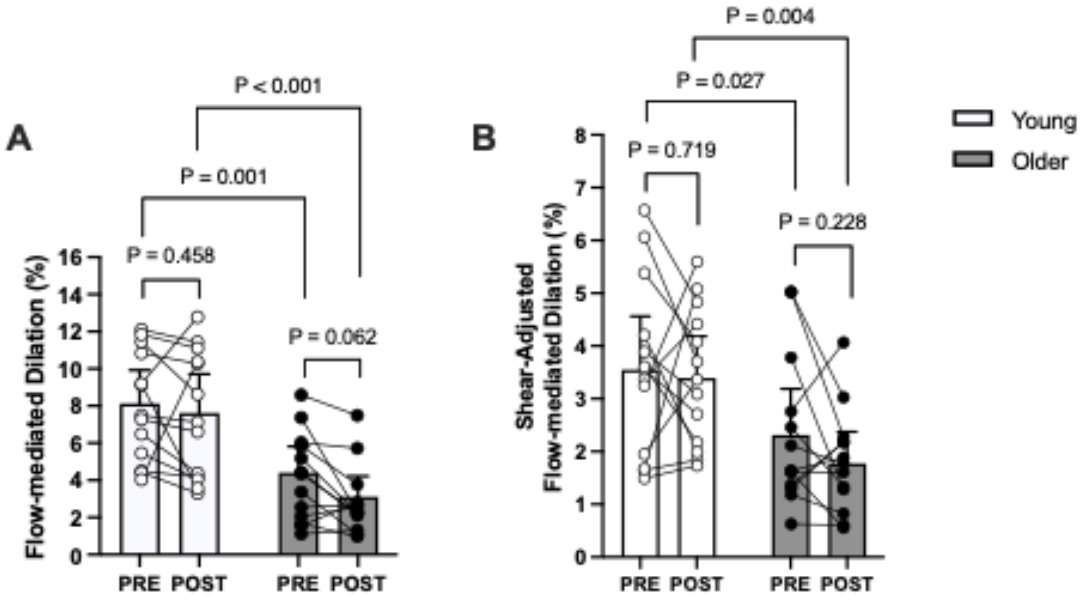


Figure 4.10. (A) Brachial artery flow-mediated dilation (FMD) expressed as percent change in diameter and (B) shear rate-adjusted FMD expressed as % change in diameter normalized to the shear stimulus measured at 24 h before (PRE) and 24 h after (POST) the start of heat exposure in young and older adults, accounting for differences in the hyperemic shear stimulus.

Table 4.4. Brachial artery flow-mediated dilation (FMD) and its hemodynamic determinants in young and older adults 24 h before (PRE) and ~24 h post (POST) heat exposure.

	Group	PRE	P value	POST	P value
Shear Rate (AUC)	Young	28608 (17572, 39643)	0.244	23300 (14417, 32183)	0.664
	Older	21889 (15045, 28733)		20807 (13012, 28603)	
Reactive Hyperemia ($mL \cdot min^{-1}$)	Young	102.9 (77.4, 128.4)	0.052	109.95 (84.68, 135.23)	0.143
	Older	142.7 (110.0, 175.4)		139.6 (102.0, 177.2)	
Baseline Diameter (mm)	Young	3.61 (3.3, 3.92)	0.013	3.61 (3.28, 3.93)	0.012
	Older	4.27 (3.82, 4.72)		4.28 (3.82, 4.73)	
Peak Diameter (mm)	Young	3.89 (3.6,0, 4.18)	0.031	3.87 (3.57, 4.17)	0.039
	Older	4.45 (3.99, 4.92)		4.41 (3.93, 4.88)	
Δ Diameter (mm)	Young	0.29 (0.24, 0.34)	0.005	0.25 (0.19, 0.31)*	0.002
	Older	0.19 (0.13, 0.25)		0.13 (0.08, 0.18)*	
Time to Peak Diameter (s)	Young	59.6 (28.7, 90.6)	0.756	36.5 (27.0, 46.0)	0.103
	Older	55.2 (38.9, 71.5)		60.1 (35.6, 84.6)	
Forearm Vascular Conductance ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	Young	1.22 (0.91, 1.52)	0.155	1.34 (0.99, 1.69)	0.234
	Older	1.54 (1.23, 1.86)		1.58 (1.16, 2.00)	

Data presented as mean with 95% CIs. P-values represent comparisons between young and older adults. *P < 0.05 PRE vs POST within groups.

Biomarkers of Inflammation, Endothelial Function, and Coagulation

Interleukin (IL)-6 increased following heat exposure (P<0.001). There was no main effect of age (P=0.684) and no age × time interaction (P=0.934), indicating a similar inflammatory response to heat in young and older adults (Fig. 4.11A). Circulating von Willebrand Factor (vWF) differed by age (P=0.005), with a main effect of time (P=0.009) and an age × time interaction (P=0.040). Serum vWF increased in older adults from baseline [28.3 ug/mL (15.1,

41.4)] to end of heating [45.2 ug/mL (22.8, 67.7), P=0.036] (Fig. 4.11B). D-dimer did not change across time (P=0.563) and there was no main effect of age (P=0.459) and no age × time interaction (P=0.100). However, D-dimer was greater in older adults [32.7 ng/mL (-8.5, 73.8)] compared to young adults [8.1 (4.1, 12.2), P=0.048] at the end of heating (Fig. 4.11C). Protein C did not change across time (P=0.331) and there was no main effect of age (P=0.893) and no age × time interaction (P=0.430) (Fig. 4.11D)

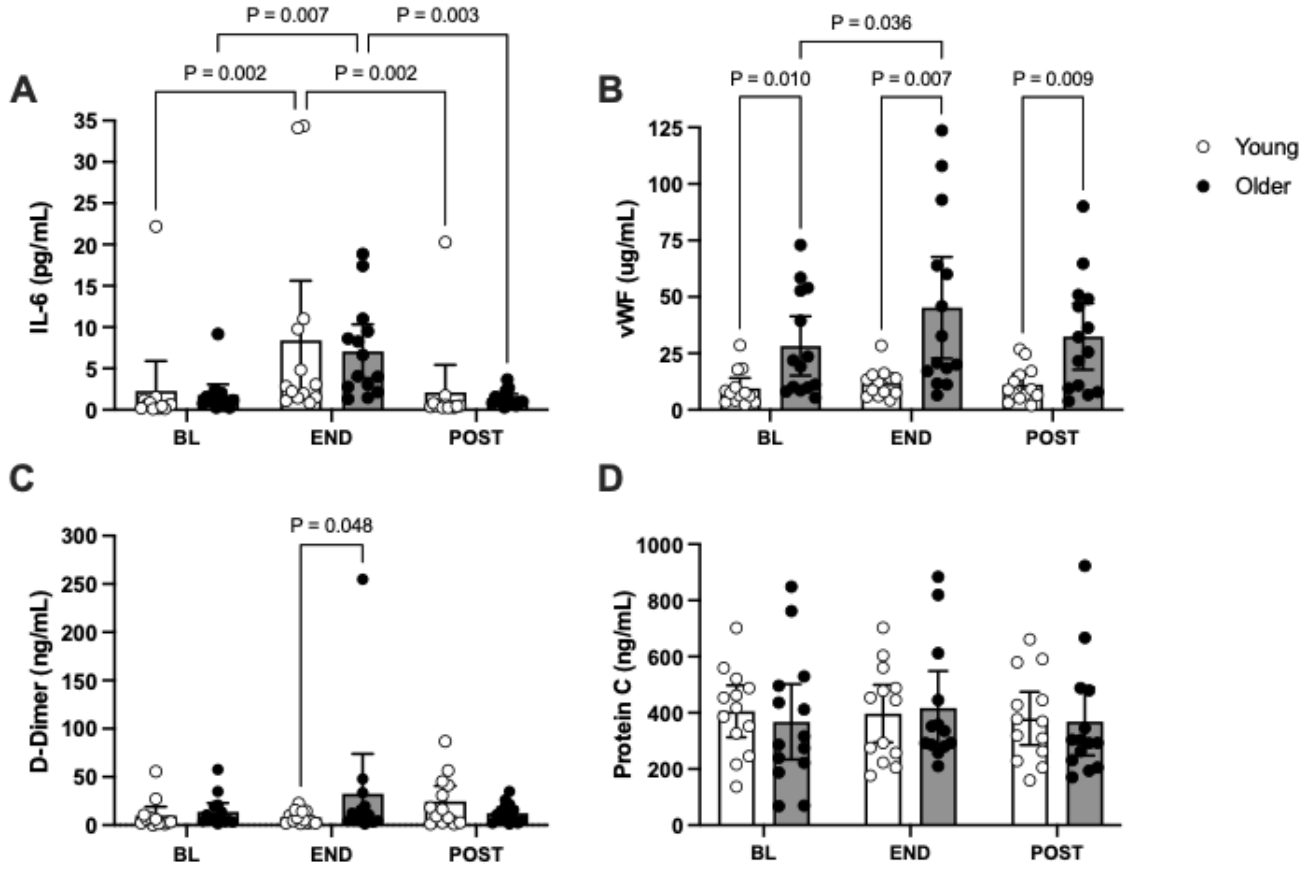


Figure 4.11. (A) Changes in serum interleukin (IL)-6, (B) serum von Willebrand Factor (vWF), (C) serum D-dimer, and (D) serum Protein C concentrations at baseline (BL), end of heating (END), and ~24 post-heating (POST) in young and older adults. Data were analyzed using repeated-measures mixed-effects models and pairwise comparisons were made using Šidák's post hoc tests. Data are presented as means with 95% confidence intervals.

Cognitive Function

Age-adjusted standard scores for NIH Toolbox tests of cognitive function across heat exposure are shown in Figure 4.12. For the Dimensional Change Card Sort (DCCS) Test, scores increased over time in both groups (P=0.004), with an increase from baseline to end of heat exposure observed in both young (P=0.007) and older adults (P=0.020; Fig. 4.12A). Despite this improvement, mean DCCS scores for both groups remained near age-normative values throughout the protocol. Pattern Comparison (PC) scores increased from baseline to halfway through heat exposure and end of heat exposure in both young and older adults (P<0.05 for all within-group comparisons; Fig. 4.12B). These improvements resulted in PC scores exceeding age-adjusted normative means at halfway through and end of heating in both groups. There was a main effect of time for the Flanker Inhibitory Control and Attention Test scores (P=0.022), but there was no difference from baseline to halfway through or end of heating in either group (Fig. 4.12C).

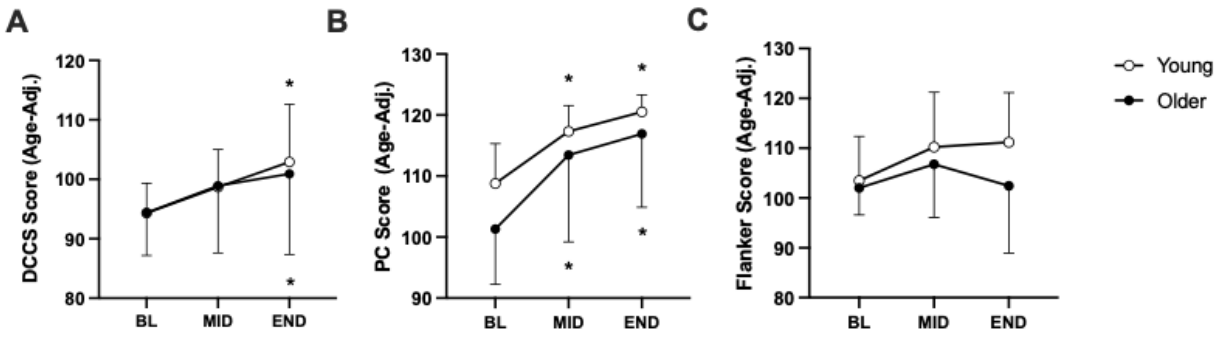


Figure 4.12. Age-adjusted (Age-Adj.) standard scores for (A) Dimensional Change Card Sort (DCCS), (B) Pattern Comparison (PC), and (C) Flanker Inhibitory Control and Attention Test at baseline (BL), mid-heat exposure (MID), and end of heat exposure or early protocol termination (END). Scores are normalized to age-matched population norms (distributed at M=100, SD=15). Age-adjusted standard scores compares the score of the test-taker to those in the NIH Toolbox nationally representative normative sample within the same age band, where a score of 100 indicates performance that was at the national average for the test-taking participant's age. Data are presented as mean with 95% confidence intervals. Asterisk (*) denotes difference from baseline within group.

DISCUSSION

The purpose of this study was to examine the thermoregulatory and cardiovascular responses to 6 h of extreme heat exposure in young and older adults and determine whether prolonged heat exposure elicits difference in vascular endothelial dysfunction, coagulation activation, and cognitive dysfunction in young versus older adults. We found that 6 h of heat exposure at 42°C and 20% relative humidity with periods of light physical activity to mimic activities of daily living elicited substantial thermal and cardiovascular strain in both young and older adults, and although the overall change in core temperature, peak core temperatures, and cumulative thermal load did not differ between age groups, fewer older adults were able to tolerate the full duration of heat exposure compared to young adults. In older adults, prolonged heat exposure was associated with evidence of endothelial injury, indicated by increased circulating von Willebrand factor, while systemic coagulation markers and cognitive function remained unaffected. Overall, these results suggest that prolonged heat exposure induces subclinical vascular stress in older adults that may precede vascular dysfunction and pathological outcomes during real-world heatwaves.

Thermoregulatory responses

A notable finding of this study is that older adults exhibited reduced tolerance to prolonged heat exposure despite experiencing a thermal load similar to that of young adults. Measures of core temperature, including peak values, end-exercise temperatures, and total thermal load expressed as area under the curve, were similar between groups. Yet a greater proportion of older adults terminated the protocol early (n=7), whereas only two younger adults terminated the protocol early. Most of the older adults terminated the protocol due to symptoms of heat-related illness such as fatigue, light-headedness, muscle cramps, or nausea, whereas one participant was removed from the environmental chamber due to hypotension, and another was removed after reaching a core temperature of 39.5°C. Thus, older adults who completed the full 6 h of heat exposure may represent a more heat-tolerant population subset, potentially underestimating the magnitude of physiological vulnerability in the broader population. These findings indicate that heat intolerance in aging is not solely determined by absolute thermal

strain, but rather by the capacity to physiologically compensate for and tolerate sustained heat stress. This supports the concept that aging is associated with diminished physiological reserve, as older adults may reach levels of thermal strain and heat intolerance at environmental exposures that remain tolerable for younger individuals.

Although core temperature responses were similar between groups, the reduced heat tolerance in older adults may be due in part to peripheral thermal responses. While mean skin temperature increased similarly in both groups (Fig. 4.7A), young adults exhibited higher skin temperatures during the final exercise bout before protocol termination compared to older adults (Fig. 4.7B; P=0.050), suggesting a greater capacity for cutaneous heat dissipation[231]. Additionally, older adults maintained lower skin temperatures despite similar thermal strain throughout heating (main effect of group: P=0.044), likely due to the age-related reductions in cutaneous vasodilation and skin blood flow[68,71,79]. This impaired ability to redistribute blood to the skin may have reduced overall heat loss, resulting in early termination, and increased the cardiovascular demands needed to maintain thermal balance.

Sweat rate did not differ between young and older adults (P=0.705; Fig. 4.9A), indicating no detectable age-related difference in sweat rate under the present study conditions. However, young adults lost a greater percentage of body mass (-3.2%) during the heat exposure compared with older adults (-2.5%) (Fig. 4.9B), reflecting greater relative dehydration. The larger reduction in percent body mass observed in young adults likely reflects differences in body size and a greater tolerance for progressive dehydration over longer durations of heat exposure. Despite a similar body surface area between age groups older adults had a greater body mass (Table 4.1), resulting in a lower surface-area-to-mass ratio, which may have reduced heat dissipation per unit mass[232] and increased reliance on cardiovascular compensation. The smaller percentage of body mass deficit in older adults likely reflects earlier withdrawal from the protocol, which limited the progression of dehydration, rather than indicating enhanced hydration status or superior heat tolerance compared to young adults.

Recent work by Meade et al.[80] evaluated physiological responses to daylong (9 h) heat exposure at 40°C and 9% relative humidity in young and older adults. Older participants stored more heat during the initial hours of exposure, such that core temperature was greater in older compared with young adults at hour 6; however, no differences in heat storage were observed between groups from hours 3-9 of the heat exposure. The authors attributed the greater early heat

storage in older adults to an age-related increase in the core temperature threshold for activation of cutaneous vasodilation and sweating, whereby once this threshold is reached, whole-body evaporative heat loss becomes sufficient to offset total heat gain [76,233]. Additionally, total blood volume declines with age [234], which may limit the ability to redistribute blood to the skin during heat exposure and contribute to altered thermoeffector responses compared with young adults. This framework may also explain the present findings. Older adults who tolerated the full 6 h of heat exposure likely achieved heat balance by the later stages of the protocol, resulting in end core temperatures comparable to those of young adults. In contrast, older adults who terminated the protocol early may have had limitations in heat-compensatory capacity during the initial phases of heat exposure, leading to early symptom-related withdrawal despite experiencing a similar absolute thermal load to younger adults. Together, these findings highlight that the observed preservation of thermoregulatory function in aging may come at the cost of increased physiological strain and reduced overall heat tolerance, which may contribute to heightened heat vulnerability during real-world heatwaves.

This is an important observation, as previous studies investigating the thermoregulatory responses to extreme heat exposure in young and older adults have implemented 3 h heat exposures and have yielded conflicting results. It has been reported that during 3 h of heat exposure at 44°C and 30% relative humidity, older adults experience greater heat gain in the absence of age-related differences in core temperature[63,84], whereas a study implementing 3 h of heat exposure at 47°C and 15% humidity where participants also performed light cycling to mimic utilizing activities of daily living reports higher end core temperatures in older compared to young adults[84]. These conflicting findings highlight that shorter-duration exposures, even those utilizing higher, yet ecologically relevant ambient conditions with physical activity to simulate activities of daily living, may be insufficient to fully capture the physiological responses that develop during prolonged heat stress in older adults. Thus, heat strain and illness without overt changes in core temperature should be considered when evaluating heat-related health risks in older adults.

Cardiovascular responses

Prolonged heat exposure resulted in progressive cardiovascular strain in both groups, with older adults displaying hemodynamic patterns that may contribute to increased heat vulnerability. Older adults exhibited higher diastolic blood pressures and blunted elevations in heart rate compared to young adults during light exercise to simulate activities of daily living at the later stages of hours 4 and 5 during heat exposure (Table 4.2). Interestingly, no differences in heart rate or diastolic blood pressure were observed during passive heat stress when participants remained seated. Thus, the higher diastolic pressures at the end of exercise at hours 4 and 5 may reflect a diminished capacity for exercise-induced vasodilation[235] and/or arterial stiffening with aging[100], as aging is associated with impaired endothelial-dependent vasodilation and increased reliance on sympathetic vasoconstrictor tone during exercise[235,236].

Additionally, the blunted increase in heart rate in older adults during exercise likely reflects diminished cardiovascular reserve and a blunted increase in cardiac output to facilitate blood flow to exercising muscle[237,238], which, when coupled with heat stress, likely exacerbates cardiovascular strain as blood pressure is typically relatively well-maintained during passive heat stress in older individuals[72]. Older adults reported higher RPEs (Table 4.3), indicating that they perceived light exercise to mimic activities of daily living during the heat exposure as more physically taxing compared to young adults, aligning with evidence of greater cardiovascular strain. Such cardiovascular demand may increase stress on the endothelium by repeatedly elevating shear forces and sympathetic activation[239–241], potentially contributing to downstream vascular dysfunction even in the absence of marked differences in core temperature.

The findings in the present study suggest that while thermoregulatory mechanisms may remain intact and older adults can maintain core temperature within acceptable limits during prolonged heat exposure, they do so with greater cardiovascular strain and diminished overall tolerance to heat. Consequently, older adults appear more likely to reach symptoms of heat illness and termination at thermal loads that younger adults can still tolerate, emphasizing a mismatch between standard indicators of thermal strain and actual functional heat tolerance with aging.

Endothelial function

Older adults exhibited lower baseline brachial artery flow-mediated dilation compared with young adults, even after normalization for shear stimulus (Fig 4.10A&B), confirming the presence of pre-existing endothelial dysfunction in older adults[102,242,243]. While prolonged heat exposure did not produce a significant decline in flow-mediated dilation in either group, there was a trend toward reduced endothelial function in older adults ~24 h post-heat exposure (P=0.062). This pattern indicates that although endothelial vasodilatory capacity appears largely preserved following a single prolonged heat exposure, aging may increase susceptibility of the endothelium to subtle or delayed heat-induced endothelial impairments that are not fully captured by brachial artery flow-mediated dilation alone. In support of this, we observed an increase in von Willebrand factor (vWF) in older adults at the end of heating (Fig. 4.11B). Von Willebrand Factor is synthesized and stored in endothelial cells[244] and is a well-established marker of endothelial activation and injury[194,195]. The elevation of vWF in older adults indicates a heat-induced perturbation of endothelial homeostasis and a potentially pro-thrombotic vascular state[244], as secretion of vWF via exocytosis of Weibel–Palade bodies[245] is a hemostatic response to vascular injury, helping initiate platelet adhesion at sites of damage by serving as a carrier for factor VIII[246]. Notably, this increase occurred despite a similar inflammatory response as observed by increases in IL-6 in both groups (Fig. 4.11A), suggesting that endothelial activation was not simply a consequence of systemic inflammation.

Heat stress can damage endothelial cells through direct thermal cytotoxicity and in more severe cases, through secondary systemic inflammatory response syndrome (SIRS)[212]. Although leukocyte count and respiratory rate were not measured in the present study, it is unlikely that our participants met the clinical criteria for SIRS, as elevations in core temperature and heart rate occurred as expected during heat stress. However, subclinical endothelial perturbation remains plausible. Specifically, damage to the glycocalyx, the endovascular barrier structure that covers the surface of endothelial cells and is critical for vascular homeostasis[212], is plausible to have occurred in our older participants, as suggested by the observed increase in vWF. The glycocalyx is involved in mechanical signal transduction, inflammation, oxidative stress, and inhibition of coagulation, and age-related thinning of the glycocalyx may increase susceptibility to heat-induced vascular stress[247].

Although flow-mediated dilation (FMD) was not significantly reduced in older adults ~24 h post-heat exposure, there was a trend toward a reduction in unadjusted FMD (%)

(P=0.063) along with elevated vWF immediately following exposure, suggesting that early endothelial activation or injury may precede overt impairments. A potential mechanistic link between these findings is a shift toward disturbed or oscillatory shear during heating, particularly during low-intensity exercise to mimic activities of daily living. Heat stress promotes cutaneous vasodilation and a redistribution of blood flow to the skin, while exercise promotes increased blood flow to exercising muscle with increased sympathetic activation to inactive vascular beds to preserve arterial pressure. Together, these competing mechanisms may increase oscillatory shear. Age-related decrements in vasodilatory capacity[102,211,242] and increased arterial stiffness[101,248] may further heighten exposure to disturbed shear in older adults, offering a plausible mechanistic link to the observed endothelial activation reflected by increased circulating von Willebrand factor. Oscillatory shear is known to increase endothelin (ET)-1[249], a potent vasoconstrictor, and proinflammatory endothelial vascular adhesion molecules (VCAM-1)[250,251], decrease endothelial nitric oxide synthase (eNOS) expression, and increase oxidative stress (ROS)[252–254], collectively impairing nitric oxide bioavailability and potentially contributing to endothelial dysfunction.

While no studies have investigated the impact of extreme heat exposure on endothelial function, a study by McCormick et al.[129] found that 9 h of heat exposure resulted in greater heat-induced cytotoxicity, as evidenced by autophagic dysfunction and increased apoptotic and inflammatory signaling in older compared to young adults. This autophagic dysregulation and heat-induced cytotoxicity may contribute to age-related heat vulnerability and the increased risk for endothelial activation and injury as observed in the present study. These findings support the idea that prolonged heat exposure directly stresses the vascular endothelium in older adults, potentially through sustained cardiovascular strain and shear stress, heat-induced cytotoxicity, and disturbances to the endothelial glycocalyx.

Coagulation

D-dimer and Protein C were selected as complementary biomarkers in the present study to assess coagulation activation and regulation during prolonged heat exposure. D-dimer is a fibrin degradation product, serving as a byproduct of blood clotting and subsequent fibrinolysis, or clot breakdown[255–257]. Thus, D-dimer represents activation of the coagulation-fibrinolytic

pathway[258], whereas Protein C is an endogenous anticoagulant that prevents excessive blood clotting[259] and is consumed in response to activated coagulation or may be functionally impaired during sustained coagulation activation[260]. We would therefore expect activation of systemic coagulation to be indicated by an increase in D-dimer levels with reductions in Protein C.

Despite evidence of endothelial activation, prolonged heat exposure did not activate systemic coagulation pathways. Protein C concentrations remained stable across time in both groups (Fig. 4.11D), and D-dimer did not increase over time. Although older adults exhibited higher D-dimer concentrations than young adults at the end of heating (P=0.048; Fig. 4.11C), this group difference is driven by a single older participant who experienced a D-dimer level of 254.80 ng/ml at the end of heating. This response occurred without substantial concurrent increases in core temperature, vWF, or IL-6 and in the setting of successful completion of the 6-hour exposure. This finding may reflect individual variability, an unmeasured preexisting pro-thrombotic tendency, or methodological influences related to serum-based D-dimer assessment and therefore should be interpreted with caution. Both Protein C and D-dimer levels were measured from serum samples in tubes containing clot activators rather than citrated plasma, which affects how these biomarkers can be interpreted in the present study. Serum-based measurement may overestimate D-dimer due to ex vivo fibrin formation and degradation during clotting; thus, direct comparison with diagnostic clinical thresholds cannot be made. As a result, both absolute Protein C and D-dimer values cannot be compared directly to clinical thresholds due to unavoidable ex vivo artifact. Overall, the absence of concomitant increases in D-dimer and reductions in Protein C suggests preserved hemostatic function during prolonged heat exposure, despite evidence of endothelial activation.

Cognitive function

Cognitive performance assessed using NIH toolbox tests for cognitive function was preserved in both young and older adults throughout prolonged heat exposure. Age-adjusted scores for executive function, processing speed, and attention remained stable or improved slightly over time (Fig. 12). Large cohort and national survey studies have linked high ambient temperature exposure with reduced cognitive performance and accelerated cognitive decline in middle-aged and older adults[90], and chronic exposure to extreme heat is associated with faster

cognitive decline among socially vulnerable older adults aged 52 years and older[91]. Previous research has shown that cognitive function declines with prolonged heat stress even beyond vulnerable populations as demonstrated by Laurent et al.[95], whose findings revealed that young individuals living in non-air conditioned buildings experienced reductions in cognitive function during a heat wave as assessed by tests examining working memory and selective attention/processing speed. Additionally, one study investigated the effects of environmental heat stress on cognitive function in older adults exposed to temperatures of 24°C and 32°C but did not find differences in cognitive performance assessed via the Cambridge Neuropsychological Test Automated Battery (CANTAB) between the two experimental temperatures[96]. However, in a subgroup of individuals exposed to greater relative humidity than those in the control session, the subgroup presented worse cognitive performance, and fitness level was associated with reduced cognitive vulnerability to heat stress. In the present study, older adults were highly active. Thus, older adults with lower fitness levels in real-world heatwaves likely face an elevated risk of cognitive dysfunction that may exacerbate adverse health outcomes associated with prolonged, extreme heat exposure.

Limitations

The present study is not without some limitations. First, the sample size was small, particularly given that several participants (n=7) terminated the heat exposure protocol early due to symptoms of heat-related illness. Although total thermal load was accounted for using area under the curve (AUC), early termination reduced total heat exposure for some participants and may have influenced the magnitude of physiological responses. Next, generalizability of these findings to real-world heat wave scenarios are limited by the controlled laboratory environment. Participants were exposed to fixed environmental conditions and were provided controlled fluid intake, which may not reflect the real-world variability in hydration practices and environmental conditions experienced during naturally occurring heat waves. However, we did incorporate activities of daily living in an effort to accurately depict the physiological strain experienced during real-world heatwaves. Additionally, the biomarkers of coagulation chosen in the present study may not optimally capture early, subclinical activation of the coagulation cascade. While D-dimer remains a sensitive indicator for fibrin formation and breakdown, it reflects later-stage

fibrin turnover rather than initiation of coagulation[261], and there are many conditions unrelated to thrombosis in which D-dimer concentrations are high[262]. Additionally, reductions in Protein C typically occur with sustained or severe thrombin generation as opposed to early activation[261,263,264]. Thus, future studies investigating early activation of coagulation may benefit from utilizing upstream biomarkers of early coagulation activation, such as thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2, which rise during the initiation phase of coagulation before fibrin formation and before D-dimer increases[265–267]. However, these analyses require high precision handling and specialized assays that may limit feasibility in long-duration protocols.

As mentioned previously, D-dimer and Protein C were analyzed from serum collected in tubes containing clotting factors rather than citrated plasma. This limits how these biomarkers can be interpreted, as the resulting concentrations cannot be directly compared to clinical reference values. However, all samples were processed under identical conditions to allow reliable within-subject and between-group comparisons despite potential ex vivo influences. Although this methodological limitation affects external comparisons, it does not negate the patterns observed in the present study but should be considered when comparing these findings to plasma-derived coagulation data.

Cognitive function was assessed using NIH Toolbox tests, which showed a clear learning effect across repeated testing. Inclusion of a thermoneutral control would have strengthened our ability to isolate the influence of heat stress on cognitive performance and determine whether the observed improvements reflected true learning or if heat exposure could have attenuated the practice-related improvements in test scores during heat stress. Future studies may benefit from using cognitive assessments with reduced susceptibility to learning and practice effects, such as reaction-time or vigilance-based tasks, as these approaches may provide greater sensitivity for detecting heat-induced impairments in cognitive function.

Conclusion

Taken together, these findings indicate that prolonged extreme heat exposure induces a complex physiological response in older individuals characterized by preserved thermoregulation, cognition, and hemostasis, but heightened cardiovascular strain and

endothelial activation. Given the observed increase in cardiovascular strain and endothelial injury among older adults coupled with dehydration, it is likely that myocardial oxygen and myocardial blood flow requirements together with heat-induced endothelial dysfunction increase the susceptibility of older individuals to myocardial ischemia as well as thrombosis during extreme heat exposure. The dissociation between thermal load and physiological tolerance highlights the importance of considering vascular and hemodynamic factors when assessing heat vulnerability in older adults. Endothelial activation in the absence of overt coagulation or cognitive impairment suggests a subclinical injury phenotype that may be exacerbated over longer, multi-day heat exposures, likely contributing to the increased cardiovascular and thrombotic risk observed in older adults during heatwaves.

CHAPTER V
KIDNEY FUNCTION AND ACUTE KIDNEY INJURY RISK DURING PROLONGED HEAT EXPOSURE IN YOUNG VS OLDER ADULTS

INTRODUCTION

As climate change progresses, extreme heat events are becoming increasingly frequent and severe, posing significant risks to human health[268–270]. The leading causes of excess hospitalizations during extreme heat events are fluid and electrolyte imbalances and acute kidney injury (AKI)[140,143,271], which is characterized by a sudden decline in kidney function[272–274]. Heat exposure increases AKI risk through multiple physiological mechanisms, including hypovolemia due to sweating-induced fluid losses, redistribution of blood flow away from the kidneys to support thermoregulation, reduced renal perfusion pressure, and heat-induced systemic inflammation and oxidative stress[140,152,158,167]. Together, these responses place substantial strain on the kidneys during prolonged heat exposure, even in the absence of evident heat illness.

Among the most vulnerable to the negative effects of extreme heat exposure are older adults, as aging is associated with reduced thermotolerance due to impairments in thermoregulatory and cardiovascular function[65,67,275]. Adults aged ≥65 years are 10 times more likely to develop AKI compared to adults <40 years[174], and in-hospital mortality among older patients with AKI ranges from 15-40%[175,176]. Age-related structural and functional changes in the kidneys also further contribute to heightened AKI risk, including reductions in total renal mass[177], attenuated renal function and water and electrolyte homeostasis[140,276], a decline in renal blood flow of ~10% per decade after the age of 40[181], glomerulosclerosis[182], and a reduction in the number and length of tubules in the kidneys[177]. Given the rapidly growing population of adults aged ≥65 years[277], there is increasing concern regarding the safety of older individuals in a warming climate.

The purpose of this study was to examine acute kidney injury risk and its physiological etiology in young versus older adults during prolonged heat exposure designed to simulate realistic heat wave conditions and activities of daily living. While previous studies have assessed AKI risk during heat exposure in older adults using core temperature-controlled or passive heating paradigms, these studies have often relied on limited biomarkers, short exposure

durations, and experimental conditions that do not reflect real-world heat wave scenarios[190–192]. To date, no study has systematically evaluated AKI risk in young and older adults during prolonged heat exposure using activities of daily living and a panel of biomarkers reflecting AKI risk, kidney function, inflammation, and oxidative stress. We hypothesized that older adults would experience greater impairments in kidney function and increases in biomarkers of AKI risk, inflammation, and oxidative stress compared to young adults during a 6 h simulated heat event. A secondary purpose of this study was to determine whether biomarkers of AKI risk remain elevated, and if kidney function remains impaired approximately 24 h following heat exposure. We hypothesized that ~24 h post-heat exposure, older adults would exhibit sustained elevations in biomarkers of acute kidney injury risk, inflammation, and oxidative stress, and a sustained reduction in kidney function compared to young adults.

MATERIALS AND METHODS

Participants

This study was approved by the Institutional Review Board at the University of Oregon. Written informed consent was obtained from all participants in accordance with the principles set forth by the Declaration of Helsinki. Thirteen young (18-30) and 13 older (60-80) adults participated in the study. The sample size here is reduced due to one participant only completing 1 hour of the heat event and not being able to provide a urine sample at the end of heating. All participants volunteered for a screening visit and 3 experimental visits described in our previous report (Chapter IV).

Table 5.1. Participant Characteristics

	Young (n=13)	**Older (n=13)**	**P value**
Males/Females	6/7	6/7	
Age (yr)	24±4	68±7	<0.001
Height (cm)	170.1±12.8	170.7±9.5	0.895
Body mass (kg)	67.7±10.1	77.4±13.6	0.049
Body mass index (kg/m^2)	23.5±3.5	26.5±3.9	0.046

Body surface area[a] (m^2)	1.79±0.18	1.89±0.20	0.165
uACR (mg/g)	15.8±7.6	16.5±8.3	0.807

Values are presented as means ± standard deviation. Data were analyzed using unpaired t tests. [a]Body surface area was calculated according to the equation by Du Bois[218]. uACR; urine-albumin creatinine ratio.

Experimental Procedure

The experimental procedures for this study are described in our other report (Chapter IV), which investigated the thermoregulatory and cardiovascular responses to prolonged heat exposure, and their effect on endothelial function, coagulation, and cognitive function in young versus older adults. Only details pertaining to the present study's aims are provided here.

Participants were given urine collection containers to collect all their excreted urine for 24 h prior to arrival for the experimental heat event visit. Participants were asked to consume at least 500 mL of water the evening before and morning of the heat event visit to ensure adequate hydration. Upon arrival at the laboratory, a baseline urine collection sample was provided to measure hydration status. Euhydration was defined as a urine specific gravity (USG) < 1.020[221]. If participants were not euhydrated (USG ≥ 1.020), they were provided 500 mL of water, and USG was re-tested before undergoing heat exposure. Following a pre-heat nude body weight measurement, a baseline (PRE) venous blood draw was then collected, and participants entered the environmental chamber set at 42°C and 20% relative humidity. These environmental conditions were selected to align with the U.S. National Weather Service criteria for issuing an Excessive Heat Warning, which occurs when the daytime heat index exceeds 40.5°C for at least a 48 hour period[225]. The selected temperature and humidity for the present study equates to a heat index of 42.4°C, which represents a "danger" level for heat-related illnesses[226,227]. Every hour, participants cycled at a pre-determined workload (3 METs) for 15 min to simulate activities of daily living and were given water (3 mL/kg body weight). A private, indoor commode was available for participants to use, and all excreted urine was collected from hours 0-3 and 3-6. At the end of the 6-hour heat event, a venous blood sample was taken, a final urine void was collected, and a post-heat nude body weight was measured and recorded. Following recovery, participants were provided with urine collection containers for an overnight urine

collection. The morning following the heat event visit, participants arrived at the laboratory for a final blood draw and collection of the urine containers.

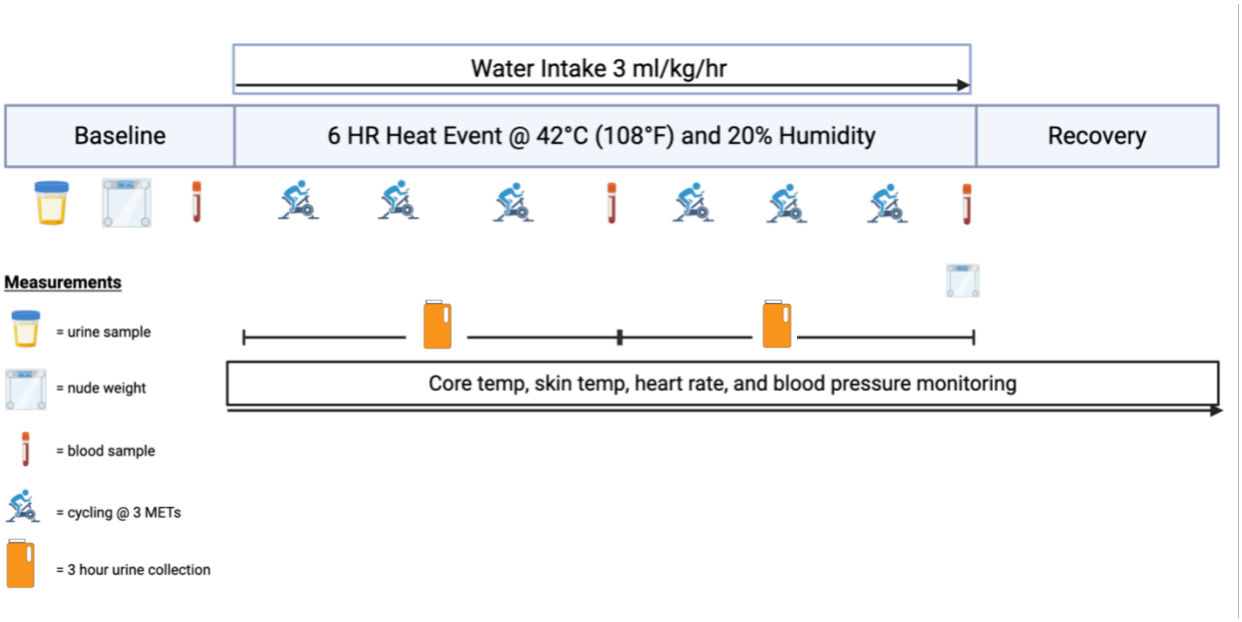


Figure 5.1. Overview of the experimental heat event. Young (n=13) and older (n=13) participants underwent a 6 hour heat exposure at 42°C and 20% relative humidity. To mimic activities of daily living, participants performed six 15 min bouts of light physical activity (~3 METs) every hour of heat exposure. Participants consumed 3 mL/kg body mass of water every hour during the heat exposure. Measurements and assessments included core and skin temperatures, heart rate, blood pressure, sweat loss, and blood and urine collections. Created with BioRender.com.

Measurements and Instrumentation

Core temperature was measured every 5 min via ingestible telemetry pill (eCelsius performance pill, BodyCap, Caen, France) swallowed the evening before the start of the heat event visit. Total thermal load expressed as the area under the curve (AUC) was calculated using the trapezoidal method over the duration of heat exposure completed by each participant. Nude body mass was measured using a scale and total sweat loss was calculated as [(pre-heat exposure body weight – post-heat exposure body weight) + (fluid consumed – urine output)] and used to calculate sweat rate: (total sweat loss/total heat exposure time). Percentage changes in body mass were calculated as [(post-heat exposure – pre-heat exposure body weight)/pre-heat

exposure body weight] × 100. Hematocrit and hemoglobin were measured from venous blood draws and used to calculate changes in plasma volume using a standard equation[278].

The day preceding the heat event visit, baseline 24 h urine collections (BL) were initiated at the start of the Pre-Vascular Function visit (described in Chapter IV). All BL participant urine voids were collected for 24 h into two light-resistant 3L containers (Medline Industries, Northfield, IL) until their visit the following day. Upon return to the lab the following day for the heat event visit, participants provided a baseline urine sample for hydration status testing, which was added to the 24 h collection containers before beginning their experimental visit. Urine was also collected from hours 0-3 of the heat event and hours 3-6 of the heat event (END) and ~17-22 h following the heat event (POST). However, urine samples collected from hours 0-3 were not included in analyses unless participants terminated the protocol early; in which case, these timed samples were considered their END urine samples. Thoroughly mixed urine samples at each respective time point were aliquoted into cryogenic tubes and frozen at -80°C until later analysis of urinary biomarkers of interest.

Urine specific gravity (USG) was measured via refractometry at all time points (BL, END, and POST) for measurement of hydration status and normalization of urinary biomarkers. Urine flow rate (UFR) was calculated as the volume of urine produced divided by the collection period (BL, END, and POST). Collection time for BL was determined as the time from the end of the Pre-Vascular Function visit to the start of the Heat Event visit (24 h). END collection time points were 3 h unless the participant terminated the heat exposure early, and collection time for POST was determined as the time from the end of the Heat Event to the start of the Post-Vascular Function visit (~17-22 h). Serum and urine creatinine were measured using commercially available assays (RayBiotech® Human Creatinine ELISA Kit, RayBiotech, Peachtree Corners, GA, USA). Glomerular filtration rate (GFR) was estimated from creatinine clearance using the following equation: (urine flow rate × urinary creatinine)/serum creatinine. Urine and serum osmolality were measured using a freezing point depression osmometer (Advanced® Model 3320 Micro-Osmometer; Advanced Instruments, Norwood, MA, USA), and free water clearance was calculated as UFR × (1 − [U_{osm} ÷ P_{osm}]), where UFR is urine flow rate, and U_{osm} and P_{osm} are the osmolality of the urine and plasma. Osmolar clearance was calculated as (U_{osm} ÷ P_{osm}) × UFR[279].

Insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) were measured in the urine with separate commercially available human IGFBP7 (RayBiotech Life, Peachtree Corners, GA) and TIMP-2 (RayBiotech Life, Peachtree Corners, GA) enzyme-linked immunosorbent assay (ELISA) kits. Biomarkers of AKI are highly developed, and the product of urinary IGFBP7 and TIMP-2 ([IGFBP × TIMP-2)] has been approved by the FDA as a tool for assessing the risk of AKI[37] outperforming over 330 biomarkers in predicting the incidence of AKI in clinical settings[154]. In renal epithelial cells, IGFBP7 and TIMP-2 are stress response proteins that induce G1 cell cycle arrest during kidney injury[155–157]. IGFBP7 is preferentially secreted in the renal proximal tubules and TIMP-2 in the distal tubules, thereby providing insight into the potential location of renal injury[37,140,158]. To examine potential differences in the etiology underlying acute kidney injury risk, inflammatory and oxidative stress markers were measured from both urine and serum samples. Monocyte chemoattractant protein-1 (MCP-1) was measured with ELISA kits (RayBiotech Life, Peachtree Corners, GA). MCP-1 is a proinflammatory cytokine expressed in acute kidney injury ([280] and is directly correlated with severity of renal injury[281]. Thioredoxin-1 (TRX-1) was measured with ELISA kits (RayBiotech Life, Peachtree Corners, GA). Urinary TRX-1 increases in response to kidney injury mediated by oxidative stress and/or hypoxia, is primarily released from proximal tubular epithelial cells, and positively correlates with the severity of tubular injury[282], whereas serum TRX-1 represents a marker for detecting conditions of excessive oxidative stress such as heart failure[283]. All biomarkers were measured at baseline (BL) before heat exposure, at the end of the 6 h heat exposure or early protocol termination (END), and at ~17-22 h post heat exposure (POST). All sample analyses and assays were completed by a single trained technician. In all instances, sample dilutions were customized for each subject and each assay kit to ensure that all concentrations were within the accuracy ranges of the assay kits. To account for differences in urine concentration, IGFBP7, TIMP-2, [IGFBP7·TIMP-2], MCP-1, and TRX-1 were normalized to USG using the following equation: $Concentration_{USG\ normalized} = Concentration_{specimen} \times (USG_{reference}^{-1} \div USG_{specimen}^{-1})$, where specimen concentration is expressed in nanograms or picograms per milliliter and $USG_{reference}$ is a population reference value for specific gravity representing “normal” or “nondiluted” urine. The $USG_{reference}$ utilized in all corrections was 1.020. In cases where the USG of the specimen was ≤1.000, USG was changed to 1.001 to allow the equation to work.

Data and Statistical Analysis

Statistical analyses were conducted using Prism 10.4.0 (GraphPad Software, Inc., San Diego, CA). Data were analyzed with a repeated-measures mixed-effects model (Age × Time) or unpaired, two-tailed t-tests as appropriate. Pairwise comparisons were made using Šidák's post hoc test when significant main effects were observed. All urinary biomarkers: TRX-1_{USG}, MCP-1_{USG}, $IGFBP7_{USG}$, $TIMP\text{-}2_{USG}$, and $[IGFBP7 \cdot TIMP\text{-}2]_{USG}$ data were not normally distributed and were thus $\log_{10}$ transformed to normally distribute the data before analysis. For all data, n = 13 in each group unless otherwise reported. Missing data were due to either failure to obtain a blood draw or urine sample and/or samples were outside of the range of detection for the specific assay kit. All data are presented as means with 95% confidence intervals. Statistical significance was set a priori to $P < 0.05$, and actual P values are reported where possible.

RESULTS

Thermoregulatory Responses

Core temperature, mean skin temperature, and mean body temperature responses at baseline, hour 3, and termination of the heat event are presented in Table 2 and increased from baseline in both young and older adults but were not different between age groups at any time point (P>0.05). Sweat rate did not differ between young and older adults (P=0.706), however, percent change (Δ) in body mass was greater in young compared to older adults (P=0.027; Table 2). The change in core temperature from baseline to end of heating did not differ between young [+0.87°C (0.60, 1.13)] and older adults [+1.10°C (0.76, 1.44), P=0.257] (Fig. 2A). However, it is important to note that 9 participants terminated the 6 h protocol early. Thus, to better represent the overall thermal load experienced by each participant, changes in core temperature are also expressed as the area under the curve (AUC), representing the total thermal load over the duration of heat exposure (°C·h). There was no difference in total thermal load (AUC) between young [2.61°C·h (1.41, 3.80)] and older adults [2.73°C·h (1.60, 3.86), P=0.875] (Fig. 2B).

Table 5.2. Thermoregulatory responses to heat stress

Outcome	Young			Older		
	BL (n=13)	3 h (n=13)	END (n=11)	BL (n=13)	3 h (n=13)	END (n=7)
Core temperature (°C)	37.2 (37.0, 37.4)	37.7 (37.5, 37.9)*	37.9 (37.7, 38.2)*	37.2 (37.0, 37.4)	38.0 (37.7, 38.2)*	37.8 (37.6, 38.0)*
Mean skin temperature (°C)	31.9 (31.6, 32.2)	38.0 (37.7, 38.2)*	37.5 (37.1, 37.9)*	31.7 (31.3, 32.2)	37.5 (37.0, 38.0)*	37.1 (36.6, 37.5)*
Mean body temperature (°C)	36.1 (36.0, 36.3)	37.8 (37.6, 38.0)*	37.9 (37.5, 38.2)*	36.1 (36.0, 36.3)	37.9 (37.6, 38.1)*	37.7 (37.5, 37.9)*
Sweat rate ($L \cdot kg^{-1} \cdot h^{-1}$)	-	-	0.38 (0.32, 0.43)	-	-	0.36 (0.31, 0.42)
Δ Body mass (%)	-	-	-3.2 (-3.6, -2.7)	-	-	-2.5 (-2.9, -2.1)‡

Data presented as mean with 95% CIs. Thermoregulatory outcomes were measured at baseline (BL) before heat exposure, halfway through heat exposure (3 h), and at the end of heat exposure (END) at hour 6 or early termination. Data were analyzed with unpaired t-tests or a repeated measures mixed-effects model (Age × Time). Post hoc analyses were completed with Šídák's multiple comparisons tests. Δ indicates change from baseline. *Different from BL ($P<0.05$); ‡Different from young ($P<0.05$).

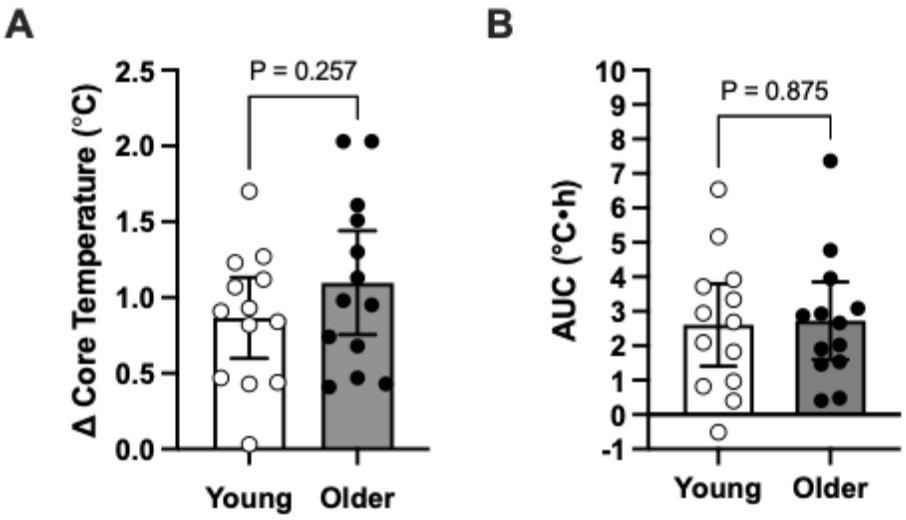


Figure 5.2. (A) Overall change (Δ) in core temperature from baseline to end of heating and (B) cumulative core temperature responses (AUC) during heat exposure in young and older adults. Thermal load is expressed as the area under the curve (AUC; °C·h), calculated over the duration

of heat exposure completed by each participant. Data were analyzed with unpaired t-tests and are presented as means with 95% confidence intervals.

Kidney Function

Renal and fluid balance responses are presented in Table 3. The decrease in plasma volume from baseline to end of heating did not differ between young [-8.3% (-13.2, -3.3)] and older adults [-9.2% (-14.2, -4.4), P=0.738). Urine specific gravity was greater in young [1.024 (1.022, 1.026)] compared with older adults [1.014 (1.009, 1.018)] at the end of heating (P<0.001) and remained elevated in young adults at ~17-22 h post heat exposure (P=0.028). Urine creatinine was also higher in young [344.8 mg/dL (295.3, 394.4)] compared with older adults [186.8 mg/dL (124.2, 249.4)] at the end of heating (P<0.001) and remained elevated in young adults at ~17-22 h post heat exposure (P=0.043). Serum creatinine was higher in older [1.07 mg/dL (0.93, 1.21)] compared with young adults [0.80 mg/dL (0.68, 0.91)] at the end of heating (P=0.003), whereas creatinine clearance was higher in young [205.7 mL/min (101.6, 309.8)] compared with older adults [72.1 mL/min (53.8, 90.5), P<0.001]. Urine osmolality was greater in young [897 mosmol·kg^{-1} H_2O (813, 980] compared to older adults [527 mosmol·kg^{-1} H_2O (395, 659)] at the end of heating (P<0.001) and at ~17-22 h post heat exposure (P=0.015), with no difference in serum osmolality between groups at any time point. Free water clearance was negative in both groups at all time points, with a larger magnitude in young [-1.0 mL/min (-1.5, -0.5)] compared to older adults [-0.4 mL/min (-0.5, -0.2)] at the end of heating (P=0.028).

Table 5.3. Renal and fluid balance responses to prolonged heat exposure

Outcome	Young			Older		
	BL	**END**	**POST**	**BL**	**END**	**POST**
ΔPlasma volume (%)	-	-8.3 (-13.2, -3.3)	-	-	-9.2 (-14.2, -4.4)	-
Urine specific gravity	1.008 (1.006, 1.010)	1.024 (1.022, 1.026)*	1.017 (1.014, 1.019)*	1.006 (1.004, 1.008)	1.014 (1.009, 1.018)*‡	1.010 (1.005, 1.015)‡
Urine flow rate (mL/min)	1.8 (1.4, 2.2)	0.5 (0.2, 0.7)*	0.7 (0.5, 0.9)*	2.0 (1.5, 2.6)	0.4 (0.3, 0.5)*	1.6 (1.1, 2.1)‡
Urine creatinine (mg/dL)	64.5 (50.4, 78.6)	344.8 (295.3, 394.4)*	153.8 (120.3, 187.3)*	52.8 (40.3, 65.3)	186.8 (124.2, 249.4)*‡	99.4 (55.5, 143.4)‡
Serum creatinine (mg/dL)	0.83 (0.75, 0.92)	0.80 (0.68, 0.91)	0.79 (0.73, 0.85)	0.92 (0.81, 1.02)	1.07 (0.93, 1.21)*‡	0.88 (0.78, 0.99)
Creatinine clearance (mL/min)	128.2 (104.2, 152.1)	205.7 (101.6, 309.8)*	139.9 (116.5, 163.2)	105.3 (89.4, 121.3)	72.1 (53.8, 90.5)‡	135.9 (95.6, 176.2)
Urine osmolality (mOsmol·kg^{-1} H_2O)	384 (301, 468)	897 (813, 980)*	690 (563, 817)*	398 (287, 509)	527 (395, 659)‡	455 (309, 601)‡
Serum osmolality (mOsmol·kg^{-1} H_2O)	286 (282, 290)	287 (283, 291)	289 (283, 294)	286 (279, 292)	286 (282, 290)	287 (281, 292)
Free water clearance (mL/min)	-0.4 (-0.7, -0.1)	-1.0 (-1.5, -0.5)	-1.0 (-1.2, -0.7)*	-0.4 (-1.1, 0.2)	-0.4 (-0.5, -0.2)‡	-0.5 (-1.2, 0.2)
Osmolar clearance (mL/min)	2.2 (1.9, 2.5)	1.5 (0.7, 2.2)	1.7 (1.4, 2.0)*	2.5 (2.2, 2.8)	0.8 (0.5, 1.0)*	2.1 (1.4, 2.8)
Albumin (g/dL)	0.032 (-0.001, 0.064)	0.130 (0.074, 0.187)*	0.057 (0.019, 0.094)	0.033 (0.010, 0.056)	0.222 (0.055, 0.390)	0.082 (0.024, 0.140)

Data presented as mean with 95% CIs. Outcomes were measured from blood and urine samples at baseline (BL) which included a 24 h urine collection, at the end of heating (END) with urine collected during the last 3 h of heating or at early termination, and after heating (POST) with urine collected during the entire period (~17-22 h). Data were analyzed with a repeated-measures mixed-effects model (Age × Time), and post hoc analyses were completed with Šídák's multiple comparisons tests. *Different from BL ($P<0.05$); ‡Different from young ($P<0.05$).

Kidney Injury Risk and Etiology

Biomarkers of AKI risk are presented in Fig. 3. Urinary [IGFBP7 · TIMP-2] increased from baseline to end of heating in both groups ($P<0.001$), with no differences between groups at any time point. IGFBP7 and TIMP-2 individually also increased over time in both groups

(P<0.001). Biomarkers of inflammation and oxidative stress are presented in Fig. 4. Urinary MCP-1 and TRX-1 increased from baseline to end of heating in both groups (P<0.001), whereas serum MCP-1 and TRX-1 did not increase over time in either group (P≥0.086). No differences between young and older adults were observed at any time point for either urinary or serum MCP-1 and TRX-1.

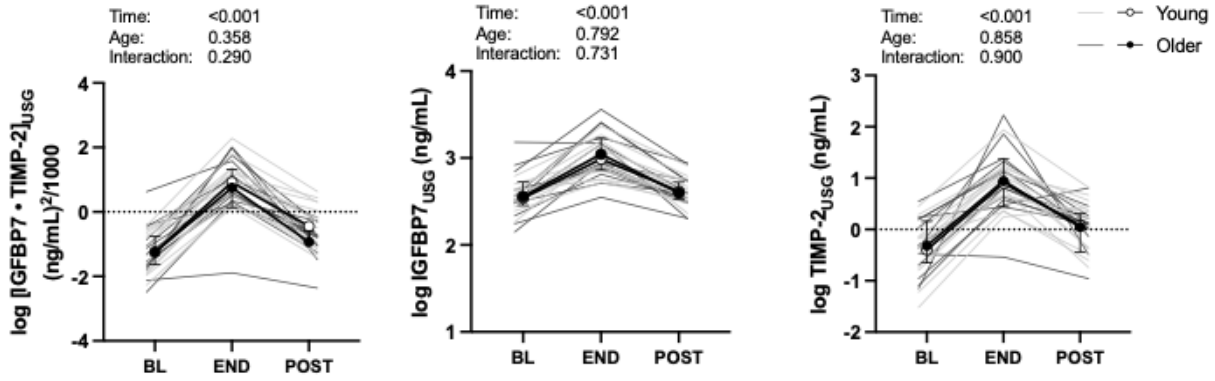


Figure 5.3. Biomarkers of acute kidney injury (AKI) risk normalized to urine specific gravity (USG). (A) Insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase [IGFBP7 · TIMP-2], (B) IGFBP7, and (C) TIMP-2. Biomarkers were measured at baseline (BL), end of the 6 h heat exposure or early protocol termination (END), and at ~17-22 h post heat exposure (POST). Data were analyzed with a repeated-measures mixed-effects model (Age × Time) and are presented as individual and means with 95% confidence intervals.

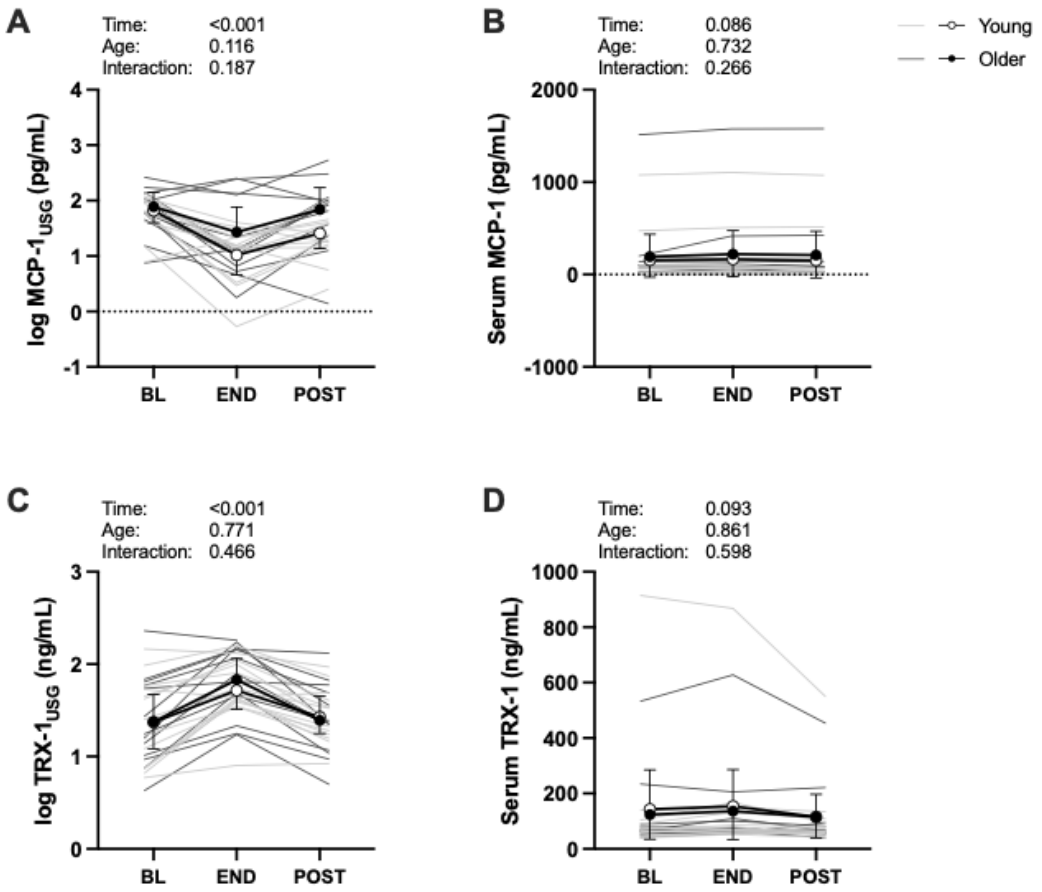


Figure 5.4. Biomarkers of inflammation and oxidative stress. (A) Urine monocyte chemoattractant protein-1 (MCP-1), (B) serum MCP-1, (C) urine thioredoxin-1 (TRX-1), and (D) serum TRX-1. Biomarkers were measured at baseline (BL), end of 6 h heat exposure or early termination (END), and ~17-22 h post heat exposure (POST). Data were analyzed a repeated-measures mixed-effects model (Age × Time) and are presented as individual and means with 95% confidence intervals.

DISCUSSION

The present study compared the effect of a 6 hour simulated heat event at 42°C and 20% relative humidity with light exercise to mimic activities of daily living on kidney function and acute kidney injury risk in young and older adults. Additionally, we sought to investigate if potential reductions in kidney function and increases in AKI risk remain elevated up ~17-22 h post-heat exposure. We observed similar increases in biomarkers of AKI risk, [IGFBP7·TIMP-2]

(Fig. 5.3A), between young and older adults, and independent increases in both IGFBP7 (Fig. 5.3B) and TIMP-2 (Fig. 5.3C) indicate elevations in AKI risk to be anatomically located in both the proximal and distal tubules. Additionally, biomarkers of renal inflammation (urinary MCP-1; Fig. 5.4A) and oxidative stress (urinary TRX-1; Fig. 5.4C) did not differ between young and older individuals. However, older adults experienced increases in serum creatinine at the end of heating whereas no change was observed in young adults (Table 5.3). Additionally, we observed decreases in kidney function as evidenced by a decline in creatinine clearance in older adults whereas creatinine clearance increased young adults (Table 5.3), and a greater reduction in free water clearance in young adults compared to old adults at the end of heating (Table 5.3), indicating a reduction in glomerular filtration rate and reduced renal concentrating ability among older adults. Biomarkers of AKI risk and its etiology did not appear to remain elevated ~17-22 h post heat exposure (Figs. 5.3 & 5.4), however kidney concentrating ability remained reduced ~17-22 h post heat exposure in older adults (Table 5.3).

Thermoregulation

Core temperature responses to 6 h of extreme heat exposure did not differ between young and older adults. As previously reported (Chapter IV) and provided here for context, the overall change in core temperature from baseline to end of heating was similar between young and older adults (P=0.257; Fig. 5.2A). However, 2 young and 5 older adults failed to complete the full 6 h heat exposure. Thus, we expressed total thermal load as area under the curve (AUC) to account for differences in total heat exposure times between age groups. Thermal AUC did not differ between young and older adults (Fig. 5.2B), indicating that older adults experienced the same thermal load as young adults despite a greater incidence of early protocol termination due to symptoms of heat-related illness. However, young adults experienced a greater level of dehydration as evidenced by a larger reduction in body mass from baseline to end of heating (Table 5.2). While similar core temperature responses and total thermal load were experienced by young and older adults, the greater level of dehydration among younger adults may have contributed to the similar elevations in AKI risk biomarkers observed in both age groups.

Acute Kidney Injury Risk and Etiology

Six hours of heat stress led to similar rises in [IGFBP7·TIMP-2] in both young and older adults, indicating no difference in AKI risk among age groups. Moreover, the similar increases in AKI risk among young and older adults were mediated by elevations in both IGFBP7 and TIMP-2 (Fig. 5.3). To our knowledge, no other study has investigated changes in [IGFBP7·TIMP-2] in both young and older adults during prolonged heat exposure. However, investigations of AKI risk in young versus older adults have assessed AKI risk using different biomarkers. In the present study, creatinine increased from baseline to end of heating by an average of 0.15 mg/dL in older adults whereas young adults experienced an average increase of just 0.03 mg/dL (Table 5.3). Specifically, one older participant experienced an increase of 0.39 mg/dL, three older participants experienced an increase of 0.29 mg/dL, and no young adults experienced increases ≥ 0.06 mg/dL. Given that the Acute Kidney Injury Network (AKIN) criteria defines Stage 1 AKI as an increase in serum creatinine ≥ 0.3 mg/dL from baseline[284], and the Kidney Disease Improving Global Guidelines (KDIGO) states that AKI is clinically diagnosed when serum creatinine increases by ≥ 0.3 mg/dL within 48 h[285], older adults displayed greater incidences/risk of developing AKI than young adults according to these criteria. In support of these findings, McKenna et al.[190] observed increases in plasma creatinine in older adults (0.10 mg/dL) whereas no change was observed in young adults following 3 h of heat exposure at 47°C and 15% relative humidity. Additionally, plasma creatinine increased in two older adults by ≥ 0.3 mg/dL at 2 hours post heat exposure. Thus, we speculate that a greater number of older adults in the present study may have reached the clinical criteria for Stage 1 AKI as serum creatinine levels may have reached ≥ 0.3 mg/dL had we taken post heating measurements up to 2 hours after termination of heat exposure. The authors attributed the greater increases in plasma creatinine to heightened thermal strain amongst older adults, as mean core temperature increased by 1.4°C compared to 0.7°C in young adults[190]. However, in the present study, changes in core temperature did not differ between young and older adults (Fig. 5.2A). Thus, it is not likely that thermal strain alone is responsible for the greater rise in serum creatinine among older adults in the present study.

In addition to other markers of AKI risk, urinary neutrophil gelatinase-associated lipocalin (NGAL) is among the most widely studied biomarkers for the detection of AKI[286]. In urine, NGAL serves as a marker of general tubular injury and in the plasma, NGAL is a general indicator of renal ischemia[287–290]. In a study by Vliora et al.[192], three hours of passive extreme

heat exposure at 44°C and 30% relative humidity led to significant increases in serum NGAL in older but not young adults, suggesting signs of acute kidney injury among older adults. However, the authors note that NGAL is widely recognized as an early biomarker of AKI[291] and is also secreted in response to increases in systemic inflammation[292]. They propose that higher basal inflammatory status among older compared to young adults may partially explain the greater NGAL response to heat stress observed with aging. Thus, NGAL is perhaps not the best indicator of AKI risk and was not measured in the present study, as [IGFBP7·TIMP-2] has been shown to outperform >330 biomarkers in predicting AKI, including NGAL[293].

In conjunction with the similar increases observed in [IGFBP7·TIMP-2], IGFBP7, and TIMP-2 among young and older adults, changes in the urinary inflammatory marker MCP-1 and urinary oxidative stress marker TRX-1 were also similar between age groups (Figs. 5.4A & 5.C). Serum measurements of MCP-1 and TRX-1 did not change in either group, indicating no evidence of systemic inflammation or oxidative stress (Figs. 5.4B & 5.D). Interestingly, urinary MCP-1 decreased during heat exposure in the present study, whereas Hess et al.[294] reported increases in urinary MCP-1 following 4 h of work and rest at wet bulb globe temperatures (WBGT) of 31.6°C, 27.3°C, and 31.7°C. Additionally, they observed increases in serum MCP-1 while the fractional excretion of MCP-1 remained unchanged, suggesting that increases in urinary MCP-1 reflected increases in circulating MCP-1 rather than local kidney production[295]. One explanation for the difference between studies is the lack of increase in serum MCP-1 observed in the present study, as Hess et al.[294] used an intermittent work-rest protocol with 30 min of walking and 30 min of rest each hour, which likely produced greater systemic inflammation. Additionally, reductions in renal perfusion and estimated glomerular filtration may limit filtration of circulating MCP-1 into the urine, contributing to the observed decrease in urinary MCP-1. Thus, the increase in TRX-1 alongside reductions in MCP-1 indicate that the observed increase in AKI risk in both groups may be driven primarily by oxidative stress rather than inflammatory signaling.

Kidney Function

Given that creatinine clearance is used to estimate glomerular filtration rate (eGFR)[140] and creatinine clearance was significantly lower in older adults compared to young adults and

young adults were able to increase creatinine clearance during heating (Table 5.3), the attenuated increase in eGFR is responsible for the observed increase in serum creatinine among older adults. GFR is an important function of the kidneys to regulate homeostatic processes such as blood pressure and water and electrolyte regulation, all of which are challenged during heat stress[140]. As GFR declines, the kidneys filter less creatinine in the blood, resulting in reduced creatinine clearance and subsequent accumulation of circulating creatinine. It is therefore not surprising that creatinine clearance was reduced in older adults compared to young, as GFR is known to decline with aging as the kidneys undergo structural and functional changes that reflect gradual loss of kidney function as a natural senescence process[296,297]. In 1999, Farquhar and Kenney[298] examined the effects of aging on GFR during acute exercise in the heat and found that absolute values of GFR were lower in older adults (~60 mL/min) compared to young adults (~78 mL/min). However, McKenna et al.[190] recently demonstrated that eGFR decreased to a similar extent in both young and older adults during ~1 h of controlled hyperthermia sufficient to raise core temperature by 2°C. Given the short duration and controlled nature of their model, the present study may better reflect alterations in kidney function during prolonged, real-world heat wave exposures.

Renal water handling also appeared to differ by age as evidenced by the lack of change in free water clearance from baseline (-0.4 mL/min) to end of heating (-0.4 mL/min) in older adults, whereas free water clearance decreased from baseline (-0.4 mL/min) to end of heating (-1.0 mL/min) in young adults (Table 5.3). Free water clearance is a sensitive indicator of tubular function and reflects renal tubular water handling[299], and a positive free water clearance in dehydrated individuals pathologically indicates that the ability of the kidneys to conserve water is impaired[140], whereas a negative value indicates reabsorption of excess free water [300]. This is further supported by the lower urine osmolality observed in older adults (527 mOsm/kg) compared to young (897 mOsm/kg; Table 5.3), suggesting a diminished ability to concentrate urine during prolonged heat stress despite controlled fluid intake (3 mL/kg/h). Although we did not observe a positive free water clearance in older individuals, the lack of change in free water clearance from baseline to end of heating indicates that older adults exhibited a blunted renal concentrating response compared to young adults during 6 h of heat exposure[301].

Recovery of AKI Risk and Kidney Function

Biomarkers of AKI risk and its etiology did not remain elevated, nor did they differ between young and older adults ~17-22 hours post heat exposure (Figs. 5.3 & 5.4). Changes in kidney function during heat exposure were largely transient. Although serum creatinine increased and creatinine clearance decreased during heating in older adults, both indices returned to baseline values by ~17-22 h post exposure (Table 3), indicating that glomerular filtration had normalized within ~24 h following heat exposure. However, young adults continued to exhibit elevated urine osmolality, urine specific gravity, and a more negative free water clearance at ~17-22 h post heat exposure (Table 5.3), indicating sustained activation of kidney water conservation mechanisms during recovery. In contrast, these variables returned to baseline in older adults. Urine osmolality and urine specific gravity remained lower in older compared to young adults during recovery (Table 5.3), consistent with well-documented age-related declines in renal concentrating ability[302–304]. However, it is important to note that fluid intake was not measured nor was it controlled for in the hours following the experimental heat event. Thus, it is possible that older adults consumed a greater amount of fluid in the ~17-22 h post heat exposure.

Limitations

Limitations regarding sample size and generalizability of our findings have been previously addressed (Chapter IV). In the present study, glomerular filtration rate was estimated using creatinine clearance derived from timed urine collections rather than gold-standard measures of glomerular filtration rate such as inulin clearance[305]. Although creatinine clearance is widely used to estimate GFR, it may overestimate true GFR due to tubular secretion of creatinine[306]. While several biomarkers of AKI risk and mechanisms of injury were measured, the present study did not include other commonly assessed markers of tubular injury such as NGAL or kidney injury molecule-1 (KIM-1), and additional markers of inflammation and oxidative stress such as interleukin-17 (IL-17a), interleukin-18 (IL-18), liver-type fatty acid-binding protein (L-FABP), or thiobarbituric acid reactive substances (TBARS). While [IGFBP7·TIMP-2] is considered superior to >330 biomarkers for assessing AKI risk[293], inclusion of additional biomarkers could have provided a more comprehensive assessment of kidney injury pathways and mechanisms during heat exposure. Moreover, biomarker

measurements were only assessed at the end of heating and ~17-22 h post exposure. Additional measurements during the early recovery period (e.g., 1-4 h post heat exposure) may have provided further insight into the time course of kidney injury and recovery, particularly given that some studies have observed peak elevations in creatinine or kidney injury biomarkers several hours after heat exposure. Finally, the participants in this study were healthy young and older adults without chronic disease. Therefore, these findings may not be generalizable to populations with comorbidities such as cardiovascular disease or diabetes, who may be more vulnerable to heat-related kidney injury.

Conclusion

In conclusion, 6 hours of heat exposure at 42°C and 20% relative humidity with light exercise to mimic activities of daily living elicited similar increases in biomarkers of AKI risk among young and older adults despite comparable thermal load achieved among older adults in a shorter amount of time, and greater dehydration-induced reductions in body mass in young adults. Reductions in MCP-1 and increases in TRX-1 suggest that increases in AKI risk in both age groups were mediated by oxidative stress. Additionally, reductions in glomerular filtration and renal water handling were observed in older but not young adults, although these changes resolved within ~24 hours. At ~17-22 h post heat exposure, age-related differences in urine concentrating capacity persisted, suggesting a reduced ability of the aging kidney to sustain water conservation mechanisms following heat stress. In the context of increasingly frequent and severe heat waves, these physiological responses may contribute to the heightened susceptibility of older adults to dehydration, kidney stress, and heat-related illness during repeated or prolonged exposure to extreme temperatures.

CHAPTER VI
TAKE AWAYS

REVIEW OF FINDINGS

Despite exhibiting similar thermoregulatory responses during 6 hours of heat stress, older adults displayed greater cardiovascular strain and reduced tolerance to heat, indicating a potentially elevated risk for heat-related illness or injury compared with younger adults. Evidence of endothelial injury was present among older adults as observed by increased circulating von Willebrand Factor at the end of heating, suggesting greater risk for the development of cardiovascular or thrombotic events during heat stress compared to young adults. No changes in cognitive function during heat stress were observed in either age group, likely due to the learning effect of the cognitive function tests performed. Interestingly, the risk of developing acute kidney injury increased similarly in both young and older adults despite young adults experiencing a greater degree of heat-induced dehydration. Although similar increases in AKI risk were observed between age groups, older adults displayed significant reductions in kidney function as evidenced by reductions in the kidneys' ability to filter waste and maintain water balance via measurements of estimated glomerular filtration rate and free water clearance. More research is needed to further elucidate the role of aging on heat-related vulnerability, as changes in thermoregulatory capacity alone may not be the primary driver for increased susceptibility to adverse health outcomes and greater risk of morbidity and mortality during heat waves.

Potential Applications

Much of the primary literature surrounding age-related susceptibility to heat has focused on the thermoregulatory capacity of older adults during heat stress. In studies utilizing temperature-controlled heat wave simulations, short durations of heat exposure (e.g., 2-3 hours) have been implemented whereby greater heat storage or larger increases in core temperature have been observed among older compared to young adults[84,307,308]. However, in conjunction with the present study, recent work by Meade et al.[80] evaluated physiological responses to

daylong (9 h) heat exposure at 40°C and 9% relative humidity in young and older adults and found that older participants stored more heat during the initial hours of exposure, such that core temperature was greater in older compared with young adults at hour six. However, no differences in heat storage were observed between groups from hours 3-9 of the heat exposure. Thus, the study by Meade et al.[80] and the present study highlight this discrepancy between previous studies, as thermoregulatory responses to longer heat exposures appear to offset the differences in core temperature or heat storage responses between young and older adults observed in shorter duration heat studies. These findings suggest that experimental protocols aimed at elucidating age-related thermoregulatory adjustments to heat exposure may not be sufficient to truly uncover age-related susceptibility to real world heat waves, whereby it is well-known that heat waves lead to increased morbidity and mortality among older adults[309]. Although impaired thermoregulation is often cited as a primary contributor to heat vulnerability in older adults, accumulating evidence suggests that age-related susceptibility to heat waves is multifactorial. Age-related reductions in endothelial function, hemostatic regulation, and renal and cellular function likely contribute to the increased vulnerability to heat-related morbidity and mortality among older adults, even when thermoregulatory responses appear relatively preserved.

Thus, in the present study, we decided to examine mechanisms associated with the adverse health outcomes that older individuals face elevated risk of developing during heat waves. Specifically, acute kidney injury, and in a broader context, the physiology associated with the development of heatstroke, whereby endothelial function and coagulation were assessed to attempt to uncover specific functions of aging that result in heightened risk of developing these health outcomes. Current research is following suit, as recent investigations have found that older adults exhibit autophagic dysfunction and greater apoptotic[129] and inflammatory signaling[192] during heat stress, further adding to our understanding of age-related heat vulnerability.

Additionally, we sought to examine the impact of prolonged heat exposure on cognitive function in young vs older adults, as the potential for heat to induce decrements in cognitive function may result in delays in thermal sensing or enacting behaviors to mitigate heat strain among older adults during heat waves, resulting in increased risk for poor health outcomes. Although no single cognitive test can fully replicate real-world thermoregulatory behaviors

during heat waves, the utilization of cognitive domains most relevant to real-world heatwave scenarios may suggest how decrements in cognitive function can result in poor health outcomes, such as attention, executive function, processing speed, and cognitive-motor integration, as deficits in these areas may directly impair an individual's ability to recognize and respond appropriately to physiological strain induced by heat stress. While most of these cognitive domains were assessed in the present study using NIH Toolbox tests for cognitive function, these tests proved to be susceptible to learning effects which reduced our sensitivity for detecting heat-induced impairments in cognitive function. Thus, we recommend other methods to assess cognitive function for future research.

A test that may be better suited for assessing behavioral alertness and sustained attention is the Psychomotor Vigilance Task (PVT), which is recognized as the gold-standard measure of deficits associated with sleep loss[310], is widely used to assess vigilance in clinical research, and has virtually no practice effects[311]. Decreases in PVT scores during heat stress may suggest the potential for a delay in the detection of internal cues such as thirst, fatigue, or dizziness, and reduced responsiveness to environmental demands among older adults during heat waves. Additionally, a Choice Reaction Time (CRT) task may be superior to assess processing speed and decision making than the NIH Pattern Comparison test as it assesses the time taken to respond to a stimulus when multiple response options are available[312] and allows for rapid stimulus recognition without a learning effect as the CRT task uses simple, fixed stimulus-response mappings and randomized trial presentation[313]. The results from this test may translate to heat wave risk by capturing delays in selecting and initiating appropriate protective behaviors, such as seeking cooler environments or maintaining adequate hydration under conditions of physiological strain. Finally, the inclusion of a dual-task paradigm (e.g., walking while performing a cognitive task) may provide additional insight into cognitive-motor integration under heat stress. Specifically, impairments in dual-task performance reflect competition for limited additional resources as the simultaneous execution of cognitive and motor tasks exceeds central processing capacity in older adults and have been associated with increased fall risk and reduced functional mobility in older adults[314]. Even more so, heat stress may result in orthostatic intolerance due to a large increase in skin blood flow and a concomitant decrease in cerebral blood flow, and the ability to safely navigate the environment while simultaneously processing

internal and external cues presents a crucial aspect of the safety of older individuals during heatwaves.

The present findings highlight the potential utility of physiological and molecular markers for identifying early indicators of heat-related health risk and improving risk assessment during extreme heat events. Elevations in biomarkers of AKI risk, including IGFBP7 and TIMP-2, indicate that prolonged heat exposure can induce subclinical kidney injury in both young and older adults, and evidence of endothelial injury via elevated circulating von Willebrand Factor (vWF) further suggests that heat exposure may initiate early vascular perturbations even when thermoregulatory responses remain largely preserved. However, given that AKI risk increased similarly in young and older adults which may be in part due to greater dehydration among younger adults whereas estimated glomerular filtration rate (eGFR) was reduced in older adults, eGFR may be an important assessment for assessing the risk of heat-related renal susceptibility among older adults. Together, these findings support the concept that biomarkers of endothelial injury and renal stress may serve as sensitive indicators of physiological strain during heat exposure before the onset of clinically detectable illness. Future research should therefore investigate whether these biomarkers or others can be used to identify individuals at heightened risk of heat-related complications (e.g., thrombosis and kidney injury) and determine whether repeated or multi-day heat exposures amplify these responses. Integrating easily measurable physiological markers into health screenings for older individuals living in high-risk locations could improve identification for individuals at heightened risk during extreme heat events and inform the development of evidence-based public health strategies aimed at reducing heat-related morbidity as heat waves become increasingly frequent and severe.

Role of the Endothelium

While thermoregulatory responses appeared to be largely preserved in older adults during 6 h of heat exposure, older individuals experienced greater cardiovascular strain and evidence of endothelial activation and injury, suggesting that vulnerability to heat may involve physiological pathways beyond impaired heat dissipation. Importantly, endothelial dysfunction may not only influence thermoregulatory capacity but may also contribute to downstream pathophysiological processes associated with heat-related illness.

The endothelium plays a central role in maintaining vascular homeostasis through regulation of vasomotor tone, hemostasis, inflammation, and microvascular perfusion[102,241,315,316]. During heat stress, cutaneous and systemic vasodilation are required to facilitate heat dissipation and maintain thermal balance[68,317,318]. These responses are highly dependent on endothelial signaling pathways, including nitric oxide (NO)-mediated vasodilation and shear-stress-induced endothelial activation[68,222,319]. In older adults, however, endothelial function is commonly impaired due to age-associated reductions in NO bioavailability, increased oxidative stress, chronic low-grade inflammation, and structural changes within the vascular wall[102,105,109,320]. As a result, the ability of the vasculature to appropriately respond to the hemodynamic and thermal demands of heat exposure may be compromised.

Endothelial injury and activation can promote vascular permeability, inflammatory signaling, and pro-thrombotic conditions that collectively increase cardiovascular and organ injury risk during extreme heat exposure[112,212,321]. These processes are particularly relevant in aging populations, where baseline endothelial dysfunction and vascular stiffness already exist[100,101,248]. Consequently, even when thermoregulatory responses appear relatively preserved, underlying endothelial dysfunction may predispose older adults to adverse cardiovascular and systemic outcomes during heat waves. Thus, these considerations suggest that the endothelium may represent a key mechanistic link between aging and increased vulnerability to heat-related morbidity and mortality. Understanding how heat stress influences endothelial function in older adults may therefore provide important insight into the physiological pathways that contribute to heat-related health risks and may help identify early vascular biomarkers of heat vulnerability, as the endothelium represents a critical aspect of cardiovascular and thrombotic risk during extreme heat stress.

Kidney Function as a Predictor

Our data also indicate that the risk of acute kidney injury increased similarly in both young and older adults, as evidenced by elevations in the cell-cycle arrest biomarkers IGFBP7 and TIMP-2. Few studies have directly compared renal responses to acute heat stress between young and older adults under controlled conditions. Most existing evidence linking heat exposure to kidney injury arises from epidemiological studies of heat waves in older

populations[144,322,323] or occupational studies in younger workers[166,187,188,324], limiting our understanding of age-specific renal responses to heat stress. To our knowledge, this is the first study to directly evaluate kidney function alongside biomarkers of acute kidney injury risk in both young and older adults during controlled, laboratory-based heat stress. Using this experimental model, we observed impairments in glomerular filtration and renal water handling following prolonged heat exposure. Additionally, renal concentration ability appeared reduced in the ~17-22 h following heat exposure, as urine osmolality and urine specific gravity remained lower in older compared to young adults during recovery. These findings highlight the potential for subclinical kidney stress during heat exposure even in healthy, aging individuals and suggests that age-related differences in renal function may influence recovery from heat stress. Future studies should therefore extend our current investigations into differences in heat-stressed kidney function between young and older adults, and how repeated or multi-day heat exposures exacerbate kidney dysfunction and lead to increased incidence of acute kidney injury among older adults.

CONCLUSIONS

In conclusion, thermoregulatory responses to heat stress appear relatively similar between healthy young and older adults during six hours of laboratory-based heat exposure with controlled water intake and light exercise to mimic activities of daily living. Despite similar thermoregulatory adjustments, older adults displayed reduced overall tolerance to heat, impaired kidney function, and physiological responses suggesting an elevated risk for cardiovascular or thrombotic events. While age-related reductions in thermoregulatory capacity have been well-characterized[65,74,84,231,325], laboratory-based heat exposures, including the present study, are inherently limited in their ability to fully capture the complex physiological challenges that occur during real-world heatwaves. Environmental heat events typically involve prolonged, multi-day exposures and interact with numerous age-related physiological changes. Thus, future research should extend beyond thermoregulatory outcomes and continue investigate mechanisms at the vascular and cellular level, including endothelial function, inflammatory and coagulation pathways, and other age-related alterations in susceptible organ systems. Additionally, this work should be extended to older individuals living with chronic conditions linked with heat-vulnerability such as diabetes, hypertension, cardiovascular disease, etc.[60,88]. Such work will be

critical for improving our understanding of why older adults experience disproportionate morbidity and mortality during extreme heat events.

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