ACUTE KIDNEY INJURY RISK IN AGING ADULTS: BIOMARKER RESPONSES TO HEAT STRESS

by

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Heat events are becoming increasingly frequent and severe due to climate change, posing a major public health issue for older adults (1). This population experiences disproportionately high rates of poor health outcomes during heat waves, including acute kidney injury (AKI), which is recognized as one of the top causes of hospitalization during heat waves (2). AKI is defined as a rapid (≤7 days) decline in kidney function, characterized by a decrease in glomerular filtration rate (GFR) and urine output (3, 4). Aging heightens this risk by limiting the body's ability to respond to thermal stress. Older adults experience impaired thermoregulation during heat exposure along with reduced renal perfusion reserve and a diminished capacity to conserve water and electrolytes (5–7), resulting in faster dehydration, slower cooling, and thus, quicker and larger rises in core temperature (5–7).

By 2050, the number of adults aged 65 years and older in the United States will nearly double (8). With more older individuals exposed to intensifying heat, determining how aging alters kidney injury pathways during heat stress is now a significant clinical priority. However, it remains unclear how the development and severity of AKI during prolonged heat exposure differs between young and older adults.

The purpose of this investigation was to evaluate age-related differences in kidney function and AKI risk during a six-hour simulated heat exposure using renal stress biomarkers. To assess these outcomes, this study measures glomerular filtration (creatinine clearance), fluid regulation (free water clearance and osmolality), the AKI risk biomarkers [IGFBP7·TIMP-2], and the etiology of renal stress (TRX-1 and MCP-1).

We hypothesize that older adults will exhibit greater impairments in kidney function, reflected by reduced glomerular filtration rate and a decline in fluid regulation. Additionally, we hypothesize that urinary [IGFBP7·TIMP-2] will increase to a greater extent in older adults compared to young adults immediately following a 6-hour heat exposure and remain elevated ~24 hours after the event. Finally, we hypothesize that older adults will demonstrate greater increases in biomarkers of oxidative stress (TRX-1) and inflammatory signaling (MCP-1).

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Literature Review

Climate Change, Heat Waves, and Older Adults

Over the past century, climate change has emerged as one of the most urgent global health crises. The frequency, duration, and intensity of heat events have increased globally due to human-driven climate change (9). According to the European Union's Copernicus Climate Change Service, the summer of 2022 was the hottest on record in Europe (1). This resulted in over 61,000 heat-related deaths, with the highest mortality occurring in adults aged 65–79 and those 80 years and older (1). Similar temperature patterns have been observed in North America, where the 2021 Pacific Northwest heat dome shattered temperature records and overwhelmed public health systems (10). This extremely hot weather places elderly individuals among the most vulnerable due to age-related alterations in thermoregulatory function. For example, one of the most devastating heat events in recent history was the 2003 European heatwave. This was the hottest summer recorded in Europe in over five centuries (11–14). Research on this heatwave revealed that most excess deaths occurred in the elderly population, with 82–92% of the excess deaths occurring among this group (15, 16).

This growing risk is compounded by demographic trends: as the global population ages, the number of individuals vulnerable to extreme heat continues to increase. By 2050, the global population of older adults is projected to surpass the population of children under 15. This "graying" of the population, combined with increasingly extreme summer temperatures, raises urgent questions about how to protect the health of aging individuals in a warming world.

Aging and Thermoregulation

Thermoregulatory Decline

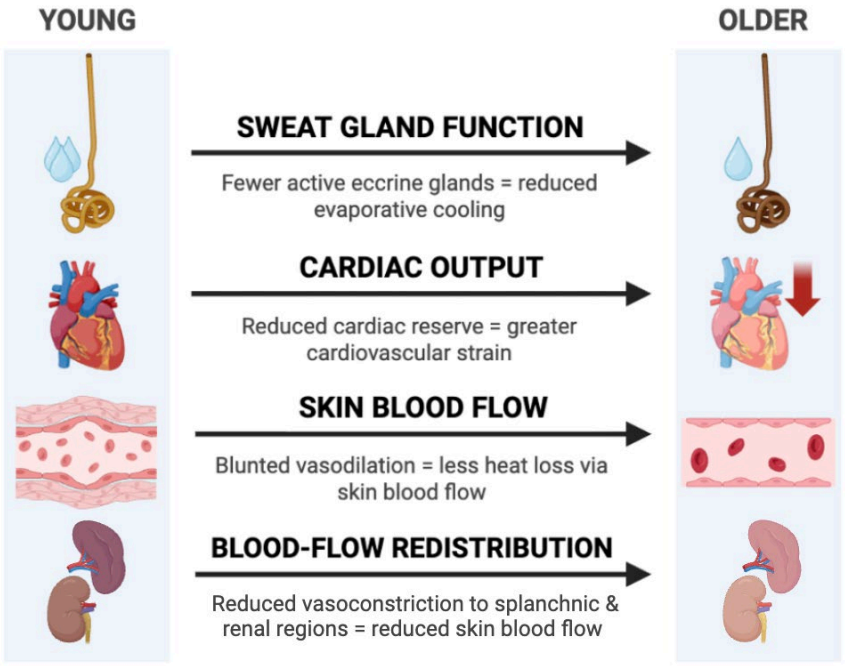


Figure 1. Age-Related Changes in Thermoregulation. Created using BioRender.com

Aging is associated with changes in thermoregulatory function (Figure 1). One of the most significant age-related changes is the reduced capacity for evaporative cooling. Older adults have fewer active sweat glands and a lower overall sweat rate, which limits their ability to dissipate heat through sweating (17). Sweating helps cool the body because as sweat evaporates from the skin, it removes heat, thus lowering core temperature. This is particularly concerning during prolonged heat exposure or physical exertion, where sweat evaporation becomes the primary method for cooling the body. Additionally, aging blunts cutaneous vasodilation due to reduced endothelial responsiveness and impaired nitric oxide signaling (18). This mechanism is essential for transferring core body heat to the periphery, where it can be lost to the environment. Impaired vasodilation delays this transfer, contributing to an increase in core body temperature.

The cardiovascular system also exhibits age-related changes that further impair thermoregulation. Older adults show reduced cardiac output and a reduced heart rate response during passive heat exposure, limiting the body's ability to redistribute blood flow to the skin for heat dissipation (19). Additionally, cardiovascular strain is increased during heat stress due to reduced cardiac reserve, requiring a greater effort relative to young adults. As a result, older adults operate at a higher percentage of their maximal heart rate to support blood flow to the skin for heat dissipation (20). These impairments result in a diminished ability to maintain safe core body temperatures during environmental heat stress.

Consequences

These physiological declines translate into measurable health risks. Older individuals are more likely to experience elevated core body temperatures during heat exposure, which can lead to adverse heat-related health outcomes (6). Furthermore, their limited ability to sweat and maintain blood pressure during states of dehydration increases the likelihood of thermal strain, hypotension, and syncope during heat exposure (4,17). Dehydration, which is a common consequence of prolonged heat exposure, can rapidly lead to kidney dysfunction, electrolyte imbalances, and delirium in older adults (21) . This risk is further amplified by medications commonly prescribed to older adults, such as diuretics, ACE inhibitors, and beta-blockers, which can impair sweating, alter blood pressure regulation, or reduce the kidney's ability to conserve water (6). In extreme cases, organ failure and death can occur (22). In addition, chronic diseases such as hypertension, diabetes, and cardiovascular disease are more prevalent in older adults and can further impair thermoregulation and increase susceptibility to heat-related complications (6). However, the present study focuses on relatively healthy older adults without any diagnosed metabolic, cardiovascular, or respiratory conditions to isolate the effects of aging itself.

Participants in the present study were also screened to ensure they were not taking medications that could alter thermoregulation, kidney function, or hydration status during heat exposure. The cumulative effects of aging, chronic disease, and impaired thermoregulation render this population highly vulnerable during extreme heat events.

Acute Kidney Injury and Heat Exposure in Older Adults

Prevalence in Older Adults

During heatwaves, AKI is a leading cause of hospitalization among older adults (23, 24). As individuals age, several changes in renal physiology reduce the kidneys' ability to maintain homeostasis under stress, increasing AKI risk. These include declines in renal blood flow, reduced glomerular filtration rate (GFR), impaired tubular function, and a diminished ability to concentrate urine (25, 26). Even at rest, renal blood flow is lower in older than younger adults, and during heat stress older adults redistribute less blood flow away from the renal and splanchnic circulations (27). This blunted redistribution reflects diminished vascular responsiveness to sympathetic signals rather than a weaker vasoconstrictor signal, leaving the aged kidney with a diminished capacity to adjust perfusion under thermal stress. From ages 30 to 80, renal mass decreases by approximately 10% per decade (28), leading to a gradual decline in kidney function and reduced ability to filter waste and maintain fluid balance. Older adults also experience a decrease in thirst perception, resulting in inadequate fluid intake (29). Together, these changes increase the likelihood of dehydration and hypovolemia (a state of decreased blood volume, often due to fluid loss), thus contributing to the heightened risk of AKI during heat exposure among older adults.

Pathophysiology of AKI During Heat Exposure

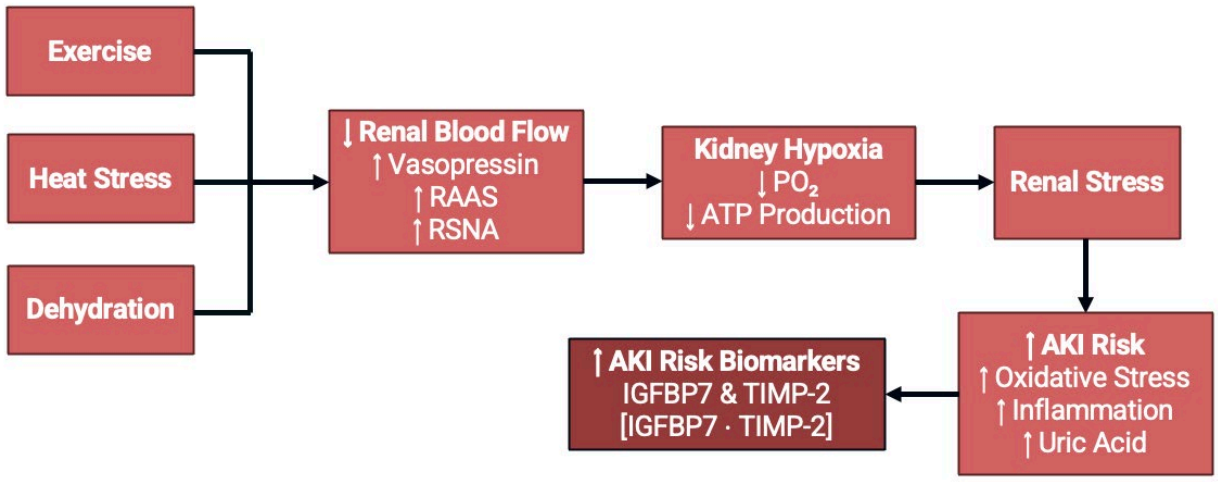


Figure 2. Etiology of AKI: Adapted from Chapman et al. 2021 (2). Exercise, heat stress, and dehydration reduce renal blood flow through increased vasopressin release, activation of the renin-angiotensin-aldosterone system (RAAS), and heightened renal sympathetic nerve activity (RSNA). This results in kidney hypoxia, as shown by decreased oxygen tension (pO_2) and adenosine triphosphate (ATP) production. This produces renal stress, leading to oxidative stress, inflammation, and uric acid accumulation that increase acute kidney injury (AKI) risk. These processes raise the clinical AKI risk biomarkers insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2).

Exercise, dehydration, and heat stress act together as the primary stimuli for AKI risk (30). As sweating persists, fluid losses lead to progressive dehydration, which reduces circulating blood volume and impairs the ability to maintain blood pressure (26, 31). In response to this combined physiological strain, the body activates several fluid-regulating and cardiovascular control systems. Figure 2 illustrates how these responses include increased release of vasopressin (32), activation of the renin–angiotensin–aldosterone system (RAAS) (33), and heightened renal sympathetic nerve activity (RSNA) (33), which constrict renal blood vessels and reduce renal blood flow (33).

As these regulatory systems conserve fluid and maintain blood pressure, they simultaneously reduce renal blood flow by narrowing blood vessels that supply the kidneys (34–

36). This restricted perfusion limits oxygen delivery to both the cortical and medullary regions of the kidney (37), and because the renal medulla already operates at a low oxygen tension (38), it becomes especially vulnerable under heat-stress (39, 40). Reduced oxygen availability causes a drop in tissue pO_2, as shown in Figure 2, forcing tubular epithelial cells to produce energy with insufficient oxygen. With limited oxygen, ATP production declines, and the kidney's energy-dependent processes become increasingly impaired. When ATP decreases, tubular cells cannot maintain normal ion gradients or structural stability, making them more susceptible to injury during prolonged heat exposure (41, 42). These early functional disruptions create conditions that lead to oxidative stress, inflammation, and uric acid build-up, all of which contribute to the initiation of AKI (43–45).

Oxidative stress arises when reactive oxygen species begin to accumulate faster than the kidney can neutralize them, overwhelming antioxidant defenses that normally protect membrane lipids, proteins, and DNA (46). As this imbalance worsens, reactive oxygen species disrupt structural stability in tubular cells and impair the transport mechanisms required for maintaining filtration (46). The resulting cellular injury increases the kidney's vulnerability during heat exposure and interacts with inflammatory and metabolic pathways that further contribute to AKI development.

Additionally, heat stress can cause renal injury, which activates an immune response aimed at repairing the kidney tissue (47, 48). However, immune cell activation can exacerbate renal injury by amplifying inflammation (47, 48). Inflammatory cells release reactive oxygen species and cytokines (30, 49), which can induce oxidative stress, thus impairing kidney blood flow and filtration (46). Oxidative and inflammatory stress can also activate cell cycle arrest pathways, which are detected by the AKI biomarkers insulin-like growth factor binding protein 7

(IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2), the two FDA-approved biomarkers used clinically to identify early tubular stress before measurable loss of kidney function (50).

Another factor that contributes to kidney stress during heat exposure is uric acid build-up. Under normal conditions, uric acid is filtered by the kidneys and reabsorbed into the blood or excreted in the urine (51). However, during dehydration and reduced glomerular filtration, uric acid levels increase in the blood due to both increased production and reabsorption (52–54). While uric acid is often used as an indication of impaired renal function, it can contribute to further injury. Increased uric acid levels can produce additional reactive oxygen species, which damage cells and trigger inflammation in kidney tissue (54). Uric acid has also been shown to increase ATP demand and directly reduce renal blood flow (2, 55, 56), which further amplifies inflammation and oxidative stress (57).

It's important to note that additional metabolic pathways that may accelerate cellular injury during heat stress, including the polyol-fructokinase (PFK) pathway which has been associated with increased oxidative stress and renal injury (58). Although this mechanism plays a role in heat-induced AKI, it is not examined in the present study due to the lack of feasible, reliable biomarkers for assessing this pathway in human participants.

Previous Research on AKI and Heat Stress

A growing body of research demonstrates that AKI risk rises during episodes of environmental heat stress. While much of the experimental work has examined young, healthy men, epidemiological and experimental studies in older adults highlight that aging may alter susceptibility to kidney injury under thermal strain.

Epidemiological studies provide strong evidence that older adults are disproportionately affected. A large case–control study in Ontario, Canada, reported that periods of extreme heat were associated with a significant increase in hospital encounters for AKI among older adults (48). In this study, heat periods were defined as three consecutive days above the local 95th percentile for maximum daily temperature. According to this classification, researchers found that during the warm season, there were about 182 more AKI cases for every 100,000 older adults each year (59). Similarly, a recent longitudinal cohort study in China used data from the China Health and Retirement Longitudinal Study to find that repeated exposure to high-intensity heat waves accelerated renal function decline in older adults. The risk of rapid kidney deterioration was most pronounced during the strongest and longest-lasting heat events, underscoring a dose–response relationship between cumulative heat exposure and AKI outcomes (60). Together, these population-level findings point to heat as an independent stressor that elevates AKI risk in older age groups.

In an experimental study investigating the effect of prolonged passive heat exposure at increasing wet-bulb temperatures, researchers demonstrated that sustained thermal strain can elevate AKI risk (61). Young, healthy men were exposed to 8 hours of rest at wet-bulb temperatures of 31 °C and 34 °C without fluid intake. The study found that the product of insulin-like growth factor–binding protein 7 and tissue inhibitor of metalloproteinases-2 [IGFBP7·TIMP-2], an FDA-approved biomarker of early renal tubular stress and AKI risk, increased significantly in both trials, with a substantially greater rise at 34 °C. This was accompanied by increases in urinary markers of oxidative stress and systemic inflammation. This suggests that thermal strain alone, even in the absence of exercise or illness, can initiate renal injury through hemodynamic and cellular pathways. However, a major limitation of this study

was that it was only done in young healthy men. It's important to assess at risk populations because older adults and individuals with chronic conditions have reduced renal perfusion reserve, impaired thermoregulation, and slower cardiovascular adjustments during heat exposure, making them more susceptible to heat-induced AKI at lower levels of stress than healthy young adults.

To directly compare responses across age groups, controlled laboratory studies have extended this work by comparing renal biomarkers to heat between older and young adults. In one experiment using a water-perfused suit to elevate core temperature by ~2 °C, both young and older participants demonstrated comparable increases in early kidney injury biomarkers such as neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) and [IGFBP7·TIMP-2], along with reductions in renal function (62). This indicates that when thermal strain is precisely matched, the acute renal response to heat is similar across age groups (62).

Additionally, one experiment exposed young and older adults to 3 h of extreme heat (dry: 47 °C, 15% RH and humid: 41 °C, 40% RH), with intermittent light activity and controlled hydration (63). They found modest but significant increases in plasma creatinine and cystatin C in older adults post-exposure, but minimal changes in the younger group. However, these differences in kidney function markers were small and provide only limited evidence that older adults may experience greater renal strain during short periods of heat stress.

Together, these studies highlight two important points. First, older adults are at greater population-level risk of AKI during heat waves, likely due to age-related declines in renal reserve, impaired thermoregulation, and higher prevalence of comorbidities. Second, controlled laboratory data suggests that under carefully matched thermal loads, the renal response may not

differ substantially between young and older individuals. This discrepancy underscores a critical gap: few experimental studies have examined prolonged heat exposures in older adults under real-world conditions.

Age-Related Considerations on AKI Risk

Interpreting AKI risk in older adults is further complicated by age-associated baseline elevations in certain kidney injury biomarkers, which can obscure the detection of acute changes (64). Additionally, older individuals have a reduced renal functional reserve, meaning that their kidneys may struggle to adapt to physiological stressors even when resting measures appear normal (65). For example, renal blood flow declines by approximately 10 percent per decade after age 40 (7), and median estimated glomerular filtration rate decreases from about 110 mL · min^{-1} · 1.73 m^2 at age 35 to approximately 80–85 mL · min^{-1} · 1.73 m^2 at age 75 in both men and women (66), alongside a 20–50 percent reduction in urine concentrating capacity (5). This reduced adaptability, coupled with chronic low-grade inflammation and impaired thermoregulation, may amplify injury responses to heat exposure (67). In summary, while AKI is increasingly recognized as a heat-sensitive outcome, more research is needed to characterize its presentation and possible interventions for the prevention in older adults.

Biomarkers of AKI Risk & Kidney Function

Biomarkers are measurable biological substances that reflect specific processes occurring in the body. To understand how heat stress affects kidney function across young and old adults, this study incorporates a variety of biomarkers to detect signs of renal stress and track changes in kidney function that lead to AKI risk. Specifically, these biomarkers can reflect if the kidney's cells are under stress, if they're filtering properly, and if processes such as inflammation or oxidative stress are active.

Table 1. Biomarkers

Biomarker	Measurement	Outcome
IGFBP7·TIMP-2	Urine	Acute kidney injury risk
Osmolality	Serum and Urine	Kidney Function
Free Water Clearance	Serum and Urine	Kidney Function
Creatinine	Serum and Urine	Kidney function (creatinine clearance)
TRX-1	Serum and Urine	Marker of oxidative stress
MCP-1	Serum and Urine	Marker of inflammation

AKI = acute kidney injury; IGFBP-7 = insulin-like growth-factor binding protein 7; TIMP-2 = tissue inhibitor of metalloproteinases 2; TRX-1 = thioredoxin-1; MCP-1 = monocyte chemoattractant protein 1.

Primary and Secondary Markers of AKI Risk

When the kidneys are under stress, the renal tubular epithelial cells (specialized cells that line the nephron) can sense physiological stress before irreversible damage occurs (68). In response, these cells halt cell division, as attempting to replicate while under metabolic stress can lead to mutated or dead cells (69). During this arrested state, the renal tubular epithelial cells release two specific proteins into the urine: IGFBP7 and TIMP-2 (70). Since this response occurs within 2-4 hours of stress (before the kidneys begin showing functional decline), these biomarkers have been approved by the FDA for clinical prediction of AKI (71).

Kidney Function Indicators

While the previous biomarkers are useful for detecting early signs that the kidney is under stress, they do not measure how well the kidney is performing its primary functions (filtration, reabsorption, and excretion of waste). To measure these processes, this study includes functional biomarkers that reflect the kidney's ability to maintain homeostasis during and after heat exposure.

Creatinine, a waste product filtered out as muscles perform their everyday activities, is filtered out of the blood by the kidneys and excreted in the urine. Measuring serum and urine creatinine allows one to estimate how effectively the kidneys are clearing waste, and tracking creatinine over time can reveal shifts in filtration capacity (72). Clinically, AKI is identified by an increase in serum creatinine of ≥ 0.3 mg $\cdot$ dL^{-1} within 48 hours ≥ 1.5 times baseline within 7 days, or reduced urine output below 0.5 mL $\cdot$ kg^{-1} $\cdot$ hr^{-1} for at least 6 hours (73). However, creatinine can be influenced by non-renal factors. For example, dehydration (which often occurs during heat stress) may elevate serum creatinine without reflecting true kidney damage (74). Older adults may have a lower baseline creatinine due to reduced muscle mass, which may mask declines in kidney function (72). Despite this limitation, creatinine is an important functional marker in this study's assessment of AKI risk.

To account for this shortcoming, the present study also measures creatinine clearance to provide a more direct assessment of renal filtration. Creatinine clearance is calculated by measuring the amount of creatinine excreted in the urine over a defined period relative to the plasma creatinine concentration. This yields an estimate of the volume of blood the kidneys fully clear of creatinine per unit time (mL $\cdot$ min^{-1}). This serves as the most common means of estimating glomerular filtration rate (eGFR), which is widely regarded as the most reliable

overall index of renal function . Because creatinine clearance accounts for individual differences in muscle mass, age, and body composition that influence baseline serum creatinine, it provides a more sensitive measure of reductions in renal filtration capacity.

Mechanistic Markers

In addition to AKI risk and functional biomarkers of kidney function, this study incorporates mechanistic biomarkers that provide insight into the underlying processes driving acute kidney injury risk during heat stress. These markers help show why injury may occur by identifying cellular pathways such as oxidative stress and inflammation.

Thioredoxin-1 (TRX-1) is an antioxidant protein that helps protect cells from oxidative damage by reducing reactive oxygen species and repairing oxidized proteins through thiol-disulfide exchange reactions (2). When oxidative stress increases, TRX-1 becomes more active or elevated as the body attempts to counteract damage (2). Measuring TRX-1 in this study helps determine whether oxidative stress responses are being activated during heat exposure, indicating that oxidative damage may be contributing to kidney stress.

Monocyte chemoattractant protein-1 (MCP-1) is a signaling protein that attracts immune cells to sites of injury. When the kidneys are stressed or damaged, MCP-1 levels rise as part of the inflammatory response. Measuring MCP-1 in this study allows us to evaluate how much inflammation contributes to kidney stress during heat exposure.

Methods

Participants

Thirteen young (18–35 y) and fourteen older (60–80 y) adults were enrolled in this study. All participants were non-smokers and free of any cardiovascular, respiratory, or metabolic disease. Participants were excluded if they were taking any of the following medications daily: aspirin, diuretics, anticholinergic agents, non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, or more than one anti-hypertensive medication.

Participants were also excluded if they met any of the following criteria:

1. Non-English speaking.
2. History of stroke, clotting disorder, or venous thrombosis.
3. History of heat illness/heat injury, orthostatic intolerance, or hypotension.
4. Currently undergoing routine heat therapy (hot tubs, saunas, hot baths).
5. Currently pregnant, breastfeeding, desiring pregnancy in the next 6 months, or undergoing treatment to increase sperm count.
6. Mobility restrictions that interfere with physical activity.
7. Pacemaker or other electrically implanted device.
8. Resting systolic BP ≥140 mmHg.
9. Resting diastolic BP ≥90 mmHg.
10. Contraindication to ingestible core-temperature pill.
11. Any ACSM absolute indication for exercise cessation.
12. Three or more ACSM relative indications for exercise cessation.
13. Contraindication to nitroglycerin use.

14. Mini mental state exam ≤ 27.

15. Body mass index <18 or >35 kg/m².

16. Urine albumin-creatinine ratio <30 mg/g.

Table 2. Subject Demographics

Characteristic	Young (n = 13)	Older (n = 14)
Sex (F/M)	7 / 6	8 / 6
Age (y)	23.85 ± 3.48	68.07 ± 6.42*
Weight (kg)	67.67 ± 10.10	77.06 ± 13.09*
Height (cm)	170.1 ± 12.81	166.9 ± 17.04
Body mass index (kg/m^2)	23.45 ± 3.54	28.41 ± 7.99*
Systolic blood pressure (mmHg)	115 ± 12.38	123 ± 12.79
Diastolic blood pressure (mmHg)	76 ± 8.48	77 ± 9.56

Data are presented as means ± SD. *P<0.05 vs Young

Study Design

After an over-the-phone pre-screening to ensure participants qualified and in-person screening to ensure participants didn't meet any of the exclusion criteria, participants completed three experimental visits at the Bowerman Sports Science Center. Visits 2, 3, and 4 occurred on consecutive days to ensure consistent environmental and physiological conditions across testing sessions. The protocol was approved by the University of Oregon Institutional Review Board.

Visit 1: Screening and Functional Capacity Exercise Test

Participants were asked to arrive having abstained from caffeine and food at least two hours before arrival. Participants were also instructed to avoid alcohol, nicotine, recreational drugs, over-the-counter supplements or medications, and any moderate-to-vigorous exercise during the 24 hours preceding their visit. After obtaining their written informed consent, participants completed a Health History Questionnaire, International Physical Activity Questionnaire, and a Mini-Mental State Exam. Additionally, body mass index (BMI) was calculated based on weight and height, resting blood pressure was measured, and a urine sample was collected to screen for pregnancy and ensure normal kidney function via urine-albumin creatinine ratio. Participants then performed a light-workload trial: they pedaled at 60-70 rpm while resistance was gradually raised from 20 W until their oxygen uptake stabilized near 10.5 $mL \cdot kg^{-1} \cdot min^{-1}$ which is equivalent to 3 metabolic equivalents (METs). The wattage that elicited this low-intensity effort was recorded and later used for the six-hour simulated heat event to stimulate activities of daily living during the visit. Then, participants were instrumented with a 12-lead ECG to monitor cardiac rhythm and screen for any cardiac abnormalities. Participants then completed a graded exercise test: following a brief warm-up, resistance increased by 10 W every minute at the same cadence until they reached 85% of their age-predicted heart rate max. The test stopped and the participant was excluded from the study if any American College of Sports Medicine absolute indications for exercise cessation were met or if 3 or more of the relative indications for exercise cessation were met (75).

Visit 2: Baseline Vascular Function Testing

After refraining from eating for at least six hours before arrival, caffeine for 12 hours, and adhering to the same 24-hour abstentions as described in Visit 1, participants underwent a

vascular endothelial function assessment, including non-invasive ultrasound techniques of flow-mediated dilation (FMD). However, these tests were not central to this thesis' focus on acute kidney injury risk and are therefore not discussed further. Participants then received a core temperature pill to ingest the evening before Visit 3 as well as two large urine collection containers to collect all voided urine between Visits 2 and 3. These samples were used to assess baseline kidney function. To control for dietary sodium intake, participants also received a standardized overnight oats breakfast and were instructed to consume 500 mL of water the night before and morning of Visit 3 to ensure adequate hydration.

Visit 3: Heat Event

After confirming that the participant was adequately hydrated (urine specific gravity ≤ 1.020), researchers collected a baseline venous blood draw, nude body weight, and placed eight skin-temperature sensors on the participant. They then entered an environmental chamber set to 42 °C and 20 % RH for 6 hours. Every 45 minutes, participants performed 15 minutes of light cycling at an intensity of 3 METs, which is a level comparable to light daily activities such as walking or doing household chores. Core temperature, skin temperature, heart rate, blood pressure, and perceptual scales (rating of perceived exertion (RPE), thermal perception, thermal comfort, and skin wetness) were recorded throughout. Cognitive function was assessed at the start of the heat event, three hours into the heat event, and at the end of the heat event. Participants consumed 3 mL of water per kilogram of their body weight every hour and received a granola bar after the 3-hour mark. Urine was collected continuously (from 0-3 hours and 3-6 hours), and venous blood draws were collected again at hours 3 and 6 of the heat event. After the heat event, a final nude body weight was obtained to calculate total sweat loss. Two urine

collection containers were provided to collect all urine excreted during the 24 hours leading up to Visit 4.

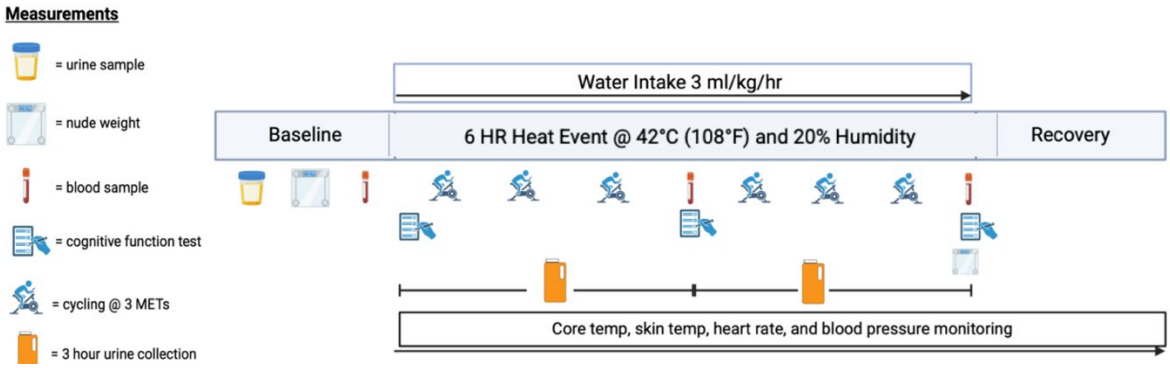


Figure 3. Simulated Heat Event Protocol. Created using BioRender.com

Visit 4: Post-Heat Vascular Function Testing

Twenty-four hours after the start of Visit 3, participants repeated FMD and GTN tests to determine the vascular effects of heat exposure and provide a final venous blood sample.

Urine Collection & Analysis

Urine Collection Protocol

At each collection timepoint, researchers collected urine into nine labeled cryovials per sample time point (PRE, BL, END, POST). This included both urine collected during the 6-hour heat event and the containers brought in from participants' overnight urine collections. All cryovials were immediately stored at -20°C for preservation until biomarker analysis.

Assay Procedure

Urine specific gravity was assessed by refractometry at baseline (BL), end of heat exposure (END), and post (POST) to measure hydration status and normalization of urinary biomarkers. Urine flow rate (UFR) was determined by dividing total urine volume by the

duration of each collection period. For BL, collection time was defined as the interval between the Pre-Vascular Function visit and the Heat Event visit (24 h). END collection time points were 3 h, unless heat exposure was terminated early. POST collection time corresponded to the time between completion of the Heat Event and the Post-Vascular Function visit (~17-22 h).

Serum and urine creatinine, as well as urinary IGFBP7, TIMP-2, MCP-1, and TRX-1 were measured using commercially available assays (RayBio® Human Creatinine ELISA Kit, RayBiotech, Peachtree Corners, GA, USA). Glomerular filtration rate (GFR) was estimated via creatinine clearance, which was calculated as: (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)Free water clearance was calculated as: urine flow rate × $(1 - [U_{osm} \div P_{osm}])$, where U_{osm} and P_{osm} represent urine and plasma osmolality, respectively. Osmolar clearance was calculated as $(U_{osm} \div P_{osm})$ × urine flow rate (76).

Data and Statistical Analysis

Statistical analyses were conducted using Prism 10.6.1 (GraphPad Software, Inc., San Diego, CA). Data were analyzed using mixed-effects models (Age × Time). When significant main effects were observed, post hoc analyses were performed using Šídák's multiple comparisons test. Single-time-point outcomes (Δ core temperature, total sweat loss, and percent change in body mass) were compared between age groups using unpaired, two-tailed t tests. All urinary biomarkers (IGFBP7, TIMP-2, MCP-1, and TRX-1) were log transformed prior to analyses for normalization of data. Statistical significance was set a priori at $a = 0.05$, and exact P values are reported where possible.

Results

Thermoregulatory Responses

Kaplan–Meier curves depicting the proportion of participants remaining during the 6-hour heat exposure are shown in Figure 4. Early terminations occurred in 2 of 13 young participants and 7 of 14 older participants (Figure 4). Changes in core temperature (T_c) over time (hr) are shown in Figure 5. T_c increased over time in both groups ($P < 0.001$) (Figure 5), did not differ between groups ($P = 0.187$) and showed no differences between groups over time ($P = 0.799$). The overall change in T_c from baseline to end of heating (ΔT_c) did not differ between young [0.866 ± 0.441 °C] and older participants [1.057 ± 0.566 °C, $P = 0.336$] (Figure 6A). There was no difference in the overall thermal load experienced between young [2.606 ± 1.979 °C·h] and older adults [2.726 ± 1.870 °C·h, $P = 0.875$] (Figure 6B). Sweat rate ($L \cdot kg^{-1} \cdot h^{-1}$) did not differ between young [0.375 ± 0.084] and older participants [0.362 ± 0.093, $P = 0.706$] (Figure 7A). Percent change in body mass differed between groups, with a greater percent change in body mass in young [−3.156 ± 0.797%] compared with older participants [−2.502 ± 0.610%, $P = 0.027$] (Figure 7B).

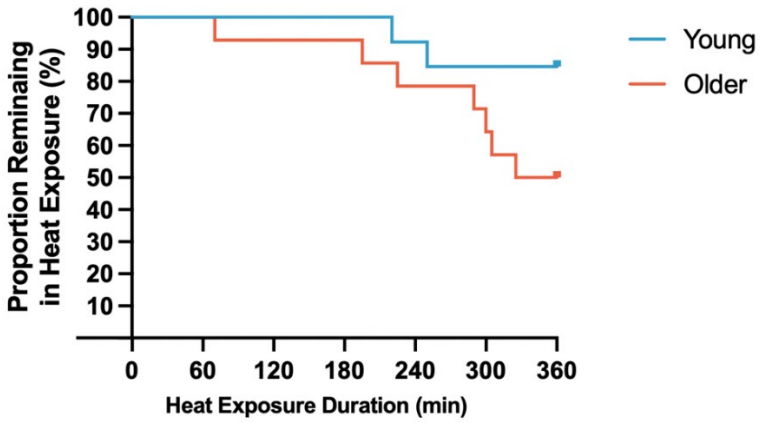


Figure 4. Kaplan–Meier curves depicting the proportion of participants remaining during prolonged heat exposure in young and older groups.

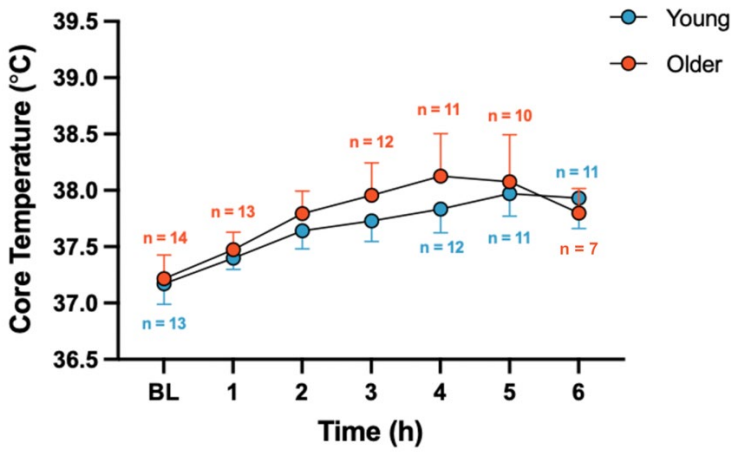


Figure 5. Time course of core temperature responses during 6-hour heat exposure in young and older participants.

Core temperature (°C) presented at baseline (BL) and each subsequent hour of heat exposure. Sample sizes (n) decrease across time points due to participant early termination of heat exposure. Only individuals who completed a given time point are included in the corresponding data.

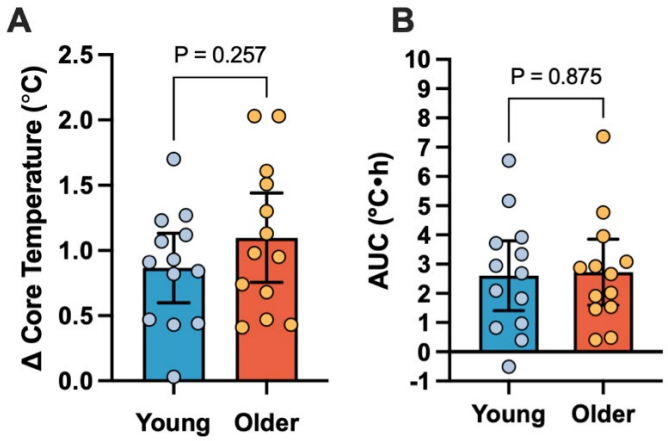


Figure 6. Comparison of peak core temperature and cumulative thermal load during prolonged heat exposure in young and older participants.

(A) Change in core temperature (Δ°C) from baseline to end of heating. (B) Area under the curve (AUC; °C·h) for core temperature, representing cumulative thermal load over time.

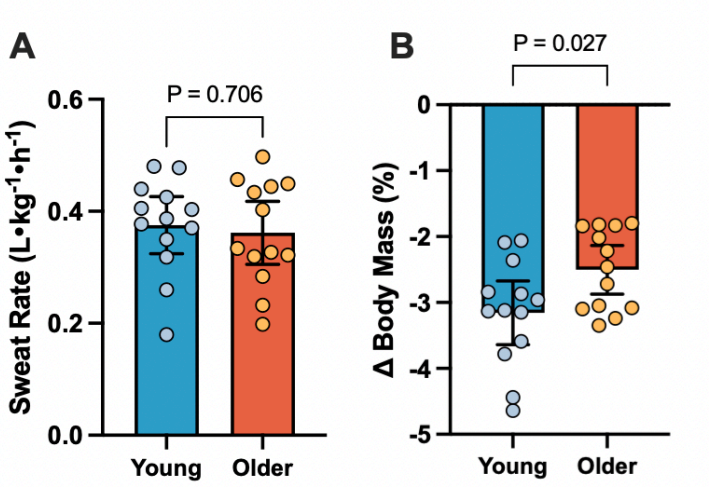


Figure 7. Comparison of sweating and percent change in body mass during heat exposure in young and older participants.

(A) Sweat rate ($L \cdot kg^{-1} \cdot h^{-1}$). **(B)** Percent change in body mass (%).

Stress and Mechanistic Markers of AKI

Urinary kidney biomarkers corrected for urine specific gravity are shown at baseline (BL), end of heating (END), and ~24 h post-heating (POST) in young and older participants. The product of insulin-like growth factor–binding protein 7 and tissue inhibitor of metalloproteinases-2 (IGFBP7•TIMP-2) increased from BL to END in both young ($P < 0.001$) and older participants ($P = 0.002$), and decreased from END to POST in both groups (young: $P = 0.002$; older: $P = 0.002$), with no difference between age groups at BL ($P = 0.906$), END ($P = 0.810$), or 24 ($P = 0.801$). Monocyte chemoattractant protein-1 (MCP-1) decreased from BL to END in young participants ($P < 0.001$) and older participants ($P = 0.003$), with a difference between groups only at POST ($P = 0.046$). Thioredoxin-1 (TRX-1) increased from BL to END in young ($P = 0.006$) and older participants ($P = 0.019$) and decreased from END to POST in young ($P = 0.003$) and older participants ($P = 0.013$), with no difference between age groups at BL ($P = 0.906$), END ($P = 0.239$), or 24 ($P = 0.819$).

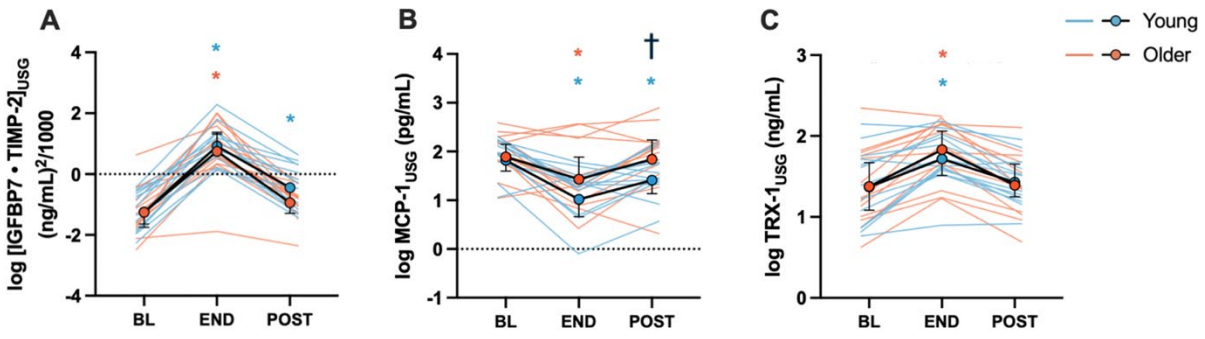


Figure 8. Urinary biomarker responses across heat exposure and recovery in young and older participants.

(A) Product of insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2 [IGFBP7·TIMP-2] normalized to urine specific gravity (USG), **(B)** monocyte chemoattractant protein-1 (MCP-1) normalized to USG, and **(C)** thioredoxin-1 (TRX-1) normalized to USG. Biomarkers were measured at baseline (BL), end of heating (END), and ~24 h post-heating (POST). *Different from BL (P<0.05); †Different from young (P<0.05).

Renal and Fluid Balance Responses

Renal and fluid balance responses to the 6-hour heat exposure in young and older adults are presented in Table 3. Urine specific gravity increased from baseline to the end of heating and was higher in young [1.024 ± 0.003] compared to older adults [1.014 ± 0.007] at the end of heating (P<0.001) and remained higher in younger adults after heating (P=0.028). Urine creatinine was higher in young [344.8 ± 82.0 mg/dL] compared to older adults [186.8 ± 103.6 mg/dL] at the end of heating (P<0.001) and remained elevated in younger adults after heating (P=0.043). Serum creatinine was higher in older [1.07 ± 0.22 mg/dL] than young adults [0.80 ± 0.19 mg/dL] at the end of heating (P=0.003). Creatinine clearance was higher in young adults [205.7 ± 172.2 mL/min] than older adults [72.1 ± 28.9 mL/min] at the end of heating (P<0.001). Urine osmolality was higher in young [897 ± 138 mOsmol·kg^{-1} H_2O] than older adults [527 ±

219 mOsmol·kg⁻¹ H_2O] at the end of heating (P<0.001) and remained higher after heat exposure (P=0.015). Free water clearance was negative in both groups at all time points and was lower in young [−1.0 ± 0.9 mL/min] compared to older adults [−0.4 ± 0.3 mL/min] at the end of heating (P = 0.028).

Table 3. Renal and fluid balance responses to prolonged heat exposure in young and older adults.

Outcome	Young BL	Young END	Young POST	Older BL	Older END	Older POST
Urine specific gravity	1.008 ± 0.004	1.024 ± 0.003*	1.017 ± 0.005*	1.006 ± 0.004	1.014 ± 0.007*‡	1.010 ± 0.009‡
Urine creatinine (mg/dL)	64.5 ± 23.3	344.8 ± 82.0*	153.8 ± 55.4*	52.8 ± 20.6	186.8 ± 103.6*‡	99.4 ± 72.7‡
Serum creatinine (mg/dL)	0.83 ± 0.14	0.80 ± 0.19	0.79 ± 0.10	0.92 ± 0.17	1.07 ± 0.22*‡	0.88 ± 0.16
Creatinine clearance (mL/min)	128.2 ± 39.6	205.7 ± 172.2*	139.9 ± 36.8	105.3 ± 25.1	72.1 ± 28.9‡	135.9 ± 63.4
Urine osmolality (mOsmol·kg⁻¹ H_2O)	384 ± 138	897 ± 138*	690 ± 210*	398 ± 183	527 ± 219‡	455 ± 242‡
Free water clearance (mL/min)	-0.4 ± 0.5	-1.0 ± 0.9	-1.0 ± 0.4*	-0.4 ± 1.0	-0.4 ± 0.3‡	-0.5 ± 1.1

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).

Discussion

The purpose of this study was to evaluate age-related differences in kidney function and acute kidney injury (AKI) risk during a six-hour simulated heat exposure by comparing kidney stress biomarkers in young and older adults. It was hypothesized that there would be age-related differences in kidney function, with older adults demonstrating greater reductions in glomerular filtration rate and impairments in fluid regulation compared to younger adults. Additionally, it was hypothesized that heat exposure would elicit greater AKI risk in older adults, evidenced by larger increases in urinary [IGFBP7·TIMP-2] following the heat event. Finally, it was hypothesized that heat exposure would elicit a greater oxidative stress and inflammatory response in older adults, reflected by larger increases in TRX-1 and MCP-1.

Despite both groups experiencing similar sweat rates and changes in core temperature, older adults had reduced heat tolerance during the protocol, with a greater number of older adults terminating heat exposure early. However, young adults experienced a greater percent reduction in body mass, indicating greater relative dehydration. Together, these findings suggest that overall thermal responses were comparable, but tolerance to prolonged heat exposure was reduced in older adults.

Older adults exhibited decreases in kidney function, as demonstrated by reduced creatinine clearance and impaired fluid regulation during heat exposure. AKI risk biomarkers [IGFBP7]·[TIMP-2] increased similarly during heat exposure in both age groups. TRX-1 increased during heating and declined after 24 hours, with no age-group differences. MCP-1 decreased over time in both groups, which was unexpected. The reason for this response is unclear, however, these findings suggest that oxidative stress may play a more prominent role in driving AKI risk in the present study. These results suggest that while healthy older and younger

adults yield similar AKI risk biomarker responses under heat stress, older adults experience greater functional decline.

Thermoregulatory Responses

Core temperature increased over time during the six-hour heat exposure in young and older adults, with no difference between age groups ($P > 0.05$). The overall change in core temperature from baseline to end of heating did not differ between young [0.866 ± 0.441 °C] and older adults [1.057 ± 0.566 °C]. These findings differ from the work of McKenna et al., which used a three-hour heat exposure model that incorporated brief activity bouts to simulate activities of daily living (63). In McKenna et al., older adults demonstrated larger increases in core temperature than young adults in both conditions: hot-dry [older: $\Delta T_c = 1.37 \pm 0.42$ °C; younger: $\Delta T_c = 0.68 \pm 0.27$ °C] and hot-humid [older: $\Delta T_c = 1.02 \pm 0.32$ °C; younger: $\Delta T_c = 0.58 \pm 0.25$ °C] (63). McKenna et al. may have observed clear age differences in core temperature, while this study did not due to the greater withdrawal from the heat exposure in older adults. As shown in the Kaplan-Meier curve (Figure 7), 50% of the older adults terminated the protocol early, resulting in shorter exposure time, thus limiting their rise in core temperature.

Given that some participants did not complete the entire 6-hour heat exposure, changes in core temperature were expressed as area under the curve (AUC) to represent total thermal load (Figure 6B). AUC did not differ between young (2.606 ± 1.979 °C·h) and older adults (2.726 ± 1.870 °C·h, $P = 0.875$). This indicates that overall thermal load was comparable between groups despite the early terminations in older adults. Because older adults experienced a similar thermal load to younger adults despite their shorter exposure time, they demonstrate a reduced capacity to tolerate prolonged heat.

Sweat rate did not differ between young [0.375 ± 0.084·kg^{-1}·h^{-1}] and older adults [0.362 ± 0.093 ·kg^{-1}·h^{-1}]. Percent change in body mass loss was greater in young adults [−3.156 ± 0.797%] than older adults [−2.502 ± 0.610%]. However, McKenna et al. observed no age-group differences in body mass loss (62). The difference here may be explained by older adults dropping out of the heat exposure, as shown in the Kaplan-Meier curve (Figure 7), resulting in a shorter exposure time and reduced sweat loss compared to young adults. Despite this greater fluid loss in young adults, AKI risk did not differ between groups. Urinary [IGFBP7·TIMP-2] increased from baseline to the end of heating in both young ($P < 0.001$) and older adults ($P = 0.002$), with no difference between groups at any timepoint. Because thermal load was also comparable, the similar AKI risk cannot be attributed to greater heat exposure, nor to greater fluid loss. Instead, these findings suggest that older adults reached the same threshold of renal stress with less physiological stress, which is consistent with an age-related increase in renal vulnerability.

Kidney Function

Creatinine clearance, derived from serum and urinary creatinine concentrations, is an estimate for glomerular filtration rate (eGFR), which measures the kidney's ability to filter waste products and maintaining electrolyte balance. During heat stress, kidneys are less effective at maintaining these hemodynamic and osmotic functions (63). Given the effect of heat stress and the decline of GFR with age (77), it follows that creatinine clearance post-heat exposure remained the same in older adults [72.1 ± 28.9 mL/min, $P = 0.672$], while it increased in young adults [205.7 ± 172.2 mL/min, $P = 0.048$]. This suggests that older adults have a reduced capacity to increase GFR under stress. Accordingly, a limited ability to increase filtration during heat stress may have contributed to the higher levels of creatinine in the blood in older adults

[1.07 ± 0.22 mg/dL, P = 0.003]. This data is supported by McKenna et al (2024), who reported that older adults, but not young adults, exhibited higher levels of creatinine after exposure to hot and dry heat (63). Together, these findings indicate that aging is associated with a reduced ability to enhance renal filtration during thermal stress.

In addition, older adults show a blunted fluid regulatory response. Free water clearance is a measure of the volume of dilute urine excreted by the kidneys, with more negative values indicating greater water reabsorption and more concentrated urine (78). Following heat exposure, free water clearance became more negative in young adults [−1.0 ± 0.4 mL/min], while older adults showed no change from baseline [−0.5 ± 1.1 mL/min]. This lack of a reduction in free water clearance in older adults suggests that aging is associated with a diminished capacity to increase water reabsorption during heat stress.

A similar pattern was observed in urine specific gravity (USG), with smaller increases at the end of heat exposure in older adults [1.014 ± 0.007] compared to young adults [1.024 ± 0.003]. In addition, older adults returned to baseline [1.010 ± 0.009], whereas young adults maintained an elevated USG [1.017 ± 0.005]. This indicates a less robust and less sustained ability to concentrate urine in response to heat stress.

Urine osmolality further reinforces this trend, as values were lower in older adults [527 ± 219 mOsmol·kg^{-1} H_2O] compared to young adults [897 ± 138 mOsmol·kg^{-1} H_2O] at the end of heating and remained lower 24 hours after the heat event. The lower urine osmolality observed in older adults reflects a reduced concentration of solutes in the urine, suggesting that age diminishes the kidneys' ability to conserve water and concentrate urine.

Acute Kidney Injury Risk

Renal biomarkers showed evidence of acute kidney injury risk during the heat event, but no age-related differences in the magnitude or time course of the response. Specifically, [IGFBP7•TIMP-2] increased from BL to END in young ($P = 0.001$) and older adults ($P = 0.002$), then declined from END to 24 in both groups ($P = 0.002$), with no between-group differences at BL ($P = 0.906$), END ($P = 0.810$), or 24 ($P = 0.802$; Fig X). Additionally, the mechanistic biomarkers indicated a temporary rise in oxidative stress during heat exposure without a corresponding inflammatory response. TRX-1 increased from BL to END and then decreased by 24 h in both age groups (Figure 8C), indicating a temporary rise in oxidative stress during heat exposure. In contrast, MCP-1 showed no effect of time or age (Figure 8B), suggesting that this stress did not elicit a measurable inflammatory response. Together, these findings indicate that the renal stress observed during this 6-hour heat event may be driven primarily by oxidative stress rather than inflammatory signaling.

These findings are consistent with prior heat-stress studies showing that urinary [IGFBP7•TIMP-2] can increase during heat exposure in healthy adults even when serum creatinine or creatinine clearance do not indicate sustained impairment. In a controlled laboratory study by Hess et al. (61), healthy young men exposed for eight hours to current and projected extreme-heat wet-bulb temperature conditions exhibited increases in urinary [IGFBP7•TIMP-2], with larger increases under the more severe heat condition (61). Similarly, during simulated occupational heat stress, Chapman et al. (52) reported increases in [IGFBP7•TIMP-2] from healthy adults during physical work in the heat. These finding suggests that these markers are sensitive to combined heat and exertional strain in otherwise healthy individuals.

While clear age-related differences in kidney function were observed, no differences in AKI risk biomarkers were detected between groups. Several factors may explain this finding. First, the older cohort was relatively healthy and screened for kidney risk factors. Participants were free of major cardiometabolic disease and were excluded for medication profiles that could meaningfully alter thermoregulation, hemodynamics, or hydration responses (e.g. diuretics, chronic NSAID use, beta-blockers, or multiple antihypertensives). This design was intended to isolate age-related effects but could also cause the older group to have a more preserved renal reserve than the broader older adult population. Second, although core temperature did not differ by age in this study, older adults exhibited reduced heat tolerance and greater physiological strain. This was evidenced by greater early termination in older adults and no difference in AUC (Figure 7), showing that older adults achieved a comparable thermal load over a shorter duration. Core temperature increased over time with no age-group difference and no interaction between age and time. Because heat-related renal stress is closely tied to the combined strain of hyperthermia and circulating volume challenges, similar thermal strain would be expected to yield similar [IGFBP7•TIMP-2] and TRX-1 responses. Additionally, protocol completion and statistical power may have contributed to the lack of age-differences. Sample size varied across outcomes and timepoints, which can reduce sensitivity to group differences and bias the sample toward participants who withstood the heat. Finally, dehydration patterns may have further decreased the age effect. Young adults experienced a greater percent loss of body mass [−3.156 ± 0.797%] than older adults [−2.502 ± 0.610%]. Even after correcting the biomarkers for urine concentration, greater dehydration can still reduce kidney blood flow and trigger vasoconstriction, which may raise AKI risk stress markers and make the young and older groups look more similar by increasing the young group response.

Limitations

There are several limitations that should be considered when interpreting these findings. First, the total sample size in this study was limited (n = 27), making it challenging to detect small to moderate age-group differences. Second, the sample size varied across timepoints, and some participants did not complete the full six-hour heat exposure, which may have biased analyses toward individuals who participated in the entire simulated heat event. Next, the older cohort was relatively healthy and screened for cardiometabolic disease and medication that could alter thermoregulation, renal hemodynamics, or hydration responses. This sample is not representative of the older population and therefore cannot be generalized to older adults at highest risk during heat waves. However, despite being healthy, the older adults in this study still demonstrated lower heat tolerance and higher early termination, suggesting that older adults have a greater vulnerability to heat stress. Finally, this study's design may not fully reflect real-world heat events. Exposures often occur over multiple days, access to cooling and fluids may be limited, and social, behavioral, and economic constraints can exacerbate heat strain and dehydration risk.

Conclusion

In conclusion, we demonstrate that controlled heat exposure increases biomarkers of AKI risk and oxidative stress, as evidenced by elevations in urinary [IGFBP7•TIMP-2]. Despite similar AKI biomarker responses between age groups, older adults exhibited greater impairments in kidney function. This includes reduced creatinine clearance and diminished fluid regulation following heat stress. Responses in TRX-1 and MCP-1 suggest that oxidative stress may play a more prominent role than inflammation in driving the renal stress response observed in this study. Together, these findings indicate that while AKI risk biomarker responses were comparable between healthy young and older adults, aging is associated with reduced renal functional resilience during heat exposure.

Despite these findings, the controlled laboratory design limits the ability to fully replicate real heat waves, where individuals may experience heat exposure over multiple days and nights. Additionally, the study population was restricted to healthy individuals without chronic disease or medications that alter thermoregulatory, cardiovascular, or renal function. Future research should therefore investigate responses to prolonged or repeated heat exposures and examine more clinically representative populations.

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