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RESEARCH ARTICLE

Don't Deny Your Inner Environmental Physiologist: Investigating Physiology with Environmental Stimuli

Glomerular filtration rate reserve is reduced during mild passive heat stress in healthy young adults

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Abstract

We tested the hypothesis that, compared with normothermia, the increase in glomerular filtration rate (GFR) after an oral protein load (defined as the GFR reserve) is attenuated during moderate passive heat stress in young healthy adults. Sixteen participants (5 women; 26 ± 2 yr) completed two experimental visits, heat stress or a normothermic time-control, assigned in a block-randomized crossover design. During the heat stress trial, core temperature was increased by 0.6°C in the first hour before commencing a 2-min cold pressor test (CPT) to assess renal vasoconstrictor responses. One-hour post-CPT, subjects ingested a whey protein shake (1.2 g of protein/kg body wt), and measurements were taken pre-, 75, and 150 min postprotein. Segmental artery vascular resistance was calculated as the quotient of Doppler ultrasound-derived segmental artery blood velocity and mean arterial pressure and provided an estimate of renal vascular tone. GFR was estimated from creatinine clearance. The increase in segmental artery vascular resistance during the CPT was attenuated during heat stress (end CPT: 5.6 ± 0.9 vs. 4.7 ± 1.1 mmHg/cm/s, P = 0.024). However, the reduction in segmental artery vascular resistance in response to an oral protein load did not differ between heat stress (at 150 min: 1.9 ± 0.4 mmHg/cm/s) and normothermia (at 150 min: 1.8 ± 0.5 mmHg/cm/s; P = 0.979). The peak increase in creatinine clearance postprotein, independent of time, was attenuated during heat stress (+26 ± 19 vs. +16 ± 20 mL/min, P = 0.013, n = 13). GFR reserve is diminished by mild passive heat stress. Moreover, renal vasoconstrictor responses are attenuated by mild passive heat stress, but renal vasodilator responses are maintained.

glomerular filtration rate; heat stress; kidney function; oral protein loading; renal blood flow

INTRODUCTION

To accommodate profound reductions in cutaneous vascular resistance while maintaining arterial pressure, passive heat stress elevates cardiac output and causes a systemic redistribution of blood flow that results in decreases in renal blood flow (1). This decline in renal blood flow may be associated with diminished kidney function, as classically demonstrated by decreases in the glomerular filtration rate (GFR; 2, 3), which is likely beneficial for fluid conservation (4). However, reductions in GFR with passive heat stress are not consistently observed (5–7). The reasons for these discrepancies are not clear. One recently proposed explanation is that GFR is only reduced when the magnitude of the physiological challenge associated with passive heat stress exceeds the ability of individual nephrons to increase filtration (i.e., hyperfiltration; 8) and/or recruit additional nephrons (9, 10) necessary to sustain GFR (11). In this instance, the GFR reserve, which is defined as the capacity to increase GFR in response to an increase in functional demand (12), would be reduced with passive heat stress, independent of whether GFR is decreased. However, to our knowledge, the effect of passive heat stress on GFR reserve has never been investigated. This knowledge gap has important public health ramifications because acute kidney injury, which is clinically defined as an abrupt reduction in GFR (13), is one of the top causes of hospitalization during heat waves (14, 15).

The most widely accepted way to examine GFR reserve is via administration of a protein load into the circulation. Specifically, GFR has been observed to increase following intravenous infusion of amino acids (10, 16) or after consuming a high protein meal (12) with the maximum increase in GFR

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occurring 2.0–2.5 h after protein introduction (12, 17). The direct mechanisms for these protein-mediated increases in GFR have yet to be fully elucidated but have been consistently demonstrated in both healthy and clinical populations (12, 17–19). It is speculated that the increased filtration of amino acids stimulates tubular sodium reabsorption that inhibits tubuloglomerular feedback and causes afferent arteriolar vasodilation, elevations in renal blood flow, and hyperfiltration that is modulated by endocrine (e.g., increased circulating glucagon) and paracrine (e.g., nitric oxide and prostaglandins) factors (8). It is unknown if heat stress modifies the extent by which GFR is increased following a protein load, which may reflect alterations in GFR reserve. This is an important consideration because when filtration capacity is challenged, as may occur during heat stress, GFR reserve can be recruited as a way to preserve GFR (8). With this background, the primary purpose of this study was to test the hypothesis that, compared with normothermia, the increase in GFR following an oral protein load (i.e., GFR reserve) will be attenuated during mild passive heat stress in humans.

METHODS

Participants

Sixteen healthy adults (5 women) participated in this study. Participant characteristics were the following: age: 26 ± 2 yr; height: 175 ± 8 cm; and weight: 76.3 ± 15.6 kg. Participants provided verbal and written informed consent after being fully informed of the experimental procedures and possible risks. Participants were eligible if they reported no known cardiovascular, metabolic, renal, or neurological diseases and self-reported to be physically active and nonsmokers. Women were not pregnant as confirmed through a urine pregnancy test, self-reported to be normally menstruating, and had no diagnosis of a menstrual cycle-specific disorder. Women were tested within the first 5 days (3 ± 1 days) of their self-identified onset of menstrual bleeding, and three of five women were on hormonal contraceptives. This study was approved by the Indiana University Institutional Review Board and was carried out according to the most recent revision of the Declaration of Helsinki, except for registration in a database.

Instrumentation and Measurements

Height was measured using a stadiometer (Holtain Limited, Seritex, Wales, UK). Nude body weight was measured using a digital scale (Sauter, Balingen, Germany). Core temperature was measured continually with an ingestible telemetry capsule (n = 12, HQ, Palmetto, FL) or rectal temperature (n = 4, Covidien, Medtronic, Minneapolis, MN) when participants were contraindicated for ingesting the telemetry capsule. Thermocouples (Omega Technologies, Inc., Westlake Village, CA) were used to continually measure mean skin temperature, which was calculated as the weighted average of six skin locations (20). Body temperature was controlled with a tube-lined water-perfused suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except for the head, hands, and feet. Heart rate was measured via a 3-lead electrocardiogram (Datex-Ohmeda, Instrumentarium, Corp., Helsinki, Finland). Beat-to-beat blood pressure was measured continually via the Penaz method (Human NIBP Nano System, ADI Instruments, Colorado Springs, CO), which was intermittently confirmed via auscultation of the brachial artery by electrosphygmomanometry (Tango M2; SunTech, Raleigh, NC). Beat-to-beat blood pressure data were corrected to the first brachial artery blood pressure measured at the start of each visit.

Renal blood velocity was measured via Doppler ultrasound (Toshiba Aplio 300, Canon Medical Systems) in the distal segment of the right renal artery (renal artery) and in the middle portion of a segmental artery in the right kidney (segmental artery) as a surrogate for renal blood flow (21, 22), using methods that have been thoroughly described previously (23). The specific segmental artery and the location of measurement within the renal and segmental arteries were the same at all time points within a participant. With participants in the left lateral recumbent position and using the coronal approach, a phased-array transducer (2.5–3.5 MHz) was held in the same location for all measurements after marking the transducer location on the skin with indelible ink during baseline measurements. In all instances, the focal zone was set to the artery's depth, and the insonation angle was $<60^{\circ}$. Mean renal and segmental artery blood velocities were indexed from the waveform envelope by the time-averaged maximum velocity and reported as the average of three cardiac cycles (13, 19, 24). All renal measurements were obtained and extracted by the same sonographer (J. A. F.). The transducer was removed from the body when measurements were not actively being obtained. Optimization and measurement of blood velocity in the renal and segmental arteries occurred within ~2 min of when the transducer was replaced on the participant during each measurement timepoint. With this approach, the within-subject test-retest coefficients of variation for blood velocity measurements were 2.8 ± 1.5% (renal artery) and 3.4 ± 1.4% (segmental artery) for the sonographer in this study. The transducer was held in place throughout the duration of the cold pressor tests (CPTs) so that image acquisition occurred within a 10 s window during each minute of the cold pressor test. Given the depth of the renal and segmental arteries, it is not possible to accurately measure vessel wall diameter. However, renal blood velocity was interpreted to reflect changes in renal blood flow as has been done previously (13, 19–21, 24–27). This was deemed reasonable because during pharmacologically induced renal vasoconstriction (22) and vasodilation (21) changes in renal artery blood flow were due to changes in blood velocity and not diameter, and because the renal and segmental arteries are conduit vessels. Renal and segmental artery blood velocity was normalized to mean arterial pressure providing an index of vascular resistance (i.e., mean arterial pressure/blood velocity).

Serum [intra-assay coefficient of variation (CV): 1.9 ± 1.6%, inter-assay CV: 2.7 ± 2.3%] and urinary (intra-assay CV: 3.7 ± 3.5%, inter-assay CV: 1.6 ± 0.8%), creatinine (Eagle Biosciences, Amherst, NH) and serum glucagon (intra-assay CV: 1.3 ± 0.9%, inter-assay CV: 1.7 ± 0.2%, RayBiotech Life, Inc., Peachtree Corners, GA) were measured in duplicate using commercially available ELISA kits. Urine flow rate was calculated as urine volume divided by the time (minutes) between each bladder void. GFR was estimated from creatinine clearance (i.e., urinary creatinine × urine flow rate ÷ serum creatinine). Urine specific gravity was measured using

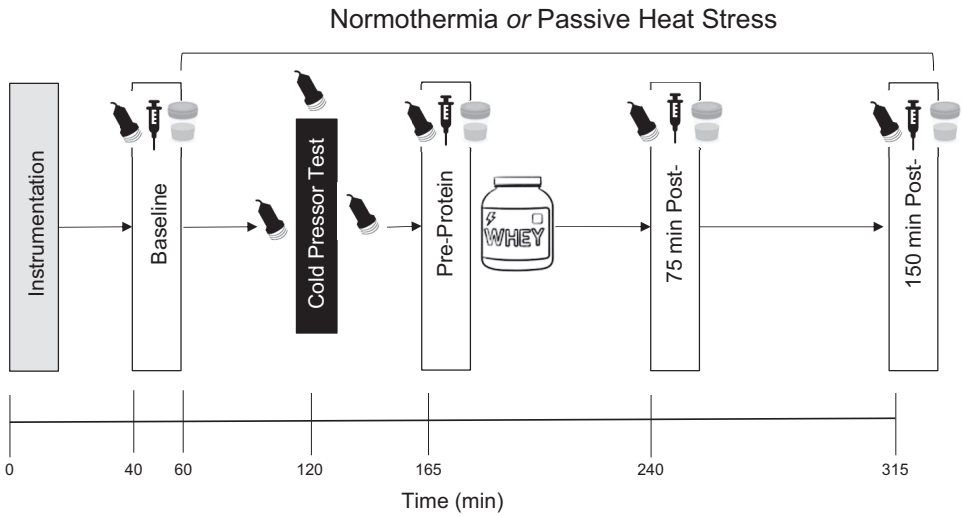

Figure 1. Schematic of the study protocol. Participants were instrumented and underwent 20 min of normothermic baseline in both trials. Renal ultrasound measurements (indicated by transducer symbols) were taken at baseline along with blood (indicated by the syringe) and urine (indicated by collection cup). Participants were then either passively heated or remained in normothermic conditions postbaseline measures. Renal ultrasound measurements were then taken after 1 h into each experimental condition at pre-CPT and during the cold pressor test. Forty-five minutes later, preprotein ultrasound measures were collected along with the collection of blood and urine samples. Participants then ingested 1.2 g/kg of whey protein. Renal ultrasound measures were collected 75 min and 150 min postprotein along with the collection of blood and urine samples. CPT, cold pressor test.

refractometry (Atago, Tokyo, Japan). Urine and plasma osmolality (Advanced Instruments, Norwood, MA) and sodium concentrations (Medica Corporation, Bedford, MA) were measured in duplicate using commercially available systems. The fractional excretion of sodium was calculated as $100 \times ([U_{Na+} \times sCr] \div [P_{Na+} \times uCr])$, where U_{Na+} and P_{Na+} represent sodium concentrations in the urine and plasma, and uCr and sCr are urinary and serum creatinine concentrations. Free water clearance was calculated as $UFR \times (1 - [Uosm \div Posm])$, where UFR is urine flow rate, and Uosm and Posm are the osmolality of the urine and plasma.

Experimental Protocol

Participants reported to the temperature-controlled laboratory (ambient temperature: 21.5 ± 1.2°C) for two experimental trials after abstaining from exercise, caffeine, and alcohol for 12 h and food for 2 h. Participants were encouraged to arrive at the laboratory well hydrated but were not given any specific fluid intake instructions. Experimental trials were completed in a block-randomized crossover design and separated by at least 72 h and no more than 40 days, which was necessary to accommodate the control of menstrual cycle phase. To minimize the effect of diet, participants were given a diet log to complete in 24 h before the first experimental visit and were instructed to replicate this diet before their second experimental trial. To control for diurnal changes, each experimental visit was completed at the same time of day (± 1 h) within a participant. Upon arrival at the laboratory for each experimental visit, participants were instructed to first void their bladder. Euhydration was confirmed using this urine sample [i.e., urine specific gravity <1.020 (Table 1)]. Participants then measured their nude body weight, consumed 250 mL of cool tap water to promote urine production, and were instrumented as outlined in *Instrumentation and Measurements*. Participants were then laid in the supine position with 34°C water perfusing the suit. After 20 min, baseline measurements were taken (i.e., a 60-s average for continuously recorded data) that culminated in the collection of a venous blood sample and voiding of the bladder. After these premeasurements, participants were either exposed to heat stress or normothermia over the next ~4.5 h. In the normothermia trial, 34°C water-perfused the suit throughout. For the heat stress trial, the goal was to achieve an elevation in core temperature by 0.8°C–1.0°C within the first hour and to maintain this elevation throughout the remainder of the experiment. Thus, 50°C water was initially perfused through the suit, but this water temperature was adjusted to maintain the core temperature as designed. This mild level of heat stress was deemed necessary to ensure most participants would complete the 4.5-h duration of the experiment (Fig. 1).

One hour after normothermia or heat stress was commenced, the cold pressor test (CPT) was performed. The cold pressor test was administered for 2 min and was executed by submerging the participant's right hand into an agitated ice slurry mixture (0.0 ± 0.2°C) up to the wrist. All continuously collected data (averaged over 30 s) and renal blood velocity were measured at precold pressor test (pre-CPT) and at 1 min and 2 min of the cold pressor test. The cold pressor test is a sympathetic maneuver that stimulates the nociceptors and subsequently increases vascular resistance (including in the renal vasculature) and blood pressure (28). The cold pressor test was used in the present study to examine the impact of mild heat stress on renal vasoconstrictor capacity during sympathetic activation.

After the cold pressor test, participants remained in a supine state and ~45 min later preprotein renal blood velocities were measured, a venous blood sample was obtained, and participants voided their bladder. Participants then drank a whey protein isolate shake (Optimum Nutrition, Inc., IL) containing 1.2 g protein/kg of their screening body weight [91.2 ± 18.8 g/kg protein in 274 ± 56 mL (3:1 ratio of water-to-protein)] of water maintained at 25.6 ± 1.2°C (normothermia)

Table 1. *Data collected during the baseline collection period*

	Normothermia	Heat Stress	*P* Value
Cardiovascular and thermal measures			
Mean arterial pressure, mmHg	81 ± 10	83 ± 6	0.371
Heart rate, beats/min	53 ± 6	54 ± 9	0.968
Core temperature, °C	37.0 ± 0.4	37.1 ± 0.4	0.965
Mean skin temperature, °C	33.4 ± 0.6	33.8 ± 1.6	0.486
Water and electrolyte homeostasis measures			
Urine specific gravity, au	1.003 ± 0.002	1.005 ± 0.005	0.251
Urine flow rate, mL/min	7.1 ± 2.8	6.0 ± 2.4	0.059
Serum osmolality, mosmol/kgH_2O	288 ± 4	288 ± 3	0.970
Urine osmolality, mosmol/kgH_2O	162 ± 124	208 ± 227	0.383
Serum sodium, mmol/L	142 ± 1	143 ± 2	0.873
Urine sodium, mmol/L	27 ± 27	31 ± 30	0.553
Kidney function measures			
Urine Creatinine, mg/dL	22.9 ± 19.8	42.5 ± 60.0	0.507
Serum creatinine, mg/dL	1.02 ± 0.19	1.05 ± 0.18	0.343
Creatinine clearance, mL/min	107 ± 56	125 ± 60	0.573
Free water clearance, mL/min	4.1 ± 2.9	3.1 ± 3.1	0.056
Fractional excretion of sodium, %	0.1 ± 0.8	0.8 ± 0.4	0.247
Renal blood velocity measures			
Renal artery blood velocity, cm/s	33 ± 8	40 ± 8	0.462
Renal artery vascular resistance, mmHg/cm/s	2.2 ± 0.6	2.2 ± 0.5	0.815
Segmental artery blood velocity, cm/s	21 ± 4	22 ± 4	0.763
Segmental artery vascular resistance, mmHg/cm/s	3.9 ± 0.8	4.0 ± 0.6	0.764
Hormonal measures			
Glucagon, pg/mL	36.0 ± 61.2	33.3 ± 51.8	0.506

Data are presented as means ± SD. Data were analyzed using two-tailed paired *t* tests. Actual *P* values are reported. All data are $n = 16$ except for urinary measures during normothermia ($n = 14$) and heat stress ($n = 15$).

and 26.1 ± 0.6°C (heat stress) within a 5-min period (normothermia: 101 ± 63 s, heat stress: 82 ± 50 s) to stimulate an increase in GFR and assess GFR reserve. An oral protein shake, instead of cooked meat, was used, as this has been shown to elevate GFR to the same extent as 1 g/kg and 2 g/kg of cooked meat (29) and because of logistical and potential gastrointestinal issues associated with the ingestion of cooked meat during heat stress. Moreover, whey protein contains an amino acid profile (30) that is similar to other protein loads that have been shown previously to stimulate increases in GFR during an amino acid infusion (17, 31). Finally, a 1.2 g/kg protein dose was used because 1.1 g/kg of protein has been found to elicit a larger increase in GFR compared with 0.6 g/kg, but this did not differ from the GFR response to a 1.3 g/kg protein dose (24). After protein ingestion, the participants resumed the supine position and renal blood velocities were measured, a venous blood sample was obtained, and participants voided their bladder at 75 min and 150 min after protein ingestion. In all instances, renal blood velocity data and venous blood samples were obtained after at least 20 min of supine rest. Data were collected at 75 min and 150 min after protein ingestion because the maximal GFR response to a protein load is observed 2.0–2.5 h after protein ingestion (18) and the maximal hemodynamic response occurs 1–3 h postingestion (32). An assessment period that encompassed these peak time periods ensured that we did not miss the close to maximal response, but also reflected a balance between our subjects' ability to produce multiple serial urine samples during the heat stress trial while collecting multiple measurements during the postprotein period. Throughout the protein testing period, all continuously recorded data were binned using a 60-s average. After the 150-min postprotein measures, all participants were cooled down, deinstrumented, and a final nude body weight was then obtained.

Data and Statistical Analysis

Upon commencing this study, to our knowledge, there were no data to estimate the sample size. Therefore, a power analysis was completed after complete creatinine clearance data sets were obtained from eight healthy adults. These data revealed a Cohen's d_z effect size of 0.80 for the peak increase in creatinine clearance after protein ingestion between heat stress (+23 ± 17 mL/min) and normothermia (+36 ± 19 mL/min). A power analysis was then carried out using this effect size and standard parameters of $1 - \beta = 0.80$ and $\alpha = 0.05$ using G*Power software (v. 3.1.9.4), which revealed we needed at least 12 complete creatinine clearance data sets to identify a significant difference in peak creatinine clearance between trials following protein ingestion using a one-tailed paired *t* test, which is consistent with our directional hypothesis. To accomplish this, we recruited eight additional healthy adults for a total sample size of 16 adults, which resulted in complete creatinine clearance data sets from 13 participants.

Continuously collected data were sampled at 1,000 Hz via a data acquisition system (PowerLab 16/35, ADInstruments). All data were analyzed with Prism software (v. 9; GraphPad Software, La Jolla, CA). Data collected during baseline, pre-CPT, and percent changes in body mass from pre- to post-testing were analyzed using two-tailed paired *t* tests. Data obtained during the CPT and during the protein period (i.e., preprotein, 75 min postprotein, and 150 min postprotein) were analyzed using repeated-measures linear mixed models. Before formal statistical analyses were completed, an outlier analysis was performed, and assumptions related to sphericity and data normality (for *t* tests) or the normality of the residuals (for the linear mixed models) were checked. Potential outliers were identified using the ROUT method

(33), using a 0.1% false discovery rate so that only definite outliers were identified. On the rare occasion when an outlier was identified, and the way it was handled, is discussed in the text of the RESULTS. When the assumption of sphericity was violated, the Geisser–Greenhouse correction was applied. Data normality and the residuals of the linear mixed model were determined to be normally distributed in all cases, and thus no corrections were necessary. When a linear mixed model revealed a significant main effect or interaction, pairwise comparisons were carried out using Sidak's test, which corrects for multiple comparisons. Consistent with the definition of GFR reserve (34), the peak increase in creatinine clearance following protein ingestion (independent of time) was calculated and analyzed using a one-tailed paired *t* test. This analysis approach was determined a priori and deemed appropriate given our directional hypothesis. A priori statistical significance was set at $P \leq 0.05$ and actual *P* values for main effects, interactions, and pairwise comparisons are reported where possible. Data are reported as means ± SD or as mean with individual values.

RESULTS

Baseline Measures

As expected, there were no differences in the cardiovascular and thermal measures ($P \geq 0.371$), water and electrolyte homeostasis measures ($P \geq 0.059$), kidney function measures ($P \geq 0.056$), or renal blood velocity measures ($P \geq 0.462$) between trials at baseline (Table 1). Likewise, serum glucagon did not differ between trials at baseline ($P = 0.506$). All data at baseline are $n = 16$ except for urinary measures where data are reported as $n = 15$ due to a contaminated urine sample (menstrual blood) during heat stress and as $n = 14$ during normothermia due to contaminated sample (menstrual blood) and a missed urine collection.

Body fluid loss (i.e., the percent change in body mass) from pre- to poststudy period was greater in heat stress (3.0 ± 0.8%) compared with normothermia (1.5 ± 0.4%, $P < 0.001$).

Cold Pressor Test

As expected, core temperature and mean skin temperature were higher during the heat stress trial at pre-CPT compared with normothermia ($P \leq 0.001$; Table 2). Mean arterial pressure did not differ pre-CPT between heat stress and normothermia but was lower in the heat stress trial at *minutes 1* and *2* of the cold pressor test ($P \leq 0.001$; Fig. 2). Segmental artery blood velocity decreased during the cold pressor test (main effect of time: $P = 0.021$) but did not differ between heat stress and normothermia ($P \geq 0.426$). An outlier was identified for segmental artery vascular resistance during the cold pressor test in the heat stress trial, and thus those data are presented as $n = 15$. Segmental artery vascular resistance pre-CPT did not differ between normothermia and heat stress and increased in both trials ($P < 0.005$) but was lower in heat stress during both minutes of the cold pressor test ($P \leq 0.042$; Fig. 2).

Protein Measures

In addition to the one participant with a contaminated urine sample during the normothermia trial and heat stress trial, one participant was unable to produce urine in the protein testing period during the heat stress trial, and one participant dropped out due to subjective discomfort in response to heat stress. Thus, during the normothermia trial, urine-dependent data are presented as $n = 15$, whereas all other data are $n = 16$. During the heat stress trial, urine-dependent data are presented as $n = 13$, whereas all other data are $n = 15$.

Heart rate was higher throughout the protein testing period in heat stress compared with normothermia ($P < 0.001$; Table 3). Despite the identification of a significant interaction ($P = 0.036$), pairwise comparisons did not reveal any differences in mean arterial pressure between normothermia and heat stress at any time during the protein testing period ($P \geq 0.392$; Table 3). Core and mean skin temperatures were higher throughout the protein testing period in the heat stress trial ($P < 0.001$; Table 3).

Serum osmolality was not different at preprotein ($P = 0.459$) but was different at 75 min postprotein ($P = 0.001$) and 150 min postprotein between trials ($P < 0.001$). Urine osmolality did not differ between trials at preprotein ($P = 0.510$) but was higher at 75 min postprotein and 150 min postprotein in the heat stress trial ($P \leq 0.002$). Serum sodium concentration was higher in the heat stress trial preprotein measures and at 150 min postprotein ingestion ($P \leq 0.037$; Table 3). Urinary sodium concentrations increased over time during the protein testing period (main effect of time: $P < 0.001$) but did not differ between the trials ($P \geq 0.515$; Table 3).

Urinary creatinine was higher in heat stress throughout the protein testing period ($P \leq 0.028$; Table 4). Serum creatinine did not differ between normothermia and heat stress at preprotein ($P = 0.979$) or 75 min postprotein ($P = 0.139$) but was higher during heat stress at 150 min postprotein ($P = 0.002$; Table 4). Glucagon was not different between trials throughout the protein testing period ($P \geq 0.440$; Table 4). Fractional excretion of sodium was lower in the heat stress trial throughout the protein testing period ($P \leq 0.001$; Table 4). Free water clearance did not differ preprotein, 75 min postprotein, or 150 min postprotein between trials ($P > 0.340$) but was different from preprotein at 75 min and 150 min postprotein in both trials ($P \leq 0.001$; Table 4).

Table 2. *Heart rate, thermal, renal artery blood velocity measures precold pressor test*

	Normothermia	Heat Stress	*P* Value
Heart rate, beats/min	52 ± 7	79 ± 14	<0.001
Core temperature, °C	37.1 ± 0.4	37.7 ± 0.4	<0.001
Mean skin temperature, °C	34.0 ± 0.6	37.4 ± 0.6	<0.001
Renal artery blood velocity, cm/s	40 ± 7	36 ± 6	0.024
Renal artery vascular resistance, mmHg/cm/s	2.1 ± 0.3	2.2 ± 0.4	0.317

Data were analyzed using two-tailed paired *t* tests and are presented as means ± SD ($n = 16$).

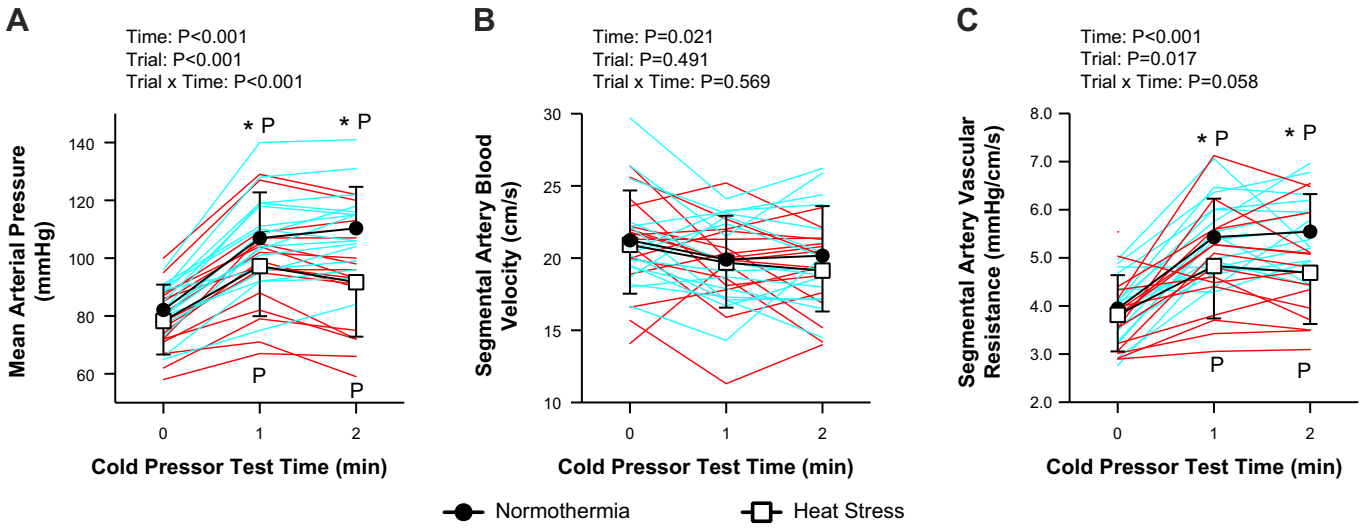

Figure 2. Mean arterial pressure (*A*), renal segmental artery blood velocity (*B*), and renal segmental artery vascular resistance pre-CPT (*C*), and every minute during the cold pressor test. Data are presented as means ± SD and individual values (normothermia: blue lines; heat stress: red lines). *Significantly different from heat stress ($P < 0.046$). ^{P}Significantly different from pre-CPT (i.e., time = 0 min; $P \leq 0.031$). Data were analyzed using a two-way linear mixed model. When a significant main effect or interaction was identified, Sidak pairwise comparisons were carried out. *P* values from the two-way linear mixed model are reported alongside pairwise comparisons (where necessary). $n = 16$ for all except for during heat stress and the segmental artery vascular resistance variable where $n = 15$ due to the identification of an outlier. Linear mixed model output with outlier included—time: $P < 0.001$, trial: $P = 0.1535$, trial × time: $P = 0.214$. CPT, cold pressor test.

Segmental artery blood velocity was not different between normothermia and heat stress at any point during the protein testing period ($P \geq 0.632$) but was elevated above preprotein levels at 75 min postprotein and 150 min postprotein in both trials ($P \leq 0.026$; Fig. 3*A*). Segmental artery vascular resistance did not differ throughout the protein testing period between normothermia and heat stress ($P \geq 0.913$) but was lower than preprotein levels at 150 min postprotein in normothermia trial ($P \leq 0.020$; Fig. 3*B*). Renal artery blood velocity did not differ throughout the protein testing period between normothermia and heat stress ($P \geq 0.363$) but was increased at 75 min postprotein and 150 min postprotein compared with preprotein in both trials ($P \leq 0.018$; Fig. 3*C*). Renal artery vascular resistance did not differ between normothermia and heat stress during the protein testing period ($P \geq 0.479$) but was decreased at 150 min postprotein only during normothermia compared with preprotein ($P \leq 0.027$; Fig. 3*D*).

Creatinine clearance did not differ between normothermia (113 ± 30 mg/dL) and heat stress (125 ± 31 mg/dL) preprotein ($P = 0.181$) or throughout the posttesting period ($P \geq 0.348$; Fig. 4*A*). Moreover, creatinine clearance preprotein did not differ from baseline in either the normothermia trial or heat stress trial ($P \geq 0.927$). In the normothermia trial, creatinine clearance at 75 min postprotein was different from preprotein ($P = 0.043$), but in the heat stress trial creatinine clearance was not different from preprotein at any time ($P \geq 0.161$; Fig. 4). The peak increase in creatinine clearance following protein ingestion (independent of time) was attenuated during heat stress whether assessed with ($P = 0.048$) or without ($P = 0.013$) an objectively identified outlier.

DISCUSSION

In support of our hypothesis, the increase in creatinine clearance in response to a standardized oral protein load was attenuated during mild passive heat stress compared with during normothermia in healthy younger adults (Fig. 4*B*). In addition, we identified that the renal vasodilator response to

Table 3. *Cardiovascular, thermal and water and electrolyte measures during the protein data collection period*

	Normothermia			Heat Stress			Linear Mixed Model		
	Preprotein	75 Min	150 Min	Preprotein	75 Min	150 Min	Time	Trial	Trial × Time
Heart rate, beats/min	53 ± 6	59 ± 7	59 ± 9	76 ± 15*	78 ± 15*	75 ± 16*	0.025	<0.001	0.075
Mean arterial pressure, mmHg	83 ± 9	80 ± 8	81 ± 9	82 ± 9	85 ± 14	84 ± 12	0.956	0.387	0.036
Core temperature, °C	37.1 ± 0.4	37.2 ± 0.4	37.3 ± 0.3	37.8 ± 0.4*	37.9 ± 0.4*	37.9 ± 0.4*	<0.001	<0.001	0.002
Mean skin temperature, °C	34.1 ± 0.6	34.3 ± 0.5	34.3 ± 0.4	37.2 ± 0.7*	36.9 ± 0.9*	35.9 ± 1.4*	<0.001	<0.001	<0.001
Urine osmolality, mosmol/kgH_2O	260 ± 85	415 ± 87	597 ± 219	357 ± 256	770 ± 217*	844 ± 206*	<0.001	0.002	0.001
Serum osmolality, mosmol/kgH_2O	291 ± 5	292 ± 4	292 ± 4	293 ± 3	298 ± 4*	300 ± 3*	<0.001	<0.001	0.004
Urinary sodium, mmol/L	59 ± 24	94 ± 21	131 ± 51	61 ± 36	111 ± 40	125 ± 54	<0.001	0.671	0.352
Serum sodium, mmol/L	143 ± 1	143 ± 1	143 ± 2	145 ± 2*	145 ± 3	146 ± 2*	0.422	<0.001	0.034

Data are presented as means ± SD. Data were analyzed using linear mixed models. Actual *P* values for the linear mixed model are presented. During the normothermia trial urine-dependent data are presented as $n = 15$, whereas all other data are $n = 16$. During the heat stress trial, urine-dependent data are presented as $n = 13$, whereas all other data are $n = 15$. *Significantly different from heat stress ($P \leq 0.014$).

Table 4. *Kidney function measures*

	Normothermia			Heat Stress			Linear Mixed Model		
	Preprotein	75 Min	150 Min	Preprotein	75 Min	150 Min	Time	Trial	Trial × Time
Heart rate, beats/min	53 ± 6	59 ± 7	59 ± 9	76 ± 15*	78 ± 15*	75 ± 16*	0.025	<0.001	0.075
Mean arterial pressure, mmHg	83 ± 9	80 ± 8	81 ± 9	82 ± 9	85 ± 14	84 ± 12	0.956	0.387	0.036
Core temperature, °C	37.1 ± 0.4	37.2 ± 0.4	37.3 ± 0.3	37.8 ± 0.4*	37.9 ± 0.4*	37.9 ± 0.4*	<0.001	<0.001	0.002
Mean skin temperature, °C	34.1 ± 0.6	34.3 ± 0.5	34.3 ± 0.4	37.2 ± 0.7*	36.9 ± 0.9*	35.9 ± 1.4*	<0.001	<0.001	<0.001
Urine osmolality, mosmol/kgH_2O	260 ± 85	415 ± 87	597 ± 219	357 ± 256	770 ± 217*	844 ± 206*	<0.001	0.002	0.001
Serum osmolality, mosmol/kgH_2O	291 ± 5	292 ± 4	292 ± 4	293 ± 3	298 ± 4*	300 ± 3*	<0.001	<0.001	0.004
Urinary sodium, mmol/L	59 ± 24	94 ± 21	131 ± 51	61 ± 36	111 ± 40	125 ± 54	<0.001	0.671	0.352
Serum sodium, mmol/L	143 ± 1	143 ± 1	143 ± 2	145 ± 2*	145 ± 3	146 ± 2*	0.422	<0.001	0.034

Data were analyzed with linear mixed model and are presented as means ± SD. During the normothermia trial, urine-dependent data are presented as n = 15, whereas all other data are n = 16. During the heat stress trial, urine-dependent data are presented as n = 13, whereas all other data are n = 15. *Significantly different from heat stress trial ($P \leq 0.028$).

an oral protein load did not differ between normothermia and heat stress (Fig. 3, *B* and *D*). Furthermore, in support of previous findings (27), we also show that the increase in renal vascular resistance to the cold pressor test during mild passive heat stress is diminished compared with normothermia (Fig. 2*C*). Collectively, these findings indicate that GFR reserve is reduced by mild passive heat stress. This observation, together with the findings that GFR did not differ between normothermia and heat stress, suggests that GFR reserve is recruited during mild passive heat stress to preserve GFR. Moreover, our findings also indicate that the renal vasoconstrictor, but not vasodilator, responses are modified by mild passive heat stress.

Glomerular Filtration Rate Reserve during Heat Stress

Independent of time, the peak increase in creatinine clearance following an oral protein load was lower during mild passive heat stress compared with during normothermia (Fig. 4*B*). Although there were no differences in absolute creatinine clearance between trials, creatinine clearance was increased at 75 min postprotein ingestion in normothermia, a response that was not evident during heat stress (Fig. 4*A*). The mechanisms underlying increases in GFR following a protein load are relatively unclear (8). Current evidence supports that postprotein administration, the increased reabsorption of amino acids and sodium in the proximal tubules reduces the distal delivery of sodium, which inhibits tubuloglomerular feedback causing afferent arteriolar vasodilation, increases in renal blood flow, and tubular hyperfiltration (8). This cascade of events appears to be modulated by a combination of metabolic, endocrine, and paracrine factors (8). Although the mechanisms by which the increase in creatinine clearance was attenuated postprotein in the heat stress trial remain largely unknown, we speculate that this response is modulated by similar mechanisms.

It is relatively well established that intake of protein promotes renal vasodilation (12), which can be measured via Doppler ultrasound (22). In the current study, the magnitude of vasodilation in the renal and segmental arteries did not differ postprotein between normothermia and heat stress (Fig. 3, *B* and *D*). Thus, it is unlikely that differences in renal or segmental artery blood flow in response to an oral protein load explain the differential rise in creatinine clearance between normothermia and heat stress. Moreover, evidence indicates that glucagon plays a permissive role in facilitating increases in renal blood flow and GFR postprotein, likely by modulating the effects of local paracrine factors (e.g., nitric oxide; 8). That said, we observed no differences in glucagon concentrations between normothermia and heat stress. Thus, it is unlikely that glucagon contributed to the differential rise in creatinine clearance between normothermia and heat stress postprotein ingestion. Therefore, it is likely that local mechanisms unable to be directly observed in the present study are responsible for the observations in the present study. For example, heat stress stimulates tubular sodium reabsorption (6), which is supported in the present study by observations of a lower fractional excretion of sodium in the heat stress trial (Table 4). Approximately 60%–70% of tubular sodium reabsorption takes place in the proximal tubules (36). Sodium concentrations are likely reduced at the macula densa during heat stress due to increased proximal tubular sodium reabsorption, resulting in tubuloglomerular feedback inhibition and increased filtration independent of a protein load. Thus, it is possible that tubuloglomerular feedback contributed to the control of GFR during heat stress based on our observations that *1*) increases in creatinine clearance postprotein were attenuated in the heat stress trial compared with during normothermia (Fig. 4*B*), and *2*) no differences in creatinine clearance were observed between heat stress and normothermia preprotein (Fig. 4*A*). By extension, these findings can also be interpreted to indicate that heat stress may utilize GFR reserve, likely via tubuloglomerular feedback mechanisms, to maintain GFR during heat stress (5) despite relatively profound reductions in global (1) and cortical (37) renal blood flow. However, it is important to note that the direct effect of heat stress on the mechanisms underlying tubuloglomerular feedback remains unexplored.

Renal Vascular Control during Heat Stress

Like our previous work (27), mild passive heat stress did not modify Doppler ultrasound-derived measures of renal or segmental artery vascular resistance at any time during the experimental protocol (Table 2, Fig. 2*C*, and Fig. 3, *B* and *D*). That said, in the present study we did identify that the magnitude of increase in segmental artery vascular resistance during the cold pressor test was attenuated in the heat stress trial compared with during normothermia (Fig. 2*C*). This finding expands upon previous work demonstrating that more severe passive heat stress sufficient to increase the core temperature by ~1.2°C attenuates the vasoconstrictor response to the cold pressor test in both the renal and segmental arteries (27) and demonstrates that similar observations can be observed

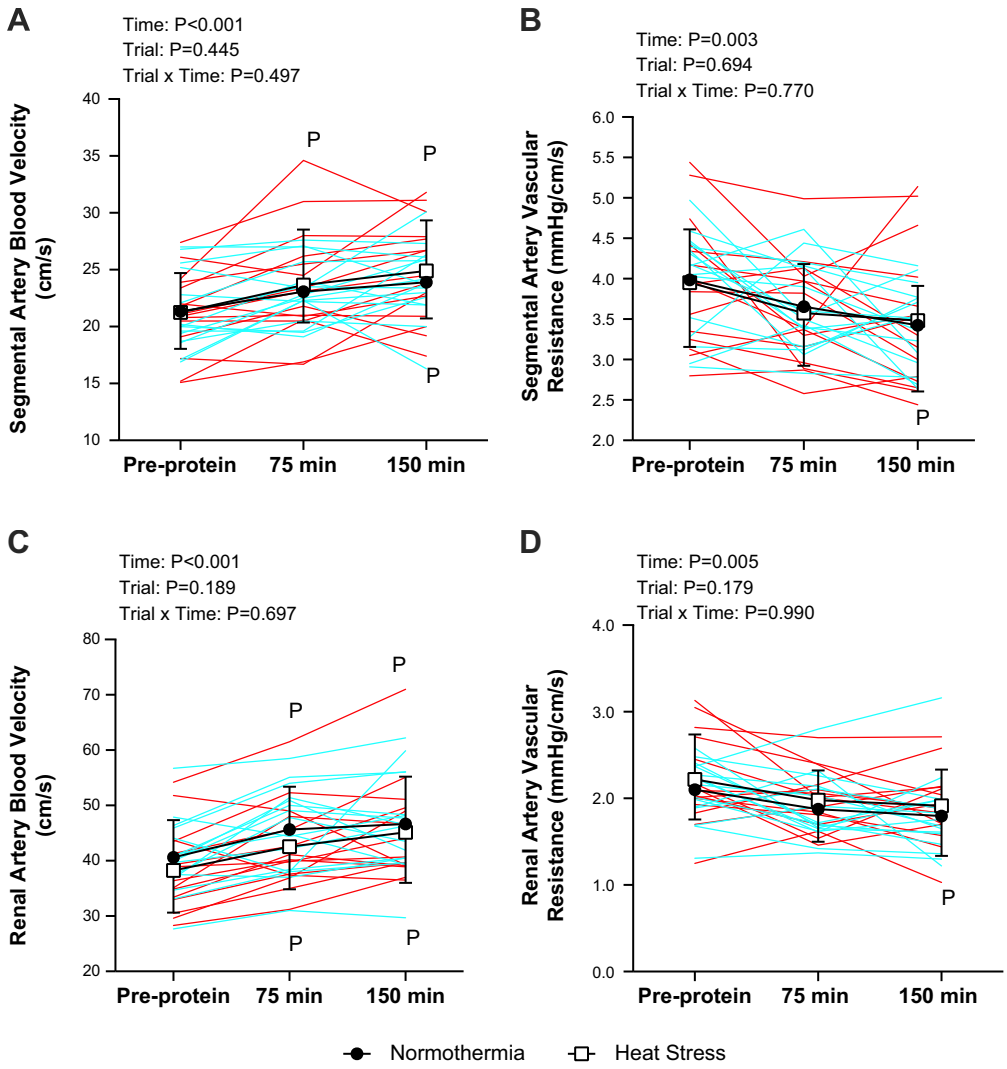

Figure 3. Segmental artery blood velocity (*A*), segmental artery vascular resistance (*B*), renal artery blood velocity (*C*), and renal artery vascular resistance preprotein (*D*), 75 min postprotein and 150 min postprotein. Data are presented as means ± SD and individual values (normothermia: blue lines; heat stress: red lines). ^{P}Significantly different from preprotein ($P \leq 0.027$). Data were analyzed using a two-way linear mixed model. When a significant main effect or interaction was identified, Sidak pairwise comparisons were carried out. *P* values from the two-way linear mixed model are reported alongside pairwise comparisons (where necessary). Normothermia: $n = 16$; heat stress: $n = 15$.

with the more modest rise in core temperature observed herein (by ~0.6°C). Our current (Fig. 2*C*) and previous observations (27) are consistent with other studies demonstrating an attenuated vasoconstrictor responsiveness to sympathetic stimuli during heat stress in nonrenal vascular beds (1). Given the similarities in observations between renal and nonrenal vasculatures we speculate that the reduction in renal vasoconstrictor responsiveness during sympathetic activation under heat stress is due to increases in local vasodilators (e.g., nitric oxide) that may induce a sympatholytic effect (38), which has been observed in conduit arteries (39) and the cutaneous circulation (1), among others. This apparent functional role for local vasodilators in the renal vasculature raises the possibility that local vasodilators are one of the mechanisms by which heat stress may stimulate tubuloglomerular feedback to maintain GFR by tapping into GFR reserve. However, this remains unexplored.

A novel observation in the present study was that the renal and segmental artery vasodilator responses to an oral protein load did not differ between the normothermic and heat stress trials (Fig. 3, *B* and *D*). Heat stress is a potent vasodilator to most vascular beds, including the skin (1) and skeletal muscle (41). Thus, observations of vasodilator responses during heat stress in vascular beds not already in a vasodilated state are relatively rare. One exception is the cerebral circulation where, after correcting for reductions in the partial pressure of arterial carbon dioxide, brain blood flow is relatively well maintained during passive heat stress (water-perfused suits, sauna; 42). In this context, our findings of a renal vasodilatory response that is not different between normothermia and heat stress corroborate observations in the brain wherein cerebral vasodilation to hypercapnia, a potent cerebral vasodilator, is unaffected by heat stress (42). The mechanisms underlying hypercapnia-induced vasodilation in the brain (e.g., effect of extracellular H^+ on vascular smooth muscle)

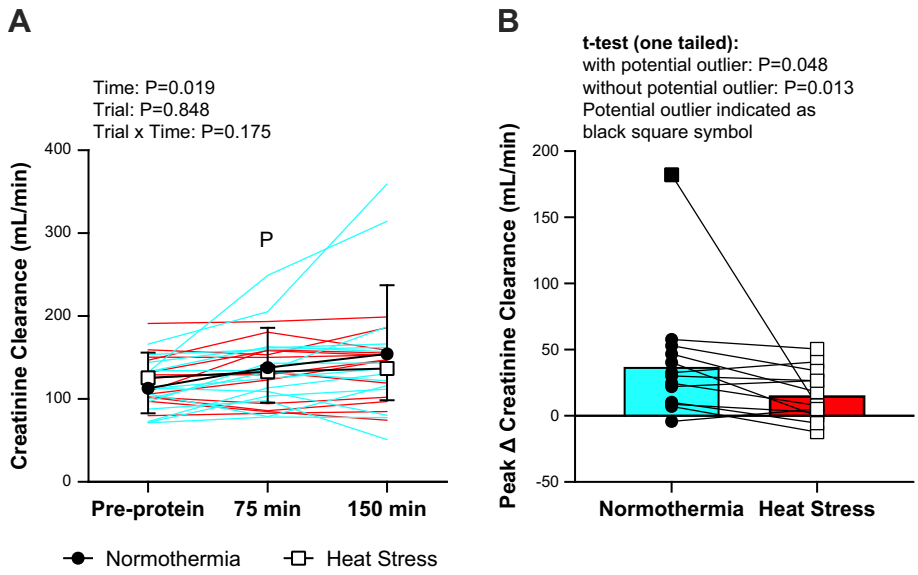

Figure 4. Creatinine clearance preprotein, 75 min postprotein and 150 min postprotein (normothermia: n = 15; heat stress: n = 13; *A*), and peak change (Δ) in creatinine clearance (n = 13 paired samples; *B*). Data are presented as means ± SD and/or individual values (normothermia: blue lines; heat stress: red lines). ^{P}Significantly different from preprotein (P = 0.043). Absolute data were analyzed using a two-way linear mixed model with Sidak post hoc comparisons. P values from the two-way linear mixed model and post hoc comparisons are reported. Peak changes in creatinine clearance were analyzed via a one tailed t test with potential outlier included and excluded.

and those underlying protein-invoked vasodilation in the kidneys (e.g., tubuloglomerular feedback inhibition; see *Glomerular Filtration Rate Reserve during Heat Stress section*) are undoubtedly different. Nevertheless, it is unlikely that heat stress impairs the vasodilator response in vascular beds not already vasodilated due to heat stress.

Methodological Considerations

There are a few factors to consider when interpreting these findings. First, we did not directly measure volumetric renal blood flow. Rather, Doppler ultrasound was used to measure renal blood velocity that was interpreted to reflect changes in renal blood flow, as has been done previously (43). To increase the reliability of the operator-dependent ultrasound measurements the intraoperator coefficient of variation of our sonographer was measured and reported to minimize limitations with regard to interpreting measurements from Doppler ultrasound (27). Nevertheless, future studies should consider incorporating more direct measures of renal blood flow and/or renal plasma flow. Second, we chose to administer a passive heating protocol with the use of water-perfused suits. Water-perfused suits allowed us to tightly control core temperature, which increased the internal validity of our study. That said, we acknowledge that ambient heat exposure is a more externally valid approach and should be used in future studies. Third, we chose to recruit young, healthy men and women, the latter of which were tested in the early follicular phase of the menstrual cycle. In the current study, we are undoubtedly underpowered to make comparisons between men and women. Thus, examination of sex differences on the outcomes measured herein remains a novel research question, particularly considering that differences in sex hormone levels (e.g., estrogen) may alter vascular function and/or control (44). Moreover, future studies should incorporate middle aged and/or older adults given the effects of age on renal function (45) and that these populations are at an elevated risk of deleterious health outcomes, including kidney injury, during heat waves (14, 15). Fourth, we acknowledge potential limitations associated with our use of creatinine clearance as a marker of GFR. Compared with inulin clearance, the gold standard for measurement of GFR, creatinine clearance can overestimate GFR due to tubular secretion of creatinine (48). That said, we chose to use creatinine clearance to estimate GFR for three primary reasons: *1*) the assumptions for creatinine clearance were not invalidated by the experimental protocol (e.g., no change in muscle mass or muscle breakdown); *2*) there is precedent in the literature for using creatinine clearance to estimate of GFR after protein ingestion (8); and *3*) the repeated measures nature of the study design (i.e., creatinine clearance was utilized in both the heat stress and normothermia trials). It may have also been useful to have conducted heat stress and normothermia trials without the administration of protein, to document fluctuations in creatinine clearance over time in the context of our study. Nevertheless, future studies should consider incorporating more direct measures of GFR. Finally, we chose to administer the same relative oral protein dose within the subject for both the heat stress and normothermia trials. This is the standard approach for assessing GFR reserve (8). However, we acknowledge that the renal physiological response to a given oral protein dose may exert different physiological responses under heat stress. Notably, however, glucagon concentrations did not differ between heat stress and normothermia, suggesting that one of the endocrine responses to protein ingestion was unaffected by heat stress. That said, future studies should consider examining the effect of protein dose on the GFR response to a protein load during different physiological states (e.g., heat stress, exercise, and dehydration, etc.). Dehydration may be particularly interesting considering that in the heat stress trial participants lost more body water than in the normothermia trial. Thus, the effect of heat stress in the absence of differences in body water remains unexplored.

Conclusions

The present study demonstrated that the increase in creatinine clearance in response to a standardized oral protein

load was reduced during mild passive heat stress in healthy young men and women compared with during normothermia. Furthermore, we showed that increases in renal vascular resistance in response to a sympathoexcitatory stimulus (i.e., the cold pressor test) were reduced during mild passive heat stress and that the renal and segmental artery vasodilator response to an oral protein load does not differ between heat stress and normothermia.

Perspectives and Significance

Heat wave frequency, intensity, and duration are increasing (49), and a top cause of hospitalization during heat waves is acute kidney injury (14, 15). Acute kidney injury is clinically defined as an abrupt reduction in GFR (13). Thus, the magnitude of reduction in GFR during acute kidney injury is reflective of the inability: *1*) of nephrons to sufficiently increase filtration and/or *2*) to recruit additional nephrons. The findings of the present study support that GFR reserve is utilized to maintain GFR during mild passive heat stress in young healthy adults. By extension, therefore, it is likely that clinically diagnosed acute kidney injury is only manifested during heat exposure when the extent of the physiological insult (i.e., heat stress) is sufficient to utilize more than 100% of the GFR reserve. This speculation is supported by observations that those populations often characterized as having a compromised GFR reserve (e.g., older adults) are also at a heightened risk of acute kidney injury during heat waves (11). Thus, this study provides initial mechanistic insights into the risk of acute kidney injury during heat stress. Additional research is needed in more at-risk populations and to examine the mechanisms by which heat stress utilizes and infringes upon the GFR reserve.

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AUTHOR CONTRIBUTIONS

J.A.F., B.D.J., C.L.C., and Z.J.S. conceived and designed research; J.A.F., M.L.W., M.C.G., H.W.H., J.M., and Z.J.S. performed experiments; J.A.F., T.B.B., and Z.J.S. analyzed data; J.A.F., M.L.W., M.C.G., H.W.H., B.D.J., C.L.C., and Z.J.S. interpreted results of experiments; J.A.F. and Z.J.S. prepared figures; J.A.F. and Z.J.S. drafted manuscript; J.A.F., M.L.W., M.C.G., H.W.H., J.M., T.B.B., B.D.J., C.L.C., and Z.J.S. edited and revised manuscript; J.A.F., M.L.W., M.C.G., H.W.H., J.M., T.B.B., B.D.J., C.L.C., and Z.J.S. approved final version of manuscript.

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