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**RESEARCH ARTICLE**

# Renal vascular control during normothermia and passive heat stress does not differ between healthy younger men and women

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## Abstract

Men are likely at greater risk for heat-induced acute kidney injury compared with women, possibly due to differences in vascular control. We tested the hypothesis that the renal vasoconstrictor and vasodilator responses will be greater in younger women compared with men during passive heat stress. Twenty-five healthy adults [12 women (early follicular phase) and 13 men] completed two experimental visits, heat stress or normothermic time-control, assigned in a block-randomized crossover design. During heat stress, participants wore a water-perfused suit perfused with 50°C water. Core temperature was increased by ~0.8°C in the first hour before commencing a 2-min cold pressor test (CPT). Core temperature remained clamped and at 1-h post-CPT, subjects ingested a whey protein shake (1.2 g of protein/kg body wt), and measurements were taken pre-, 75 min, and 150 min post-protein. Beat-to-beat blood pressure (Penaz method) was measured and segmental artery vascular resistance (VR, Doppler ultrasound) was calculated as segmental artery blood velocity ÷ mean arterial pressure. CPT-induced increases in segmental artery VR did not differ between trials (trial effect: $P = 0.142$) nor between men (heat stress: 1.5 ± 1.0 mmHg/cm/s, normothermia: 1.4 ± 1.0 mmHg/cm/s) and women (heat stress: 1.4 ± 1.2 mmHg/cm/s, normothermia: 2.1 ± 1.1 mmHg/cm/s) (group effect: $P = 0.429$). Reductions in segmental artery VR following oral protein loading did not differ between trials (trial effect: $P = 0.080$) nor between men (heat stress: −0.6 ± 0.8 mmHg/cm/s, normothermia: −0.6 ± 0.6 mmHg/cm/s) and women (heat stress: −0.5 ± 0.5 mmHg/cm/s, normothermia: −1.1 ± 0.6 mmHg/cm/s) (group effect: $P = 0.204$). Renal vasoconstrictor responses to the cold pressor test and vasodilator responses following an oral protein load during heat stress or normothermia do not differ between younger men and younger women in the early follicular phase of the menstrual cycle.

**NEW & NOTEWORTHY** The mechanisms underlying greater heat-induced acute kidney injury risk in men versus women remain unknown. This study examined renal vascular control, including both vasodilatory (oral protein load) and vasoconstrictor (cold presser test) responses, during normothermia and heat stress and compared these responses between men and women. The results indicated that in both conditions neither renal vasodilatory nor vasoconstrictor responses differ between younger men and younger women.

*heat stress; oral protein load; renal vasoconstriction; renal vasodilation; sex differences*

## INTRODUCTION

During heatwaves, there is a higher incidence of acute kidney injury (AKI) (1). Observational evidence indicates that men are at greater risk for heat-induced AKI compared with women (1, 2). These observations are generally consistent with observations in the nonheat-stressed state where AKI mortality is twice as high in men (3, 4) and premenopausal women have the lowest incidence of AKI (4). The precise mechanisms underlying sex differences in AKI risk remain relatively unexplored. During heat stress, it is hypothesized that heat-induced AKI is caused by a supply-demand mismatch that is characterized by a comparatively low renal oxygen supply (i.e., renal blood flow) relative to tissue oxygen demand (5). During heat stress, renal blood flow is reduced via both neural (i.e., renal sympathetic nerve activation) and humoral (i.e., increases in circulating vasopressin, angiotensin II, etc.) mechanisms (5), whereas renal oxygen demand is increased due to the reabsorption of sodium (6). Collectively, the resulting milieu results in oxidative stress and inflammation, both of which have been shown to be positively associated with elevations in AKI risk during heat stress (7). Thus, excessive renal vasoconstriction is likely an important contributor to heat-induced AKI risk (5).

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Estrogen enhances protection against renal oxidative stress (8) and activates pathways promoting nitric oxide (9), a potent vasodilator, which may provide protection in women during perturbations that could lead to AKI (4). For example, the vasodilator-promoting actions of estrogen on nitric oxide may protect against excessive renal vasoconstriction or more readily promote renal vasodilation in premenopausal women compared with men (9). Thus, it is possible that the control of renal blood flow during heat stress differs between men and women. That renal vascular control may differ between younger men and women during heat stress is indirectly supported by four observations while in a normothermic state. First, a lower magnitude of elevation in blood pressure during the cold pressor test (CPT) has been observed in younger women compared with younger men (10). Notably, the CPT is a sympathetic maneuver that stimulates the nociceptors and subsequently increases vascular resistance (including in the renal vasculature) and blood pressure (11). Second, younger women display attenuated femoral artery vasoconstriction during the CPT compared with younger men (12). Third, compared with younger men, younger women have numerically greater increases in renal vascular conductance during an oral protein load (13), a renal vasodilatory stimulus (14). Fourth, younger women have a ~50% lower increase in renal artery vascular resistance during a handgrip exercise, another sympathoexcitatory maneuver, compared with men (13). Importantly, however, in this latter study (13), statistical comparisons were not carried out between men and women. Nevertheless, it remains reasonable to hypothesize that the vascular vasoconstrictor responsiveness to sympathetic stimulation in renal, peripheral, and other vascular beds is lower in younger women compared with younger men, whereas the renal vasodilator response is likely greater in women. However, to our knowledge, this hypothesis has never been directly examined during normothermia or heat stress.

With this background, an understanding of renal vascular control, which involves both vasoconstrictor and vasodilator responses, is necessary to understand differences in heat-induced AKI risk between men and women. As alluded to previously, vasoconstriction or vasodilation in the renal vasculature can be assessed via changes in vascular resistance in response to a sympathetic stimulus (e.g., the CPT) (11) or an oral protein load (14). Therefore, we tested the hypotheses that during moderate passive heat stress: *1*) the renal vasodilator response after ingestion of an oral protein load will be greater in younger women compared with younger men, and *2*) the renal vasoconstrictor response to the CPT will be greater in younger men compared with younger women. To our knowledge, renal vascular control in men and women has not been comprehensively assessed using this approach during normothermia. Therefore, we also assessed these same responses during a normothermic condition, which also served as a time control.

## METHODS

### Participants

Upon study design, there were no direct data to inform differences in renal vascular control between men and women. That said, previous work has identified that the blood pressure response differs between men and women during the CPT (10), which resulted in an effect size (*f*) of 0.71. Using this effect size, $\alpha = 0.05$, and $1 - \beta = 0.80$, an a priori power analysis [G-Power v.3.1.9.4 (15)] indicated that we needed at least 12 men and 12 women to complete the study to detect a significant interaction in a mixed-model (group × time) ANOVA.

Twenty-five participants (12 women and 13 men) completed the study. The participant characteristics are listed in Table 1. Participants provided written informed consent after being fully informed of the experimental procedures and possible risks. Participants were eligible if they were between the ages of 18 and 40 yr, reported no known cardiovascular, metabolic, renal, or neurological diseases, and were physically active, nonsmokers, and not taking any medication with direct effects on the cardiovascular system. Women were not pregnant as confirmed through a urine pregnancy test, and self-reported to be normally menstruating and had no diagnosis of a menstrual cycle-specific disorder. Women participants were tested within the first 5 days (4 ± 1 days) of their self-identified onset of menstrual bleeding and 3 of 12 women were on hormonal contraceptives [2 oral contraceptive pills and 1 intrauterine device (IUD)]. 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. It should be noted that a portion of these data from 16 subjects (11 men and 5 women) have been published previously to test a related but different hypothesis (16).

### 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 the 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, and euhydration was

**Table 1.** *Participant characteristics*

| | Men | Women | Unpaired *t* Test |
|---|---|---|---|
| *n* | 13 | 12 | |
| Age, yr | 26 ± 3 | 23 ± 3 | **0.012** |
| Height, cm | 180 ± 4 | 166 ± 7 | **<0.001** |
| Body mass, kg | 82.0 ± 13.3 | 64.8 ± 7.4 | **<0.001** |
| Body mass index, kg/m$^2$ | 25.3 ± 3.8 | 23.4 ± 2.1 | 0.125 |
| Systolic blood pressure, mmHg | 118 ± 8 | 112 ± 7 | 0.080 |
| Diastolic blood pressure, mmHg | 73 ± 9 | 69 ± 8 | 0.214 |

Data are presented as means ± SD. Data were analyzed using two-tailed unpaired *t* tests. Bold type indicates statistical significance.

**Table 2.** *Baseline measures*

| | Normothermia | | Heat Stress | | Linear Mixed Model | | |
|---|---|---|---|---|---|---|---|
| | **Women ($n$ = 12)** | **Men ($n$ = 13)** | **Women ($n$ = 12)** | **Men ($n$ = 13)** | **Group** | **Trial** | **Group × Trial** |
| Core temperature, °C | 37.1 ± 0.2 | 37.0 ± 0.4 | 37.1 ± 0.2 | 37.1 ± 0.4 | 0.673 | 0.265 | 0.633 |
| Mean skin temperature, °C | 33.4 ± 0.5 | 33.3 ± 0.7 | 33.4 ± 0.5 | 33.4 ± 0.7 | 0.933 | 0.625 | 0.565 |
| Urine specific gravity | 1.007 ± 0.005 | 1.009 ± 0.006 | 1.007 ± 0.006 | 1.011 ± 0.007 | 0.205 | 0.294 | 0.596 |
| Heart rate, beats/min | 55 ± 5 | 55 ± 7 | 59 ± 13 | 55 ± 10 | 0.604 | 0.365 | 0.385 |
| Mean arterial pressure, mmHg | 85 ± 7 | 83 ± 11 | 85 ± 6 | 86 ± 9 | 0.732 | 0.367 | 0.444 |
| Renal artery blood velocity, cm/s | 40 ± 7 | 39 ± 7 | 39 ± 7 | 39 ± 7 | 0.825 | 0.928 | 0.647 |
| Renal artery vascular resistance, mmHg/cm/s | 2.2 ± 0.4 | 2.2 ± 0.5 | 2.2 ± 0.4 | 2.2 ± 0.5 | 0.922 | 0.759 | 0.990 |
| Segmental artery blood velocity, cm/s | 21 ± 4 | 21 ± 3 | 20 ± 4 | 21 ± 4 | 0.566 | 0.682 | 0.890 |
| Segmental artery vascular resistance, mmHg/cm/s | 4.2 ± 0.8 | 3.9 ± 0.7 | 4.3 ± 0.7 | 4.2 ± 0.9 | 0.387 | 0.382 | 0.515 |
| Testosterone, ng/mL | 2.1 ± 0.8 | 21.3 ± 22.0 | 2.2 ± 0.8 | 21.3 ± 22.4 | **0.007** | 0.931 | 0.754 |
| Estradiol, pg/mL | 65.7 ± 26.0 | | 71.8 ± 22.2 | | Paired $t$ test: $P = 0.060$ | | |
| Progesterone, ng/mL | 12.9 ± 16.1 | | 15.7 ± 26.2 | | Paired $t$ test: $P = 0.220$ | | |

Data are presented as means ± SD. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (trial) or via two-tailed paired *t* test. Bold type indicates statistical significance.

confirmed using this urine sample [i.e., urine specific gravity < 1.020 (Table 2)]. Participants then measured their nude body mass, consumed 250 mL of cool tap water, and were instrumented as outlined earlier. 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. Following these premeasurements, participants were either exposed to heat stress or normothermia over the next ~4.5 h. For normothermia, 34°C water perfused the suit throughout. For the heat stress trial, the goal was to achieve an elevation in core temperature by 0.8–1.0°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 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.

One hour after commencing normothermia or heat stress, the CPT was performed (Fig. 1). The CPT 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 atpre-CPT and at 1 min and 2 min of the CPT. The CPT was used in the present study to examine the impact of biological sex on renal vasoconstrictor capacity during sympathetic activation.

Following the CPT, participants remained in a supine state, and ~45 min later pre-protein renal blood velocities were measured. Participants then drank a whey protein isolate shake (Optimum Nutrition) containing 1.2 g protein/kg of their screening body mass [men: 98.4 ± 16.0 g/kg protein in 295 ± 48 mL (3:1 ratio of water-to-protein), women: 77.7 ± 8.9 g/kg protein in 233 ± 27 mL (3:1 ratio of water-to-protein)] of water maintained at 25.8 ± 1.3°C (men) and 25.6 ± 0.5°C (women) within a 5-min period (men: 123 ± 66 s, women: 120 ± 64 s) to stimulate the vasodilator response. An oral protein shake, instead of cooked meat, was used as this has been shown to vasodilate to the same extent as 1 g/kg and 2 g/kg of cooked meat (17) and because of logistical and gastrointestinal issues associated with the ingestion of cooked meat during heat stress. Moreover, whey protein contains an amino acid profile (18) that is consistent with those that have been shown previously to stimulate increases in renal vasodilation during an amino acid infusion (14, 19). Following protein ingestion, the participants resumed the supine position, and renal blood velocities were measured at 75 min and 150 min following protein ingestion (Fig. 1). Data were collected at these intervals

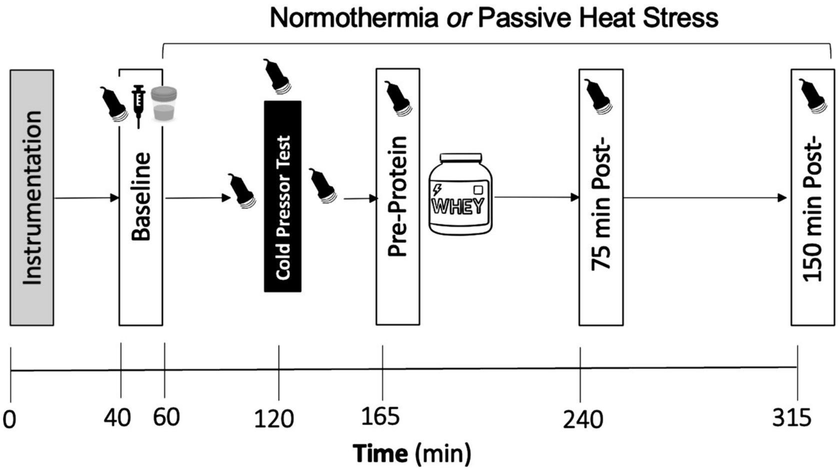

**Figure 1.** Schematic of the study protocol. Participants were instrumented and underwent 20 min of normothermic baseline in both trials. Renal ultrasound measurements (indicated by transducer symbols) were taken at baseline along with blood (indicated by the syringe) and urine (indicated by collection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

because the hemodynamic response occurs 1–3 h post-ingestion (20). 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 the ability to continue tolerating the heat stress and ensuring more than one data collection period post-protein. Throughout the protein testing period, all continuously recorded data were binned using a 60 s average. One male participant became too uncomfortable to continue following protein ingestion in the heat stress trial. Following the 150 min post-protein measures, participants were cooled (if necessary), deinstrumented, and a final nude body mass was then obtained.

## Instrumentation and Measurements

Height was measured using a stadiometer (Holtain Limited, Seritex, Wales, UK). Nude body mass was measured using a digital scale (Sauter, Balingen, Germany). Core temperature was measured continually with an ingestible telemetry capsule ($n = 20$, HQ, Palmetto, FL) or rectal temperature ($n = 5$, 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 locations (21). 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, Helsinki, Finland). Beat-to-beat blood pressure was measured continually via the Penaz method (Human NIBP Nano System, ADInstruments, 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, using methods that have been thoroughly described previously (22). The 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, which was marked 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 (23–25). All renal measurements were obtained and extracted by the same sonographer (J.A.F.). 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 ± 1.3% (segmental artery) for the sonographer in this study. The transducer was held in place throughout the duration of the CPTs so that image acquisition occurred within a 10-s window during each minute of the CPT. 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 (1, 14, 16, 19, 21, 23, 26–28). This was deemed reasonable because pharmacologically induced changes in renal blood flow (28, 29) were due to changes in renal artery blood velocity and not diameter (29). Renal and segmental artery blood velocity were normalized to mean arterial pressure to provide an index of vascular resistance (i.e., mean arterial pressure ÷ blood velocity).

Serum estradiol [intra-assay coefficient of variation (CV): 4.6 ± 2.4%, Eagle Biosciences, Amherst, NH, sensitivity: 10 pg/mL, range: 20–3,200 pg/mL] and serum progesterone (intra-assay CV: 14.1 ± 8.9%, Eagle Biosciences, Amherst, NH, sensitivity: 0.1 ng/mL, range: 0.3–60.0 ng/mL) were measured in women, whereas serum testosterone (intra-assay CV: 6.0 ± 5.3%, Eagle Biosciences, Amherst, NH, sensitivity: 0.02 ng/mL, range: 0.08–16.7 ng/mL) was measured in blood samples obtained at baseline (pre-heat stress) in both men and women. In all instances, samples were measured in duplicate using commercially available ELISA kits. Urine specific gravity was measured using refractometry (Atago, Tokyo, Japan).

## Data and Statistical Analysis

Continuously collected data were sampled at 1,000 Hz via a data acquisition system (PowerLab 16/35, ADInstruments). Data collected during baseline, pre-CPT, and pre-protein were analyzed using linear mixed models with an independent factor of group (men vs. women) and a repeated factor of trial (heat stress vs. normothermia). Data obtained during the CPT (i.e., pre-CPT, 1 min of the CPT, and 2 min of the CPT) and during the protein period (i.e., pre-protein, 75 min post-protein, and 150 min post-protein) were analyzed using linear mixed models with an independent factor of group (men vs. women) and a repeated factor of time within both the heat stress and normothermia trials. During the CPT, the peak change in mean arterial pressure and segmental vascular resistance and the nadir in segmental blood velocity were calculated, whereas during the protein period, the peak change in renal and segmental artery blood velocity and the nadir in renal and segmental artery vascular resistance were calculated. These calculated data were compared using linear mixed models with an independent factor of group (men vs. women) and a repeated factor of trial (heat stress vs. normothermia), allowing for direct comparisons between men and women during heat stress and normothermia. Estradiol and progesterone concentrations in women between trials were analyzed using two-tailed paired $t$ tests, whereas participant characteristics were compared via two-tailed unpaired $t$ tests.

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 (of the linear mixed models) were

**Table 3.** *Pre-cold pressor test measures*

| | Normothermia | | Heat Stress | | Linear Mixed Model | | |
|---|---|---|---|---|---|---|---|
| | Women ($n$ = 12) | Men ($n$ = 13) | Women ($n$ = 12) | Men ($n$ = 13) | Group | Trial | Group × Trial |
| Core temperature, °C | 37.2 ± 0.3 | 37.0 ± 0.4 | 37.9 ± 0.2 | 37.8 ± 0.4 | 0.222 | **<0.001** | 0.303 |
| Mean skin temperature, °C | 33.9 ± 0.5 | 33.8 ± 0.6 | 37.3 ± 0.4 | 37.5 ± 0.7 | 0.941 | **<0.001** | 0.348 |
| Heart rate, beats/min | 55 ± 6 | 55 ± 11 | 86 ± 17 | 83 ± 13 | 0.750 | **<0.001** | 0.652 |
| Mean arterial pressure, mmHg | 83 ± 8 | 84 ± 9 | 76 ± 7 | 80 ± 13 | 0.422 | **0.027** | 0.665 |
| Renal artery blood velocity, cm/s | 41 ± 6 | 40 ± 6 | 37 ± 6 | 35 ± 5 | 0.481 | **0.007** | 0.644 |
| Renal artery vascular resistance, mmHg/cm/s | 2.1 ± 0.3 | 2.2 ± 0.4 | 2.1 ± 0.4 | 2.3 ± 0.5 | 0.474 | 0.255 | 0.526 |
| Segmental artery blood velocity, cm/s | 21 ± 4 | 21 ± 3 | 21 ± 3 | 21 ± 3 | 0.977 | 0.384 | 0.744 |
| Segmental artery vascular resistance, mmHg/cm/s | 4.0 ± 0.9 | 3.0 ± 0.6 | 3.8 ± 0.6 | 3.9 ± 0.9 | 0.384 | 0.688 | 0.737 |

Data are presented as means ± SD. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (trial) or via two-tailed paired $t$ test. Bold type indicates statistical significance.

checked. 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 the linear mixed model revealed a significant interaction, pairwise comparisons were carried out using Sidak's test, which corrects for multiple comparisons. 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. All data were analyzed with Prism software (v.10; GraphPad Software, La Jolla, CA). Data are reported as means ± SD and individual values where possible.

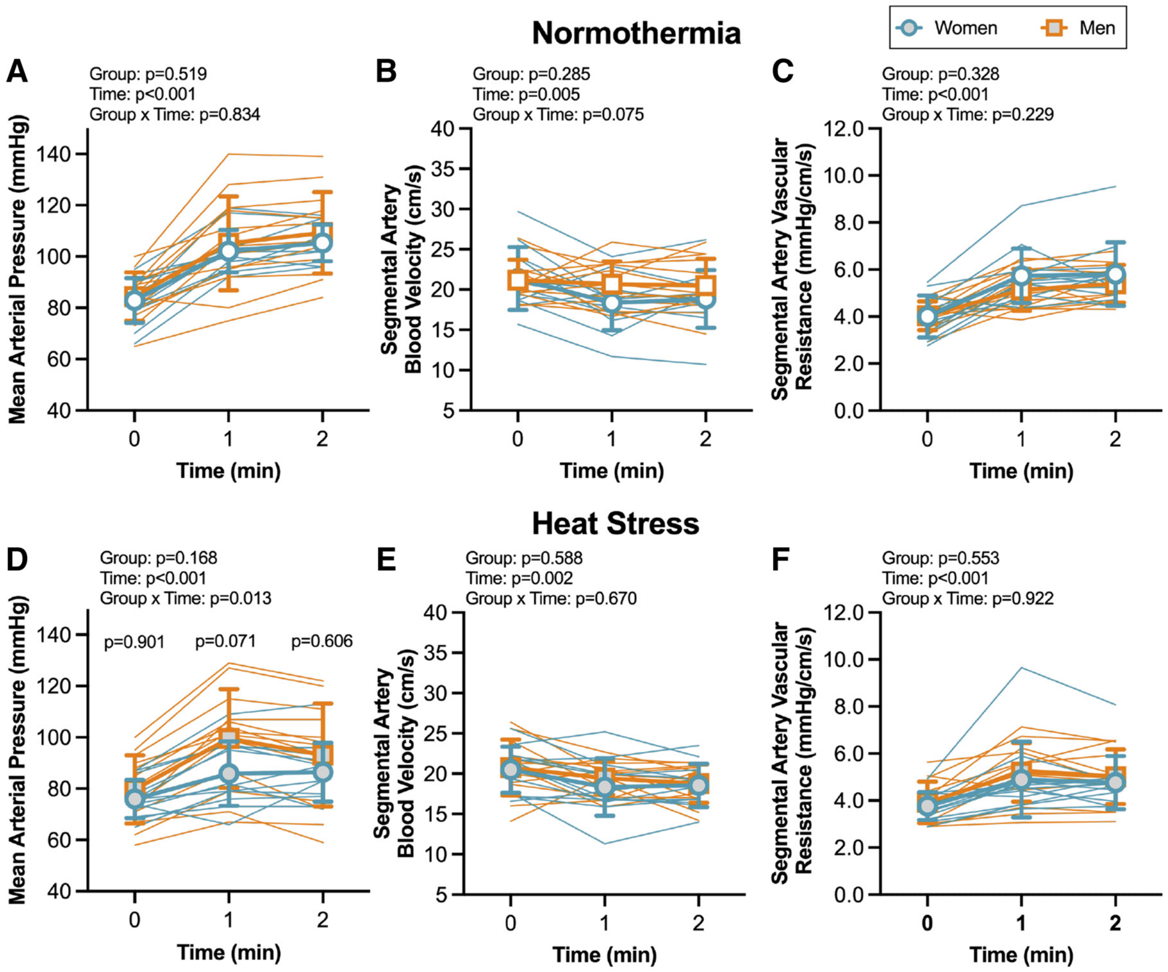

**Figure 2.** Mean arterial pressure (*A*), segmental artery blood velocity (*B*), and segmental artery vascular resistance (*C*) during the cold pressor test during normothermia (*top*) and mean arterial pressure (*D*), segmental artery blood velocity (*E*), and segmental artery vascular resistance (*F*) during heat stress (*bottom*). Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (time). When the linear mixed model revealed a significant interaction, pairwise comparisons were carried out using Sidak's test and these $P$ values are presented in the figure. $n$ = 12 women and 13 men. Data are presented as means ± SD and individual values.

## RESULTS

### Baseline

Baseline measures did not differ between trials nor between men and women ($P \geq 0.060$), except serum testosterone concentrations, which were higher in men compared with women (main effect of group: $P = 0.007$, Table 2). The percent change in body mass loss from pre- to post-experimental visit was greater in the heat stress trial (main effect of trial: $P < 0.001$) but did not change differently between men (normothermia: 1.5 ± 0.5%, heat stress: 2.9 ± 0.7%) and women (normothermia: 1.6 ± 0.4%, heat stress: 2.9 ± 0.8%) (group × trial: $P = 0.589$).

### Cold Pressor Test

Pre-CPT, core temperature, mean skin temperature, and heart rate were all higher in the heat stress trial (main effect of trial: $P < 0.001$), whereas mean arterial pressure and renal artery blood velocity were lower in the heat stress trial (main effect of trial: $P \leq 0.027$). However, these variables did not differ between men and women (main effect of group: $P \geq 0.222$; group × trial: $P \geq 0.303$, Table 3). During the CPT in both normothermia and heat stress trials, mean arterial pressure increased (main effect of time: $P < 0.001$), segmental artery blood velocity decreased (main effect of time: $P \leq 0.005$), and segmental artery vascular resistance increased (main effect of time: $P < 0.001$, Fig. 2). Although the magnitude of increase in mean arterial pressure was blunted with heat stress (main effect of trial: $P < 0.001$), the magnitude of changes in segmental artery blood velocity and vascular resistance did not differ between normothermia and heat stress (main effect of trial: $P \geq 0.142$) or between men and women (main effect of group: $P \geq 0.192$, Fig. 3).

### Protein

Pre-protein, core temperature, mean skin temperature, and heart rate were all higher in the heat stress trial (main effect of trial: $P < 0.001$), whereas mean arterial pressure was lower in the heat stress trial (main effect of trial: $P = 0.047$), but none of these variables changed differently between men and women (group × trial: $P \geq 0.153$, Table 4). Pre-protein, segmental artery blood velocity in the normothermia trial was lower in women versus men ($P = 0.031$). Although segmental artery blood velocity and vascular resistance did not differ between normothermia and heat stress in men ($P \geq 0.361$), in women segmental artery blood velocity was lower ($P = 0.025$) and vascular resistance was higher ($P = 0.008$) in normothermia versus heat stress (Table 4). Following protein ingestion, segmental and renal artery blood velocities increased (main effect of time: $P < 0.001$) and vascular resistance decreased (main effect of time: $P \leq 0.027$) in both normothermia (Fig. 4) and heat stress (Fig. 5) trials. Only segmental artery blood velocity in the normothermia trial differed between men and women, and this occurred at that pre-protein time point ($P = 0.025$), otherwise, these variables did not change differently between men and women (group × time: $P \geq 0.070$). The magnitude of changes in segmental and renal artery blood velocity and vascular resistance did not differ between normothermia and heat stress (main effect of trial: $P \geq 0.080$) nor between men and women (main effect of group: $P \geq 0.096$, Fig. 6).

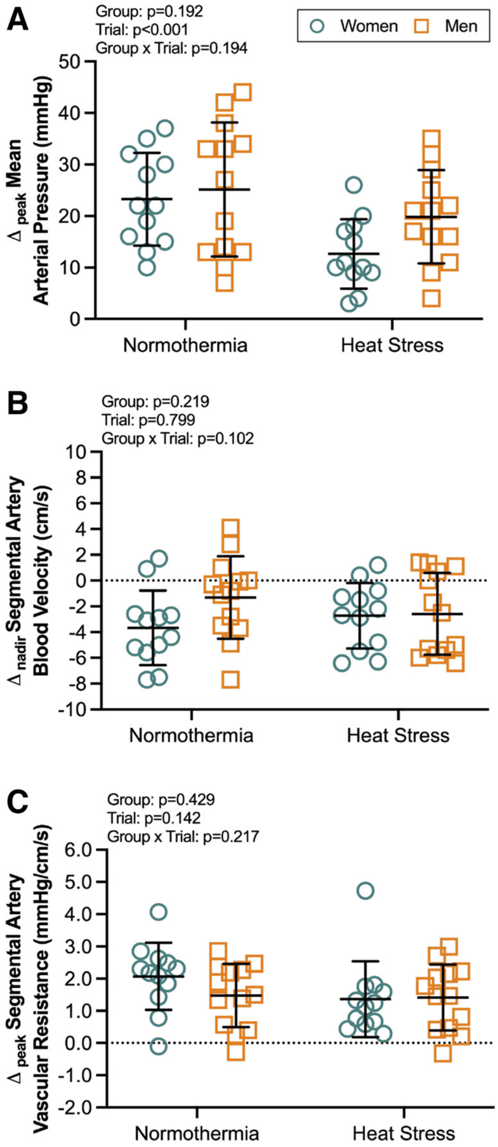

**Figure 3.** The peak change (Δ) in mean arterial pressure (*A*), nadir in segmental artery blood velocity (*B*), and peak segmental artery vascular resistance (*C*) during the cold pressor test during normothermia and heat stress. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (trial). $n = 12$ women and 13 men. Data are presented as means ± SD and individual values.

## DISCUSSION

In contrast to our hypothesis, the increase in segmental artery vascular resistance during the CPT did not differ between younger men and women during mild passive heat stress (Fig. 2*F*) or normothermia (Fig. 2*C*), and the peak increase in vascular resistance did not differ between the heat stress and normothermic conditions (Fig. 3*C*). Similarly, the renal vasodilator response to an oral protein load did not differ between men and women during heat stress (Fig. 5, *D* and *E*) or normothermia (Fig. 4, *D* and *E*), and the magnitude of renal vasodilation did not differ between the heat stress and normothermic conditions (Fig. 6, *C* and *D*). These data indicate that renal vascular control, as assessed in the present study, is unlikely to differ between healthy younger men and younger women tested in the early follicular phase of the menstrual cycle during both mild passive heat stress and normothermia.

As expected, renal vascular resistance, as assessed in the renal segmental artery, increased during the CPT in men and women during both passive heat stress (Fig. 2*F*) and normothermia (Fig. 2*C*). Heat stress blunted the magnitude of increase in mean arterial pressure in both men and women (Fig. 3*A*), as has been observed previously (11). However, there was no difference in the magnitude of changes in segmental artery vascular resistance between normothermia and heat stress (Fig. 3*C*). This is contrary to what we have reported previously (16, 23). Based on this previous work, we expected to observe an attenuated renal vasoconstrictor response to the CPT during heat stress, which has been speculated to be due to increases in local vasodilators (e.g., nitric oxide) that may induce a sympatholytic effect in the renal vasculature (30). The mechanisms explaining our observations are not readily apparent from the current study. However, we speculate that the severity of heat stress was too mild (e.g., increased core temperature by ∼0.7°C vs. +1.2°C) to consistently elicit attenuated renal vasoconstrictor responses to the CPT. For example, we likely observed increased variability in the cardiovascular response to the CPT by using a less severe heat stress. Nevertheless, the current study does not support that sympathetically mediated renal vasoconstriction differs between men and women during normothermia or mild passive heat stress.

Based on previous evidence (10), it is somewhat surprising that CPT induced increases in blood pressure during both normothermia and passive heat stress did not differ between younger men and women. That said, our findings are consistent with a previous study that has demonstrated a similar increase in blood pressure and muscle-sympathetic nerve activity between younger men and women (12). However, it is interesting to note that there is evidence supporting attenuated vasoconstrictor responses in forearm (31) and leg (12) vascular beds during sympathetic stimulation in younger women compared with younger men. Therefore, this study adds to the literature by demonstrating that differential sympathetic-induced renal vasoconstriction is unlikely to differ between younger men and younger women during the early follicular phase in normothermia or passive heat stress.

Consistent with our previous studies (13, 16), we have identified that renal vascular resistance, as assessed in both the renal and segmental arteries, decreased with an oral protein load in men and women during both passive heat stress (Fig. 5) and normothermia (Fig. 4). Moreover, the magnitude of renal vasodilation did not differ between passive heat stress and normothermic conditions, which is also consistent with previous findings (16) and also did not differ between men and women (Fig. 6). The renal vasodilation elicited by an oral protein load is believed to be primarily caused by tubuloglomerular feedback mechanisms, with the hormone glucagon playing a key role in modulating local paracrine factors, namely nitric oxide —a potent vasodilator (32). However, the findings presented herein, combined with previous work (16), support that passive heat stress has little impact on glucagon bioavailability or bioactivity, and its downstream effects on the kidneys. It is somewhat surprising that there were no differences in protein-induced renal vasodilation between younger men and younger women, given the potent role of estrogen (E2) in nitric oxide production and nitric oxide-dependent vasodilation (33). Thus, these findings support that differences in sex hormones, particularly estrogen, between men and women are unlikely to modulate the local bioavailability/bioactivity of nitric oxide and/or its downstream effects within the kidneys following an oral protein load. Caveats to this conclusion, however, are that we did not measure estradiol in the men, and we also only tested the women at a time when estrogen is at its lowest levels (i.e., the early follicular phase of the menstrual cycle). That said, although we did not measure estradiol in our men, we think it is unlikely that the men would have estrogen levels approaching that observed in our women (e.g., the average adult male has ∼four fold less E2 concentration than the average adult female) (34). Nevertheless, it is important to note that it is unlikely that heat stress alters the vasodilator response in vascular beds [e.g., the brain (35)] unresponsive to the heat stimulus. The current study extends these observations by identifying that this effect is unlikely to be modified by biological sex during the low estrogen phase of the menstrual cycle when compared with men.

## Methodological Considerations

There are a few important factors to consider when interpreting the results of the current study. First, we did not directly measure volumetric renal blood flow. Rather, Doppler ultrasound was used to measure renal blood velocity, which was interpreted to reflect changes in renal blood flow, as has

**Table 4.** *Pre-protein measures*

| | Normothermia | | Heat Stress | | Linear Mixed Model | | |
|---|---|---|---|---|---|---|---|
| | **Women ($n$ = 12)** | **Men ($n$ = 13)** | **Women ($n$ = 12)** | **Men ($n$ = 13)** | **Group** | **Trial** | **Group × Trial** |
| Core temperature, °C | 37.2 ± 0.2 | 37.0 ± 0.4 | 37.9 ± 0.2 | 37.8 ± 0.4 | 0.310 | **<0.001** | 0.754 |
| Mean skin temperature, °C | 34.0 ± 0.5 | 34.0 ± 0.7 | 37.3 ± 0.5 | 37.3 ± 0.6 | 0.945 | **<0.001** | 0.930 |
| Heart rate, beats/min | 56 ± 4 | 56 ± 9 | 84 ± 17 | 77 ± 15 | 0.460 | **<0.001** | 0.190 |
| Mean arterial pressure, mmHg | 83 ± 7 | 87 ± 9 | 77 ± 7 | 85 ± 9 | **0.043** | **0.047** | 0.252 |
| Renal artery blood velocity, cm/s | 41 ± 6 | 41 ± 6 | 39 ± 6 | 37 ± 8 | 0.800 | **0.041** | 0.670 |
| Renal artery vascular resistance, mmHg/cm/s | 2.1 ± 0.4 | 2.2 ± 0.3 | 2.0 ± 0.4 | 2.4 ± 0.5 | 0.251 | 0.077 | 0.153 |
| Segmental artery blood velocity, cm/s | 19 ± 3* | 22 ± 3 | 22 ± 4N | 21 ± 3 | 0.310 | 0.343 | **0.009** |
| Segmental artery vascular resistance, mmHg/cm/s | 4.6 ± 0.9 | 4.0 ± 0.6 | 3.6 ± 0.7N | 4.3 ± 0.9 | 0.860 | 0.107 | **0.008** |

Data are presented as means ± SD. Data were analyzed using linear mixed models with one between participant factor (group) and one within participant factor (trial). When the linear mixed model revealed a significant interaction, pairwise comparisons were carried out using Sidak's test. *Different from men ($P = 0.031$); N different from normothermia ($P \leq 0.025$). Bold type indicates statistical significance.

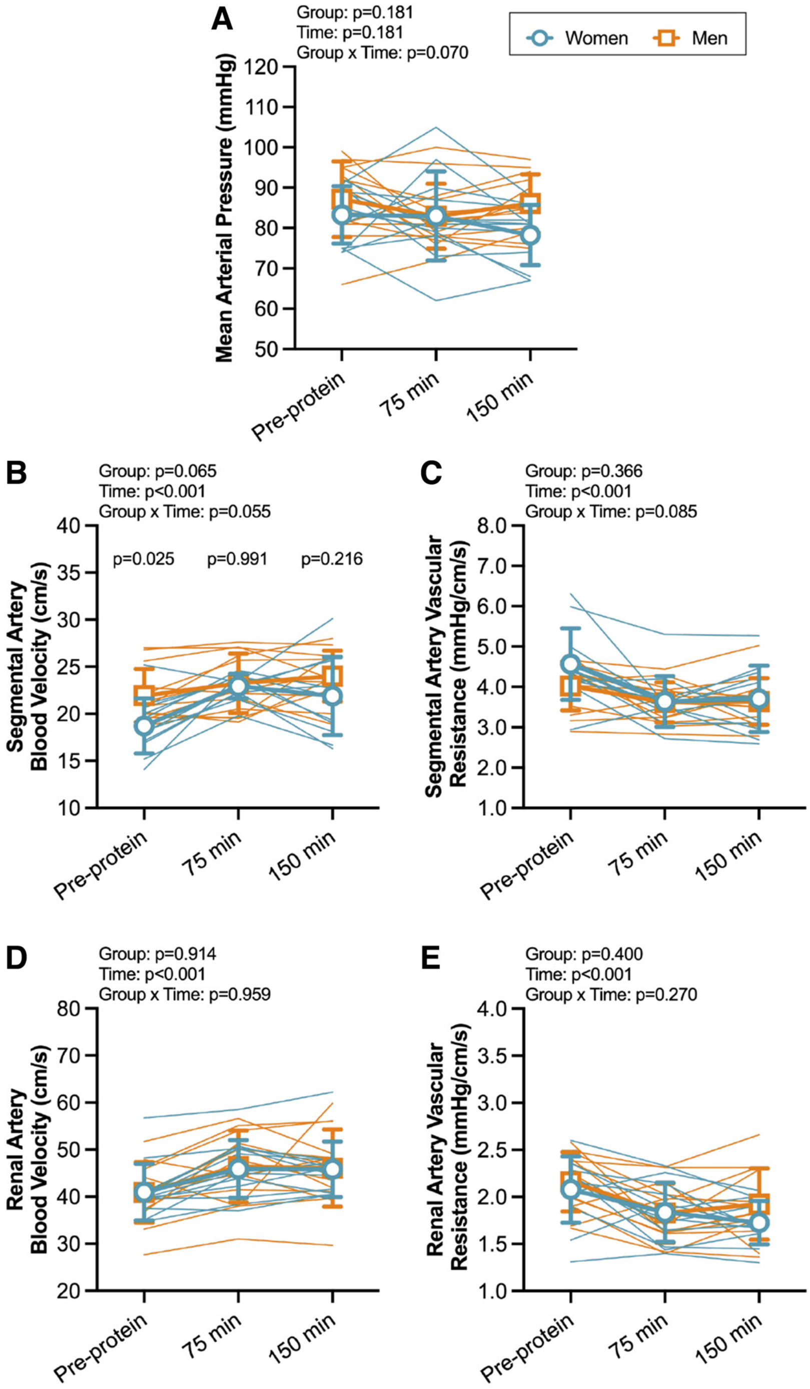

**Figure 4.** Mean arterial pressure (*A*), segmental artery bloodvelocity (*B*), segmental artery vascular resistance (*C*), renal artery blood velocity (*D*), and renal artery vascular resistance (*E*) during the protein period during normothermia. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (time). When the linear mixed model revealed a significant interaction, pairwise comparisons were carried out using Sidak's test and these *P* values are presented in the figure. $n = 12$ women and 13 men. Data are presented as means ± SD and individual values.

been done previously (13). To increase the reliability of the operator-dependent ultrasound measurements, the intraoperator coefficient of variation of our sonographer was measured and reported to aid in the interpretation of measurements obtained from Doppler ultrasound (23). 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. That said, we acknowledge that ambient heat exposure is a more externally valid approach and should be used in future studies. Third, we recruited younger, healthy men and women, the latter of which were tested in only the early follicular phase of the menstrual cycle. Future studies should examine renal vascular control in other populations and in women across multiple menstrual cycle phases. Finally, we chose to administer the same relative oral protein dose within the subject for both the heat stress and normothermia trials. Dehydration may be particularly interesting considering that in the heat stress trial participants lost more body water than in the normothermia trial. This is particularly important because dehydration induced by fluid restriction has recently been shown to attenuate both the increase in creatinine clearance (an index

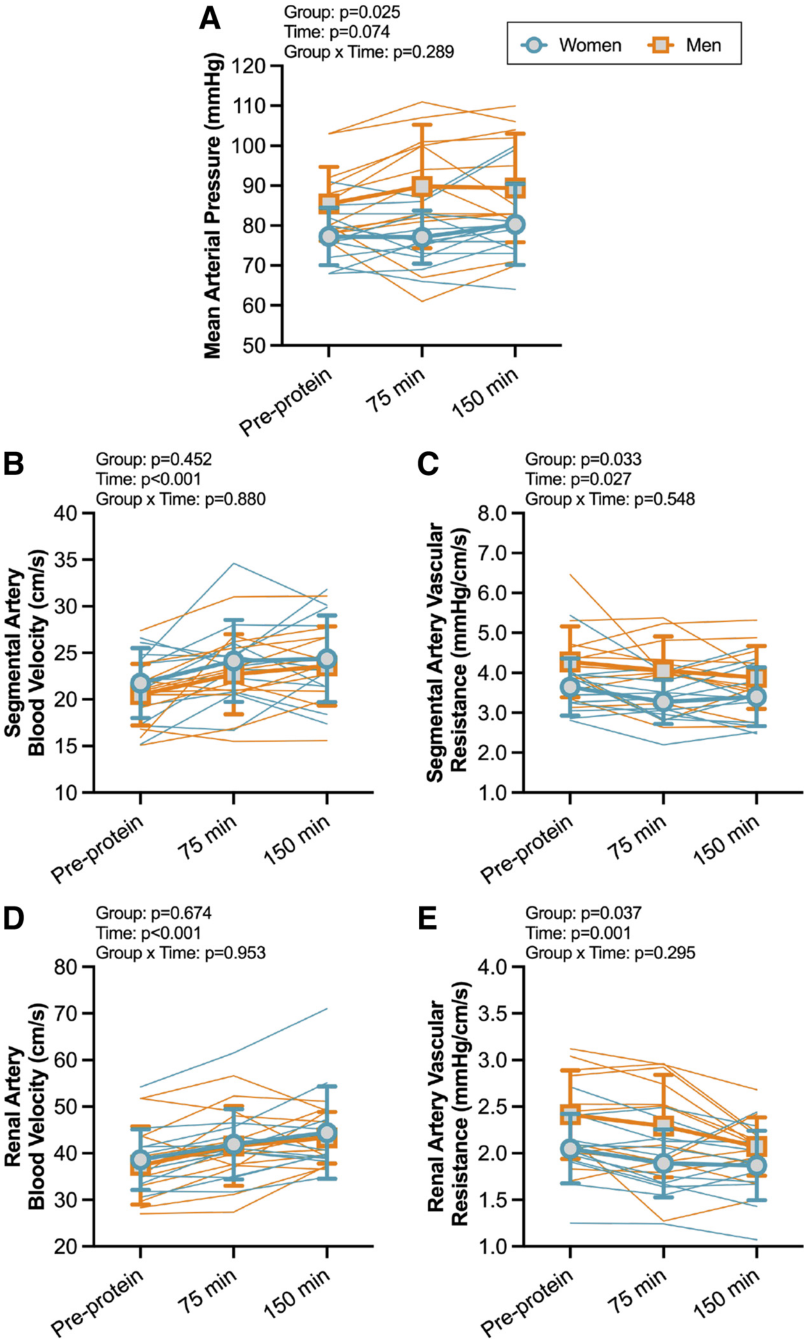

**Figure 5.** Mean arterial pressure (*A*), segmental artery blood velocity (*B*), segmental artery vascular resistance (*C*), renal artery blood velocity (D), and renal artery vascular resistance (*E*) during the protein period during heat stress. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (time). When the linear mixed model revealed a significant interaction, pairwise comparisons were carried out using Sidak's test. *n* = 12 women and 12 men. Data are presented as means ± SD and individual values.

of glomerular filtration rate) during an oral protein load and renal vasoconstriction during a sympathoexcitatory stimulus (i.e., exercise pressor reflex) (13). Interestingly, and consistent with the findings presented herein, dehydration had no impact on the magnitude of renal vasodilation following oral protein load. This being said, it is notable that the effect of heat stress on renal vascular control in the absence of differences in body water remains unexplored.

## Conclusions

Renal vasoconstrictor responses to the CPT and vasodilator responses following an oral protein load during heat stress or normothermia do not differ between younger men and younger women in the early follicular phase of the menstrual cycle. Therefore, sex-dependent differences in renal vascular control are unlikely to contribute to sex differences in acute kidney injury risk during mild hyperthermia in younger adults when females are tested in the low estrogen phase of the menstrual cycle (follicular phase).

## Perspectives and Significance

The frequency and intensity of heatwaves is increasing (36). A top cause of hospitalization during heatwaves is AKI, with some evidence of sex differences in the incidence of heat-induced AKI in both younger (1) and older adults (2). Interestingly, few studies have thoroughly investigated the basic physiological differences in renal structure and function between men and women (37). Thus, there is consensus

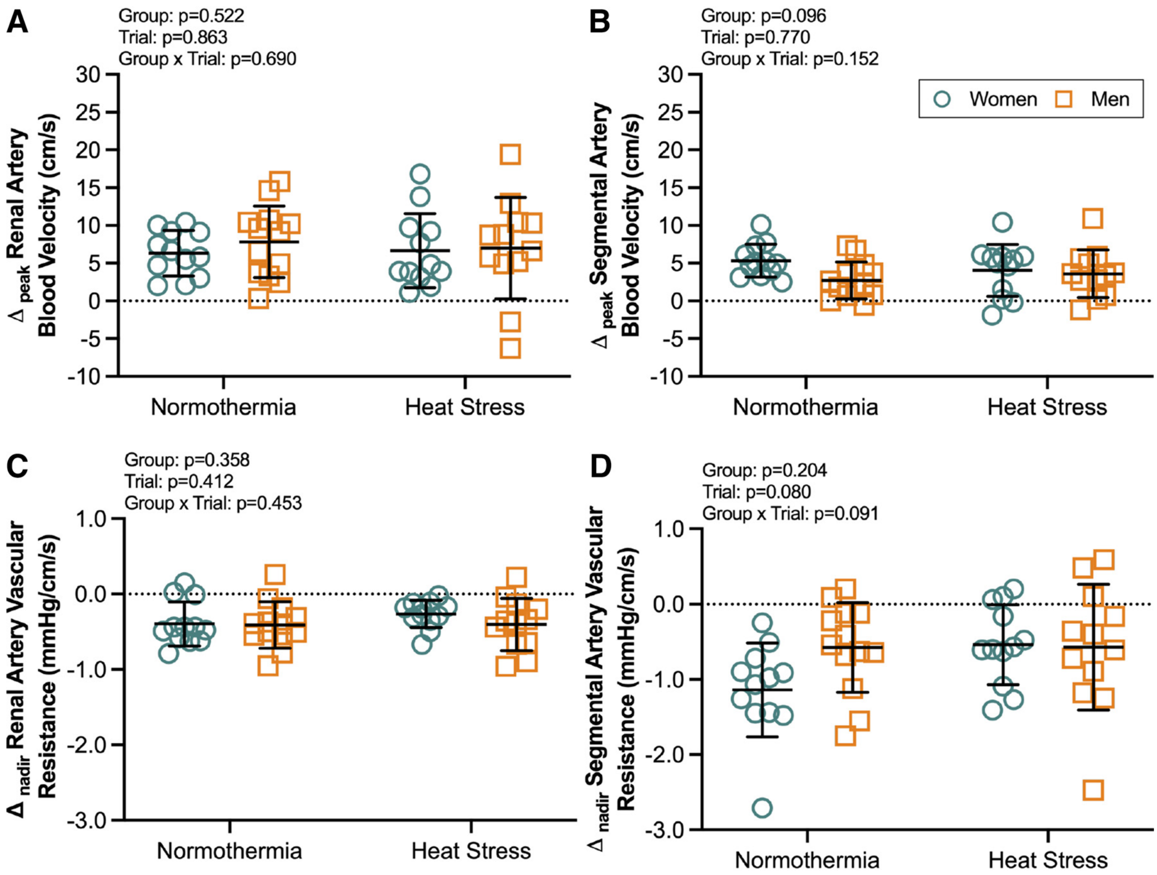

**Figure 6.** The peak change (Δ) renal artery blood velocity (*A*), peak change (Δ) in segmental artery blood velocity (*B*), nadir in renal artery vascular resistance (*C*), and nadir in segmental artery vascular resistance (*D*) during the protein period during normothermia and heat stress. Data were analyzed using linear mixed models with one between-participant factor (group) and one within-participant factor (trial). Normothermia: $n = 12$ women and 13 men, heat stress: $n = 12$ women and 12 men. Data are presented as means ± SD and individual values.

that there is a relatively poor understanding of the mechanisms that lead to observed sex differences in progression and risk of kidney diseases (4). By extension, there is little information regarding sex differences in AKI risk during nonheat stress or heat stress situations. The results of the current study indicate that renal vascular control, which involved an assessment of both sympathetically mediated vasoconstrictor and oral protein-mediated vasodilator responses, does not readily explain differences in heat-induced AKI risk between younger men and women. Moreover, differences in renal vascular control during normothermia are unlikely to contribute to the differential risk of AKI between younger men and younger women in a nonheat-stressed state. However, it is important to note that our observations are specific to healthy, younger women assessed during the early follicular phase of the menstrual cycle and to younger men. Thus, future work should focus on different at-risk populations (e.g., peri- or postmenopausal women, people with hypertension, etc.) because a greater understanding of sex differences in renal physiology (and pathophysiology) is necessary for the development of effective sex-specific therapies in both men and women (37).

## DATA AVAILABILITY

Data will be made available upon reasonable request.

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## DISCLOSURES

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## AUTHOR CONTRIBUTIONS

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

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