american physiological society

JOURNAL OF APPLIED PHYSIOLOGY

J Appl Physiol 139: 863–872, 2025.
First published August 29, 2025; doi:10.1152/japplphysiol.00405.2025

RESEARCH ARTICLE

Distinct physiological responses to heat acclimation in males and females lead to similar thermal adaptations in both sexes

Gabrielle E. W. Giersch,[1] Andrew M. Greenfield,[1] Shaun C. Brazelton,[1,2] Billie K. Alba,[1] Phillip O. Bodurtha,[1,2] Aaron R. Caldwell,[3] Thomas A. Mayer,[1] Timothy A. Murray,[1] Benjamin J. Ryan,[1] Roy M. Salgado,[1] Afton D. Seeley,[1] and Nisha Charkoudian[1]

[1]*U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts, United States;* [2]*Oak Ridge Institute of Science and Education, Oak Ridge, Tennessee, United States; and* [3]*Fay W. Boozman College of Public Health, University of Arkansas for Medical Sciences Northwest, Springdale, Arkansas, United States*

Abstract

Although there are known differences in mechanisms of thermoregulation between males and females, it is unclear whether or how these alter physiological adaptations during heat acclimation. Our goal was to evaluate possible sex differences in responses to a 10-day controlled work rate heat acclimation (HA) protocol. We studied 27 young healthy individuals (16 females). Volunteers underwent 10 days of HA at a controlled work rate (walking at 5 km·h^{-1}, 2% grade, 40°C, 40% relative humidity, 1.34 m·s^{-1} windspeed for 120 min). Core temperature (T_{core}) and heart rate (HR) were measured continuously. Whole body sweat rate (WBSR) was calculated from pre- and post-exercise nude body mass. Blood samples were taken pre-exercise on *days 1* and *10* (HAD1 and HAD10) to evaluate HSP72. Males and females successfully (and similarly) adapted to HA, as assessed by progressive decreases in peak T_{core} (males: HAD1 38.69 ± 0.48 vs. HAD10 38.30 ± 0.28°C, $P < 0.001$; females: HAD1 38.90 ± 0.49 vs. HAD10 38.46 ± 0.45°C, $P < 0.001$). Peak HR was higher in females throughout HA, but adapted to HA in both groups (males: HAD1 141 ± 16 vs. HAD10 127 ± 11 beats/min, $P < 0.001$; females: HAD1 170 ± 17 vs. HAD10 152 ± 17 beats/min, $P < 0.001$). WBSR increased in both groups (males: HAD1 0.73 ± 0.23 vs. HAD10 0.92 ± 0.23 L·h^{-1}, $P < 0.001$; females: HAD1 0.65 ± 0.15 vs. HAD10 0.72 ± 0.12 L·h^{-1}, $P = 0.041$) but was higher in male on HAD3-HAD5. Across HA, HSP72 increased similarly between males and females. These results suggest that males and females have a similar ability to adapt to a 10-day exercise-HA protocol but appear to do so via distinct physiological mechanisms.

NEW & NOTEWORTHY We evaluated sex differences in the magnitude and time course of physiological adaptations to heat stress using a generalizable and practical protocol (i.e., standardized work rate in the heat). Men and women had similar adaptations in core temperature, despite greater sweating in the men. We also report for the first time that intracellular heat shock protein 72 increases similarly in males and females during heat acclimation.

core temperature; sex differences; thermoregulation

INTRODUCTION

Heat illnesses are a regular and potentially serious risk for individuals who participate in outdoor physical activity, such as military personnel, athletes, and laborers. As temperatures get progressively warmer, heat illnesses will become more pervasive. Heat acclimation (HA) is the integrative physiological process by which the body systematically adapts to repeated heat exposures, resulting in lower body temperatures, lower heart rates, and increased sweating rates during exercise (1). These adaptations enhance heat dissipation, decrease cardiovascular strain, and can lower the risk of exertional heat illnesses (1–3). The beneficial adaptations gained from HA and the integrative physiological mechanisms supporting these adaptations have been extensively studied over the past several decades (1, 4, 5).

Sex differences exist in thermoregulation, as evidenced by both biophysical (e.g., body size differences) and thermoregulatory (e.g., lower sweating rates in females at very high workloads) differences between males and females (6–8). Men, on average, are larger than women and have greater body surface area (BSA), lower BSA to mass ratio (BSA:mass), lower percent body fat, and greater lean mass. These factors may contribute to differences in heat production, dissipation, and storage between sexes, highlighted by a recent review (9). Although there is evidence to suggest that there are differences in thermoregulatory mechanisms between males and females (6, 8, 10), these differences do not appear to alter practical outcomes, such as heat illness risk (11–13).

Previous data regarding time course or magnitude of HA differences between males and females are limited and inconsistent. Horstman and Christensen (14) suggested that

Correspondence: G. E. W. Giersch (gegiersch@gmail.com).
Submitted 13 May 2025 / Revised 11 June 2025 / Accepted 20 August 2025

female adapt more quickly or to a greater degree than male as measured by core temperature (T_{core}) changes, with a similar impact on performance parameters during HA in hot, humid conditions. In contrast, in a different study, men exhibited T_{core} adaptations faster than women, whereas female showed more rapid sweating adaptations (15). Some of the discrepancies in these findings may be related to fitness differences between groups (16) or to differences in body size and biophysical parameters (15). With increasing numbers of females in athletics, military, and laborer populations, further investigation is warranted to ensure appropriate recommendations are being made to optimize HA protocols for all individuals.

HA has been shown to improve cellular thermotolerance to heat stress, as observed by increases in heat shock proteins (HSP) (17). HSPs are molecular chaperone proteins that increase in response to stressors (such as heat and hypoxia) and provide cytoprotection during stressful exposure (18, 19). HSP upregulation during HA is thought to provide additional protection in response to heat and other potential stressors (20, 21). Previous work has shown that 10 days of HA upregulate HSP72, particularly in individuals with the largest adaptive response to HA (17). These data were collected in eight individuals, one of whom was female. Thus, evaluation of sex differences in the previous sample was not possible. The HSP response between sexes to heat stress remains conflicting in the literature, where previous research has indicated that males have a greater propensity to upregulate HSPs (22), whereas evidence in a murine model suggested an upregulation of HSP72 in female mice and rats, relative to males, and that the upregulation was related to estrogen fluctuations (23, 24). Compelling sex differences have also been observed with acute exposure to heat stress, with males demonstrating higher HSP72 upregulation compared with females (22). Taken together, these data suggest a possible difference in HSP responsiveness between males and females to heat stress and potentially, HA.

The purpose of the present study was to quantify the integrative physiological responses of males and females to a 10-day practical, controlled work-rate HA protocol. We tested the hypothesis that males and females would adapt similarly to a 10-day controlled work rate HA protocol.

METHODS

Participants and Ethical Approval

This study was approved by the US Army Medical Research and Development Command Institutional Review Board (M-10929). All volunteers gave written and informed consent, and this investigation adhered to all aspects of the Declaration of Helsinki (Clinicaltrials.gov Registration No. NCT05292170). This investigation adhered to the human subjects protection policies outlined in US Army Regulation 70-25 and US Army Medical Research and Development Command Regulation 7-25.

Subjects

Twenty-seven (n = 16 women) participants completed this investigation. Inclusion criteria ensured participants: exercised at least two times per week or passed their most recent US Military fitness assessment, had no history of heat illness, or no history of fluid/electrolyte imbalance, had no history of orthostatic intolerance within the previous 3 years, and no nicotine use within the previous four months. Females were not restricted to participating during any specific phase of the menstrual cycle or contraceptive utilization in the interest of maintaining external/ecological validity. Female volunteers were asked to complete a menstrual history questionnaire that was used to confirm that all female volunteers were naturally cycling (i.e., had a menstrual cycle every 25–35 days and had not missed more than two periods in the last 12 mo) or to collect information on contraceptive utilization.

Study Design

Volunteers reported to the laboratory 13 times to complete anthropometric measures, a maximal aerobic test ($\dot{V}o_{2max}$) to evaluate fitness status before and after HA, and a 10-day HA protocol. Study design and visits are displayed in Fig. 1. Testing took place in Natick, MA between late-October and early-April to avoid possible confounding with natural seasonal acclimatization.

Anthropometric Testing

Volunteers reported to the laboratory for the first day of testing and completed a menstrual history questionnaire (females only) and activity and background survey. Height, nude body mass, body composition analysis using a dual energy X-ray absorptiometry scan (DEXA, GE Lunar iDXA, GE Healthcare, Madison, WI), and three-dimensional (3-D) scan (SS20 Scanner, Size Stream, Cary, NC) for measure of body surface area (BSA) were also completed. Volunteers self-reported their race and ethnicity (Table 1).

$\dot{V}o_{2max}$ Testing

Before HA testing (within 72 h before beginning) and following HA (within 96 h of HAD10), volunteers underwent a maximal oxygen consumption ($\dot{V}o_{2max}$) test to evaluate fitness status. Volunteers ran on a treadmill, starting between 8.9–10.5–6.5 kph at 2% grade, where speed increased by 0.8 kph every 3 min until volitional exhaustion with continuous metabolic measures (TrueOne 2400, ParvoMedics, Sandy, UT). $\dot{V}o_{2max}$ criteria included respiratory exchange ratio (RER) >1.1, rating of perceived exertion (RPE) >17, or an increase in ventilation without a concomitant increase in oxygen consumption ($\dot{V}o_2$).

Heat Acclimation Protocol

Volunteers reported to the laboratory for 10 HA visits over 14 days. For all volunteers, the first 4 days of testing were consecutive, and there was no more than one consecutive day of rest within the 10-day protocol. During the HA testing, volunteers arrived at the laboratory with their first morning urine sample and followed appropriate instructions to ensure hydration status (urine specific gravity, USG < 1.025). If volunteers did not provide their first morning urine sample, a spot sample was collected upon arrival to the laboratory (between 0600 and 0900). In the event the USG was

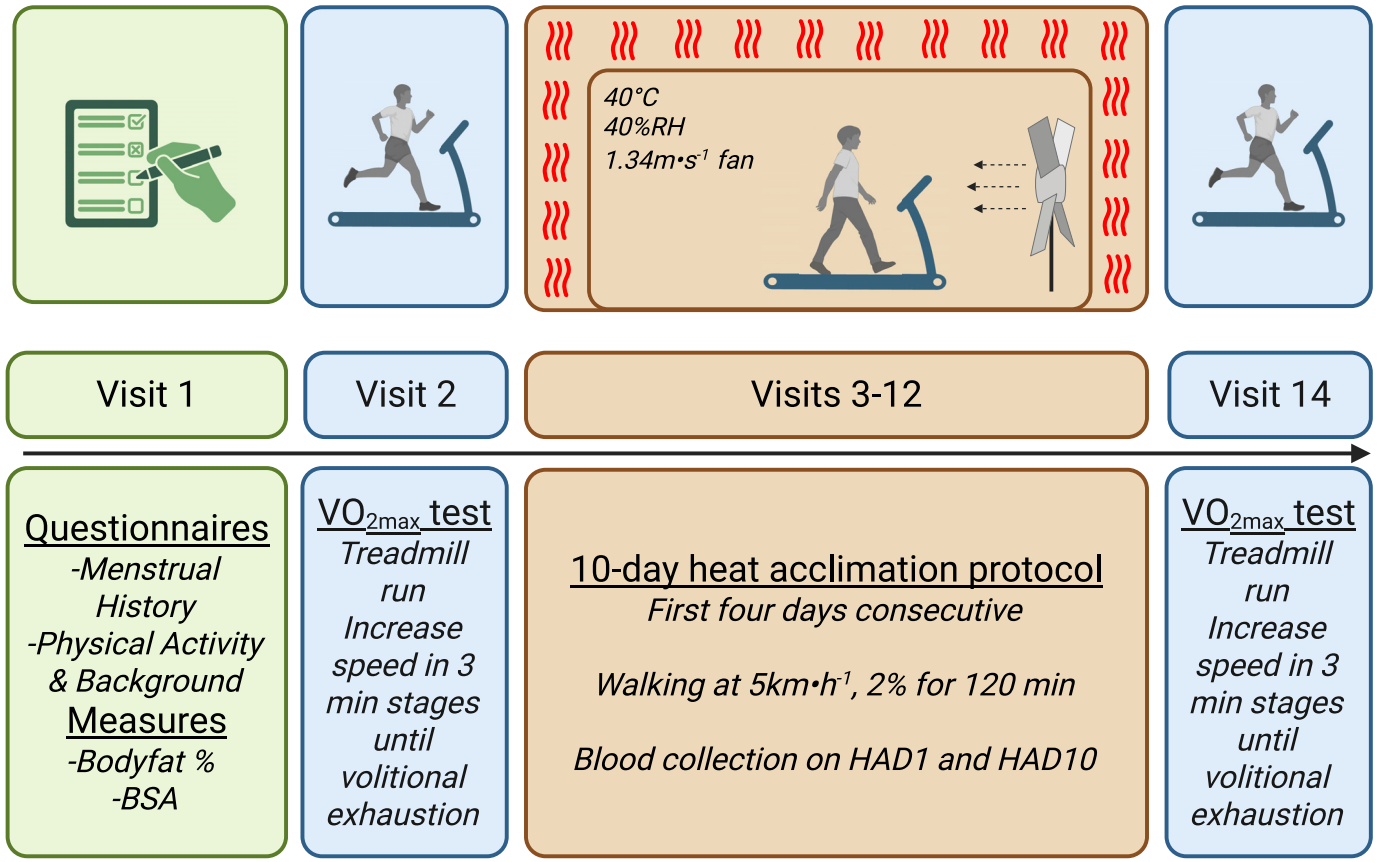

Figure 1. Study timeline. Figure created with a licensed version of BioRender.com.

>1.025, volunteers were given 500 mL of water to consume before beginning the exercise trial. On *days 1* and *10* of HA (HAD1 and HAD10), volunteers underwent a venous blood draw after remaining seated for 20-min before exercise to quantify intracellular heat shock protein concentration (HSP72).

Before starting exercise, volunteers self-inserted a telemetric pill (BodyCap, eCelsius, France) as a suppository for assessment of T_{core} (rectal temperature). Nude body mass was assessed (Adam Equipment, CPWPlus-200, Oxford, CT) before and after exercise to quantify total sweat loss each day and whole body sweating rate (WBSR), accounting for fluid consumed and urine produced during the exercise session. Volunteers were also fitted with a telemetric heart rate (HR) strap (Polar H10 HR puck; M430 watch, Polar Electro, Woodbury, NY) and wireless skin temperature sensors (iButton, Maxim Integrated Products Inc., San Jose, CA) on four sites: chest, deltoid, thigh, and

Table 1. *Subject characteristics*

	Females (n = 16)	Males (n = 11)
Age, yr	24 ± 5	22 ± 5
Height, cm	162.0 ± 6.3*	178.0 ± 7.3
Nude body mass, kg	67.54 ± 8.24*	82.85 ± 15.13
% Body fat	36.25 ± 6.24*	26.36 ± 5.69
BSA, m^2	1.774 ± 0.128*	1.983 ± 0.178
BSA:mass	0.02645 ± 0.0018*	0.02432 ± 0.0025
Pre-HA $\dot{V}o_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	35.9 ± 3.4*	47.8 ± 4.9
Post-HA $\dot{V}o_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	36.5 ± 3.8*	46.0 ± 4.7
Race/Ethnicity		
White/Hispanic or Latino	5 (31.25%)	1 (9.09%)
White/Not Hispanic or Latino	5 (31.25%)	4 (36.36%)
Black/Not Hispanic or Latino	2 (12.5%)	1 (9.09%)
Asian/Not Hispanic or Latino	1 (6.25%)	2 (18.18%)
American Indian or Alaskan Native/Not Hispanic or Latino	1 (6.25%)	0 (0%)
Other/Hispanic or Latino	1 (6.25%)	2 (18.18%)
Other/Not Hispanic or Latino	1 (6.25%)	1 (9.09%)

Values are means ± SD. BSA is body surface area; BSA:mass, is body surface area to mass ratio. *Differences between sexes $P < 0.05$. Other includes individuals who did not report race, or who reported multiple races.

calf (25). Upon entering the heat chamber, volunteers walked on a treadmill at 1.39 $m \cdot s^{-1}$, 2% grade, with 1.34 $m \cdot s^{-1}$ windspeed for 120 min in 40°C, 40% RH. Core temperature (T_{core}) and mean weighted skin temperature [MWT_{sk}, (25)] were measured continuously, and HR, RPE, (26), and thermal sensation scale [TSS, (27)] were recorded every 5 min. Volunteers were provided 200 mL of fluid every 20 min of exercise, with fluid volume measured at each timepoint (Ohaus, ES6R, Parsippany, NJ).

If volunteers' core temperature reached 39.5°C ($n = 14$ trials, $n = 5$ volunteers, 4 F), they became too fatigued to continue exercise ($n = 4$ trials, $n = 3$ volunteers, 3 F), or they experienced lightheadedness necessitating an early exit ($n = 1$ trial, 1 M), duration of time in the heat chamber was accounted for in calculations of WBSR. Individuals whose T_{core} reached 39.5°C were immediately removed from the heat chamber and began a monitored recovery with fan cooling that lasted until their T_{core} reached 38.5°C. Ice sheets and towels were available for cooling during each testing visit, in the event participants requested additional cooling or were experiencing symptoms associated with heat-related illness, but were not utilized by any volunteers. T_{core}, HR, MWTsk, RPE, TSS, and WBSR adaptation were evaluated as a change from HAD1.

Isolation of Peripheral Blood Mononuclear Cells and Immunoblotting

Venous blood was collected into a 10 mL EDTA-treated collection tube (BD Vacutainer, Franklin Lakes, NJ) pre-HAD1 and pre-HAD10. Peripheral blood mononuclear cells (PBMCs) were isolated using methods previously described (28, 29). The PBMC pellet was stored in −80°C for analysis of HSP72. Intracellular proteins were extracted from PBMCs, as previously described (28). Protein (8 μg) was loaded into gels, separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred onto polyvinylidene difluoride membranes. Following transfer, a total protein strain (Memcode, Cat. No. 24585, Thermo Fisher Scientific) was used to confirm equal protein loading and transfer. Three volunteers' samples were excluded from analysis due to unequal total protein staining [see Supplemental Fig. S2*A*–S2*F*, (30)]. A primary antibody for HSP72 (HSP70/HSP72 polyclonal antibody, Cat. No. ADI-SPA-812, Enzo Biochem Inc., New York, NY) was used to determine HSP72 protein abundance. An internal standard (pooled composite sample from multiple volunteers) was loaded in the last two lanes of each gel, and HSP72 abundance was evaluated relative to the internal standard. This approach allows for comparison across gels, specifically in this case, between sexes. Three gels were utilized to evaluate all data across this investigation, and volunteers were randomly assigned across wells and gels.

Statistical Analysis

Linear mixed-effect models were used to assess sex differences, both as a group and over the time course. The model parameters were estimated using restricted maximum likelihood, and the Kenward–Roger method was used to estimate the variance-covariance of the fixed effects (31). *Equation 1* shows the linear mixed-effect model:

$$y_{it} = \beta_0 + \beta_1 \text{sex}_i + \beta_2 \text{time}_{it} + \beta_3 \text{sex}_i \times \text{time}_{it} + \beta_4 \text{bsa} : \text{mass}_{it} + \delta_i + \mu_t + \varepsilon_{it}. \quad (1)$$

Where y_{it} represents T_{core}, ΔT_{core}, WBSR, $WBSR \cdot m^{-2}$, ΔWBSR, Δ $WBSR \cdot m^{-2}$, HR, ΔHR, MWT_{sk}, RPE, TSS, and H2P72 for subject i at time point t, sex_i is the sex of subject i where females take a value of 1 and males 0, time_t is a fixed effect to control for observed and unobserved differences that remain constant across individuals but may vary over the time course (we include time as a categorial variable to account for the discrete nature of each trial to allow for comparisons between time points. We included a table of results in the supplemental files where time is a continuous variable for comparison), bsa:mass_{it} is the BSA:mass and is included to evaluate differences between males and females that were not related to body size differences (this variable is not included in the models where the outcome is $WBSR \cdot m^{-2}$ since BSA is already adjusted for in that variable, HSP72, scaled by BSA), δ_i is a random effect for subject i controlling for unobserved heterogeneity across subjects, and μ_t is a nested random effect of time point t for subject i. In addition, for models where HSP72 is the outcome, the nested random effect is excluded, and an additional random effect was included to control for unobserved differences in the Western blot gels.

For each model, marginal effects of sex were calculated for each outcome variable over the time course. Marginal effects show the discrete difference in the outcome variable between males and females at each time point. Statistical significance was set a priori at $P < 0.05$. Significance markers shown in Figs. 2 and 3 come from the P values of the marginal effects. The results from the calculated marginal effects and regression can be found in the Supplemental Tables S1–S4. For all analyses, except relative ($WBSR \cdot BSA^{-1}$) differences were evaluated while controlling for BSA:mass. Data were analyzed using Stata (SE Version 18.0, StataCorp LLC, College Station, TX). An a priori power calculation was conducted using R (version 4.0.3) with β = 80% showed that $n = 10$ females and $n = 10$ males would be necessary to detect T_{core} differences between sexes. Given the variability in female sex hormones expected, we purposefully sought to oversample female volunteers to account for fluctuating female sex hormones and maintain ecological validity.

RESULTS

Females were significantly shorter and lighter and had lower BSA and higher BSA:mass, with higher body fat %, and lower $\dot{V}O_{2max}$ (Table 1). Baseline T_{core} was not significantly affected by HA in either sex (males: HAD1 37.10 ± 0.24 vs. HAD10 37.01 ± 0.21°C; females: HAD1 37.18 ± 0.56 vs. HAD10 37.14 ± 0.22°C, $P > 0.05$). Acclimation was confirmed in both groups via decreased peak T_{core} (males: HAD1 38.69 ± 0.48 vs. HAD10 38.30 ± 0.28°C, $P < 0.001$; females: HAD1 38.90 ± 0.49 vs. HAD10 38.46 ± 0.45°C, $P < 0.001$), decreased peak HR (males: HAD1 141 ± 16 vs. HAD10 127 ± 11 beats/min, $P < 0.001$; females: HAD1 170 ± 17 vs. HAD10 152 ± 17 beats/min, $P < 0.001$), increased WBSR (males: HAD1 0.73 ± 0.23 vs. HAD10 0.92 ± 0.23 $L \cdot h^{-1}$, $P < 0.001$; females: HAD1 0.65 ± 0.15 vs. HAD10 0.72 ± 0.12 $L \cdot h^{-1}$, $P = 0.041$), and increased $WBSR \cdot m^{-2}$ (males: HAD1 0.33 ± 0.14 vs. HAD10 0.46 ± 0.09

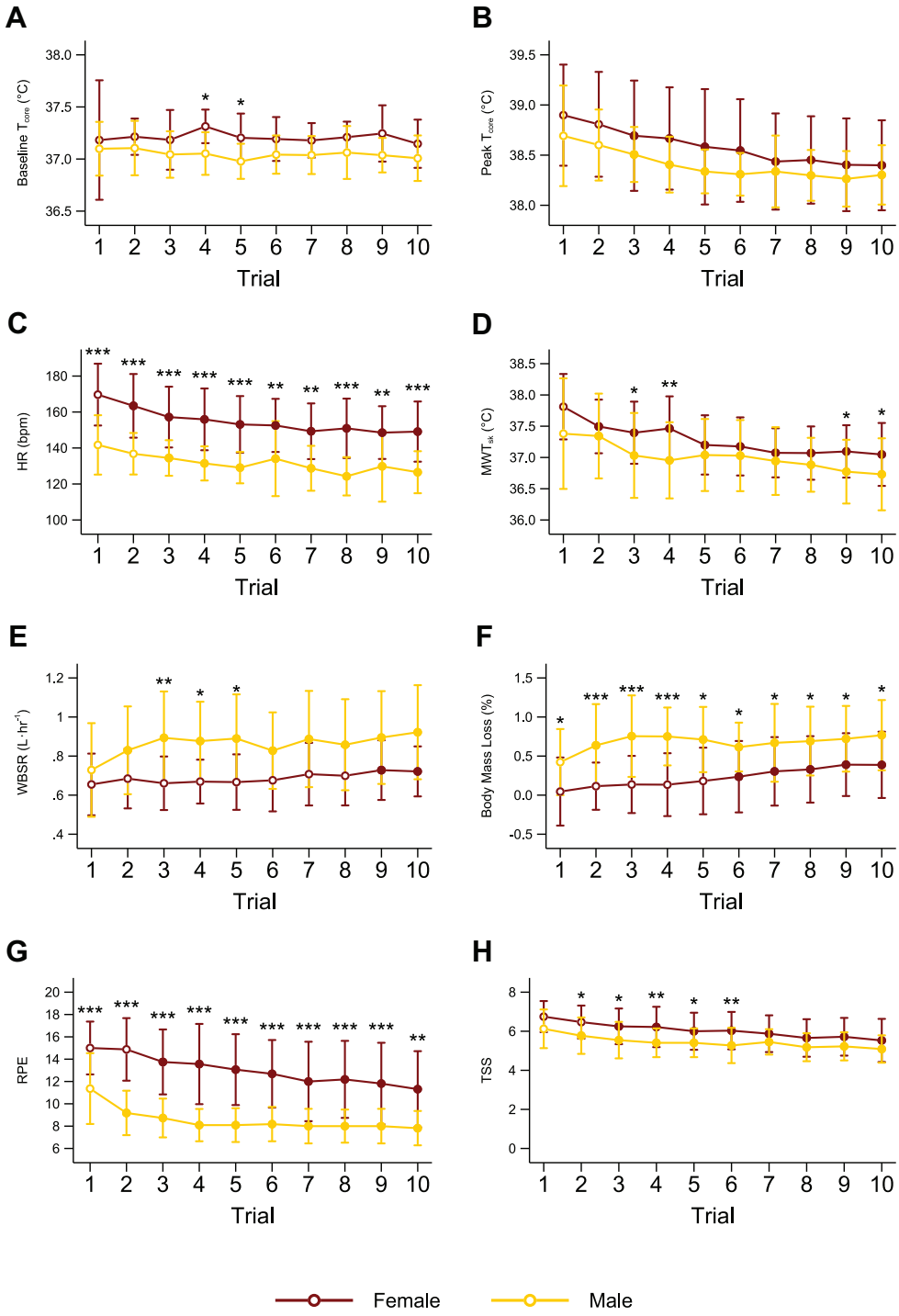

Figure 2. Measures between men and women for: Baseline core temperature (T_{core}) (*A*), peak core temperature (*B*), highest recorded heart rate (HR) (*C*), mean weighted skin temperature (MWT_{sk}) (*D*), whole body sweating rate (WBSR) (*E*), WBSR per body surface area (BSA) (*F*), rating of perceived exertion (RPE) (*G*), thermal sensation scale (TSS) (*H*). Represented as mean ± standard deviation. Linear mixed effects models were used to calculate differences between men ($n = 11$) and women ($n = 16$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Closed symbols represent a significant difference from HAD1 ($P < 0.05$) within each sex.

$L \cdot h^{-1} \cdot m^{-2}$, $P < 0.001$; females: HAD1 0.37 ± 0.07 vs. HAD10 0.40 ± 0.05 $L \cdot h^{-1} \cdot m^{-2}$, $P = 0.032$).

Adaptation was evaluated as a change (delta) from HAD1 for T_{core}, HR, WBSR, and WBSR·m^{-2} only (Fig. 3). There were no differences between males and females for the degree of adaptation for T_{core} or HR (Fig. 3, *A* and *B*, respectively). Both males and females observed statistically significant decreases in peak T_{core}, relative to HAD1, beginning on HAD3 (Fig. 2*B*). Males saw increased adaptation in both absolute WBSR from HAD3-HAD10 and WBSR·m^{-2} from HAD3, HAD4, and HAD6-HAD10 (Fig. 3, *C* and *D*, respectively). Males exhibited increases in WBSR and WBSR·m^{-2} starting on HAD2, but females did not exhibit increased WBSR and WBSR·m^{-2} until HAD9 (Fig. 2, *E* and *F*, respectively).

Baseline T_{core} only differed between males and females on HAD4 and HAD5 (Fig. 2*A*). There were no differences in peak T_{core} between males and females over the course of 10 days of heat acclimation (Fig. 2*B*). Females had significantly higher peak HR over each day of heat acclimation (Fig. 2*C*).

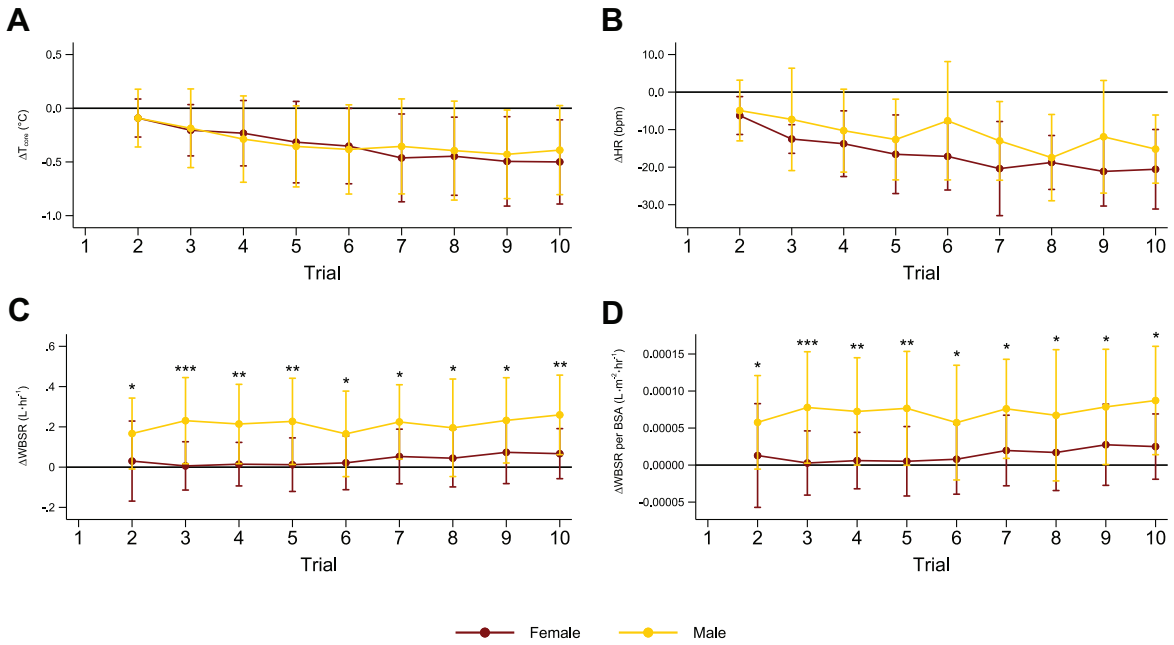

Figure 3. Measures of heat acclimation as a change from HAD1 for: peak core temperature (*A*), heart rate (*B*), whole body sweat rate (WBSR) (*C*), and WBSR·m^{-2} (*D*). Data are represented as mean and 95% CI. Linear mixed effects models were used to calculate differences between men ($n = 11$) and women ($n = 16$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Females had higher MWTsk than male on HAD1, HAD3-HAD4, and HAD9 (Fig. 2*D*). Females also had significantly lower sweating rate than males from HAD3-HAD5 both absolutely and relative to BSA (Fig. 2, *E* and *F*, respectively). There were no additional differences in WBSR between men and women. Perceptual measures were significantly impacted by sex, with females having higher RPE across all days of HA (Fig. 2*G*). For RPE, males observed a significant decrease from HAD1 to HAD2 ($P < 0.001$), and females observed a significant decrease in HAD3 ($P < 0.001$). TSS was also higher in women, but only from HAD2-HAD6 (Fig. 2*H*). For TSS, the relationship was similar with males observing a significant decrease from HAD1 to HAD2 ($P < 0.001$) and females on HAD3 ($P < 0.001$).

HSP72 isolated from PBMCs showed an ~13% increase from HAD10 compared with HAD1 (1.625 ± 0.294 vs. 1.436 ± 0.388 AU, $P = 0.023$), respectively. There was no significant impact of sex on HSP72 adaptations to HA (Fig. 4).

DISCUSSION

The major new findings of the present study were that there were no differences in T_{core}, responses, or in T_{core} adaptations to HA between males and females, throughout a 10-day controlled work rate HA protocol. Interestingly, females had consistently higher HR (consistent with potentially lower stroke volume/smaller body size) and lower WBSR on intermediate HA days, but this did not impair their ability to demonstrate successful HA in a manner quantitatively similar to that seen in men. Lower T_{core} is the most impactful adaptation resulting from HA and can enhance performance during heat stress and decrease the risk of developing heat-related illness (1). We did observe a larger BSA:mass ratio in females relative to men, which may have contributed to the ability of females to maintain lower T_{core} despite lower sweating rate on some days. In addition, we report for the first time that in both females and males, there was no impact of HA on $\dot{V}o_{2max}$.

The lack of sex differences in absolute measures of T_{core} (Fig. 2, *A* and *B*) as well as T_{core} adaptation (Fig. 3*A*) in our sample contrasts with previous reports (15, 32). Interestingly, the previous studies that showed a greater difference between the sexes had several methodological approaches that might have contributed to our contrary findings. First, the previous work utilized a method of inducing HA called "isothermic HA," where the rate of work is altered throughout the HA protocol to maintain a specific exercise T_{core} throughout exercise. Instead, the present study utilized treadmill walking at a controlled work rate to improve practicality and ease of use for the general population. Our findings suggest that the ability to adapt is not governed by sex, with both males and females adapting similarly in T_{core}. However, with a greater thermal stimulus (i.e., isothermic HA), differences in T_{core} adaptation have been observed between sexes (15). In addition, in the present study, both males and females demonstrated reductions in peak T_{core} from HAD1 on HAD3, suggesting a similar timeline of adaptation between sexes, also in contrast to previous work (2, 15).

The second major difference is that the two studies referenced above leveraged a cycling modality, whereas we used treadmill walking. In terms of research design, there are advantages and limitations to both approaches, but the use of treadmill walking is more practical and relevant to many occupations and tasks that are typically performed in the heat. Furthermore, walking is a load-bearing exercise (unlike cycling), which may augment the extent to which the BSA:mass ratio has a biophysical influence on the ability to dissipate heat with more skeletal muscle required for load-

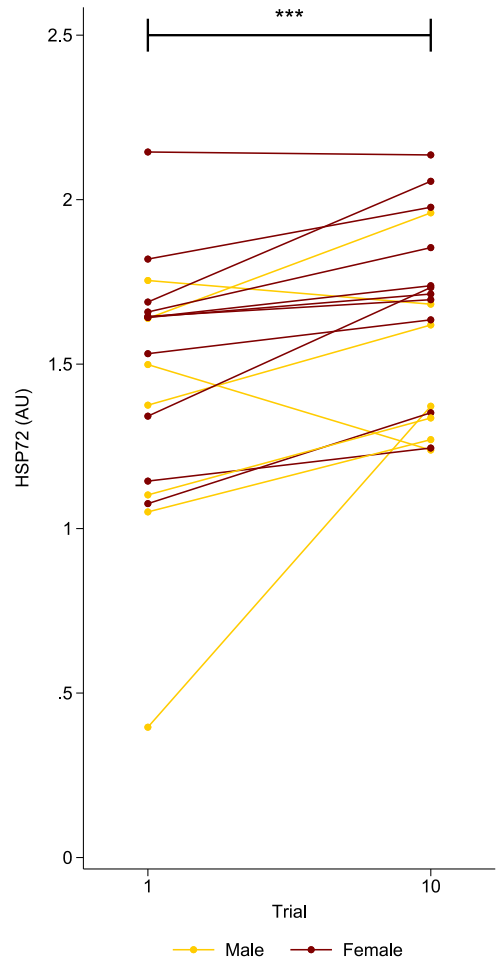

Figure 4. HSP72 results for all volunteers at HAD1 and HAD10 and representative Western blot images from male (*A*) and female (*B*) volunteers (HAD1 represented on the *left* and HAD10 represented on the *right*). Linear mixed effects models were run on $n = 19$ ($n = 12$ females) to quantify HSP72 response to heat acclimation. ***$P < 0.001$.

bearing activities in the heat (33). In our study population (and in general), females were smaller, with a larger BSA:mass ratio, giving them a biophysical advantage for heat dissipation in this compensable environment. This advantage may have been less apparent in previous studies that used cycling as their mode of exercise during HA (15). It is important to note that the practical protocol utilized in this investigation likely yielded different levels of absolute metabolic heat production between sexes, although this was not measured in this investigation. Previous work has shown that heat production has explained differences in thermoeffector function, particularly between sexes (7), and it is possible that absolute heat production may explain some of the differences observed in this investigation, including the differences in WBSR between sexes.

We observed higher HR in females throughout HA and lower WBSR (absolute and relative to BSA) on HAD3-HAD5. Both findings may be related to the overall smaller body size in the women. Females tend to have higher HR for a given exercise workload, particularly in the heat, due to smaller stroke volumes associated with their smaller body size. Although we did not measure cardiac output in the present study, cardiac output must increase substantially during exercise in the heat, both to supply the working muscles and to address the thermoregulatory demands for increased skin blood flow (34). This demand for increased cardiac output would have required a higher HR in the women. The relatively lower fitness status (lower $\dot{V}o_{2max}$) of females likely also contributed to the higher HR. As such, our findings are consistent with the conclusions of Horstman and Christensen (14), who observed higher HR in females during HA, and with those of Mee et al. (15), where there were no sex differences in HR adaptations between males and females during HA when the two groups were of similar fitness status.

The lower WBSR responses in females may also have been indirectly related to smaller body size. As noted earlier, the higher BSA:mass ratio in the women, combined with load-bearing exercise in a compensable environment, meant that they had more surface area for heat dissipation relative to the amount of body mass generating heat. We have recently shown that higher BSA:mass is associated with a lower risk for exertional heat stroke in a retrospective, population-based analysis (35). Our present data may be consistent with those epidemiological findings in the sense that less sweating is "needed" due to greater surface area available for heat dissipation relative to the amount of muscle mass generating heat.

Interestingly, sweating rate adapted slower and to a lesser degree in females (Fig. 3, *C* and *D*), which is inconsistent with previous conclusions that females may adapt to sweating earlier than males during HA (9). Previously, Mee et al. (15) observed an increase in WBSR relative to BSA in females after only 5 days of isothermic HA (maintaining body temperature at 38.5°C for ~90 min) on a cycle ergometer (15). Possible reasons for the discrepancy in findings includes *1*) the (higher) heat load associated with the isothermic HA protocol could be a driver for these differences (36); and *2*) in the previous work, both sexes had similar relative fitness status, whereas the females in our sample were less fit than the males (lower $\dot{V}o_{2max}$). However, given the small number of research studies investigating sex differences in thermoregulation during HA, more research is warranted to elucidate potential differences in sweating rate throughout the course of an HA regimen.

Increased sweating rate is a primary and impactful adaptation that results from HA. Although sweating is a powerful mechanism for heat dissipation in humans, it is also a major source of water loss, contributing to dehydration. Dehydration itself can lead to excessive hyperthermia, decreases in exercise performance, and increased risk of exertional heat illness (37–39). As such, the optimal approach for exercise in the heat is to sweat enough to maintain heat balance but not so much that the sweat is "wasted" (i.e., drips off the skin or is not needed/used for heat dissipation). In our present results, the females appeared to have a more efficient thermoregulatory approach, in that they maintained core temperature adaptations throughout HA while apparently requiring less of a sweating response to do so (see BSA:mass discussion in previous two paragraphs). In addition, whether or not we controlled for BSA:mass in the statistical model comparing sexes across timepoints, the WBSR was higher on some days in men, suggesting that this efficiency of sweating is not due to body size alone.

Previous work by Avellini et al. (40), showed increased sweating rate following 10 days of HA in both males and females matched for BSA:mass and fitness status, though males had a larger proportionate increase in sweating rate relative to women. Similar to our present work, the study by Avellini et al. (40) did not find significant differences between males and females in core temperature responses. Conversely, following humid HA, Buono et al. (41) observed increases in WBSR in men, but not women, and increases in local sweat rate in both males and females with pilocarpine administration (41). This is possibly related to hidromeiosis (i.e., a reduction in sweat gland activity with wet skin) and differences in sweat production in humid heat as previously observed by Shapiro et al. (42). However, hidromeiosis is most prominent in cases with very high skin saturation, which is possible given the environmental conditions in this investigation, and importantly, skin wettedness was not directly evaluated in this investigation. Certainly, in humid environments, reduced reliance on sweating may be beneficial to reduce ineffective sweat loss that could contribute to dehydration, decreased performance, and increased risk of illness.

Cellular tolerance to heat stress has been previously described in detail with increases in HSP72 observed to lead to "thermal memory" following HA (20). In our sample, HSP72 increased in baseline measures on HAD10 relative to HAD1, with no differences between males and females. The magnitude of change (13%) is similar to a previous study in which ambient temperature was higher and HSP72 was elevated by 17% (17). Our novel finding that there were no sex differences in the increase in HSP72 protein following HA is consistent with previous work showing no sex differences in HSP72 mRNA (43). This is unsurprising given the similar T_{core} measures throughout the HA protocol. Thus, current evidence supports the conclusion that males and females increase HSP72 protein abundance similarly in response to HA.

Experimental Considerations

In the present work, although we provide strong evidence of similar thermal acclimation in males and females via distinct physiological mechanisms, we recognize the following experimental considerations. First, we chose to use a controlled work rate protocol, rather than a controlled hyperthermia protocol or an intensity based on metabolic heat production. Previous work using controlled hyperthermia (adjusting the workload throughout HA to maintain a similar high T_{core} throughout) has shown a greater magnitude of adaptations (15, 32). For the present experimental design, we chose to use a standardized work rate as a practical protocol that might be more usable in the general population and thereby have higher external validity. This is a limitation given the differences in metabolic heat production that were possible between sexes; however, this was not assessed in this protocol and thus cannot be confirmed. Second, the quantification of partitional calorimetry during the testing was outside the scope of the present study. Therefore, we were not able to quantify the relative contributions of the various components of the heat balance equation throughout the heat acclimation protocol. Third, females were not scheduled based on their menstrual cycles or hormonal concentrations. We recognize that there are clear arguments both for and against controlling for the menstrual or oral contraceptive cycle when scheduling studies in females (44, 45). For the present study, the logistical constraints of such a time- and labor-intensive study precluded our ability to do so. We also submit that this approach allowed us to draw conclusions about women's ability to adapt to exercise heat stress outside the constraints of a specific cycle phase or hormonal concentration. Another important factor for consideration is that the 10 days of HA were not entirely consecutive in this protocol. This was done based on weather considerations, practicality/convenience for participants, and to allow for adequate recovery. Although all volunteers had similar protocol schedules, they were not identical (i.e., some individuals had a rest day after 4 consecutive days, some after 5).

Conclusions

We observed similar adaptation in T_{core} between young, healthy males and females throughout 10 days of controlled work rate HA in a 40°C/40% RH environment. Males appeared to have a greater sweating rate and sweating rate adaptation, both absolute and relative to body surface area, whereas females had a higher HR during exercise heat stress. HSP72 increased (pre-exercise) on *day 10* of HA relative to *day 1*. These findings suggest that females and males show similar thermal adaptations in terms of magnitude and time course during a 10-day HA protocol, although there were distinct differences between the sexes in physiological mechanisms (thermoeffector function and cardiovascular strain). We conclude that a similar controlled work rate HA protocol is effective for both males and females to adapt to exercise in the heat.

DATA AVAILABILITY

Data are available from the corresponding author upon reasonable request and approval of a data-sharing agreement.

SUPPLEMENTAL MATERIAL

Supplemental Fig. S1 https://doi.org/10.6084/m9.figshare.29630510.v1.

Supplemental Fig. S2: https://doi.org/10.6084/m9.figshare.29630522.v1.

Supplemental Tables S1–S4: https://doi.org/10.6084/m9.figshare.29630045.v3.

ACKNOWLEDGMENTS

We acknowledge the volunteers who participated in this study, along with Dr. Samantha Goldenstein, K. Riley Connor, Benjamin Fry, David Gonzales-Rojas, Kathryn McCarthy, and Mina Shaw for assistance with data collection; Mark Plamper, Dr. Kentaro Oki, Shauna Ward, and Jermaine Ward for expertise in immunological analysis. We thank Dr. Abigail Colburn for her critical review of the manuscript. Figure 1 created with a licensed version of BioRender.com.

GRANTS

This work was supported by Military Operational Medicine Research Program (MOMRP): Grant No. MO220032 (to G.E.W.G.).

DISCLAIMERS

The views, opinions, and/or findings contained in this article are those of the authors and should not be construed as an official

United States Department of the Army position, or decision, unless so designated by other official documentation. Approved for public release, distribution unlimited. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.E.W.G., A.M.G., A.R.C., B.J.R., R.M.S., and N.C. conceived and designed research; G.E.W.G., A.M.G., S.C.B., B.K.A., P.O.B., A.R.C., T. A. Mayer, B.J.R., R.M.S., A.D.S., and N.C. performed experiments; G.E.W.G., A.M.G., S.C.B., T. A. Murray, B.J.R., and R.M.S. analyzed data; G.E.W.G., A.M.G., S.C.B., B.J.R., R.M.S., and N.C. interpreted results of experiments; G.E.W.G. and T. A. Murray prepared figures; G.E.W.G. drafted manuscript; G.E.W.G., A.M.G., S.C.B., B.K.A., P.O.B., A.R.C., T. A. Mayer, T. A. Murray, B.J.R., R.M.S., A.D.S., and N.C. edited and revised manuscript; G.E.W.G., A.M.G., S.C.B., B.K.A., P.O.B., A.R.C., T. A. Mayer, T. A. Murray, B.J.R., R.M.S., A.D.S., and N.C. approved final version of manuscript.

REFERENCES

1. **Periard JD**, **Racinais S**, **Sawka MN.** Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. *Scand J Med Sci Sports* 25, *Suppl* 2: 20–38, 2015. doi:10.1111/sms.12408.
2. **Périard JD**, **Travers GJS**, **Racinais S**, **Sawka MN.** Cardiovascular adaptations supporting human exercise-heat acclimation. *Auton Neurosci* 196: 52–62, 2016. doi:10.1016/j.autneu.2016.02.002.
3. **Racinais S**, **Sawka M**, **Daanen H**, **Périard JD.** Heat acclimation. In: *Heat Stress in Sport and Exercise: Thermophysiology of Health and Performance*, edited by Périard JD, Racinais S. Springer, 2019, p. 159–178.
4. **Pandolf KB.** Time course of heat acclimation and its decay. *Int J Sports Med* 19, *Suppl* 1: S157–S160, 1998. doi:10.1055/s-2007-971985.
5. **Roberts MF**, **Wenger CB**, **Stolwijk JA**, **Nadel ER.** Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol Respir Environ Exerc Physiol* 43: 133–137, 1977. doi:10.1152/jappl.1977.43.1.133.
6. **Gagnon D**, **Crandall CG**, **Kenny GP.** Sex differences in postsynaptic sweating and cutaneous vasodilation. *J Appl Physiol (1985)* 114: 394–401, 2013. doi:10.1152/japplphysiol.00877.2012.
7. **Gagnon D**, **Jay O**, **Lemire B**, **Kenny GP.** Sex-related differences in evaporative heat loss: the importance of metabolic heat production. *Eur J Appl Physiol* 104: 821–829, 2008. doi:10.1007/s00421-008-0837-0.
8. **Gagnon D**, **Kenny GP.** Sex differences in thermoeffector responses during exercise at fixed requirements for heat loss. *J Appl Physiol (1985)* 113: 746–757, 2012. doi:10.1152/japplphysiol.00637.2012.
9. **Wickham KA**, **Wallace PJ**, **Cheung SS.** Sex differences in the physiological adaptations to heat acclimation: a state-of-the-art review. *Eur J Appl Physiol* 121: 353–367, 2021. doi:10.1007/s00421-020-04550-y.
10. **Shapiro Y**, **Pandolf KB**, **Goldman RF.** Sex differences in acclimation to a hot-dry environment. *Ergonomics* 23: 635–642, 1980. doi:10.1080/00140138008924778.
11. **Belval LN**, **Giersch GEW**, **Adams WM**, **Hosokawa Y**, **Jardine JF**, **Katch RK**, **Stearns RL**, **Casa DJ.** Age- and sex-based differences in exertional heat stroke incidence in a 7-mile road race. *J Athl Train* 55: 1224–1229, 2020. doi:10.4085/1062-6050-539-19.
12. **Giersch GE**, **Taylor KM**, **Caldwell AR**, **Charkoudian N.** Body mass index, but not sex, influences exertional heat stroke risk in young healthy men and women. *Am J Physiol Regul Integr Comp Physiol* 324: R15–R19, 2023. doi:10.1152/ajpregu.00168.2022.
13. **Kazman JB**, **Nelson DA**, **Ahmed AE**, **Deuster PA**, **O'Connor FG**, **Mancuso JD**, **Lewandowski SA.** Risk for exertional heat illness among US army enlistees: climate indexes, intrinsic factors and their interactions. *Br J Sports Med* 59: 231–240, 2025. doi:10.1136/bjsports-2024-108441.
14. **Horstman DH**, **Christensen E.** Acclimatization to dry heat: active men vs. active women. *J Appl Physiol Respir Environ Exerc Physiol* 52: 825–831, 1982. doi:10.1152/jappl.1982.52.4.825.
15. **Mee JA**, **Gibson OR**, **Doust J**, **Maxwell NS.** A comparison of males and females' temporal patterning to short- and long-term heat acclimation. *Scand J Med Sci Sports* 25, *Suppl* 2: 250–258, 2015. doi:10.1111/sms.12417.
16. **Weinman KP**, **Slabochova Z**, **Bernauer EM**, **Morimoto T**, **Sargent F 2nd.** Reactions of men and women to repeated exposure to humid heat. *J Appl Physiol* 22: 533–538, 1967. doi:10.1152/jappl.1967.22.3.533.
17. **McClung JP**, **Hasday JD**, **He J**, **R, Montain SJ**, **Cheuvront SN**, **Sawka MN**, **Singh IS.** Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. *Am J Physiol Regul Integr Comp Physiol* 294: R185–R191, 2008. doi:10.1152/ajpregu.00532.2007.
18. **Horowitz M.** Heat acclimation: phenotypic plasticity and cues to the underlying molecular mechanisms. *J Therm Biol* 26: 357–363, 2001. doi:10.1016/S0306-4565(01)00044-4.
19. **Yamada P**, **Amorim F**, **Moseley P**, **Schneider S.** Heat shock protein 72 response to exercise in humans. *Sports Med* 38: 715–733, 2008. doi:10.2165/00007256-200838090-00002.
20. **Horowitz M.** Heat acclimation, epigenetics, and cytoprotection memory. *Compr Physiol* 4: 199–230, 2014. doi:10.1002/cphy.c130025.
21. **Yamada PM**, **Amorim FT**, **Moseley P**, **Robergs R**, **Schneider SM.** Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. *J Appl Physiol (1985)* 103: 1196–1204, 2007. doi:10.1152/japplphysiol.00242.2007.
22. **Gillum T**, **Kuennen M**, **Gourley C**, **Dokladny K**, **Schneider S**, **Moseley P.** Sex differences in heat shock protein 72 expression in peripheral blood mononuclear cells to acute exercise in the heat. *Int J Endocrinol Metab* 11: e8739, 2013. doi:10.5812/ijem.8739.
23. **Shinohara T**, **Takahashi N**, **Ooie T**, **Ichinose M**, **Hara M**, **Yonemochi H**, **Saikawa T**, **Yoshimatsu H.** Estrogen inhibits hyperthermia-induced expression of heat-shock protein 72 and cardioprotection against ischemia/reperfusion injury in female rat heart. *J Mol Cell Cardiol* 37: 1053–1061, 2004. doi:10.1016/j.yjmcc.2004.09.006.
24. **Voss MR**, **Stallone JN**, **Li M**, **Cornelussen RN**, **Knuefermann P**, **Knowlton AA.** Gender differences in the expression of heat shock proteins: the effect of estrogen. *Am J Physiol Heart Circ Physiol* 285: H687–H692, 2003. doi:10.1152/ajpheart.01000.2002.
25. **Ramanathan NL.** A new weighting system for mean surface temperature of the human body. *J Appl Physiol* 19: 531–533, 1964. doi:10.1152/jappl.1964.19.3.531.
26. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377–381, 1982.
27. **Gagge AP**, **Stolwijk J**, **Hardy J.** Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res* 1: 1–20, 1967. doi:10.1016/0013-9351(67)90002-3.
28. **Salgado RM**, **Coffman KE**, **Bradbury KE**, **Mitchell KM**, **Yurkevicius BR**, **Luippold AJ**, **Mayer TA**, **Charkoudian N**, **Alba BK**, **Fulco CS**, **Kenefick RW.** Effect of 8 days of exercise-heat acclimation on aerobic exercise performance of men in hypobaric hypoxia. *Am J Physiol Regul Integr Comp Physiol* 319: R114–R122, 2020. doi:10.1152/ajpregu.00048.2020.
29. **Zuhl M.** *The Effect of Oral Glutamine Supplementation On Gut Permeability and Heat Shock Protein Regulation in Runners with a History of Gastrointestinal Distress* (Dissertation). University of New Mexico, 2013.
30. **Moritz CP.** Tubulin or not tubulin: heading toward total protein staining as loading control in western blots. *Proteomics* 17, 2017. doi:10.1002/pmic.201600189.
31. **Kenward MG**, **Roger JH.** Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53: 983–997, 1997.

32. **Kirby NV**, **Lucas SJE**, **Lucas RAI.** Nine-, but not four-days heat acclimation improves self-paced endurance performance in females. *Front Physiol* 10: 539, 2019. doi:10.3389/fphys.2019.00539.
33. **Foster J**, **Hodder SG**, **Lloyd AB**, **Havenith G.** Individual responses to heat stress: implications for hyperthermia and physical work capacity. *Front Physiol* 11: 541483, 2020. doi:10.3389/fphys.2020.541483.
34. **Rowell LB.** Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* 54: 75–159, 1974. doi:10.1152/physrev.1974.54.1.75.
35. **Taylor KM**, **Giersch GE**, **Caldwell AR**, **Epstein Y**, **Charkoudian N.** Relation of body surface area-to-mass ratio to risk of exertional heat stroke in healthy men and women. *J Appl Physiol (1985)* 136: 549–554, 2024. doi:10.1152/japplphysiol.00597.2023.
36. **Gibson OR**, **Mee JA**, **Tuttle JA**, **Taylor L**, **Watt PW**, **Maxwell NS.** Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales. *J Therm Biol* 49-50: 55–65, 2015. doi:10.1016/j.jtherbio.2015.02.005.
37. **Sawka MN**, **Coyle EF.** Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exerc Sport Sci Rev* 27: 167–218, 1999.
38. **Sawka MN**, **Latzka WA**, **Matott RP**, **Montain SJ.** Hydration effects on temperature regulation. *Int J Sports Med* 19, *Suppl* 2: S108–S110, 1998. doi:10.1055/s-2007-971971.
39. **Sawka MN**, **Montain SJ**, **Latzka WA.** Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol A Mol Integr Physiol* 128: 679–690, 2001. doi:10.1016/s1095-6433(01)00274-4.
40. **Avellini B**, **Kamon E**, **Krajewski J.** Physiological responses of physically fit men and women to acclimation to humid heat. *J Appl Physiol Respir Environ Exerc Physiol* 49: 254–261, 1980. doi:10.1152/jappl.1980.49.2.254.
41. **Buono MJ**, **Leichliter Martha S**, **Heaney JH.** Peripheral sweat gland function, but not whole-body sweat rate, increases in women following humid heat acclimation. *J Therm Biol* 35: 134–137, 2010. doi:10.1016/j.jtherbio.2010.01.004.
42. **Shapiro Y**, **Pandolf KB**, **Avellini BA**, **Pimental NA**, **Goldman RF.** Physiological responses of men and women to humid and dry heat. *J Appl Physiol Respir Environ Exerc Physiol* 49: 1–8, 1980. doi:10.1152/jappl.1980.49.1.1.
43. **Mee JA**, **Gibson O**, **Tuttle J**, **Taylor L**, **Watt P**, **Doust J**, **Maxwell N.** Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation. *Temperature (Austin)* 3: 549–556, 2016. doi:10.1080/23328940.2016.1214336.
44. **Stanhewicz AE**, **Wong B.** Counterpoint: Investigators should not control for menstrual cycle phase when performing studies of vascular control that include women. *J Appl Physiol* 129: 1117–1119, 2020. doi:10.1152/japplphysiol.00427.2020.
45. **Wenner MM**, **Stachenfeld NS.** Point: investigators should control for menstrual cycle phase when performing studies of vascular control that include women. *J Appl Physiol (1985)* 129: 1114–1116, 2020. doi:10.1152/japplphysiol.00443.2020.