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Original Research

An acute naproxen dose does not affect core temperature or Interleukin-6 during cycling in a hot environment

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ABSTRACT

Non-steroidal anti-inflammatory drugs' anti-pyretic and anti-inflammatory effects has led some individuals to theorize these medications may blunt core body temperature (Tc) increases during exercise. We utilized a double-blind, randomized, and counterbalanced cross-over design to examine the effects of a 24-h naproxen dose (3–220 mg naproxen pills) and placebo (0 mg naproxen) on Tc and plasma interleukin-6 (IL-6) concentrations during cycling in a hot or ambient environment. Participants ($n = 11$; 6 male, 5 female; age = 27.8 ± 6.5 years, weight = 79.1 ± 17.9 kg, height = 177 ± 9.5 cm) completed 4 conditions: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat). Dependent measures were taken before, during, and immediately after 90 min of cycling and then 3 h after cycling. Overall, Tc significantly increased pre- (37.1 ± 0.4 °C) to post-cycling (38.2 ± 0.3 °C, $F_{1.7,67.3} = 150.5$, $p < 0.001$) and decreased during rest (37.0 ± 0.3 °C, $F_{2.0,81.5} = 201.6$, $p < 0.001$). Rate of change or maximum Tc were not significantly different between conditions. IL-6 increased pre- (0.54 ± 0.06 pg/ml) to post-exercise (2.46 ± 0.28 pg/ml, $p < 0.001$) and remained significantly higher than pre-at 3 h post- (1.17 ± 0.14 pg/ml, 95% $CI = -1.01$ to -0.23, $p = 0.001$). No significant IL-6 differences occurred between conditions. A 24-h, over-the-counter naproxen dose did not significantly affect Tc or IL-6 among males and females cycling in hot or ambient environments.

Introduction

During exercise, particularly in warm environments, working muscles produce metabolic heat that must be dissipated to maintain a safe core body temperature (Tc; ~37 °C). Thermoregulation is controlled by the hypothalamus, which elicits several responses to cool the body. Cardiovascular responses are key to thermoregulation, where peripheral vasodilation and central vasoconstriction shunts warm blood from the core and working muscles to the skin.[1] Together, the cardiovascular and renal systems regulate blood pressure and plasma volume to allow the person to maintain their performance while also trying to dissipate metabolic heat. When the cardiovascular system is compromised, thermoregulation begins to fail and Tc increases, resulting in hyperthermia. Several factors may compromise cardiovascular function. For example, dehydration leads to low blood pressure which stimulates fluid and sodium retention in the kidneys and systemic vasoconstriction.[1] Systemic vasoconstriction would inhibit the person's ability to dissipate metabolic heat. In some cases, increased Tc may cause individuals to experience exertional heat syncope (i.e., fainting) or exhaustion (e.g., headache, dizziness). When Tc reaches critical levels, a person can experience more serious symptoms of exertional heat stroke (EHS).[2]

A life-threatening condition, EHS is characterized by severely elevated Tc (> 40.5 °C) and central nervous system dysfunction (e.g., aggression, loss of consciousness, irrational behavior). The critical temperature to induce EHS differs between individuals, and EHS is

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Abbreviations

Control	Placebo and ambient condition
EHS	Exertional heat stroke
GI	Gastrointestinal
Heat	Placebo and heat condition
HR	Heart rate
IL-6	Interleukin-6
NpxHeat	Naproxen and heat condition
Npx	Naproxen and ambient condition
NSAID	Non-steroidal anti-inflammatory drug
RH	Relative humidity
Tc	Core body temperature
Usg	Urine specific gravity
VO_{2max}	Maximal oxygen consumption

documented in cool environments,[3] suggesting heat exposure alone is not the cause and EHS can develop because of compromised physiological systems. Gastrointestinal (GI) and immune system disturbances likely drive EHS.[4] In addition to metabolic heat exposure, exercise-induced hypoxia (due to shunted blood from the GI tract) and mechanical pressures damage the GI epithelial barrier.[5,6] Increased GI permeability allows endotoxin – gram-negative bacteria also known as lipopolysaccharide – to leak from the GI tract into the bloodstream.[4] In healthy individuals, liver and immune responses remove circulating endotoxin and the person can continue physical activity. If the person has a compromised response, endotoxin builds up in the blood (i.e., endotoxemia) and pro-inflammatory mediators are released to neutralize the offender. These pro-inflammatory mediators (e.g., cytokines) promote necrosis, vasodilation, fever, decrease plasma volume, and further induce GI damage.[7–9] If unmitigated and physical activity continues, the person may experience a systemic inflammatory response that perpetuates EHS and/or multi-system organ failure.[4]

The anti-pyretic and anti-inflammatory effects of non-steroidal anti-inflammatory drugs (NSAIDs) have led some individuals to believe these medications may blunt Tc increases during exercise, theoretically preventing exertional hyperthermia or heat illness.[10] NSAIDs target cyclo-oxygenase (COX), which is responsible for catalyzing prostaglandins from arachidonic acid. COX-1 is inherently present in tissues throughout the body and yields prostaglandins that regulate gastric mucosa, platelet aggregation, and renal blood flow. COX-2 is present in small amounts and upregulated during injury/illness by inflammatory mediators to produce prostaglandins that promote pain, inflammation, and fever.[11,12] NSAIDs reduce fever by inhibiting the inflammatory and fever signals to the hypothalamus.

Human and animal investigations evaluating the ability for NSAIDs to alter Tc and inflammatory markers during exercise are limited and conflicting.[13–15] The possibility exists that NSAIDs may compromise thermoregulation and exacerbate Tc increases by altering GI, liver, renal, and/or cardiovascular functions.[15–20] Among 19 ultra-marathon runners, NSAID use was not correlated to the inflammatory cytokine interleukin-6 (IL-6) and had no effect on Tc.[21] However, Tc was assessed aurally,[21] which is an inaccurate Tc assessment in exercising individuals.[22] Only aspirin and ibuprofen have been evaluated in well-designed, randomized controlled trials. The majority of investigations found that Tc did not differ between NSAID and placebo.[23–26] These investigations utilized males and females ~20–40 years old, who exhibited a maximum aerobic capacity (VO_{2max}) of ~40–65 mL/kg/min, and lean to average body fat percentage (~10–24%). The exercise type and intensity varied considerably between studies and included running, walking, or cycling for 45–60 min at set speeds, inclines, or %VO_{2max}.[23–26] Environment also varied between studies, ranging from cool (~18–22 °C)[25,26] to hot (~40 °C) temperatures. A recent investigation utilizing aspirin in older, less aerobically fit adults showed significant Tc increases during passive heat exposure, compared to placebo, Tc remained higher during 120 min of cycling in a warm environment.[18] Elevated Tc was attributed to aspirin decreasing skin blood flow, which prevented heat dissipation[18]; no measures were taken to determine whether aspirin had an anti-inflammatory effect.

NSAIDs are commonly used among the general public and physically active persons,[27–30] yet a lack of investigations examining NSAID effects exist, particularly non-aspirin medications, on physiological responses during exercise. As part of a larger study, we sought to determine the acute dose effects of naproxen on Tc during exercise and assessed plasma IL-6 to determine whether any Tc difference could be attributed to an inflammatory mechanism. Based on NSAIDs' potential adverse effects on the GI tract, we hypothesized naproxen would increase Tc and plasma IL-6 concentrations significantly during exercise in the heat compared to placebo controls.

Materials and methods

Participants

Seventeen participants (12 males, 5 females) began the study; 6 participants withdrew due to difficulty, time commitment, or an unrelated injury. Our final sample size was 11 participants (6 male, 5 female; demographics in Table 1). Specific information on inclusion and exclusion criteria is presented in a previous publication[31]; briefly, participants were free from chronic diseases, moderately endurance-trained (based on a graded cycling VO_{2max} test), and were not using analgesic or anti-inflammatory medications or treatments during the study. The study was conducted in accordance with the Declaration of Helsinki and the University of South Carolina's Institutional Review Board (PRO00024476). All participants read and signed an approved informed consent form prior to data collection.

Experimental conditions

We used a double-blind, randomized and counter-balanced design. All participants completed 4 experimental conditions: 1) placebo and ambient (Control); 2) placebo and heat (Heat); 3) naproxen and ambient (Npx); and 4) naproxen and heat (NpxHeat). The 24-h naproxen dose was 3 pills with 220 mg of naproxen sodium per pill. We chose this naproxen dosage based on previous investigations examining similar 24-h NSAID dose effects on GI and inflammatory responses.[32–34] The placebo was 3 pills with 0 mg of naproxen sodium per pill. Capsules were compounded to look the same to blind participants and primary investigators to naproxen or placebo trials. Participants were randomized into a trial order and prepared capsules in coded, sealed envelopes. We provided take-home instructions to participants with directions for when to take the pills – 1 pill each at 16 h, 8 h, and immediately before exercise – and to take each pill with 8 oz of water and not with food. These instructions were intended to maximize potential naproxen induced effects within safe prescription guidelines. The exercise trials took place in either a hot

Table 1
Participant demographics (mean ± standard deviation).

	Aggregate (n = 11)	Male (n = 6)	Female (n = 5)
Age (years)	27.8 ± 5.7	28.7 ± 5.3	26.8 ± 6.5
Weight (kg)	79.1 ± 17.9	88.4 ± 14.0	67.9 ± 16.5
Height (cm)	177.0 ± 9.5	183.2 ± 5.3	169.5 ± 7.8[a]
Body fat (%)	15.2 ± 8.3	10.7 ± 4.0	20.6 ± 9.1[b]
VO_{2max} (mL/kg)	41.4 ± 5.7	43.6 ± 5.3	38.7 ± 5.3

Abbreviations: VO_{2max} = maximum oxygen consumption.
[a] Significantly less than males ($F_{1,9}$ = 11.856, p =0.007).
[b] Significantly greater than males ($F_{1,9}$ = 5.954, p = 0.037).

environmental chamber (35.7 ± 1.3 °C, 53.2 ± 3.2% relative humidity) or ambient laboratory (22.7 ± 1.8 °C, 52.4 ± 5.5% relative humidity). A minimum of 7 days separated each experimental trial to ensure there were no residual effects of naproxen or exercise prior to the next trial.

Instruments and protocols

Demographics

Age, sex, and height were self-reported. Weight and body fat percentage were measured using a bioelectrical impedance analysis scale (Tanita SC-331S Body Composition Monitor, Tanita Co., Tokyo, Japan).

Cycle exercise protocol

During the screening session, we used the VO_{2max} test to familiarize participants with the stationary cycle ergometer (Monark Ergomedic 828E, Monark Exercise AB, Vansbro, Sweden). To maintain consistency throughout trials, seat and handlebar position were noted and the participant used the same cycle ergometer. For each trial, participants completed a 90-min cycle protocol. Before starting a 3-min warm-up, researchers provided participants with their target heart rate (HR) corresponding to 70% VO_{2max} to maintain during 80 min of steady-state cycling.[35] The mean target HR for participants = 125 ± 7.1 bpm. Heart rate was continuously monitored using Polar HR monitors (Polar Electro Inc., Lake Success, NY). Participants were able to see the HR monitor and researchers provided verbal cues to assist participants in maintaining HR throughout. Following 80 min, participants cycled 10 min at maximum effort. To encourage participants to maintain maximum effort, research assistants gave verbal encouragement throughout the 10 min. For additional motivation, the top 5 mileages completed during the 10 min were posted on a board in the laboratory, and researchers encouraged participants to try to "get on the board" or beat a certain mileage. The exercise protocol ended with a 5-min cool-down.

Core temperature

We measured Tc using rectal thermometry (Doric 450 Series digital thermometer, VAS Engineering, Inc., San Diego, CA). Participants inserted the probe 10 cm past the anal sphincter. Baseline Tc was assessed approximately 10 min before beginning the cycle protocol while participants were still in an ambient environment. Participants were continuously monitored and Tc recorded every 5 min during exercise. Participants were not allowed to continue a trial if Tc exceeded 40 °C. The last exercise Tc measure occurred at 90 min (conclusion of the 10-min maximum effort). Core temperature was recorded at 5 min (active bike cool-down) and 10 min post-exercise (5-min seated cool-down). Participants' Tc was measured at 15 min post-exercise and then every 15 min after that during the 3-h rest period until the participant reached baseline (pre-exercise Tc) or the rest period concluded.

Inflammatory cytokines

Blood was collected from a cubital vein pre-, post-, and 3-h post-exercise into two 6 ml K_2EDTA vacutainer tubes and inverted several times to mix. IL-6 was assessed using enzyme linked immunosorbent assay high sensitivity kits (R&D Systems human IL-6 Quantikine ELISA kit, R&D Systems, Inc., Minneapolis, MN). IL-6 was chosen because IL-6 is a prominent pro-inflammatory cytokine that induces other acute-phase immune responses,[36] and elevated IL-6 is observed in EHS patients[37] and during exercise in the heat.[35,38]

Hydration, nutrition, and physical activity

We attempted to control factors known to affect thermoregulation and inflammatory responses, including nutrition, hydration, and recent physical activity. To maintain hydration during exercise, participants drank 3.5 ml/kg of water every 15 min. Hydration status was measured at pre-, post-, and 3-h post-exercise using urine specific gravity (Usg) by a handheld clinical refractometer (model REF 312, Atago Company Ltd., Tokyo, Japan). Euhydration was defined as Usg ≤1.020.[39] To control for pre-data collection dietary and physical activity influences, participants tracked the diet and physical activity for 3 days prior and 1 day after data collection using an online nutrition software FoodProdigy (ESHA Research, Salem, OR). Participants were encouraged to maintain a similar diet and physical activity habits during the 24 h prior to each data collection session.

Procedures

Approximately 72 h before data collection, we contacted participants with instructions to refrain from intense, vigorous exercise[40] and to begin their diet and physical activity logs. Approximately 24 h before data collection, participants were given a sealed envelope with 2 pills and instructions. We also reminded participants to refrain from consuming alcohol and exercising and to maintain normal sleep behaviors. Participants were encouraged to consume fluids, such as water, to ensure euhydration upon arriving for data collection. Lastly, we instructed participants to consume a small meal (e.g., bagel, peanut butter and jelly) at least 2 h before arriving to ensure no food was in the stomach.

Upon arrival at the laboratory for data collection, we verbally asked participants if they took the 2 pills and then provided them their third pill. We obtained a urine sample to verify participants were euhydrated,[41] and obtained a baseline Tc and blood sample. Participants completed the 90-min cycling bout and researchers continuously monitored Tc and HR. After cycling, participants rested for 5 min where post-blood was collected. Participants then rested for 3 h in a semi-reclined/seated position in a laboratory set at room temperature (23 °C and 56% RH). Throughout the rest, researchers recorded Tc every 15 min. Researchers collected blood at the end of 3 h.

Statistical analysis

IBM SPSS Statistics (version XXII; IBM Corporation, Armonk, NY) were used for all analyses. the significance level was set at $p < 0.05$. A-priori power calculations using standard deviations for Tc and IL-6 indicated a sample size of 8 was necessary to achieve a statistical power of 0.8. We obtained appropriate statistical power with our participant number ($n = 11$), and this number is consistent with previously published research.[32,35,42,43] Descriptive statistics (mean and standard deviations) for all dependent variables were calculated. A one-way ANOVA assessed differences in demographics (age, height, weight, body fat %) between conditions and sex. Two repeated-measures ANOVAs assessed Tc during exercise [4 (condition) x 20 (cycling)] and rest [4 (condition) x 9 (rest)]. Regression analysis examined the rate of Tc increase for each experimental condition. For IL-6, we conducted a 4 (condition) x 3 (time: pre-, post-, and 3-h post-cycling) repeated measures ANOVA. Greenhouse-Geisser corrections were used for Tc and IL-6 when sphericity was violated. We conducted post-hoc analysis with Bonferroni corrections on significant main effects.

Results

Table 1 presents participant demographics. Height and body fat percentage were significantly different between sexes. Five of 6 female participants used oral contraceptives. One participant reported a history of exertional heat illness. All participants began experimental trials euhydrated (Usg = 1.012 ± 0.005) and remained euhydrated to post- (1.011 ± 0.008) and 3 h post-exercise (1.007 ± 0.006). As mentioned, this study was part of a larger project; we previously reported no significant differences between experimental conditions for hydration, maximum HR, perceived GI symptoms (e.g., cramping, bloating, nausea), or performance measures.[31,41] Dietary analysis revealed no significant differences in calories, protein, fats, and carbohydrates 1-day prior to data collection. No differences were found in macronutrient intake the morning of the trials (i.e., small meal prior to arriving) between conditions. The overall morning average consumption = 253.8 ± 169.5 cal,

protein = 8.2 ± 5.5 g, fats = 7.3 ± 8.0 g, and carbohydrates = 39.8 ± 27.6 g. Physical activity level 24-h before remained low to sedentary and was not significantly different between conditions. Based on maintaining a euhydrated status and the lack of difference in hydration, nutrition, and physical activity between trials, we are confident that Tc changes are not attributed to increased cardiovascular strain due to hypohydration, inadequate or differences in nutrition, or fatigue or immune responses from recent physical activity.

Core temperature

Fig. 1 presents Tc changes throughout the exercise and rest for each experimental condition. Overall, Tc significantly increased pre- (37.1 ± 0.4 °C) to post-exercise (38.2 ± 0.3 °C, $F_{1.7,67.3} = 150.5$, $p < 0.001$). During rest, Tc significantly decreased over time ($F_{2.0,81.5} = 201.6$, $p < 0.001$) and reached baseline by 75 min post-exercise. Starting at 60 min of cycling, mean Tc was higher in heat trials compared to ambient (Fig. 1) and remained higher throughout rest. However, no statistically significant Tc differences existed between experimental conditions at any time point. Fig. 2a–d illustrates individual participants' Tc, overall mean, and regression statistics for each condition during exercise. Both Heat and NpxHeat followed a linear regression model with no difference in slope between conditions. Similar results were found for Tc during rest, where no significant differences in slope between conditions were found.

IL-6

We did not have complete blood data sets for 4 participants due to the inability to complete blood collection at 3-h post-cycling. Therefore, we present IL-6 results for 7 participants only. Differences in IL-6 between pre-, post-, and 3-h post-exercise are presented in Fig. 3. No significant differences were found between conditions at any time point. A significant change in mean IL-6 occurred over time ($F_{1.4,48} = 41.8$, $p < 0.001$), with IL-6 increasing pre- (0.54 ± 0.06 pg/ml) to post-exercise (2.46 ± 0.28 pg/ml, 95% $CI = -2.62$ to -1.23, $p < 0.001$) and remaining significantly higher than pre-at 3-h post- (1.17 ± 0.14 pg/ml, 95% $CI = -1.01$ to -0.23, $p = 0.001$). For each condition, post-exercise IL-6 was significantly higher than pre- and 3 h post-cycling (Fig. 3). At 3-h post-exercise, both Npx (1.06 ± 0.73 pg/ml) and Heat (0.97 ± 0.49 pg/ml) were significantly higher compared to pre-exercise Npx (0.44 ± 0.29 pg/ml, 95% $CI = 0.05$ to 1.18, $p = 0.038$) and Heat (0.44 ± 0.29 pg/ml, 95% $CI = 0.13$ to 0.93, $p = 0.018$).

Discussion

Due to anti-pyretic and anti-inflammatory effects, some individuals theorize NSAIDs can blunt Tc rise during exercise.[10] This conjecture remains unsupported by research, which in part may be explained by the different mechanisms between exertional hyperthermia and fever. However, it is also plausible that NSAIDs' adverse effects on the GI tract and cardiovascular system may negate any thermoregulatory benefit during exercise.[10] Aspirin and ibuprofen are highly accessible and used in clinical practice; therefore, they are predominately used in scientific investigations. Naproxen, another commonly used NSAID in clinical practice, has not been investigated. We sought to determine whether a 24-h, over-the-counter naproxen dose would affect thermoregulation and inflammation during moderate-intense exercise. In contrast to our hypothesis, naproxen had no effect on Tc or IL-6 during exercise in either an ambient or hot environment.

Our results are consistent with findings in males administered 7.8 g of sodium salicylate who walked in a hot environment,[42] and with males administered aspirin acutely (1.5 h pre-exercise) or chronically (daily doses for 2 and 7 days) before walking in ambient conditions.[43] These studies[42,43] and ours are in contrast to Bruning et al.,[18] who showed, compared to placebo, low dose aspirin (81 mg daily for 7 days) significantly increased Tc during 30 min of passive heat stress.[18] Increased Tc remained significantly greater throughout 2 h of moderate-intense cycling and was attributed to peripheral vasoconstriction that prevented individuals from dissipating heat.[18] Blunted skin blood flow is understandable considering aspirin's efficacy as a cylooxygenase-1 inhibitor, altering vascular function and irreversibly mediating platelets.[44]

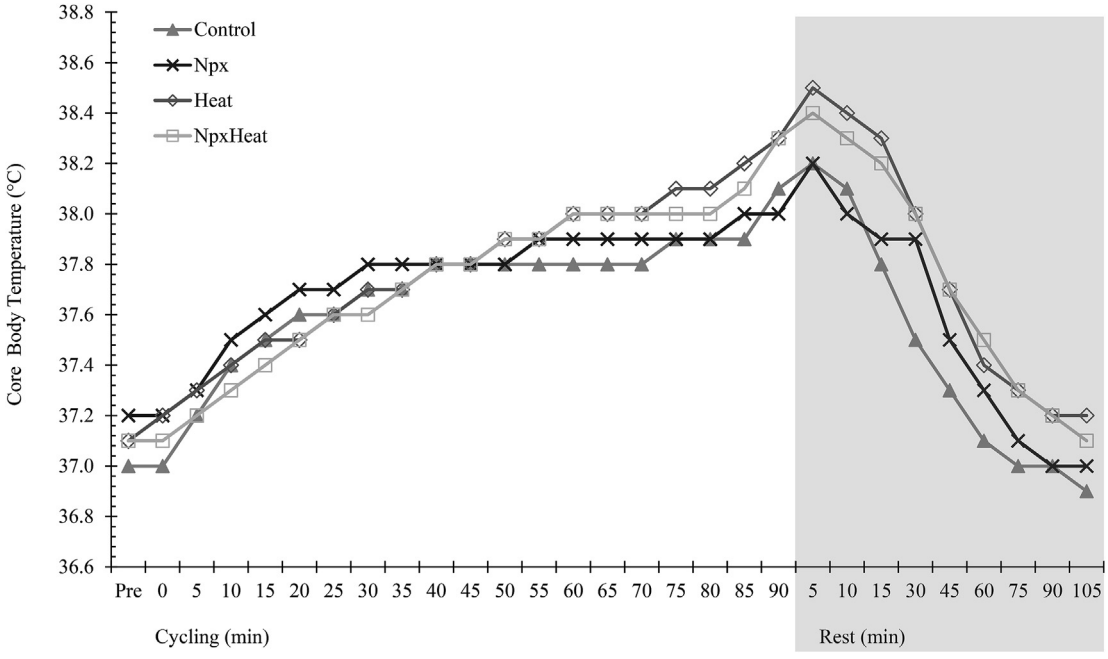

Fig. 1. Mean core body temperature during cycling and rest for experimental conditions. Significant increase in core body temperature during exercise ($F_{1.7,67.3} = 150.5$, $p < 0.001$) and decrease during rest ($F_{2.0,81.5} = 201.6$, $p < 0.001$). No significant difference between experimental conditions. Abbreviations: Control = placebo and ambient environment, Heat = placebo and hot environment, Npx = naproxen and ambient environment, NpxHeat = naproxen and hot environment.

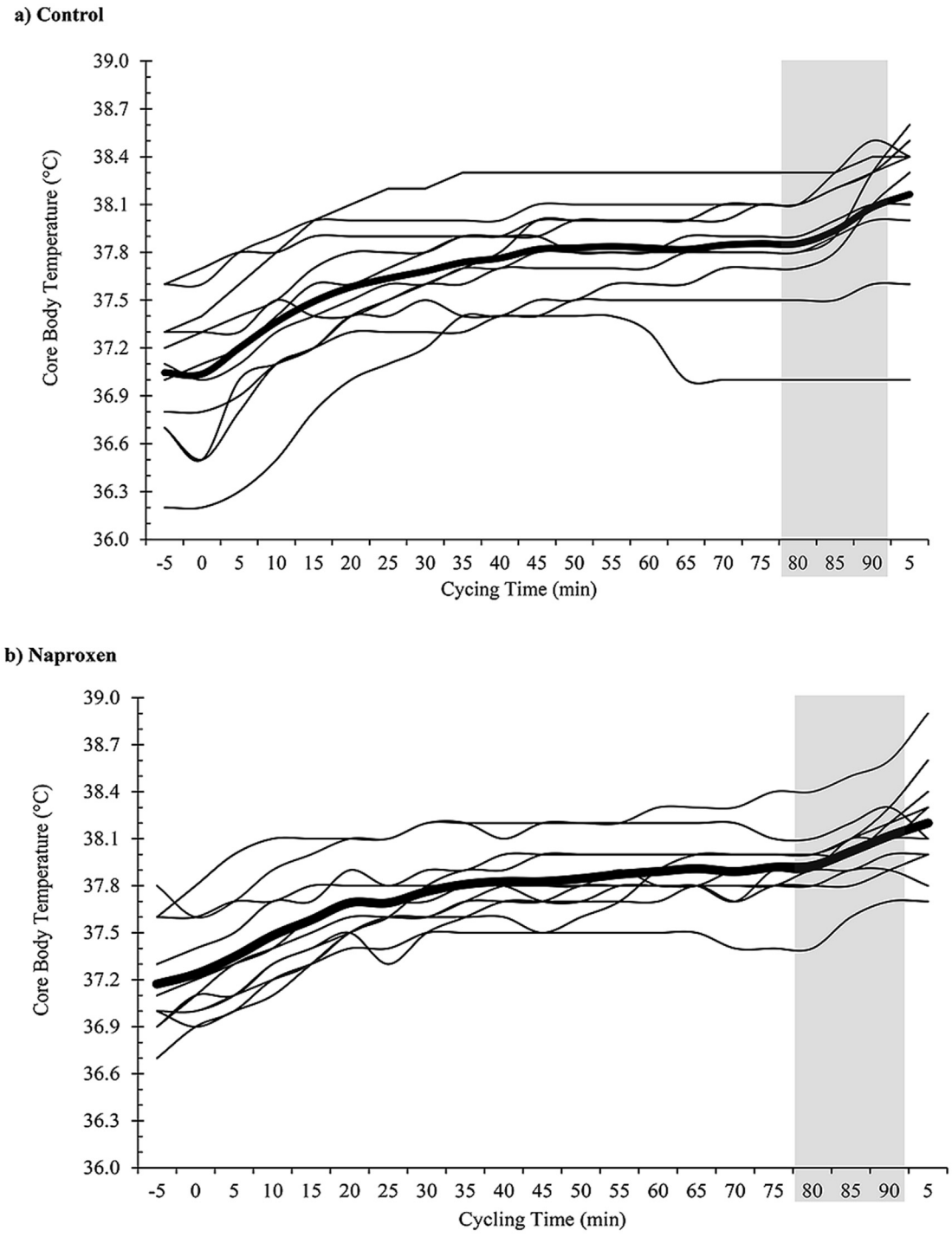

Fig. 2. Regression and participant core temperature during cycling for a) Control, b) Naproxen, c) Heat, and d) Naproxen and Heat. Bold line represents mean core temperature for condition. Shaded area indicates 10-min cycling at maximum effort. Final 5 min is an active cool down. Final Control model: $\hat{y} = 0.03x + 37.3$; $r^2 = 0.59$; 95% *CI* = 37.1, 37.5; $p = 0.001$. Final Naproxen model: $\hat{y} = 0.02x + 37.4$; $r^2 = 0.58$; 95% *CI* = 37.2, 37.6; $p = 0.002$. Final Heat model: $\hat{y} = 0.01x + 37.4$; $r^2 = 0.54$; 95% *CI* = 37.2, 37.6; $p < 0.001$. Final Heat and Naproxen model: $\hat{y} = 0.01x + 37.4$; $r^2 = 0.56$; 95% *CI* = 37.2, 37.6; $p < 0.001$.

Compared to aspirin, naproxen has a greater affinity toward cyclo-oxygenase-2,[45] making naproxen more effective at reducing pain and inflammation and less effective at mediating cardiovascular function.[46] Because skin blood flow was not measured, we are unable to make a definitive statement whether naproxen elicited any effect through peripheral vasoconstriction. Based on our lack of finding Tc changes, the acute naproxen dosage likely does not cause skin blood flow changes. With higher dosages (i.e., prescription strength) or longer use (i.e., 7 days), the possibility exists that we would have found a similar response as Bruning et al.[18]

Our overall IL-6 increase pre-to post-exercise was expected and similar to other scientific investigations using moderate-intense cycling.[47] Our lack of significant IL-6 changes between naproxen and placebo is consistent with studies showing neither 1000 mg of aspirin[48] nor 400 mg of ibuprofen[49] significantly affected plasma inflammatory cytokine concentrations. Ibuprofen's effects on exercise-driven

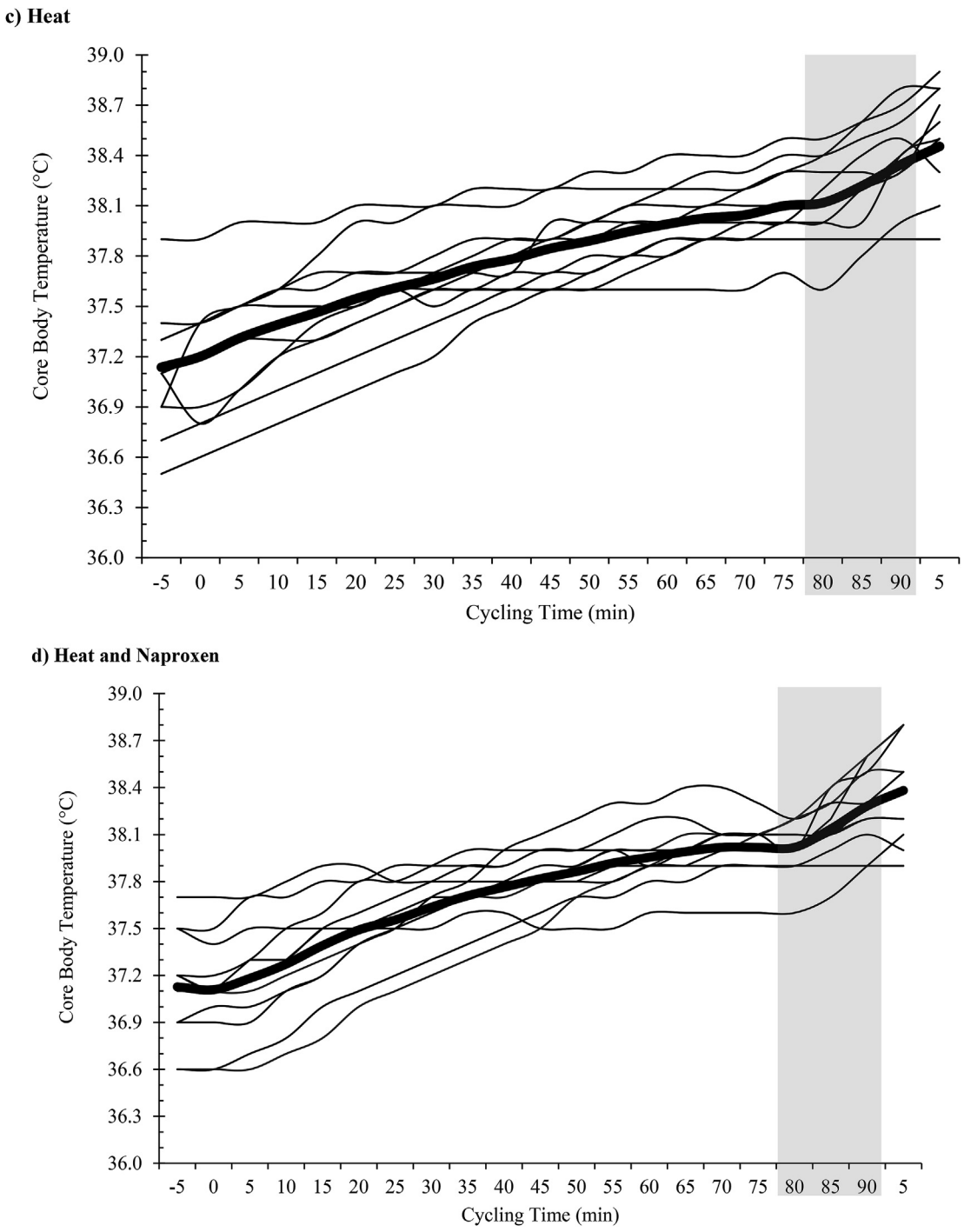

Fig. 2. (*continued*).

inflammation appear to be based on dosage. While 400 mg 2 h prior to ultra-distance running made no difference in IL-6,[49] 600 mg the afternoon prior and 1200 mg on the day of an ultra-distance race resulted in significantly higher post-race IL-6.[34] We found 3–220 mg of naproxen over 24 h prior to exercise did not significantly increase or decrease IL-6 compared to placebos. If dosing was increased and/or extended across multiple days, a significant effect may have been found. Our relatively large IL-6 standard deviations, particularly post-exercise among males (Appendix A, Figure A), likely prevented us from identifying significant effects. Skeletal muscle contraction during exercise produces a high amount of IL-6.[50] Male participants did not weigh significantly more than females, but males had greater muscle mass, as indicated by their significantly lower body fat percentage (Table 1). Furthermore, participants' work output during the 10-min maximum cycling effort likely varied. A greater muscle induced IL-6 response would be seen among participants with more muscle mass and if they were working harder.

As previously mentioned, a Tc of 40.5 °C does not necessarily trigger EHS, and individual risk factors (e.g., medication or supplement use, recent illness, poor cardiovascular conditioning, inadequate sleep) can perpetuate someone experiencing EHS at a Tc $<$ 40.5 °C or in a relatively cool environment. The complex EHS etiology requires looking at individual variability to understand how, together, these factors may influence EHS risk. For instance, one participant, who did not use oral contraception and was in the luteal phase when she completed the

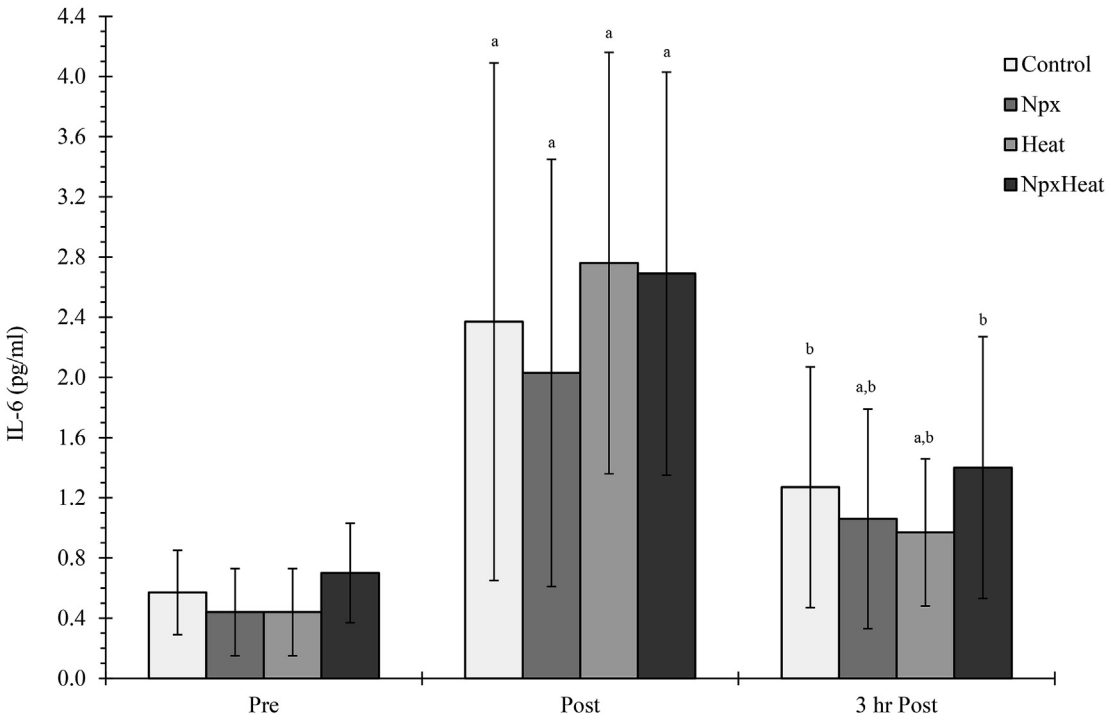

Fig. 3. Mean interleukin-6 (IL-6) at pre-, post-, and 3 h post-cycling for experimental conditions. $n = 7$. [a]Significantly higher than pre-within condition ($p < 0.04$). [b]Significantly lower than post-within condition ($p < 0.04$). Abbreviations: Control = placebo and ambient environment, Heat = placebo and hot environment, Npx = naproxen and ambient environment, NpxHeat = naproxen and hot environment.

NpxHeat trial, experienced her highest post-exercise Tc and IL-6. This response was not found during the luteal phase in any other female participant. This female was also the one participant who reported a previous exertional heat illness, had the highest body fat percentage, and the lowest VO_{2max} among females. Another consideration for individual risk factors is in the participants who were unable to complete their trials. A female participant during the NpxHeat trial experienced hypotension and severe nausea; her trial was stopped. Although her pre-trial measures were within normal limits, upon inquiry, the participant reported feeling tired and having a GI illness within a few days before this trial. One male participant was unable to begin a data collection trial due to extreme GI symptoms and illness after initiating the naproxen dose; they later withdrew from the study. These examples support the need to inquire about NSAID use in physically active persons and educate individuals about potential adverse events, particularly if they also present with other known exertional heat illness risk factors.

Limitations and future research

Our primary limitation was using a constant HR during 80 min of cycling rather than using the work rate corresponding to 70% VO_{2max}. Using constant HR decreased the cycling intensity during heat trials due to cardiovascular shifts in response to thermal stress (increased HR and blood pressure). If we had used work rate or power output corresponding to 70% VO_{2max}, the increased cardiovascular strain, particularly in the heat, would have raised Tc and increasing the difficulty to determine the direct effects of naproxen in the different trials. While many exercise sessions are completed at a set work rate or intensity (e.g., min per mile), our results are useful for individuals who may use a set HR to pace themselves during exercise. Another study limitation is only measuring IL-6 in 7 participants, which is one less than required for appropriate statistical power and could have prevented us from identifying significant differences between conditions. Tumor necrosis factor-alpha is another prominent pro-inflammatory cytokine that increases several hours after exercise[51] and would provide insight into naproxen's effects over the 24 h post-cycling. Another concern is the inclusion of both females who used and females who did not use oral contraception. We did not initially control for this based on previous research showing Tc increases similarly during moderate-intense exercise in the heat for both oral contraceptive and non-oral contraceptive users.[52] We also did not initially control for the menstrual cycle phase. Post-hoc, we identified female participant's menstrual phase for each trial, and no significant difference existed in resting Tc in either the follicular or luteal phase. We acknowledge the possibility that including both oral contraceptive and non-oral contraceptive users and not conducting all trials during the same menstrual phase (i.e., luteal) for all female participants affected our results. Finally, despite our attempts to control certain factors (e.g., nutrition), we assume participants were honest with completing the diet and physical activity logs and complying with recommendations to avoid certain foods, medications, and activities.

Future research should examine non-aspirin NSAIDs in higher doses, particularly evaluating prescription strength and long-term use (e.g., 14 days). We also encourage investigations that consider obtaining outcome measures during peak plasma drug concentrations. Aspirin peaks quickly (about 30 min) while naproxen peak plasma concentrations occur 1–3 h after administration. We administered the last naproxen dosage approximately 1 h before the cycling protocol began. Presumably, any drug effect on Tc would correspond with peak concentrations, and while it is possible our peak concentrations occurred after the exercise protocol, we did not see a difference between trials during the 3-h rest period. Scientific investigations are warranted in hot environments using greater cycling intensity and other exercise modes. Running is shown to place additional strain on the GI tract,[53] which may exacerbate responses to our various trials. Considering that GI barrier integrity is associated with EHS models, future research should examine plasma biomarkers for changes in GI permeability related to NSAID use and exercise in the heat. We also suggest research on potential sex differences. Our low participant number did not allow us to evaluate possible sex differences. Studies in individuals with musculoskeletal injuries or compromised immune system are also pertinent. Our study participants were non-heat

acclimatized, moderately endurance trained, euhydrated, and presumably had adequate nutrition and sleep prior to trials. Future investigations need to examine how individuals taking NSAIDs who are hypohydrated, have inadequate nutrition, poor sleep, poor endurance training, or who have other risk factors that place additional strain on thermoregulation are affected during physical activity.

Conclusions

Our results suggest taking a 24-h naproxen dose prior to completing moderate-intense exercise in either ambient or hot environments does not significantly increase Tc or IL-6 in healthy, moderately endurance-trained, euhydrated individuals. We also suggest an acute naproxen dose before exercise does not appear to provide a thermoregulatory benefit via an anti-pyretic or anti-inflammatory mechanism. Based on the lack of available research in this area and well-established knowledge that NSAIDs' can adversely affect the cardiovascular and GI systems,[18,19,32,33] we encourage physically active persons and clinicians working with physically active populations to be conscious of the potential negative effects NSAID have on thermoregulation during exercise, particularly if the person has predisposing factors.

Submission statement

This manuscript has not been published and is not under consideration for publication elsewhere.

Authors' contributions

Conceptualization and Study Design: DME, JMD, SCLC, TMTM, CCE; Data Acquisition: DME, TMTM, CEP, CCE; Data Analysis and Interpretation: DME, JMD, SCLC, TMTM; Drafting Manuscript: DME; Critical Revision of Manuscript: DME, JMD, SCLC, TMTM, CEP, CCE; Final Approval of Manuscript: DME, JMD, SCLC, TMTM, CEP,CCE.

Ethical approval statement

The study was conducted in accordance with the Declaration of Helsinki and the University of South Carolina's Institutional Review Board (PRO00024476). All participants read and signed an approved informed consent form prior to data collection.

Conflict of interest

Portions of the study were supported by funding from the American College of Sports Medicine Foundation and the National Athletic Trainers' Association Research and Education Foundation. The agencies had no role in the study design, data collection or analysis, or the writing and submitting for publication. The authors have no other conflict of interests to disclose.

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APPENDIX A

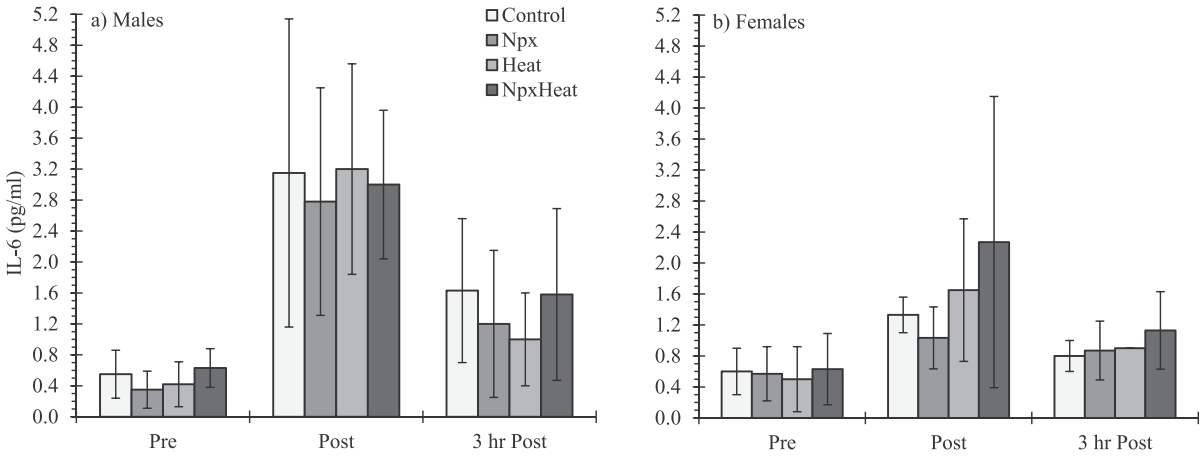

Fig. A. Mean interleukin-6 (IL-6) pre-, post-, and 3 h post-cycling for a) males (n = 4) and b) females (n = 3). Abbreviations: Control = placebo and ambient environment, Heat = placebo and hot environment, Npx = naproxen and ambient environment, NpxHeat = naproxen and hot environment.

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