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Effect of Ibuprofen on Markers of Acute Kidney Injury, Intestinal Injury, and Endotoxemia after Running in the Heat

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Abstract

Purpose: To test the hypothesis that ibuprofen ingestion exacerbates markers of acute kidney injury (AKI), gastrointestinal (GI) injury, and endotoxemia after running in the heat.

Methods: Using a randomized double-blind crossover design, eleven physically active individuals (six women) ingested 600 mg of ibuprofen or placebo 12- and one-hour prior to running one-hour in a heated chamber (35 °C, 20%–60% R.H.) at an intensity of 60% $\dot{V}O_{2peak}$. Blood and urine samples were collected pre-, post-, and one-hour post-exercise to assess cytokines and markers of AKI, GI injury, and endotoxemia.

Results: One hour of running in the heat increased markers of AKI (urinary product of IGFBP7•TIMP2 [Placebo: $\Delta 1.8 \pm 0.8 \log_{10}(\text{ng/ml})^2/1000$, Ibuprofen: $\Delta 1.8 \pm 0.9 \log_{10}(\text{ng/ml})^2/1000$], urinary NGAL, and serum cystatin C), GI damage (I-FABP [Placebo: $\Delta 631 \pm 446$ pg/ml, Ibuprofen: $\Delta 576 \pm 455$ pg/ml]), and inflammatory cytokines (TNF α [Placebo: $\Delta 5.2 \pm 3.5$ pg/ml, Ibuprofen: $\Delta 6.2 \pm 4.9$ pg/ml], IL-6, IL-10, and MCP-1), but these changes were not exacerbated by ibuprofen ingestion. There were effects of time ($p < 0.001$) and condition ($p = 0.03$) for serum IL-8, with greater concentrations in the ibuprofen (pre: 11.4 ± 5.1 pg/mL, post: 15.5 ± 7.3 pg/mL) trials than placebo (pre: 9.7 ± 4.2 pg/mL, post: 11.7 ± 5.4 pg/mL). There were no effects of time or condition on markers of endotoxemia (LBP [Placebo: $\Delta -1.2 \pm 3.2$ $\mu\text{g/ml}$, Ibuprofen: $\Delta 1.0 \pm 1.6$ $\mu\text{g/ml}$], sCD14).

Conclusions: These findings indicate that ibuprofen ingestion does not worsen intestinal or renal injury experienced during one hour of exercise in the heat, but increases pro-inflammatory IL-8.

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Keywords

CYTOKINE; EXERCISE; EXERTIONAL HEAT STRESS; NSAID

INTRODUCTION

Endurance athletes frequently use non-steroidal anti-inflammatory drugs (NSAIDs) to reduce pain and inflammation induced by overuse injuries including iliotibial band friction syndrome, medial tibial stress syndrome, and Achilles tendonitis (1) (2) (3). Ibuprofen is an NSAID that inhibits cyclooxygenase isoforms 1 and 2 (COX-1 and COX-2), which are responsible for the conversion of arachnoid acid into prostaglandins (4). When synthesized, prostaglandins serve many functions throughout the body in the promotion of pain, fever, and inflammation (5) (6). Although ibuprofen use and consequential inhibition of prostaglandin synthesis are acutely beneficial for athletes by mitigating inflammation and soreness, there are also side effects that may impair performance, training adaptations, and health (7).

In the kidney, decreased prostaglandin synthesis via ibuprofen causes vasoconstriction of the afferent arterioles, thereby decreasing renal perfusion (8). This response may increase risk of acute kidney injury (AKI) (9), causing a sudden loss of the kidney's ability to excrete waste and regulate water and electrolyte balance (10). AKI is typically caused by renal ischemia, hypoxia, or nephrotoxic substances (11) and is characterized by a decreased glomerular filtration rate (GFR), leading to a buildup of waste products in the blood that would otherwise be excreted in urine (11). Over time, AKI is known to increase mortality, with this risk being dependent on the severity of AKI (12). Prolonged physical activity in the heat, as seen in endurance athletes and many workers (i.e. construction, agricultural workers, firefighters), increases AKI risk due to vasoconstriction in the afferent arterioles of the kidney primarily via increased renal sympathetic nerve activity (13). The resultant decrease in renal blood flow causes localized ischemia within the kidney. Similarly, inhibiting the synthesis of prostaglandins via ibuprofen and decreasing renal perfusion may exacerbate renal strain experienced during physical activity in the heat. Limited research has investigated the combined effects of ibuprofen ingestion and exercise in a hot environment on AKI. In a field-based study, McDermott et al. (2018) found that 600 mg of ibuprofen prior to a cycling event (either a 100- or 161-kilometer distance, 5.2 ± 1.2 hour completion time) in hot conditions (33.2 °C, 38.4% R.H.) had no effect on markers of AKI (serum creatinine and neutrophil gelatinase-associated lipocalin; NGAL) compared to placebo condition. While this study did not find an effect of ibuprofen, it is important to highlight that serum creatine and serum NGAL are less specific for detecting early renal cellular stress and injury than other markers (15). Also, core temperature was not assessed and this project relied on data collected in the field, which may have masked the full effects of ibuprofen due to a lack of ability to control for potential confounding variables.

In addition to the potential for renal injury, a well-known side effect of ibuprofen use is gastrointestinal (GI) distress, with symptoms including nausea, indigestion, and abdominal pain (16). Indeed, ibuprofen ingestion prior to exercise exacerbates symptoms of GI distress

and biomarkers of GI damage and permeability (17) (18) (19). As a result of ibuprofen use, prostaglandin inhibition decreases intestinal blood flow and can directly injure the intestinal barrier via mitochondrial damage and uncoupling of oxidative phosphorylation (20) (21) (22). Intestinal barrier dysfunction increases permeability, allowing microbial contents to translocate from the intestinal lumen into systemic circulation, reaching various tissues throughout the body (23). Of particular concern is endotoxemia, the translocation of lipopolysaccharides (LPS) from gram-negative bacteria. Circulating LPS can activate innate immune cells, leading to local and systemic inflammatory responses, including upregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF α) (24). Elevated circulating LPS and associated inflammation are linked to sepsis, metabolic disorders, increased risk of heat-related illness, and AKI (25) (26) (27) (28) (29) (30) (31). There is also evidence of a bidirectional relationship between intestinal permeability and AKI, mediated by changes within the immune system (31) (32) (28). Heat strain, often caused by exercise or physical activity in high environmental temperatures, may increase GI permeability and represents another major risk factor for endotoxemia (33) (34). Therefore, athletes and other individuals who engage in physical activity in warm climates may be at greater risk of experiencing damage to renal and intestinal tissues. With many physically active individuals regularly consuming NSAIDs and the increasing environmental temperatures due to global warming (35), it is important to understand the effects of taking ibuprofen prior to exercise in a hot environment.

Although McDermott et al. (2018) found no differences in AKI markers for a long, cycling race, it remains unknown how ibuprofen ingestion prior to moderate-intensity, one hour exercise in a hot environment impacts markers of AKI and GI injury. Therefore, the two aims of this study were to assess the effects of ingesting ibuprofen prior to running in the heat (35 °C, 20%–60% R.H.) on change in markers of 1) AKI and kidney function and 2) intestinal damage and endotoxemia. We hypothesized that ibuprofen ingestion would result in exacerbated kidney and GI injury following exercise in the heat, inducing a greater immune response.

METHODS

Subject Recruitment

Twelve physically active adults (six males, six females) between the ages of 18 and 45, who self-reported engaging in greater than 150 minutes per week moderate-intensity physical activity in the past six months, were recruited for this study. All testing and data collection were conducted at the Exercise Physiology Laboratory at the University of New Mexico during the summer months (June-August). All subjects resided in Albuquerque, New Mexico, and reported regularly exercising outdoors in the heat. Participants were excluded if they had a history of heat illness, stomach ulcers, were taking blood thinners, were pregnant (assessed prior to each visit using a urine pregnancy test), or had known allergies to NSAIDs. Participants came for a total of three visits including baseline testing and two experimental trials: one while consuming 600 mg of ibuprofen 12- and one-hour prior to exercise, and one while consuming an equivalent amount of corn starch (placebo) at the same time points. The protocol and procedures were approved by the local university

institutional review board (protocol #2210021198), were registered as a Clinical Trial (Clinical Trials ID: [NCT06247462](#)), and were conducted in accordance with the Declaration of Helsinki.

Baseline Testing

During their initial visit, participants read and signed an informed consent document and completed a PAR-Q to be screened for health risks. Resting blood pressure was measured manually by the same researcher and subjects were excluded if their systolic and diastolic blood pressure were greater than 140 or 90 mmHg, respectively. If participants met the criteria to be in the study, height was measured with no shoes on using a stadiometer (SECA, seca 216, Chino, Calif., USA). While wearing a t-shirt and shorts, body weight and body composition were measured using a bioelectrical impedance scale (InBody770, InBody USA, Cerritos, CA). After a 10-minute running warm-up at a self-selected intensity, participants completed a baseline, graded exercise test on a treadmill to measure peak oxygen consumption ($\dot{V}O_{2peak}$). The testing protocol was individualized, with either speed or grade increasing every minute, with a goal for the test to last between eight and 12 minutes (36). The graded exercise test was completed when the participant reached volitional fatigue. No other procedures were used to verify true $\dot{V}O_{2max}$, thus the term $\dot{V}O_{2peak}$ is used throughout. Participants then underwent a 10-minute recovery period where they walked at a self-selected intensity. Finally, participants were instructed to run on the treadmill for a series of three-minute stages to determine the exercise intensity corresponding to 60% of their $\dot{V}O_{2peak}$. This intensity was used for the exercise during the experimental trials. Oxygen consumption measured throughout the maximal exercise test and subsequent identification of 60% of $\dot{V}O_{2peak}$ was measured using eight-breath averages from the same metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT), which had been calibrated in accordance with manufacturer guidelines prior to each trial.

Experimental Trials

In a double-blind, randomized, crossover design, participants consumed either 600 mg of ibuprofen or placebo (corn starch) at both 12 hours and one hour prior to their exercise trials. A member of the research team not involved in the data collection or analysis was responsible for blinding and randomization using a random number generator. No confirmation of blinding was assessed. Participants came to the laboratory at least 48 hours after their baseline testing for these trials and were asked to refrain from using NSAID's for one week prior to experimental trials. Participants were instructed to consume capsules, which appeared identical between conditions, 12 hours prior to their morning exercise trial (which began between 7:00 and 9:00 AM), and to adequately hydrate. Participants consumed a standardized breakfast provided by the researchers, containing between 400 and 600 calories (85–92 g carbohydrate, 5–12 g protein, 5–9 g fat), two hours prior to their exercise trial. Breakfasts were chosen by participants from a list of options which included selecting one carbohydrate/grain (oatmeal or granola bars), one fruit (banana or three clementines), and one juice (apple or orange). The breakfast chosen by each participant was matched during the second and third visit. Upon arriving at the laboratory one hour prior to exercise testing, participants ingested 600 mg of ibuprofen or placebo in unmarked capsules, then voided their bladder, and urine was assessed for hydration status by determining if the

urine specific gravity (USG) was ≤ 1.020 (37). If subjects did not meet this criterion, trials were rescheduled. Next, participants waited for one hour without consuming any fluids and urine was again collected and the volume was measured to determine urine flow rate (UFR). This urine was also measured for USG and stored for later analysis.

Participants sat for 10 minutes before a blood draw was taken to collect serum and plasma. Next, nude body weight was measured, and participants were equipped with a heart rate monitor (Polar H10, Kempele, Finland) around their chest. Participants inserted a rectal thermistor 10 cm past the anal sphincter (Level 1 esophageal/rectal temperature probe, Smiths Medical, Minneapolis, MN, USA), which was connected to a receiver (4600 precision rectal thermometer, YSI Temperature, Dayton, OH). The participants then began their exercise trials, consisting of 60 minutes of treadmill running at 60% $\dot{V}O_{2peak}$ in a temperature-controlled environment set at 35 °C (Placebo: 35.1 ± 0.9 °C, Ibuprofen: 35.2 ± 0.9 °C, $p = 0.37$) and 45% relative humidity (Placebo: $44.0\% \pm 14.0\%$, Ibuprofen: $45.8\% \pm 11.9\%$, $p = 0.22$). The treadmill speed and grade were maintained constant during and between the trials, respectively. During the exercise trials, measures of core temperature, heart rate, rating of perceived exertion (38), thermal strain and comfort (39), dry bulb temperature, and humidity (QUESTemp QT-44, TSI QUESTemp, Shoreview, MN) were recorded at baseline and every ten minutes. At 30 minutes, participants ingested three milliliters of cool tap water (~ 12 °C) per kilogram of body weight. The rationale of the water temperature was to simulate what athletes (i.e., runners) usually experience during races at the water stations. As a safety measure to reduce the risk of exertional heat illness, exercise trials were immediately terminated if participants reached a core temperature of greater than 40.0 °C. GI symptoms were recorded via a visual analog scale, adapted from Gaskell et al., (2019), at pre-morning ingestion, pre-exercise, 30-minutes, post-exercise, and one-hour post-exercise. The scale included 18 GI symptoms classified as upper (belching, heartburn, upper abdominal bloating, stomach pain, urge to regurgitate), lower (flatulence, lower abdominal bloating, urge to defecate, abnormal stool), or other (nausea, dizziness, stitch) GI symptoms, on a scale from 0–10 where 0 indicates no symptoms; 1–3 indicates very mild symptoms; 4–6 indicates severe symptoms; and 7–10 indicates very severe symptoms. Scores from all time points were combined to assess incidence of GI symptoms.

After the exercise trials, participants were removed from the heat chamber and after 10 minutes in a seated position, a venipuncture to collect blood was performed. Urine was then collected, followed by nude body weight measurement. For the following hour, participants ingested a volume of water equivalent to the change in body weight from pre- to post-exercise. After this hour, another blood draw was conducted, and urine was collected to measure USG and UFR. The same experimental protocols with the alternate treatment were repeated for the final visit at least seven but no more than 28 days later (average of 16 ± 7 days between trials).

Analyses of Urine and Blood Samples

Urine was collected pre-, post-, and one-hour post-exercise and was immediately analyzed to measure volume for UFR and to manually assess USG using a refractometer (Cole-Parmer, RSA-BR90A, Vernon Hills, IL). After these initial analyses, urine was transferred to two mL

tubes and stored at -80°C until later analyses. Blood was collected into serum separating and EDTA vacutainer tubes through venipuncture from the antecubital vein immediately pre-, post- and one-hour post-exercise. To measure hematocrit, whole blood was transferred in triplicate to microcentrifuge tubes and centrifuged for five minutes. They were then analyzed via visual inspection and recorded as the nearest whole number using a micro-hematocrit reader (Micro-Capillary Reader No. 2201, International Equipment Company, Needham Heights, MA). Whole blood for serum samples was left to clot for approximately 30 minutes before centrifugation. Blood samples were centrifuged at 2200 rpm for 15 minutes at 22°C to separate plasma and serum, respectively. They were then pipetted into two mL tubes respectively and stored at -80°C until later analyses.

Concentrations of intestinal fatty acid binding protein (I-FABP) (Hycult Biotech, Uden, The Netherlands), lipopolysaccharide binding protein (LBP) (Hycult Biotech, Uden, The Netherlands), and soluble cluster of differentiation 14 (sCD14) (Hycult Biotech, Uden, The Netherlands) were measured at pre- and post-exercise timepoints in the plasma using enzyme-linked immunosorbent assay (ELISA) kits to assess intestinal damage (I-FABP) and endotoxemia (LBP, sCD14). To assess AKI, ELISA kits were used to measure concentrations of insulin-like growth factor-binding protein 7 (IGFBP7) (RayBiotech Life, Peachtree Corners, GA), tissue inhibitor of metalloproteinase 2 (TIMP-2) (RayBiotech Life, Peachtree Corners, GA), and neutrophil gelatinase-associated lipocalin (NGAL) (BioPorto, Gentofte, Denmark) in urine, while serum cystatin C (RayBiotech Life, Peachtree Corners, GA) was also analyzed to assess renal function at pre-, post-, and one-hour post-exercise timepoints. Urine samples were centrifuged and separated prior to analysis. Samples were diluted prior to analyses for I-FABP (1:2), LBP (1:1000), sCD14 (1:120), IGFBP7 (1:50), TIMP-2 (1:60), NGAL (1:250), and cystatin C (1:5000) to ensure the obtained values were on the standard curve for each ELISA. The product of IGFBP7 and TIMP-2 was calculated as this is a valid AKI marker approved by the US Food and Drugs Administration (NephroCheck[®]), where values greater than $0.3\text{ ng/mL}^2/1000$ are considered an indication of increased risk for experiencing AKI in the following 12 hours (41) (42) (43). While the Kidney Disease Improving Global Outcomes guidelines define clinical AKI as a urine output of $< 0.5\text{ mL/kg/hour}$ for at least six hours or an increase of serum creatine $\geq 0.3\text{ mg/dL}$ within 48 hours or 1.5-fold from baseline in seven days, these are normal physiological responses to exertional heat stress and may overestimate AKI (15). Thus, the aforementioned markers were used as they are more specific to renal damage rather than physiological changes in response to exertional heat stress and may be more accurate in assessing subclinical AKI (15). All ELISA analyses were completed according to manufacturer guidelines. Serum samples for pre- and post-exercise were sent to a commercial laboratory (Eve Technologies, Calgary, Canada) for analysis of a 15-plex assay of cytokines (Assay HDF15). Serum samples used to determine cytokine concentrations were not diluted prior to analyses. All assays were analyzed in duplicate, and intra-assay coefficient of variations for all markers were $<10\%$.

Data Analyses

To determine the sample size necessary to detect differences in the urinary NGAL response between placebo and ibuprofen conditions, an *a priori* power analyses was conducted using

the effect size ($\eta^2 = 0.33$), an α -level of 0.05, power of 0.80 ($1 - \beta$), and assuming a correlation among repeated measures of 0.50. The effect size was used from a previous study by Semen et al. (2020), who investigated if runners that consumed NSAIDs prior to a race had greater increases in urinary NGAL. The analysis revealed a sample size of eight was required to detect differences in the NGAL response. A required sample size of eight subjects was also determined to detect differences using the same parameters and an effect size of $\eta^2 = 0.30$ for I-FABP based off of a study by Van Wijck et al. (2012) investigating the impact of ibuprofen on GI injury during exercise in thermoneutral conditions. Power analyses were completed using G-Power version 3.1.9.6 software.

Two-tailed paired sample t-tests were conducted to assess differences in peak core temperature and change in body weight between ibuprofen and placebo conditions. Changes in cytokines and markers of intestinal damage and endotoxemia were tested using two-way repeated measures analyses of variance (ANOVA) tests for main effects of condition (ibuprofen, placebo) and time (pre-, post-exercise), as well as an interaction between time and condition. The same testing was done to analyze changes in kidney injury and function for [IGFBP7•TIMP2], NGAL, and cystatin C, but peak post-exercise values were used instead of post-exercise. Peak post-exercise values were defined as the highest value from either the immediately post- or one-hour post-exercise timepoints. Peak values were analyzed rather than both post- and one-hour post-exercise for markers of AKI, as the aim of the study was to determine peak kidney injury rather than the time-course response. Mixed-effects models were used for the aforementioned variables in the case of missing data points. Markers with missing data points included LBP (1 data point: pre-exercise placebo), IL-1 β (two data points: pre-, post-exercise ibuprofen, same subject), and IL-6 (five timepoints: one subject x all timepoints, one pre-exercise ibuprofen), which were missing due to falling outside the range of the standard curve.

Two-way repeated measures ANOVAs were used to assess changes in USG, UFR and hematocrit at pre-, immediately post-, and one-hour post-exercise timepoints. Changes in core temperature throughout the 60-minute exercise trials were compared using a mixed-effects analysis testing for the main effects of time (recorded at 10-minute intervals), condition (ibuprofen, placebo), and interaction between time and condition. This mixed-effects model was used to account for missing core temperature values, which occurred due to rectal probe malfunction during periods of testing. There were three times, from two trials (both placebo), when core temperature was not recorded. Any significant effects found during ANOVA or mixed effects model testing were further explored by comparing differences between different condition and time points using Tukey's multiple comparisons *post hoc* test.

Due to non-normally distributed data assessed via visual inspection of Q-Q plot, analyses for IL-1Ra, IL-4, IL-5, IL12p40, IL-13, interferon-gamma (IFN- γ), [IGFBP7•TIMP-2], and NGAL were completed after log₁₀ transformation. The totaled GI symptoms were compared between conditions using a non-parametric Wilcoxon signed-rank test to handle non-normally distributed data. Incidence of GI symptoms was defined as a score of one or greater and is reported as a percentage. All statistical analyses listed above were completed using GraphPad Prism 9.4 (GraphPad Software Inc., La Jolla, CA, USA). Using RStudio

(version 2021.09.2, R Development Core Team, Vienna, Austria), repeated measures correlations were conducted to assess the relationships between peak core temperature and the change (Δ) in LBP, sCD14, TNF α , IL-8, log₁₀[IGFBP7•TIMP2], and cystatin C from pre- to post- or peak post-exercise values. Effect size from pre-to post-exercise was calculated using Cohen's d for all intestinal, immune, and AKI markers. An alpha level of 0.05 was set to determine significance for all tests.

RESULTS

Subject Characteristics and Adherence

Twelve participants (six females) were screened for this study. One male subject was removed due to reporting to trials dehydrated, as initial assessment of USG upon arrival to the laboratory was under the threshold of 1.020, however the pre-exercise USG assessed just prior to exercise was over this threshold. One participant, during their placebo trial, reached a core temperature of 40.0 °C prior to completing the 60-minute exercise protocol, and was removed from the heat chamber at minute 55 of running. Participant characteristics can be found in Table 1.

Core Temperature, Change in Body Weight, and Hematocrit

There were no differences in peak core temperature between the ibuprofen (38.9 0.6 °C) and placebo (39.0 0.5 °C) conditions ($p = 0.73$) (Figure 1B). Similarly, there was no effect of condition ($p = 0.62$) or interaction of condition and time ($p = 0.78$) on core temperature during the 60-minute exercise intervention; however, there was an effect of time ($p < 0.001$), with core temperature significantly increasing at each 10-minute interval ($p < 0.05$) (Figure 1A). There was a similar loss in body weight between the placebo (1.76% 0.72%) and ibuprofen (1.82% 0.59%) conditions ($p = 0.27$). There was no effect of time ($p = 0.07$), condition ($p = 0.98$), or interaction ($p = 0.40$) for hematocrit. Baseline hematocrit was 45% $\pm$ 4% in the placebo condition and 45% $\pm$ 4% in the ibuprofen condition. For the placebo condition, post-exercise hematocrit values were 46% $\pm$ 6% and 46% $\pm$ 5% for immediately post- and one-hour post-exercise, respectively, while values for the ibuprofen condition were 46% $\pm$ 3% and 47% $\pm$ 5%.

Renal Function and Markers of Acute Kidney Injury

There was a significant time effect for UFR ($p < 0.001$), with decreases from pre- (473 $\pm$ 185 mL/hour) to post-exercise (77 $\pm$ 65 mL/hour), but there was no significant interaction effect ($p = 0.63$) or main effect of condition ($p = 0.62$). Similarly, there was a significant time effect of USG ($p < 0.001$), with increases from pre- (1.005 $\pm$ 0.004) to post- (1.012 $\pm$ 0.008), to one-hour post-exercise (1.017 $\pm$ 0.007), but there was no significant interaction effect ($p = 0.71$) or main effect of condition ($p = 0.60$). Post-hoc analyses showed that USG was only different between pre- and one-hour post-exercise in the placebo condition ($p < 0.001$), whereas both the post- ($p = 0.03$) and one-hour post-exercise ($p < 0.001$) values were significantly greater than the pre-exercise timepoint in the ibuprofen condition. There was an increase of urinary log₁₀ [IGFBP7•TIMP-2], from pre- to peak post-exercise (Placebo: Δ 1.8 $\pm$ 0.8 log₁₀(ng/ml)²/1000, Ibuprofen: Δ 1.8 $\pm$ 0.9 log₁₀(ng/ml)²/1000) with no difference between conditions (Figure 2, A and B). Exploring the nonlog transformed

data, four of the 11 (36%) participants in the placebo condition reached a peak post-exercise concentration greater than the NephroCheck® threshold of 0.3 ng/mL²/1000, whereas six (55%) participants surpassed this value in the ibuprofen condition. There was an increase of urinary log₁₀ NGAL from pre-exercise to peak post-exercise values (Placebo: $\Delta 0.7 \pm 0.5$ log₁₀ ng/ml, Ibuprofen: $\Delta 0.9 \pm 0.4$ log₁₀ ng/ml), with no difference between conditions, and no interaction effect (Figure 2, C and D). Using the non-transformed data, there was only one trial, in the placebo condition, where a participant's urinary NGAL surpassed the clinically used threshold of 150 ng/mL, indicative of increased risk of AKI (45). There was a significant main effect of time for serum cystatin C (Placebo: $\Delta 0.11 \pm 0.14$ mg/l, Ibuprofen: $\Delta 0.21 \pm 0.28$ mg/l), but there was not an interaction effect, or main effect of condition (Figure 2, E and F).

Gastrointestinal Symptoms and Markers of Damage and Endotoxemia

Results from the GI symptoms questionnaire can be found in Table 2. While not significantly different, the ibuprofen condition consistently had greater levels of incidence for upper, lower, other, and overall GI symptoms. Severity of symptoms throughout testing were low, as the highest reported symptom across all conditions and timepoints was a four out of 10, corresponding to “severe symptoms,” while all other reported symptoms were between one and three, corresponding to “very mild symptoms.” Running in the heat resulted in increased intestinal cell injury indicated by an effect of time for I-FABP (Placebo: $\Delta 631 \pm 446$ pg/ml, Ibuprofen: $\Delta 576 \pm 455$ pg/ml) (Figure 3, A and B). Three of the 11 (27%) participants in the placebo condition had a change in I-FABP greater than 1000 pg/mL which has been proposed be a clinically significant threshold for increased risk of microbial translocation and endotoxemia (46). Only one participant (9%) surpassed this value in the ibuprofen condition. There was no significant effect of time, condition, or interaction for either marker of endotoxemia (LBP [Placebo: $\Delta -1.2 \pm 3.2$ µg/ml, Ibuprofen: $\Delta 1.0 \pm 1.6$ µg/ml], sCD14 [Placebo: $\Delta 58 \pm 172$ ng/ml, Ibuprofen: $\Delta 119 \pm 216$ ng/ml]) (Figure 3, C-F). Results for circulating cytokines can be found in Table 3. One hour of running in the heat increased concentration of several circulating cytokines including IL-1Ra, IL-6, IL-8, IL-10, MCP-1, and TNFα, indicated by a significant effect of time ($p < 0.05$). Three cytokines were found to have a significant effect of condition, as ibuprofen increased circulating IL-8 and decreased IL-4 and IL-13. There was also a significant interaction effect for IL-13, and a *post hoc* analysis showed that the post-exercise timepoint was significantly lower in the ibuprofen condition compared to the placebo condition ($p = 0.002$). There was a significant interaction effect for IL-5, but *post hoc* testing revealed no differences between timepoints.

Relationship between core temperature and markers of AKI, intestinal injury, and inflammation

Of the seven variables assessed to investigate the relationship between peak core temperature and gut-cytokine-kidney crosstalk, there were four significant correlates found (Figure 4). A positive relationship was found between peak core temperature and change in log₁₀IGFBP7•TIMP2. Change in both AKI markers (log₁₀IGFBP7•TIMP2 and cystatin C) were positively associated with changes in TNFα. Lastly, there was a positive relationship between changes in sCD14 and IL-8.

DISCUSSION

The primary aims of this study were to assess the acute effects of ibuprofen ingestion prior to running in the heat on markers of AKI and renal function as well as intestinal damage and endotoxemia. Our primary findings were that 1) running at 60% $\dot{V}O_{2peak}$ for one hour in the heat increases markers of intestinal damage, immune response, and AKI risk while decreasing renal function, 2) ibuprofen ingestion prior to exercise does not exacerbate these responses, with the exception of IL-8 and 3) there are moderate, positive relationships separately connecting cytokine response to intestinal injury (sCD14 and IL-8) and kidney injury (TNF- α and IGFBP7•TIMP2/cystatin C). These findings indicate that ibuprofen may not worsen renal and GI injury in recreationally active individuals who perform one hour of exercise in the heat.

In this study, ibuprofen did not exacerbate the increase in markers of AKI (i.e., IGFBP7•TIMP2, NGAL, cystatin C). In the kidney, COX-1 inhibition results in increased renal blood flow and decreased blood pressure, while COX-2 inhibition causes the opposite response (47). The effect of ibuprofen on renal tissue is complex as it inhibits both COX isoforms, however epidemiological research indicates an adjusted odds ratio of 2.25 for new ibuprofen users experiencing AKI compared to non-users (48). We utilized IGFBP7•TIMP2 to assess AKI risk, as this is a marker approved by the US FDA in critically ill patients and has been shown to best predict AKI compared to other common biomarkers (43). Additionally, the combined IGFBP7 and TIMP-2 serves as a pre-injury biomarker, assessing combined damage to the proximal (IGFBP7) and distal (TIMP-2) renal tubules (43). To our knowledge, only one previous experimental study has investigated whether there are exacerbated markers of AKI under such conditions, and our study is the first to do so in a controlled laboratory setting. McDermott et al. (2018) utilized a field-based, randomized, placebo-controlled methodology to compare markers of AKI in subjects who consumed either 600 mg of ibuprofen or placebo prior to competing either a 100- or 161-kilometer (5.2 ± 1.2 hours) cycling event in the heat (33.2°C , 38.4% R.H.). Similar to our study, they found that there were no significant differences between the ibuprofen and placebo groups for markers of kidney damage (serum NGAL) or function (serum creatinine). However, both measures significantly increased from pre- to post-event. We utilized serum cystatin C rather than creatinine to assess renal function, as a systematic review by Wołynec et al. (2020) suggests that serum cystatin C may be a superior marker of GFR during exercise compared to serum creatinine, which is altered by muscle damage associated with the exercise. We also analyzed urinary NGAL to assess overall kidney injury and found no effect of ibuprofen. These findings do not align with Semen et al. (2020), who found that runners that used NSAIDs, specifically ibuprofen and/or naproxen, had higher levels of urinary NGAL after racing either 10- or 21.1-kilometers in a cool environment ($4\text{--}7^{\circ}\text{C}$). The lack of effect from ibuprofen on kidney injury markers and function may be related to the large degree of thermal and renal strain induced by our experimental protocol, as participants reached a peak core temperature of $\sim 39^{\circ}\text{C}$ on average and there were large effects of time noticed for IGFBP7•TIMP2, NGAL, cystatin C, UFR, and USG. This large effect may have overshadowed any impact of ibuprofen on the renal system.

Risk for AKI is increased by hyperthermia and dehydration, (49) which increase renal sympathetic nerve activity, causing decreased renal blood flow and resultant ischemia, inflammation, and oxidative stress at renal tissue (13). Indeed, Sato et al. (2019) demonstrated in mice that a 1 °C increase in core temperature, independent of environmental temperature, increased renal tubular injury, interstitial infiltration of monocytes and macrophages, and worsened albuminuria. We found a positive relationship between peak core temperature and IGFBP7•TIMP2 ($r=0.60$), which is slightly higher than a similar relationship ($r=0.42$) found by Hess et al. (2023) while investigating AKI risk when adhering to occupational heat stress recommendations. This risk may be mediated by cytokines, as we show that IGFBP7•TIMP2 and cystatin C were positively associated with circulating TNF- α . Previous research suggests that overexpression of TNF- α may contribute to ischemic renal injury. In mice, Gao et al. (2013) found that diabetic mice expressed greater kidney dysfunction and expression of TLR-4 mediated TNF- α expression after renal ischemia compared to non-diabetic mice, however treatment of TNF- α antibody resulted in renal protection.

The most common side effect of ibuprofen is GI distress; however, its use is still prevalent in athletes, occupational laborers, and military personnel (53) (54) (55) (56). While not statistically significant, we found that rates of incidence for upper, lower, other, and overall GI symptoms were at least twice as high in the ibuprofen condition compared to placebo. However, the overall severity of these symptoms experienced were low, with all reported symptoms except one being considered “mild.” Previous research in various environmental conditions has indicated that ibuprofen ingestion prior to exercise leads to significant increases in GI damage. Van Wijck et al. (2012) found that 400 mg of ibuprofen the evening prior and one-hour prior to cycling for one-hour at 70% of maximum workload capacity resulted in greater increases in circulating I-FABP and urinary lactulose/rhamnose (L/R) ratio, a marker of small intestinal permeability. A similar study conducted in our laboratory found that 600 mg of ibuprofen 12- and one-hour prior to exercise resulted in elevated levels of circulating I-FABP, LBP, sCD14 and urinary L/R ratio after cycling for one-hour in hypobaric hypoxia (19). Contrasting with these studies, we did not see a significant effect of ibuprofen ingestion prior to exercise on markers of GI damage (I-FABP), or endotoxemia (LBP, sCD14). Interestingly we did observe that LBP appeared to decrease slightly from pre-to post-exercise in the placebo condition ($d = -0.366$), while it slightly increased in the ibuprofen condition ($d = 0.299$). One difference in design between these studies and ours was that in their studies, an overnight fast preceded experimental trials, whereas participants in our study were given a standardized breakfast the morning prior to exercise. There are several proposed mechanisms by which NSAIDs contribute to GI damage, with many of which centered around NSAID-induced decreases in microvascular blood flow to splanchnic tissues, resulting in tissue hypoxia and consequential damage (20). In our study, this damaging response may have been attenuated by postprandial hyperemia to GI tissues (57).

A consequence of endotoxemia is activation of the innate immune system, specifically resulting in increased inflammatory cytokines, such as TNF α mediated by Toll-like receptor 4 (TLR-4) activation (58) (24). Spinaz et al. (1991) found that ibuprofen ingestion prior to induction of endotoxemia, characterized by increased circulating LPS due to intestinal

barrier damage, resulted in an increased concentration of circulating TNF α , indicating that ibuprofen exacerbates the inflammatory response to endotoxemia. However, we found no significant effect of ibuprofen on several classic circulating inflammatory cytokines including TNF α , MCP-1, IL-1 β , IL-1Ra, IL-6, IFN- γ or IL-10. Vingren et al. (2023) found that 600 mg of ibuprofen prior to a 164-km cycling race in a hot (33.3 ± 3.5 °C) environment resulted in a decreased response of anti-inflammatory cytokine IL-10, while not altering several other cytokines including pro-inflammatory TNF- α . A limitation of our study was that we were only able to assess these cytokines in circulation, and there may have been different responses at local tissues, as it has been shown that NSAID administration upregulates TLR-4 activation in the small intestinal mucosa (61).

A notable finding of our study was that IL-8 concentrations were greater in the ibuprofen condition compared to placebo, and change in IL-8 was positively associated with change in sCD14 ($r=0.62$). IL-8 is a chemokine that recruits neutrophils to tissue promoting inflammation after tissue damage or infection, (62). Elevated circulating IL-8 has been associated with several conditions including cardiovascular, liver, and metabolic diseases (63) (64) (65). As several tissues and mechanisms contribute to regulation of IL-8, we are unsure why ibuprofen had this effect. In fact, COX-2 is known to upregulate IL-8 in several tissues (66)(67), which would suggest that ibuprofen use would decrease IL-8 concentrations.

There are limitations to this study which may have impacted the results and application of data to real-world scenarios. First, it is possible that we were underpowered, particularly for measuring LBP, which was close to an interaction effect ($p = 0.07$) where participants in the placebo condition slightly decreased from pre- to post peak-exercise values ($d = -0.366$), and those in the ibuprofen conditions slightly increased ($d = 0.299$). Second, this study was conducted in young, healthy runners which may limit the application of these results, particularly because NSAID-related AKI is seen at higher rates in elderly individuals (65+ years old) (68). Future research is needed in other populations who use NSAIDs while performing physical activity in the heat (e.g., military personnel, agricultural workers, and elderly athletes). Another limitation was that we did not measure changes in hemoglobin and plasma volume, which may affect concentrations of circulating blood markers. Additionally, we did not track or control for menstrual cycles, which may have impacted baseline core temperature. Finally, the results from this study only demonstrate the response to acute use of ibuprofen, whereas prolonged or chronic use of NSAIDs may lead to different findings.

CONCLUSIONS

This was the first study to assess the effects of ibuprofen on kidney and GI injury after exercise in the heat, as well as the cytokine response. Our primary finding was that ibuprofen ingestion prior to running in the heat did not worsen markers of AKI, intestinal damage, or endotoxemia in moderately trained runners, however circulating IL-8 was increased. Independent of ibuprofen, markers of AKI were positively associated with peak core temperature and TNF- α , while there was also a positive relationship noted between sCD14 and IL-8. Additionally, one hour of running in the heat, independent of ibuprofen ingestion, increased inflammatory cytokines and markers of AKI and GI damage. These findings

demonstrate that ibuprofen use by young, healthy, active individuals who exercise in the heat may not worsen renal and GI injury.

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Conflict of Interest and Funding Source:

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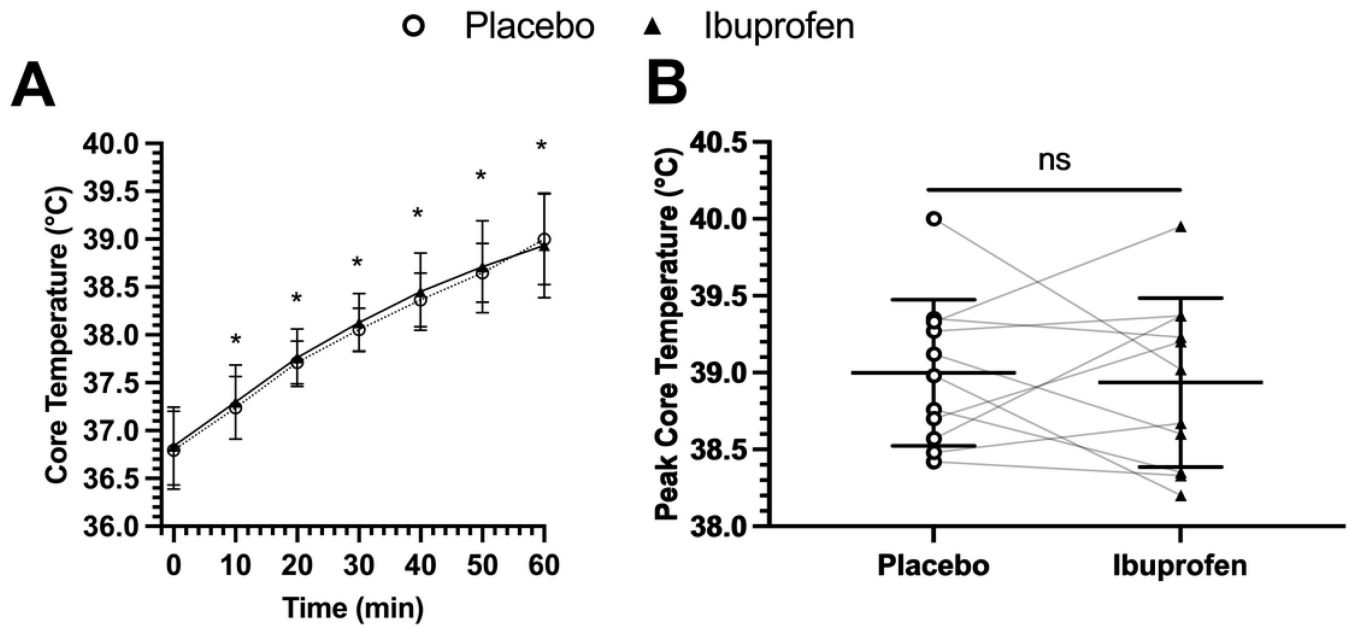


Figure 1.

Core temperature by condition (A) during 10-minute intervals over the 60-minute trial, and (B) peak core temperature.

Note: *indicates significant difference from previous 10-minute timepoint ($p < 0.05$), regardless of condition.

ns = no significant difference ($p > 0.05$).

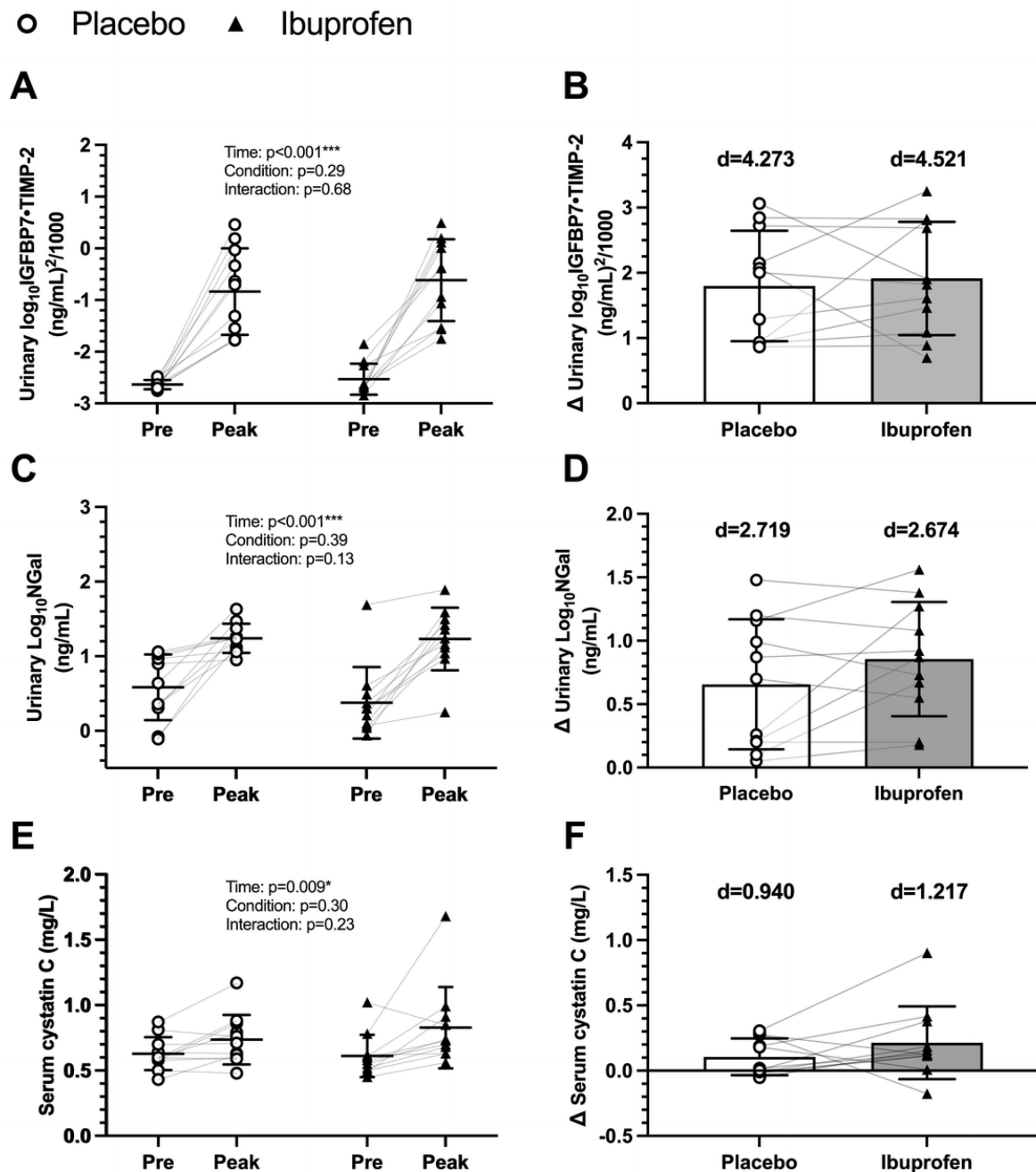


Figure 2.

Changes in markers of kidney injury (A, B) and kidney function (C) pre- to peak post-exercise values and a comparison of change (Δ) in the same markers (B, D, F) between placebo and ibuprofen conditions. Data are presented as individual data points and mean $\pm$ standard deviation, with individual responses shown in grey (N=11).

Note: significant main effect of time ($p < 0.05$) but not condition or interaction for urinary and serum markers ($p > 0.05$). * indicates significant difference of $p < 0.05$, *** indicates significant difference of $p < 0.001$.

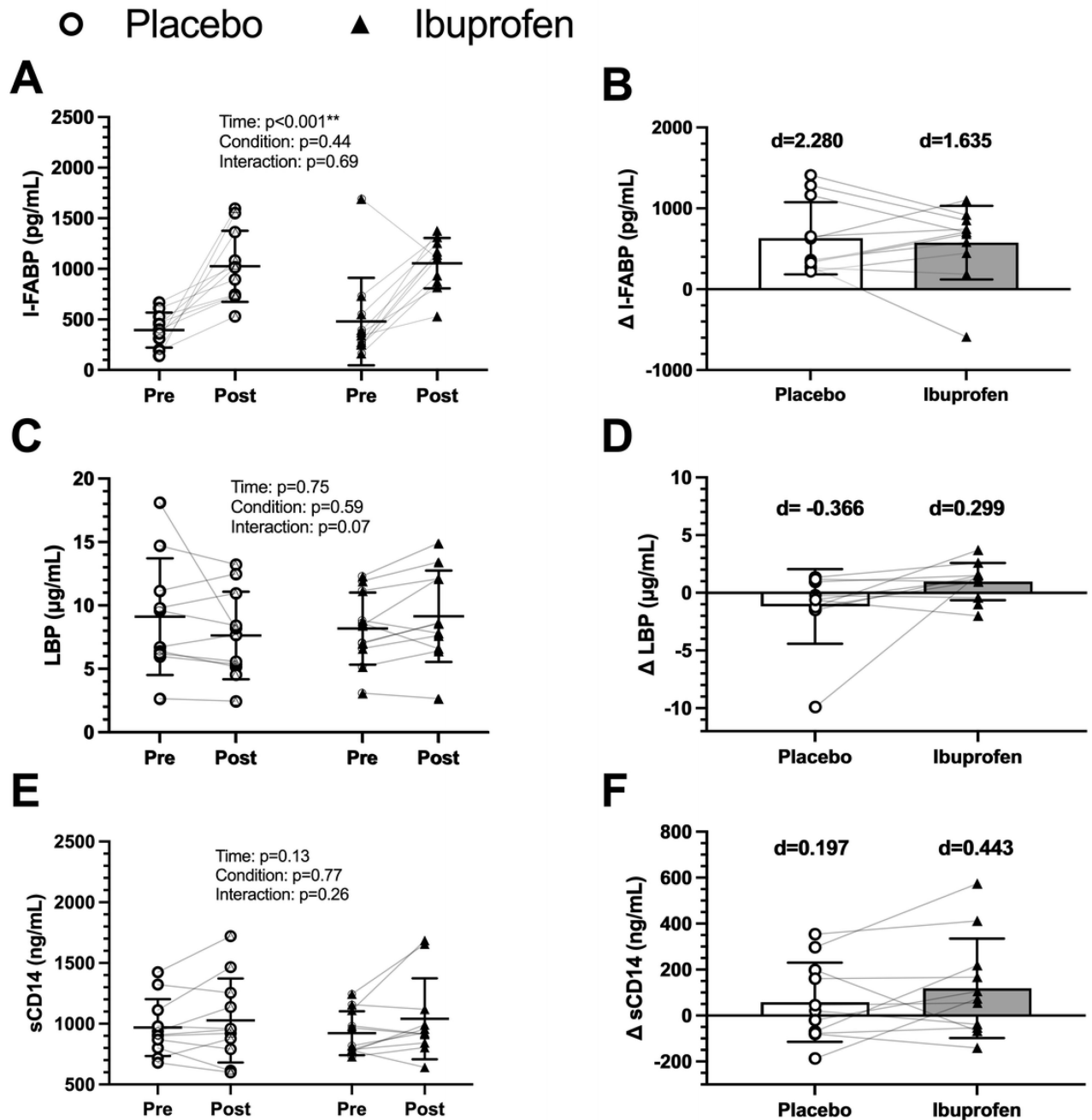


Figure 3.

Changes in plasma markers of intestinal damage and endotoxemia from pre- to post-exercise in the heat (A, C, E), and a comparison of change (Δ) in the same markers by condition (B, D, F). Data are presented as individual data points and mean $\pm$ standard deviation, with individual responses shown in grey (N=11).

Note: * indicates $p < 0.05$, *** indicates $p < 0.001$

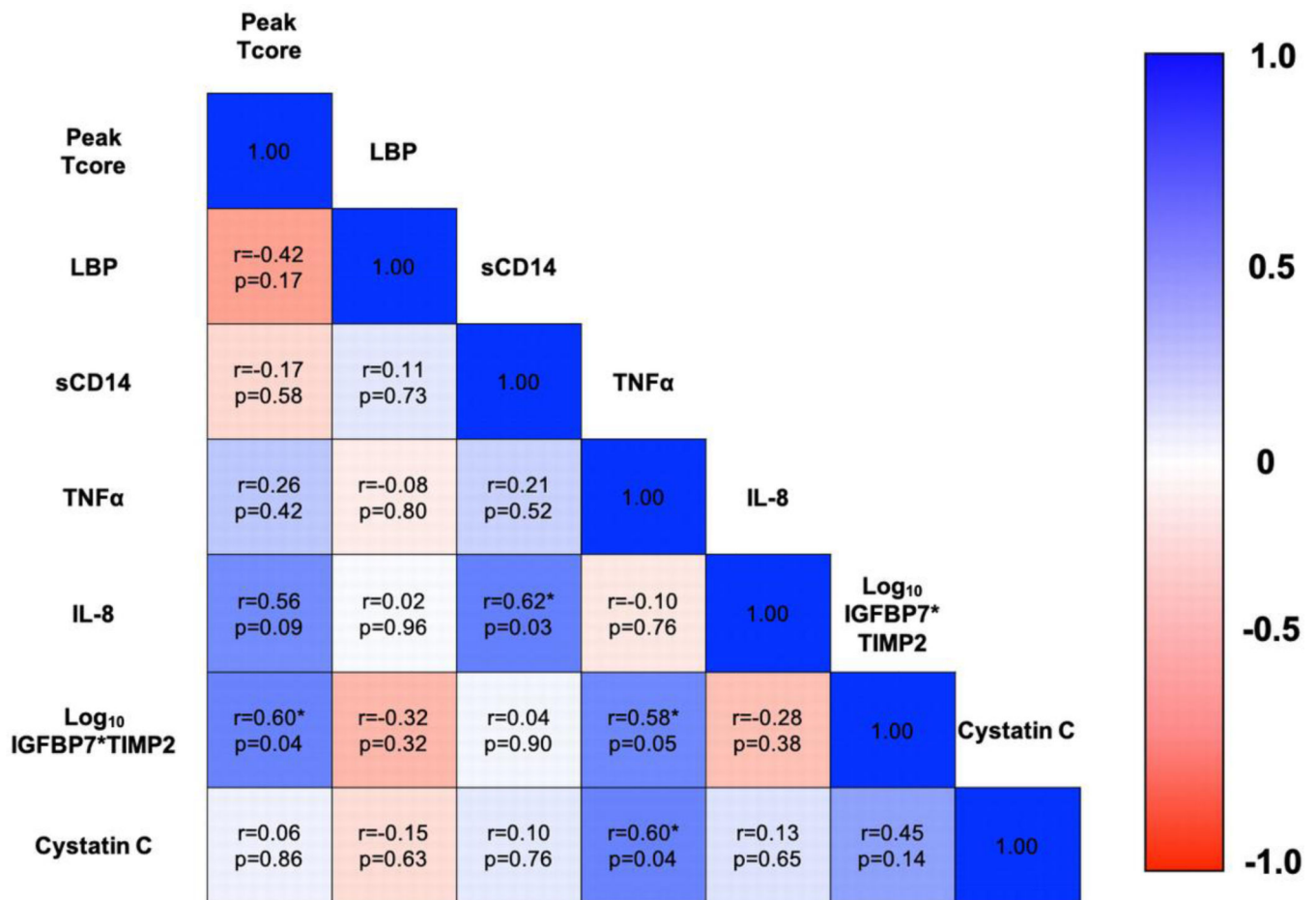


Figure 4. Repeated-measures correlation matrix assessing the strength of linear relationship[®] between peak core temperature and the change in (Δ) lipopolysaccharide binding protein (LBP), soluble cluster of differentiation 14 (sCD14), tumor necrosis factor- alpha (TNF α), interleukin-8 (IL-8), product of insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2), and cystatin C (N=11). Note: * indicates $p < 0.05$

Table 1.

Participant characteristics (N=11)

	Males (n=5)	Females (n=6)	Total (N=11)
Age (years)	30.2 ± 8.0	32.0 ± 3.7	31.2 ± 5.8
Height (cm)	183.1 ± 2.3	166.7 ± 7.4	174.1 ± 10.2
Weight (kg)	80.6 ± 11.6	60.4 ± 5.2	69.6 ± 13.4
Body Fat (%)	15.2 ± 6.5	15.9 ± 9.5	15.6 ± 7.9
$\dot{V}O_{2peak}$ (mL/kg/min)	50.5 ± 6.0	49.8 ± 8.0	50.1 ± 6.9

Note: $\dot{V}O_{2peak}$, peak oxygen uptake.

Table 2.
Gastrointestinal symptom scores for experimental conditions (N=11).

Symptoms	<u>Placebo</u>		<u>Ibuprofen</u>		p-value
	Incidence	Median (range)	Incidence	Median (range)	
Upper	36%	0 (0–10.5)	73%	2 (0–3)	0.25
Lower	18%	0 (0–5.5)	46%	0 (0–3)	0.22
Other	9%	0 (0–1)	18%	0 (0–4)	0.25
Overall	36%	0 (0–17)	82%	3(0–6)	0.18

Table 3.

Serum cytokine concentrations (pg/mL) between placebo (PLA) and caffeine (CAF) conditions before (Pre) and after (Post) 60-minutes of running in the heat (N=11).

<i>Cytokine</i>		Pre Mean ± SD	Post Mean ± SD	Cohen's d	Time p-value	Condition p-value	Interaction p-value
IL-1β	PLA	13.39 ± 14.12	13.42 ± 13.35	0.003	0.32	0.93	0.24
	IBU	12.16 ± 11.19	13.91 ± 11.55	0.15			
Log ₁₀ IL-1Ra	PLA	0.78 ± 0.30	0.86 ± 0.30	0.27	0.03 *	0.25	0.67
	IBU	0.74 ± 0.34	0.81 ± 0.35	0.19			
Log ₁₀ IL-4	PLA	0.07 ± 0.39	0.07 ± 0.39	0.007	0.79	0.03 *	0.50
	IBU	0.02 ± 0.37	0.01 ± 0.37	-0.04			
Log ₁₀ IL-5	PLA	0.69 ± 0.33	0.64 ± 0.53	-0.11	0.30	0.34	0.05 *
	IBU	0.60 ± 0.55	0.59 ± 0.56	-0.02			
IL-6	PLA	0.96 ± 1.77	2.96 ± 3.77	0.68	0.02 *	0.10	0.17
	IBU	0.60 ± 0.92	2.07 ± 2.65	0.74			
IL-8	PLA	9.71 ± 4.27	11.67 ± 5.40	0.40	<0.001 **	0.03 *	0.07
	IBU	11.36 ± 5.06	15.49 ± 7.29	0.66			
IL-10	PLA	3.13 ± 2.48	4.09 ± 3.11	0.34	0.02 *	0.27	0.20
	IBU	3.07 ± 1.98	4.75 ± 3.75	0.56			
Log ₁₀ IL12p40	PLA	1.68 ± 0.30	1.72 ± 0.34	0.13	0.15	0.36	0.67
	IBU	1.65 ± 0.25	1.68 ± 0.32	0.12			
Log ₁₀ IL-13	PLA	1.75 ± 0.45	1.80 ± 0.42	0.11	0.69	0.02 *	0.02 *
	IBU	1.73 ± 0.45	1.71 ± 0.48 †	-0.06			
Log ₁₀ IFN-γ	PLA	0.41 ± 0.49	0.40 ± 0.52	-0.02	0.48	0.23	0.53
	IBU	0.38 ± 0.48	0.34 ± 0.53	-0.08			
MCP-1	PLA	335.78 ± 106.17	394.99 ± 124.76	0.51	<0.001 **	0.21	0.41
	IBU	313.33 ± 110.32	387.27 ± 121.39	0.64			
TNF-α	PLA	38.65 ± 17.96	43.83 ± 18.20	0.29	<0.001 **	0.67	0.56
	IBU	37.71 ± 18.63	43.86 ± 16.30	0.35			

Notes: PLA indicates placebo condition, IBU indicates caffeine condition.

* indicates p<0.05

** indicates p<0.001

† indicates significant difference from same timepoint of alternate condition (p<0.05)